Eberhard Nieschlag · Hermann M. Behre ·
Sabine Kliesch · Susan Nieschlag *Editors*

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Male Reproductive Health and Dysfunction

Fourth Edition

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Preface

Since the publication of the third edition of *Andrology: Male Reproductive Health and Dysfunction* in 2009, andrology has established itself as an interdisciplinary specialty both clinically and scientifically. This is also reflected in the fact that the original 33 chapters in the third edition have now evolved into 50. The importance of the biological basis for the subject of andrology is reflected in the expanded initial chapters. The many cross-references to other chapters form a continuous bridge to the clinical chapters of the fourth edition.

Many of the original authors from the "Münster School" are also present in the fourth edition. New authors can be found among the authors of the current edition because of their specific expertise. In order to provide access to the relevant literature, the bibliographies are focused on the relevant current reference publications.

We thank the authors who completed their chapters in sometimes difficult times. Special thanks go to Ms. Susanne Sobich, Senior Editor, Ms. Alessandra Born and to the staff of Springer-Verlag for their patience. A special acknowledgment goes to Ms. Maria Schalkowski, who was already a member of the third edition team.

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Scope and Goals of Andrology

1

Eberhard Nieschlag and Hermann M. Behre

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Abstract

Andrology encompasses all areas of medicine and science dealing with the reproductive functions of the male under physiological and pathological conditions. It focuses on infertility and hypogonadism, as well as on sexual aspects, including erectile dysfunction, male senescence (the aging male) and male contraception. In this chapter infertility is treated as one of the main areas of andrology, with a special emphasis on diagnosis and therapy of the male in the context of the infertile couple. The chapter deals with the possibilities of male contraception as the complementary aspect of reproduction.

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1.1 Definition and Status of Andrology

Andrology is defined as the branches of science and medicine dealing with reproductive functions of the male under physiological and pathological conditions (Statutes of the European Academy of Andrology n.d.).

If this definition were to be interpreted in the context of sociobiology, considering reproduction the central task of life to which the entire organism is devoted (e.g., Dawkins 2006), andrology would be a broad field. Generally and also for the purpose of this book, andrology is considered the science and practice of dealing with male reproductive functions and their disturbances in the strict sense. Following the

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definitions of the WHO, **male reproductive health** is the subject of andrology (Fig. 1.1).

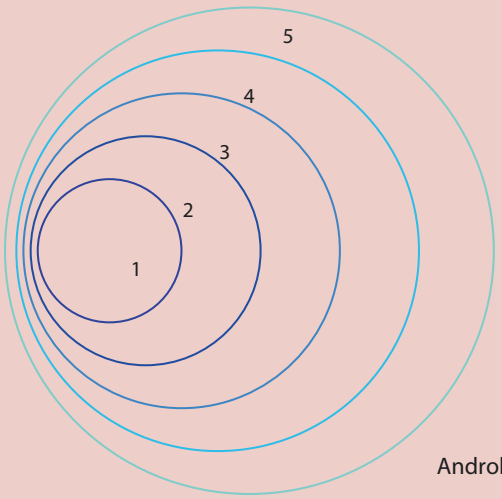


Fig. 1.1 Symbolic representation of development and scope of andrology

1.2 Andrology, Gynecology, Reproductive Medicine: Reproductive Health

Every layman knows that a man and a woman are necessary to produce offspring. However, an infertile couple does not necessarily know whether the affliction lies on the male or the female or both sides. Therefore, it would be sensible for the couples to turn to a physician in a discipline dealing holistically with problems of infertility.

As plausible as this idea may be to the layman, medicine has so far hardly taken this idea into account. Thus, the individual partners of the couple with an unfulfilled desire to have children still mostly seek out doctors from various disciplines in order to be diagnosed and treated. It is relatively easy for the woman to choose a competent doctor, as the traditional field of gynecology is widely known and represented. At least in Germany, one of the three subareas of gynecology and obstetrics has the title "**Reproductive Medicine** and Gynaecological Endocrinology", a term which is now also recognized by laypersons and used in the media. There are also numerous fertility centers throughout the country. However, the gynecologists working in these centers are not qualified in andrology, so their reproductive medicine remains incomplete or they have to work closely with andrologists in order to offer the couple the full range of reproductive medicine.

Until now, it has been more difficult for the man: if he suspected problems with his fertility, he did not immediately know whom to turn to. Thus, 93% of women with an unfulfilled desire for progeny turn to a gynecologist, while 44% of male partners turn to a urologist or 12% to a general practitioner, but 36% do not know whom to consult and fail to consult a physician at all (Münster et al. 2018).

However, with the (model) further training regulations (WBO) passed in 2003, the German Federal Medical Association (BÄK) introduced andrology as a sub-speciality (BÄK WBO 2019a). As the name suggests, it is a subspeciality pursuant to additional training in the fields of urology, dermatology, and endocrinology/ internal medicine, which can currently be acquired after 12 months of further training.

Thus, **andrology** has become an established medical field in Germany, so that the title "**andrologist**" may now be used by qualified physicians and is increasingly recognized by the general public. There are now 1657 physicians who are allowed to use the additional title of andrologist, of whom about two-thirds work in private practice and the remaining in inpatient care (BÄK Statistics 2019b).

So far, in the rest of Europe, the professional title of andrologist has only been recognized in Poland and Estonia and in Italy in connection with the specialist in endocrinology. In addition, Egypt and Indonesia are among the few countries where andrology is represented as a specialty. In order to achieve better care in male reproductive health with all its partial aspects, better training of medical students and physicians in andrology must be achieved (De Jonge and Barratt 2019; Cairo Consensus Workshop Group 2020). The **European Academy of Andrology (EAA)**, founded in 1992, is dedicated to this goal in Europe and has 30 training centers throughout Europe, five of them in Germany (in Bonn, Giessen, Halle, Leipzig, and Münster).

The **European Academy of Andrology (EAA)**, founded in 1992, has established over 30 training centers in Europe. These centers offer an 18-month course in which qualified physicians can acquire the specialty training evaluated according to the credit point system and can pass the required EAA examination (EAA Homepage 2020). The EAA has adopted a pacemaker function for official recognition of andrology throughout Europe. The acceptance of the EAA training objectives by the German Federal Medical Board is an acknowledgement of this goal.

From the point of view of the afflicted couple, a specialized interdisciplinary field of **reproductive medicine** would seem to offer a solution. A few such services are in fact avail-

able at universities or in individual practices. It is, however, seldom that both partners of an infertile couple are cared for by one person; usually an interdisciplinary team consisting of an andrologist and a gynecologist working together represents reproductive medicine. At the same time, there is a tendency to recognize that, because reproductive medicine has become so complex and comprehensive over recent decades, a discipline encompassing both sexes is imaginable. Especially those gynecologists working in this field have distanced themselves so far from oncology and obstetrics that they can conceive of an independent **specialty of reproductive medicine including andrology**.

The **World Health Organization (WHO)** considers the couple as a single entity in its definition of "**reproductive health"**, which it defines as freedom from disease and disturbances of reproductive functions, both in the male and in the female. As part of its concept of reproductive health, the WHO postulates that reproduction should take place in an environment of physical, mental, and social well-being. In addition, in its demand for self-determination of the number of children by the couple, it assumes that *both* partners have free access to reliable contraceptive methods.

As desirable as care for the infertile couple by only one attending physician may appear, it should not be overlooked that, apart from the initial consultation and those eventually occurring during the course of treatment, the actual investigations of husband and wife do not necessarily proceed in synchrony. Only when techniques of assisted reproduction, which, however, are still only appropriate for selected couples, are applied, is closest cooperation mandatory. Moreover, andrology covers certain aspects of male reproductive functions which can be treated largely without recourse to the partner, e.g., **replacement therapy in hypogonadism, delayed pubertal development, sexual and erectile dysfunction, contraception, and male senescence**. For this reason, in **addition to gynecology**, the part of reproductive medicine dealing with the male, i.e., **andrology**, is indispensable. Moreover, the field of reproductive gynecology is so comprehensive that it cannot adequately deal with problems of the male as well. While not losing sight of the goal of an integrated field of reproductive medicine, at present, the development of **gynecology and andrology** as separate fields seems to offer the most advantages, not forgetting, however, that **both fields should cooperate most closely** in the care of the infertile couple, e.g., within the framework of a **center for reproductive medicine**. When treatment consists of assisted reproduction, guidelines of the German Federal Medical Board on Assisted Reproduction (2018) require that the male partner be examined by an andrologist prior to treatment. The German societies for gynecology, reproductive medicine and andrology belonging to the Association of Scientific Societies (AWMF) have stated in a joint guideline as a basic requirement that the male partner must be investigated by a certified andrologist before any assisted fertilization treatment is performed (AWMF 2019). For practical purposes, this means that an andrologist be part of every center of reproductive medicine.

1.3 Infertility, Subfertility, Sterility, Fecundity: Definition of Terms

When speaking of disturbed fertility, certain concepts must be introduced and defined. It must also be taken into consideration that terminology may change over time.

Fertility refers to the capability to conceive or induce a pregnancy. **Fecundity** refers to the probability of producing a live birth arising from a given menstrual cycle. **Infertility** is the term used when a couple fails to induce a pregnancy within 1 year of regular unprotected intercourse. **Primary infertility** defines the condition when no pregnancy at all has been achieved, and **secondary infertility** means no further pregnancies have occurred. The term **infertile** can be applied to both men and women.

In addition to infertility, the term **sterility** is also used. Historically, it is an even older term. However, **infertility** is the more general term which may also include sterility (Vander Borght and Wyns 2018). **"Infertility"** is doubtless the most accurate description for childlessness. "Sterility", on the other hand, is a term with additional meanings as well (e.g., in the context of hygiene). "Infertility" also has the advantage of making less of a value judgement and avoids terminological ambiguity, for infertility and sterility are not separable nosological entities. The change in usage is reflected by the fact that, up to 1982, the database Medline used "**sterility"**; since then "**infertility"** has been in use as a headline word.

One objection to general use of the concept of "infertility" as opposed to "fertility" is that what is meant may actually be **subfertility**, as basically, the ability to sire or conceive may actually exist, e.g., with another partner. However, here too it is difficult to delineate sharply between definitions.

For these reasons in this volume we use the term **infertility** to refer to disturbed fertility in general and we speak of it when, within 1 year of regular, unprotected intercourse, no pregnancy occurs. One can discriminate between primary and secondary infertility, depending on whether a pregnancy has once been induced or not.

1.4 The Infertile Couple as Target Patients

Even if medical care of the female suffering from disturbed fertility is much better organized than that of the man, an analysis of the distribution of causes of disturbed fertility shows that, in up to half of the couples wishing offspring, the male may be implicated (Fig. 1.2). Disturbances in fertility may remain latent for years and only become evident when a couple develops a firm desire for a child. It is of particular importance that the suboptimal reproductive capacities of one partner may become evident because of the infertility of the other partner. This demonstrates the **interdependence of male and female reproductive functions**, as shown schematically in Fig. 1.3.

In order to evaluate the effects of limited reproductive functions, it is important to know the **time span** within which a "normal" couple of group 1 (Fig. 1.3) will conceive. In unselected women attending the delivery ward of a larger German municipal hospital, 70% conceived within the first 6 months and 90% conceived within the first 12 months of unprotected intercourse (Knuth and Mühlenstedt 1991; Louis et al. 2013).

However, this rate decreases steadily with the **age of the female partner**. As historical data show, in women older than 25 years, pregnancy occurs in 80% of couples only within 20–28 months (Bender 1953). In women whose husbands are azoospermic and who submitted to donor insemination, a rapid decline of fecundity could be found after the age of 30 (Van Noord-Zaadstra et al. 1991). This is attributable to the declining ability of the oocyte to be fertilized. Results from assisted reproduction show that the pregnancy rates of the female partner clearly decline after the age of 35 (Fig. 1.4). If a woman wants to have only one child, she can wait until the age of 35 to plan her first pregnancy. However, if she wants to have two or three children, she must plan her first pregnancy no later than 31 or 28 years of age (Habbema et al. 2015).

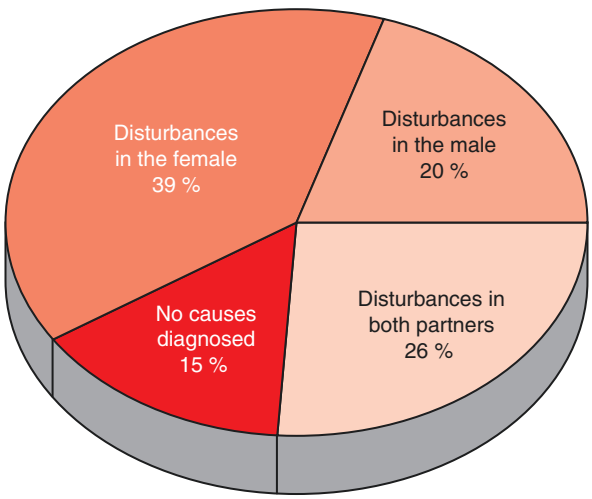


Fig. 1.2 Distribution of causes of involuntary childlessness between men and women

However, the **age of the male partner** also influences the occurrence of pregnancy. The "**time to pregnancy**" **(TTP)**, an important parameter for characterizing the fertility of a couple, increases when the male is over 40 independent of the woman's age (see Chap. 25).

Moreover, the **frequency of coitus** plays an important role. When both semen parameters and female factors are normal, the interval to conception decreases with the frequency of coitus as long as sperm production is not exhausted. Partners complaining of involuntary childlessness of more than 12 months' duration and in whom andrological factors have been excluded achieve a maximum conception rate when coitus takes place 3–4 times per week (McLeod et al. 1955). When sperm production is limited, however, this direct relationship is no longer valid.

Also the **timing of coitus** is of great importance. Most conceptions occur on the day of ovulation and the two preceding days, few conceptions, if intercourse takes place on days 3–5 before ovulation, but no conceptions after the day of ovulation (Wilcox et al. 1995).

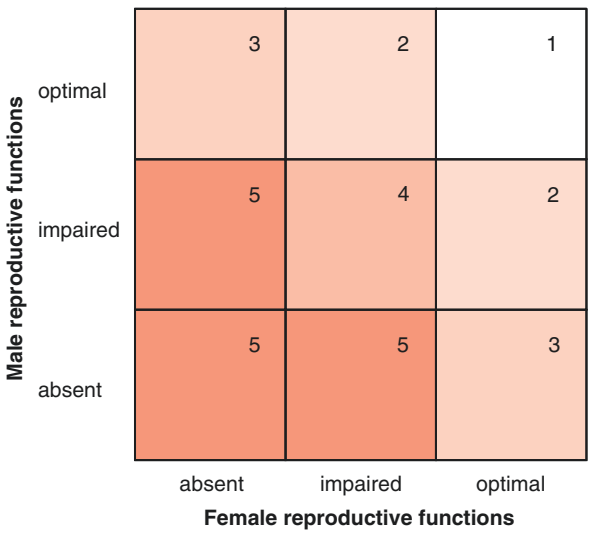


Fig. 1.3 Interdependence of male and female reproductive functions: (1) Couples from group 1, with both partners having optimal reproductive functions, will not consult a physician because of childlessness. (2) The suboptimal functions of one partner in couples of group 2 will probably be compensated for by optimal functions of the other partner. These couples are probably more prevalent in the general population than their representation in fertility clinics may suggest. (3) In couples of group 3, treatment will be concentrated only on one partner and treatment by only either gynecologist or andrologist will suffice. (4) Both partners of groups 4 and 5 require treatment. Therapeutic success, i.e., pregnancy, will be achieved more rapidly the more intensive and coordinated the medical care is. Precisely, these couples benefit most from treatment in a center for reproductive medicine where physicians have both gynecological and andrological trainings.

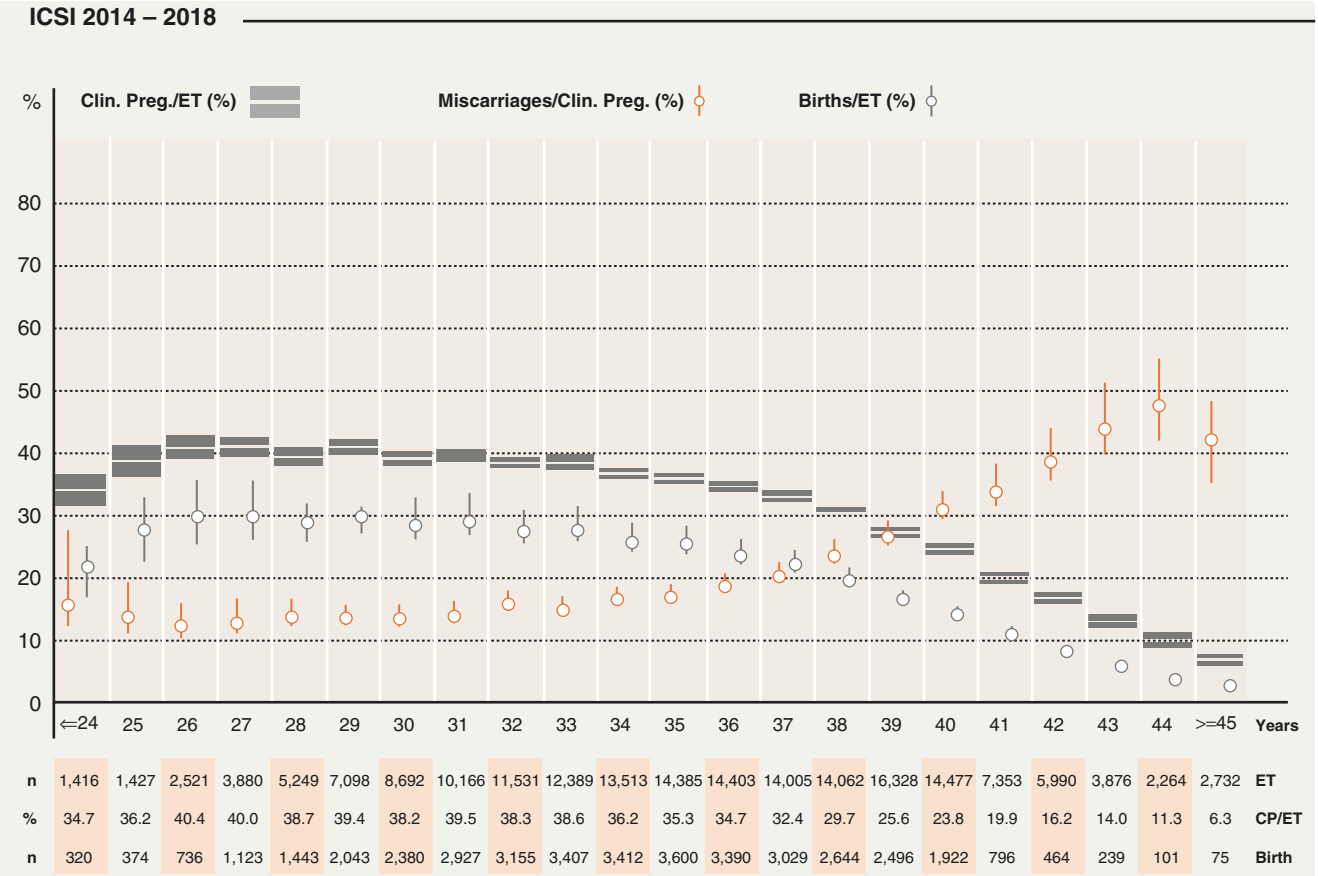


Fig. 1.4 Impact of female age on the success of ICSI treatments in Germany 2014–2018: Clinical pregnancies, miscarriages, and live births (German IVF Registry 2020)

It follows that **younger couples** should be examined only after they have tried to found a family for at least 1 year. Should the **woman** be **over 35 years or the male partner has any fertility problems,** investigations may be initiated after 6 months. (AWMF 2019). In industrialized nations, married childless couples tend to have on average increasing age. Whereas in Germany age at marriage was 22.7 for women and 25.9 for men in 1975, by 2018 it had increased to 32.1 for women and 34.6 for men (Destatis 2019). The average age of couples visiting the fertility clinics in Germany rose correspondingly. Thus, the average age of the female partner at her first visit to a reproductive center in Germany rose from 1997 to 2018 from 32.6 to 35.2 and from 35.2 to 38.4 for men. (Fig. 1.5).

The interdependencies of male and female reproductive functions described above should provide reason enough to examine both partners simultaneously in the event of involuntary childlessness. Both partners should be examined with the same degree of thoroughness. Good medical practice requires a full anamnesis; careful physical examination followed by all necessary technical and laboratory investigations. The recent Guideline of the societies for gynecology, reproductive medicine and andrology in Germany, Austria, and Switzerland requires the investigation of the male partner by a certified andrologist as standard procedure (AWMF 2019).

The **entity represented by the couple** with disturbed fertility must not be ignored. For this reason, although this volume deals primarily with andrology, it also provides an overview of diagnosis and therapy of female infertility (see Chap. 41).

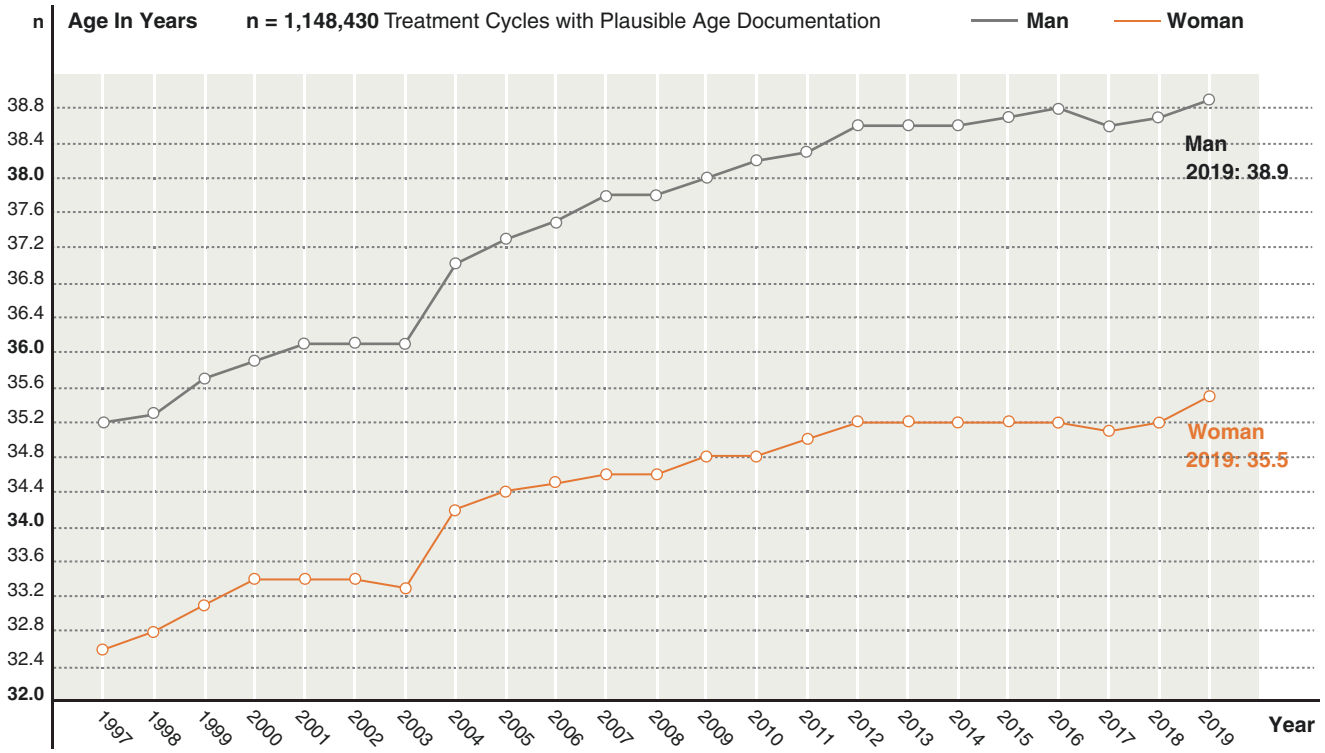


Fig. 1.5 Mean age of women and men 1997–2019 treated in Germany by assisted reproduction (German IVF Registry 2020)

1.5 Prevalence of Infertility

Data on the **prevalence of infertility** vary considerably and a few are reliable. There are considerable geographical differences. An analysis of reports from 190 countries and regions showed that couple infertility has the highest prevalence in South Asia, Sub-Saharan Africa, North Africa, and the Middle East (Mascarenhas et al. 2012). A representative survey of 15–44 year-old women in the USA showed an infertility rate of 15.5% (Thoma et al. 2013). In the United Kingdom, a survey of 15,000 16–74 year-old women and men found infertility in 12.5% of women and 10.1% of men (Datta et al. 2016). For Germany, there are no definitively confirmed data on the prevalence of infertility. In a detailed study of 2194 women and men aged 39–41 years, a point prevalence of infertility of 7.9% in women and 7.5% in men was found (Passet-Wittig et al. 2016). The point prevalence describes the currently existing infertility of women and men—in contrast to the lifetime prevalence of infertility, which in turn indicates the probability of being affected by infertility during the reproductive phase, but, in contrast to the point prevalence, does not indicate how many women and men are acutely affected at a certain age.

Lifetime prevalence of infertility (primary and secondary) is estimated to be up to and even above 15% of couples of reproductive age (Bruckert 1991; Juul et al. 1999). The difference between East and West Germany also continues to exist 30 years after reunification (DESTATIS 2019). On the other hand, the curves of different normal collectives constructed from the "time-to-pregnancy" have hardly changed over centuries (Fig. 1.6). Thus, the prevalence of male infertility is significantly higher than that for diabetes mellitus (types I and II), which is considered as an endemic disease.

Beyond the care of the individual couple, exact figures on fertility and infertility are of great importance for health policy and economic issues. Since previous studies have only ever considered smaller segments of the population retrospectively and applied different definitions of infertility, prospective studies with clear criteria are called for, although these would have to cover longer periods of time, which researchers shy away from (te Velde et al. 2017).

The incidence of each infertility disorder is discussed in relation to the different conditions in the following chapters.

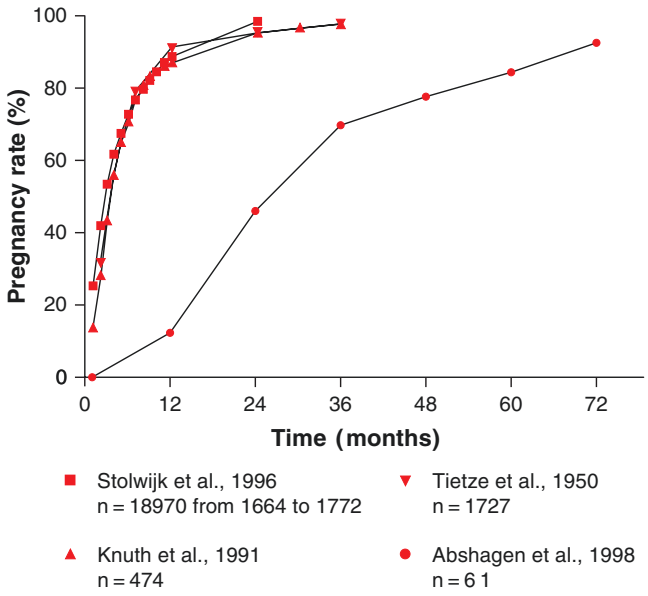


Fig. 1.6 Cumulative pregnancy rates of different normal populations in different epochs. Stolwijk et al. (1996) investigated Canadian church registers from the 17th and 18th centuries, Tietze et al. (1950) analyzed a population in New York, and Knuth and Mühlenstedt (1991) interviewed mothers of newborns in a maternity ward in Germany. For comparison, pregnancy rates of untreated couples with sperm surface antibodies in seminal plasma are shown (Abshagen et al. 1998)

1.6 Evidence-Based Andrology = Rational Andrology

There are many reasons why so much time passed before andrology developed into an independent specialized medical discipline. One important reason is that, until recently, diagnostic and therapeutic measures had not reached a critical mass great enough to justify establishment of an independent field. It is a fact that research efforts investigating the physiology and pathology of male reproductive functions and hormones were not undertaken systematically before the 1960s and pathophysiological concepts explaining individual diseases only gradually emerged. One factor contributing to the situation was also that andrological diagnostic techniques lacked standardization and tended to produce vague diagnoses. At least 30% of cases of disturbed male fertility still remain etiologically unclear and are referred to as **idiopathic infertility** (see Chaps. 4 and 39).

These shortcomings, characterizing andrology as well as other specialities, often meant that the physician's personal authority and experience tended to become the dominating factors in management decisions. The situation makes it tempting for some physicians to apply innumerable empirical therapeutic procedures whose effectiveness remains uncertain. Many errors in judgement—not only in andrology—can be attributed to this attraction to meddlesome but unproven treatments.

With the advance of basic research and scientific thinking in andrology, the dilemma became obvious to those actively practicing clinical andrology and a "**new andrology"** operating on scientific thinking and rationally based medical practice was called for. "**Evidence-based andrology"** developed simultaneously with the beginnings of "**Evidence-based Medicine" (EbM)**, which is increasingly becoming a pervasive force in all fields of medicine. It marks a gradual shift of paradigm in clinical medicine.

> The term "evidence-based medicine" signifies that clinical decisions must be based on results from controlled clinical studies and applied statistics and not rely predominantly on intuition, empiricism, and traditional protocols (Cochrane Collaboration n.d.; Djulbegovic and Guyatt 2017).

Whereas they remained rare in the 60 s, today **controlled, prospective, randomized and, if possible, double-blind clinical studies** are the accepted standard for evaluating the effectiveness of a diagnostic or therapeutic measure. No medication, no diagnostic test, and no interventional measure should be incorporated into clinical practice if its effectiveness has not been proven by appropriate controlled studies. The highest degree of evidence is achieved when several controlled studies deliver identical results of a meta-analysis.

While the clinical andrologist found it particularly difficult to incorporate this **shift of paradigm into the decision-making process**, the exponential increase in clinical studies concerning infertility treatment in the 1990s showed that this concept is finally becoming established in andrology as well.

The details of carrying out **controlled clinical studies** cannot be dealt with here. The most important elements are the studies' design and statistical evaluation. Over and beyond the problems generally caused by performing controlled studies in other fields of medicine, studies on infertility treatment face the particular difficulty that not **one** patient is being dealt with, a **second** participant must not only fulfill strict inclusion criteria, but is in fact the person in whom the end-point, pregnancy, occurs. The fact that pregnancy rates are naturally relatively low and that therefore large numbers of patients must be followed over prolonged periods of time creates special problems in controlled clinical studies on infertility treatment.

"Evidence-based medicine" also subjects **pathophysiological concepts**, on which diagnosis and therapeutic measures are based, to **critical examination** and assumes that not all pathophysiological concepts must be correct **a priori**. This is supported by experience from basic research showing that errors do in fact occur, that research results may be

translated prematurely into clinical strategies, thus giving rise to conceptual "short circuits". Examples of this are demonstrated by the various ill-founded treatments of idiopathic male infertility (see Chap. 39). The situation is even more complex in controlled studies on male contraception, where the aim is to prevent pregnancy as an "adverse event". For this reason, most such studies are limited to sperm counts as a surrogate parameter (Nieschlag 2010).

Evidence-based medicine is based essentially on scientific foundations of medicine. It does, however, require that the implementation of a scientific concept into a clinical strategy is rationally comprehensible and stands up to scrutiny by a controlled clinical study. In this respect, Evidence-Based Medicine can best be translated by the term **"Rational Medicine"** and, for the purpose of our subject, by "**Rational Andrology**".

Such medicine can rationally justify its decisions and summarize them in **guidelines** that provide the physician with clear directions for diagnostic and therapeutic measures and can be subjected to **quality control**. In this book, the individual chapters will always take into account the guidelines that are now also valid for andrology.

One of the important components of evidence-based andrology is **standardized diagnostics**, making results within one laboratory as well as between laboratories comparable. In this respect, **the WHO Laboratory Manual for the Examination and Processing of Human Semen** (5th edition 2009) is a standard work and provides the basis for all andrological laboratory diagnoses. Notwithstanding that Chap. 9 of the present volume briefly describes semen analysis as a laboratory technique, the **WHO Manual must be considered as an appendix to this volume**. The methodology described provides the basis for both internal and external quality control in the andrology laboratory. It is to be hoped that other areas of andrological diagnostics will be standardized to further buttress evidence-based andrology.

As long as there is no therapy for the causal treatment of male infertility, the concept of rational andrology remains endangered. When, at the conclusion of diagnostic procedures, only techniques of assisted reproduction **(ICSI** and **TESE**) remain as possible solutions, the time and effort required for careful diagnosis may seem exaggerated. This short-sighted conclusion must be rejected emphatically, as many treatable pathologies may be discovered by meticulous investigations. A drastic example are testicular tumors of which 200 were discovered among infertile men at an andrology clinic, but only because thorough diagnosis including ultrasound was performed.

While it is desirable that andrology increasingly be given a firm scientific base and that diagnostic and therapeutic measures be tested by their results, it must not be forgotten that **the patient or couple** requiring care remains the **central point of medical attention**. This includes providing time for extensive counselling, for clarifying physical and pathological facts, for explaining diagnostic findings and therapeutic measures, for answering questions concerning sexuality, and for exploring the importance of a child to each partner and to the couple. The patient must be convinced by practice that precisely these aspects are of greatest importance to the physician, whereas scientific validity of his management must be the unspoken precondition of professional expertise underlying patient-doctor interaction.

The **placebo-effect of medical advice and attention** must never be underestimated. This assumes that the **placebo be defined** as a measure lacking a specific effect, but which will nevertheless have a significantly greater influence on the desired outcome than no measure at all (Jütte 2011; Colloca and Barsky 2020). It must be stressed that the placebo so defined has no negative connotation, of which it is often accused. The meaningfulness of such a placebo-effect becomes clear from results of a controlled study on therapy for varicocele: patients subjected to surgical or angiographic intervention showed the same pregnancy rates as those only counselled and examined at regular intervals (Nieschlag et al. 1998).

Knowing the placebo-effect of medical attention and applying it in treatment strategies is just as much a part of evidence-based andrology as its scientific basis.

When judging the success of therapy, it should also be considered that infertility does not represent an absolute diagnosis, but that factors related to time may play an important role. In the course of time, pregnancies may occur spontaneously, without any medical intervention. When couples on the Dutch island of Walcheren consulting a primary care center for infertility were left "untreated", after 2 years the spontaneous pregnancy rate was 40% (Snick et al. 1997) (Fig.1.7). When patients at a tertiary

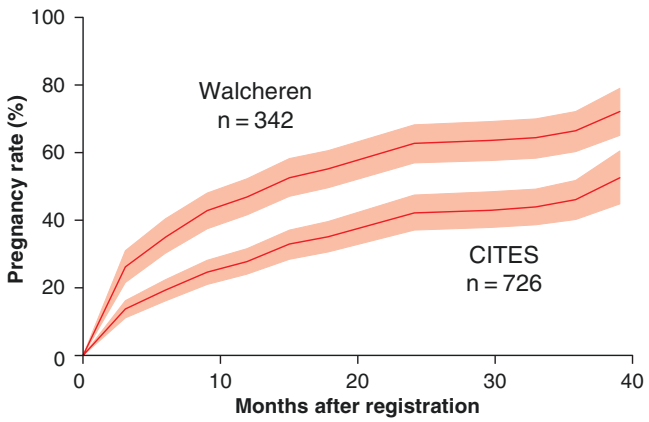


Fig. 1.7 Cumulative pregnancy rates in two populations of diagnosed, but untreated, couples in centers of primary care (Walcheren) and of tertiary care (Canadian Infertility Therapy Evaluation Study CITES, Collins et al. 1995). Only pregnancies resulting in live births were computed (from Snick et al. 1997)

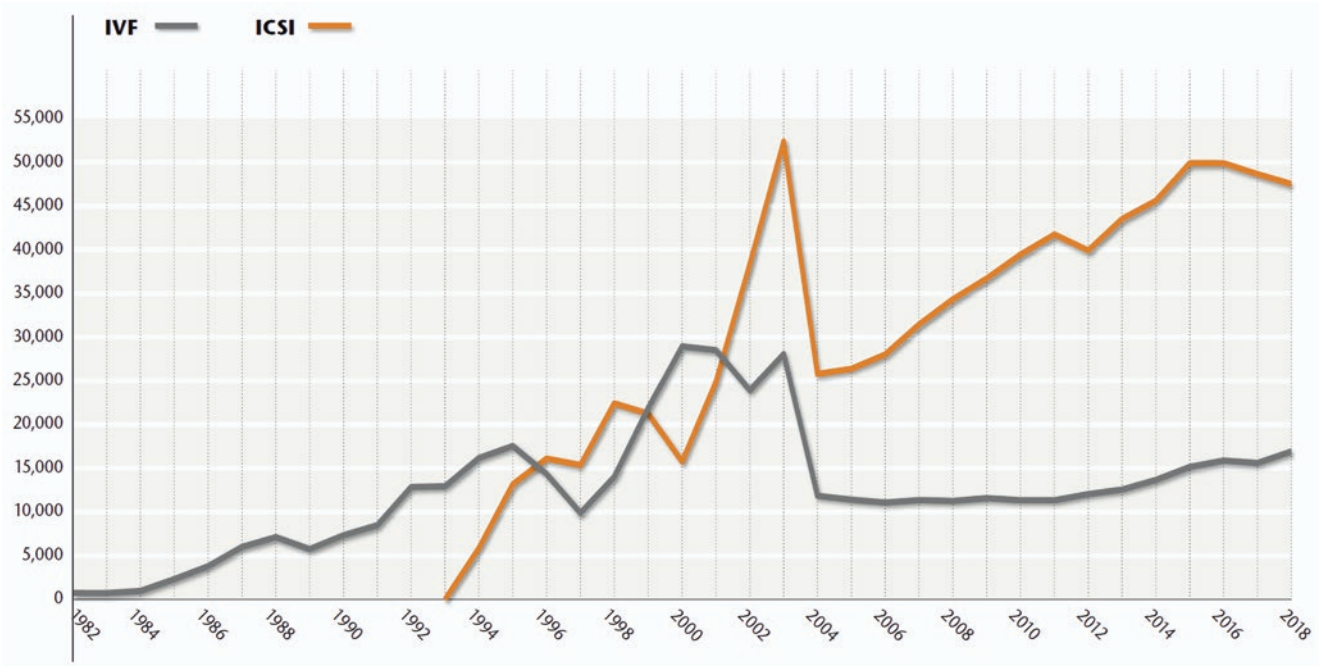
fertility center were treated similarly, after the 2-year observation period the pregnancy rate was 20% (Collins et al. 1995) The figures show that the selection of couples plays an important role and that even at a specialized center spontaneous pregnancies occur. Such occurrences must be taken into consideration when judging therapeutic measures; they are the basis for models predicting the chances for pregnancies (Collins et al. 1995).

1.7 The Crisis in Andrological Research

Without doubt, andrology has experienced an enormous upswing since the 1960s, thanks to intensified translational research. In Germany, the German Research Foundation (DFG) with its Collaborative Research Centres and Priority Programmes and the Max Planck Society (MPG) with a Clinical Research Group were particularly involved in these efforts. Patient care has also benefited from this. New testosterone preparations and recombinant gonadotropins were developed for substitution, phosphodiesterase-5 inhibitors for the treatment of erectile dysfunction were introduced, genetic causes of infertility and hypogonadism were elucidated, the influence of environmental factors and occupational exposures on reproductive health became a central topic, new imaging methods for the representation of the reproductive organs were developed, a standardization of ejaculate analysis was established and sperm function tests were developed.

However, hardly any new therapeutic procedures for the treatment of male infertility have been developed. With the introduction of in vitro fertilization, which was initially intended to treat female infertility, attempts were initially made to treat male infertility to a limited extent. This changed abruptly in 1994 when it became possible to fertilize eggs with individual sperm cells and "intra-cellular sperm injection" (ICSI) was introduced as a method of treating male infertility. In the meantime, more ICSI treatments for male indications than IVF procedures for female indications are carried out worldwide, as can be demonstrated by the German IVF Register (DIR 2019): of 64,441 ART treatments in 2018, 47,518 = 74% were ICSI treatments (Fig. 1.8).

As successful as ICSI treatment may be, it must not be forgotten that it is only a symptomatic and not a causal therapy without differentiation of the causes. Early warning is



	1982	1986	1990	[...]	2002	2003	2004	[...]	2014	2015	2016	2017	2018
IVF	742	3,806	7,343	*For values from 1991 to 2001 see www.deutsches-ivf-register.de*	23,936	28,058	11,848	*For values from 2005 to 2013 see www.deutsches-ivf-register.de*	13,675	15,164	15,879	15,606	16,923
ICSI					38,370	52,376	25,785		45,605	49,897	49,893	48,641	47,518
Total	**742**	**3,806**	**7,343**		**62,306**	**80,434**	**37,633**		**59,280**	**65,061**	**65,772**	**64,247**	**64,441**

Fig. 1.8 Number of ovum pick-up cycles (OPU) 1982–2018 in Germany (2018) 16,923 OPUs were performed for IVF and 47,518 OPUs for ICSI (German IVF Registry 2020)

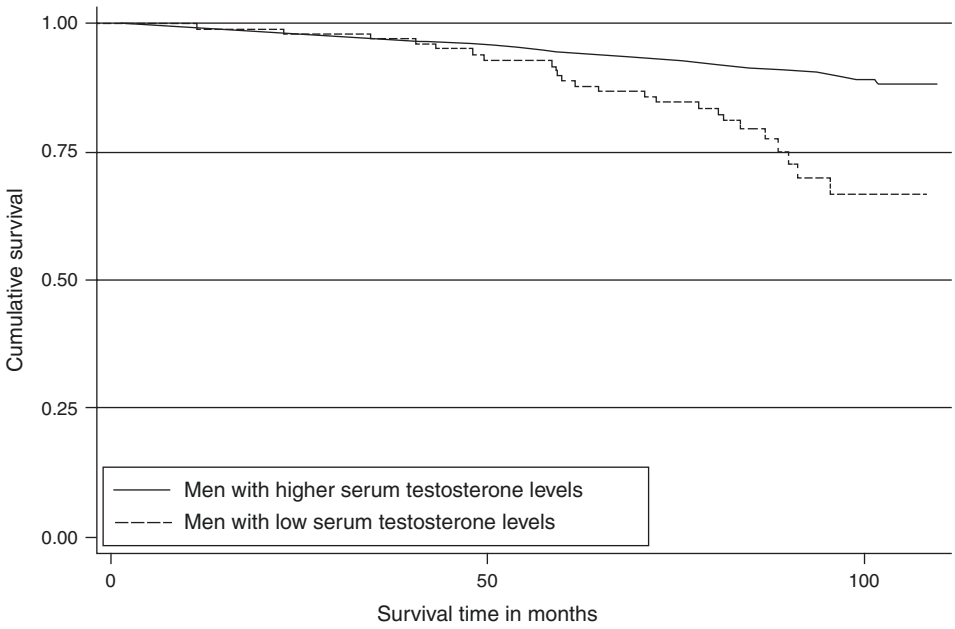


Fig. 1.9 Mortality of 1954 men aged 20–79 years whose testosterone was measured at the beginning of the study and their mortality was recorded in the following 7 years. Mortality is higher in men <8.7 nmol/L than in men >8.7 nmol/L (Haring et al. 2010)

that this treatment could stifle research into the causes (Nieschlag 1997). Today this development has been recognized as a general problem (te Velde et al. 2017; Barratt et al. 2018; Cairo Consensus Workshop Group 2020). For not only does ICSI circumvent the need for andrological research, but male infertility is treated at the expense of women. For the woman must assume the burdens, side effects, and risks of ICSI, which are also associated with an increased risk of premature births, low birth weight, and malformations of the cardiovascular and musculoskeletal system on the part of the infant (von Wolff and Haaf 2020). In addition, the economically lucrative ICSI may lead to premature decisions before other options are exhausted (te Velde et al. 2017).

The threat to andrological research is occurring precisely at a time when it is becoming increasingly clear that infertility and hypogonadism are not isolated phenomena, but indicators of increased morbidity and mortality. Thus, life expectancy correlates positively with serum testosterone levels in aging men (Haring et al. 2010; Fig. 1.9). It is known that Klinefelter syndrome is associated with an accumulation of comorbidities and thus increased mortality compared to the general population (Bojesen and Gravholt 2011; see Chap. 21). Infertile men are more susceptible to general diseases as shown by a higher rate of hospital admissions (Latif et al. 2017; Fig. 1.10). In particular, infertile men have increased rates of cardiovascular, metabolic, neoplastic, and autoimmune diseases (Murshidi et al. 2020). The Covid pandemic has also shown that hypogonadal men, especially those with obesity-related functional hypogonadism, have higher mortality rates (Giagulli et al. 2020).

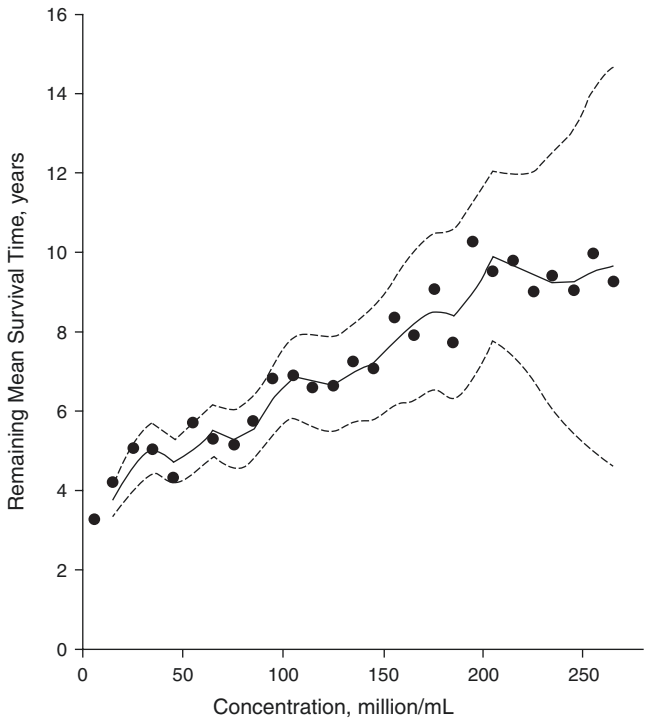


Fig. 1.10 Length of period prior to first hospitalization in relation to sperm concentration in 4712 infertile men in Denmark (1977–2010): Morbidity (and mortality) is correlated with sperm concentrations (Latif et al. 2017)

Against this background, intensive research in the field of andrology would be required, not only to investigate the causes of infertility and hypogonadism, but also to investigate the links with general health. It is also noted that, unlike for women, there are no adequate prevention or early

detection programmes for men. In Germany, until the abolition of compulsory military service, the medical examination for physical fitness fulfilled this function in part, at least for young men. Since then, there have been no systematic screening programmes for men in the various age groups. The call for such programmes, for men at national and international levels, as set out by the WHO (2020) in its "Global Strategy for Women's, Children's and Adolescent's Health 2016-2030" is becoming ever stronger (Rovito et al. 2017).

The public health aspects of andrology are becoming more and more prominent and require more research and research programmes. As andrology is a highly interdisciplinary field, goal-oriented research requires centers that bring together the participating basic and clinical subdisciplines (biology, biochemistry, molecular biology, genetics, endocrinology, immunology, andrology, gynecology, and reproductive medicine), of which there are only a few to date.

1.8 The Special Case of Male Contraception

Providing male contraceptive methods is one of the **tasks of andrology**. Here the question arises whether the andrologist (or the specialist for reproductive medicine) is not at odds with himself if, on the one hand, he treats disturbed fertility and contributes to increasing birth rates, and on the other hand, provides contraceptive methods, thus influencing birth rates negatively.

The **apparent contradiction** is easily resolved as it is a matter of two sides of the same coin. Once the reproductive system has been understood, it can be influenced both positively and negatively. Andrology and reproductive medicine **do not in the first instance** concern themselves with the **politics of population control**. Rather they are primarily directed towards the individual and strive to help the individual couple to improve their affected reproductive functions, or to control them if they are not required. In this fashion, reproductive medicine should help to reduce the suffering experienced by the couple wanting a child, while simultaneously creating the prerequisites allowing the couple to freely determine the size of its family which is a decisive factor of **Reproductive Health** as defined by WHO. Finally, creating the medical preconditions also provides the means of curbing the world's overpopulation with its ecological and social problems as a by-product of care for the individual patients and their voluntary rights to reproductive freedom and family planning. As male contraceptive methods in particular are lacking, research leading to the development of such methods appears strongly needed. However, male contraception research is particularly difficult and full of hindrances (Chaps.48 and 49).

Reproduction can be considered as compensation for death. If medical progress allows increasing numbers of people to reach reproductive age, and if, during periods of increasing birth rates, the date of death continues to be pushed forward, thus leading to overpopulation, then medicine must also provide contraceptive methods in order to maintain or restore **the balance between reproduction and death.** Andrology must contribute to this goal.

Key Points

- Andrology comprises all areas of medicine and natural sciences that deal with male reproductive functions under physiological and pathological conditions and can thus be characterized in short as "male reproductive health".
- Focal points of andrology are infertility, hypogonadism, sexual dysfunction, the aging male, and male contraception.
- Although andrology is a recognized independent field in Germany and some other countries, the andrologist must work very closely with the gynecologist on infertility issues and consider the infertile couple as a unit.
- As infertility affects about 15% of couples, infertility disorders must be considered a widespread disease.
- Hypogonadism and erectile dysfunction are well treatable today, thanks to intensive research.
- Nevertheless, andrological research is in crisis, as ICSI is considered a universal treatment for infertility and a need for research in andrology is considered unnecessary.
- This also affects research in the field of male contraception in order to make a male contribution to family planning and to contain the overpopulation of the earth.

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Part I

Physiologic Basis

Physiology of Testicular Function

2

Joachim Wistuba, Nina Neuhaus, and Eberhard Nieschlag

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Abstract

The male gonads, the testes, fulfill two essential functions. They are the site of spermatogenesis. The male gametes, the sperm, are produced here. As important is their second function as an endocrine gland that synthesizes androgens. Androgens are necessary for the formation of the typical male phenotype. The male gonad develops during the embryonic phase when the SRY gene is expressed in sex differentiation and a testosterone signal occurs.

Testicular tissue is divided into two main compartments: the seminiferous tubules, which are surrounded by peristaltically active peritubular cells, and house the germinal epithelium where the germ cells differentiate through meiosis into haploidization to finally form spermatozoa. The second compartment consists of the interstitium, containing the Leydig cells, which synthesize steroids and produce androgens. In immunological terms, the further differentiated germ cells are protected by the blood–testis barrier, which is formed by the Sertoli cells. The Sertoli cells also provide the niche of spermatogonial stem cells which are unipotent adult stem cells and drive spermatogenesis lifelong after puberty onset. The complex processes of germ cell maturation are regulated by numerous factors, including cytokines, transcriptional and growth factors, but especially by endocrine, paracrine, and autocrine signals.

The endocrine function is part of the hypothalamic-pituitary-gonadal axis. Numerous hormones and receptors act along this axis in a balanced feedback mechanism. Primarily, the system is driven by kisspeptin and pulsatile GnRH controlling the secretion of the pituitary gonadotropins LH and FSH, which in turn induce effects on the testes. LH stimulates the production of testosterone, whilst FSH acts on Sertoli cells and provokes an inhibin response. Testosterone, which is present in the testes in much higher concentrations than in the serum, and inhibin in turn regulate gonadotropin secretion in negative feedback, resulting in an endocrine control loop along the hypothalamic-pituitary-gonadal axis. FSH and androgen action are essential for quantitatively and qualitatively complete spermatogenesis.

Testosterone and its various metabolites, especially dihydrotestosterone and estradiol, are necessary for the normal male development of numerous target organs, including bone metabolism, muscles, hair and beard growth, vocal register, fat distribution, and certain brain functions.

Disorders of testicular function can have various causes, such as genetic or disease-related. Such disorders lead to prenatal disorders of sex differentiation and postnatally to further pathological consequences such as infertility or hypogonadism, which are associated with numerous specifically male diseases.

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2.1 Introduction

The testes fulfill two essential functions: the production and supply of male gametes and the synthesis and secretion of male sex hormones.

This chapter presents the physiological basis underlying the dual function of the male gonads. The facts below apply predominantly to the situation in humans. Animal experimental findings have been used when human data are not available or cannot be collected for ethical reasons, and to clarify concepts and theories. The description of the organization of the testes and the physiological principles of germ cell maturation is based on conditions in the human testis and provides the basis for understanding the endocrine and local regulation of testicular function. Additionally, testicular histology is described in detail in Chap. 11. The hypothalamic-pituitary-testicular regulatory feedback mechanism, the control unit of the physiological regulation of testicular function, and a regulatory system in the classical endocrine sense, are explained. Local regulatory mechanisms play an important role in the "fine-tuning" of germ cell maturation. The chapter ends with a detailed description of the synthesis and biological effects of androgens in the organism.

2.1.1 Functional Organization of the Testes

The male gametes as well as the male steroid hormones (androgens) are produced in the testes. **Spermatogenesis** covers all processes involved in the development of the gametes. **Steroidogenesis** comprises all those reactions that lead to the production of the steroid hormones. Spermatogenesis and steroidogenesis take place in two compartments of the testis that are separated according to morphological and functional criteria: the tubular compartment, which consists of the **seminiferous tubules (tubuli seminiferi)** and the **interstitial compartment (interstitium)**, the space between the seminal tubules (Figs. 2.1 and 2.2). Although spatially

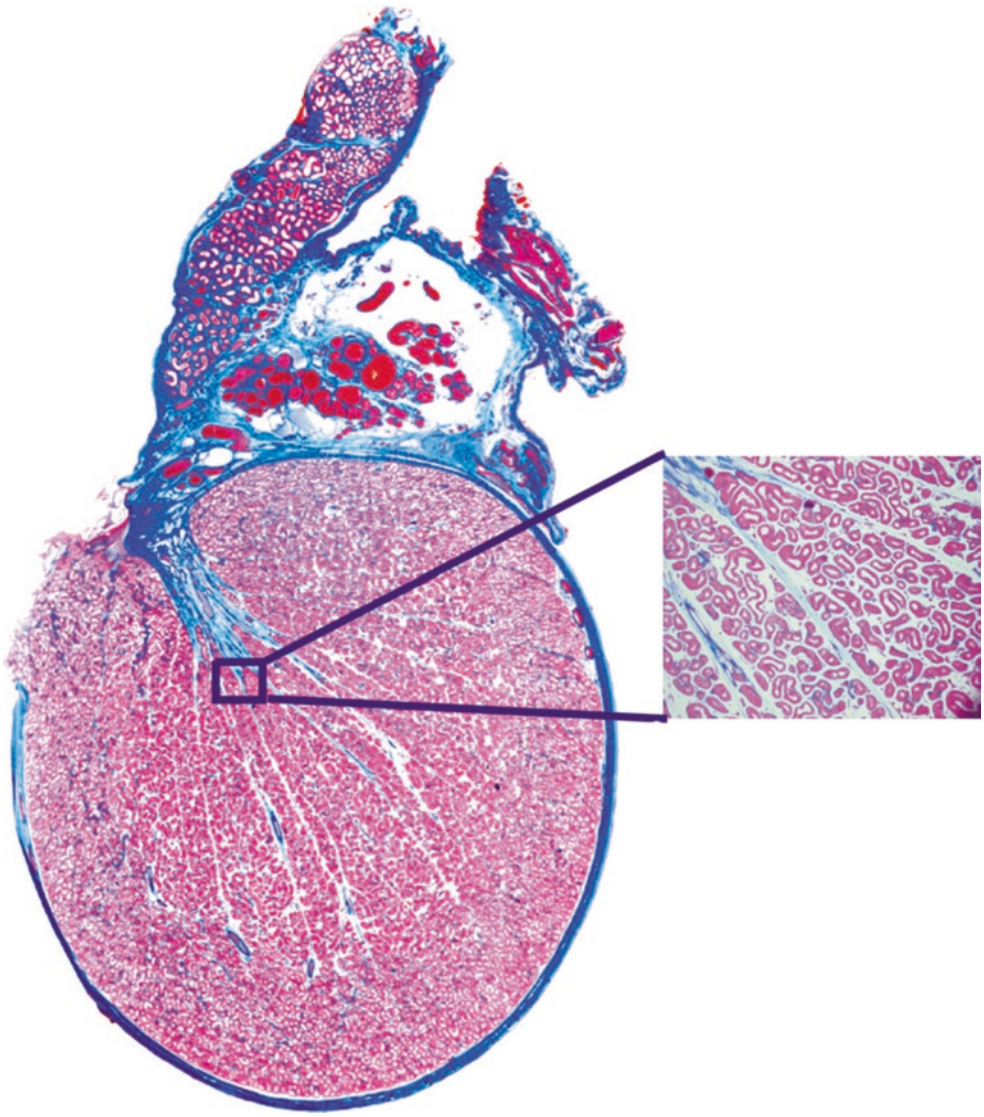


Fig. 2.1 Transversal section of an entire human testis. The section also includes parts of the efferent ducts and the epididymis. The lobular architecture of the testis is evident. (Courtesy of Prof. Dr. A.F. Holstein, Institute of Anatomy, University of Hamburg)

separated, both compartments are closely related to each other. The integrity of both compartments is a prerequisite for the qualitatively and quantitatively normal production of sperm. The function of the testes and thus the functions of its compartments are primarily influenced by structures of the diencephalon and pituitary gland (**endocrine regulation**). These endocrine effects are mediated and controlled by local factors (**paracrine and autocrine regulation**) and play an important role.

2.1.1.1 Interstitial Compartment

In the interstitial compartment, the most important cells are the **Leydig cell**s, which produce testosterone and insulin-like factor 3 (INSL3). In addition to Leydig cells, the interstitial compartment also contains loose connective tissue, cells of the immune system, blood vessels, nerves, and lymph vessels. In the human testis, 12–15% of volume is occupied by the interstitial compartment. About 10–20% of the interstitial volume is accounted for by Leydig cells (approx. 200,000,000) (Petersen et al. 1996).

2.1.1.1.1 Leydig Cells

The cells described by Franz Leydig (1821–1908) in 1850 produce and secrete the most important male sex hormone, **testosterone**. According to developmental, cytological, and functional aspects, different types of Leydig cells are distinguished: **"stem" Leydig cells** as the (less differentiated) precursor cell type, and two different populations, namely **fetal Leydig cells** with terminal differentiation in the fetal testis and **adult Leydig cells** as fully differentiated adult cells of the differentiated gonad (Shima 2019; Mäkelä et al. 2019; Rotgers et al. 2018). Fetal Leydig cells become neonatal Leydig cells at birth but degenerate after birth. Some fetal Leydig cells also appear to be able to dedifferentiate to serve as stem cells for adult Leydig cells. Continued formation of fetal Leydig cells into the postnatal phase may lead to induction of pathological dysfunction of adult Leydig cells (Shima 2019). Fetal Leydig cells produce androgens and are thus essential for the differentiation of derivatives of the Wolffian ducts and external genital organs (Mäkelä et al. 2019).

Adult Leydig cells are rich in smooth endoplasmic reticulum and mitochondria with tubular cristae. These cytological characteristics are typical of steroid hormone-producing cells and are also found in other cells active in steroidogenesis such as the adrenal gland and the ovary. Other prominent cytoplasmic components are lipofuscin granules, the final product of endocytosis and lysosomal degradation, and lipid droplets containing the precursors required for testosterone synthesis. The so-called **"Reinke" crystals** are often found in adult Leydig cells. These are globular protein conglomerates. The function of these crystals is still not fully understood. It has been reported that they are more common in cryptorchidism, but there is also evidence that they may be artificially lost due to fixation and their normal abundance cannot be accurately determined (Mesa et al. 2015; Soerensen et al. 2016).

The **proliferation rate** of Leydig cells in the adult testis is very low and influenced by LH. The ontogenesis of Leydig cells has not been conclusively clarified, but most of the evidence suggests that progenitor cells are derived from the mesonephros with a regulatory involvement of Sertoli cells (Mäkelä et al. 2019; Rotgers et al. 2018). In the adult testis, Leydig cells develop from perivascular and peritubular mesenchymal-like cells. This process is mainly controlled by LH but also by growth and other differentiation factors from Sertoli cells.

2.1.1.1.2 Macrophages, Lymphocytes, and Nerve Fibers

In addition to Leydig cells, the interstitial compartment also contains macrophages and lymphocytes, which are part of the cellular immune defense. Roughly, for every 10–50 Leydig cells, there is one **macrophage**. In terms of function, Leydig cells and macrophages are in close contact and, through intercytoplasmic protrusions, also in direct physical connection to each other (Heinrich and De Falco 2020). Macrophages are involved in the regulation of proliferation, differentiation, and the steroid production of Leydig cells by secretion of stimulators and inhibitors (e.g., cytokines, 25-hydroxy-cholesterol, reactive oxygen species (ROS), interleukin, and TGF). Conversely, products of Leydig cells also regulate the function and activity of macrophages, such as testosterone and colony-stimulating factor 1 (CSF1).

Altogether, according to the current state of research, the considerable importance of testicular macrophages, not only for the immune system but also for the correct function of other somatic cells of the testis, as well as for the formation and function of the stem cell niche can be considered as confirmed (Heinrich and De Falco 2020). The immunology of the testis is described in detail in Chap. 28.

2.1.1.2 Tubular Compartment

Spermatogenesis takes place in the tubular compartment. In healthy testes, this compartment accounts for 60–80% of the organ volume. It contains the germ cells and two somatic cell types, the **peritubular cells** and the **Sertoli cells**. Connective tissue septum divides the testis into about 250–300 lobules (Fig. 2.1) with 1–3 very bulky spermatozoa per lobule. On average, the human testis contains about 600 sperm tubules of 30–80 cm length.

2.1.1.2.1 Peritubular Cells

The seminiferous tubules are surrounded by a lamina propria, which consists of a basement membrane, a collagen fiber layer, and the **peritubular cells** (myofibroblasts). Myofibroblasts are lowly differentiated myocytes with the ability to contract spontaneously. These cells surround the tubule in several concentric layers (up to 6), each separated by a collagen fiber layer (Fig. 2.2). The cells of the inner and

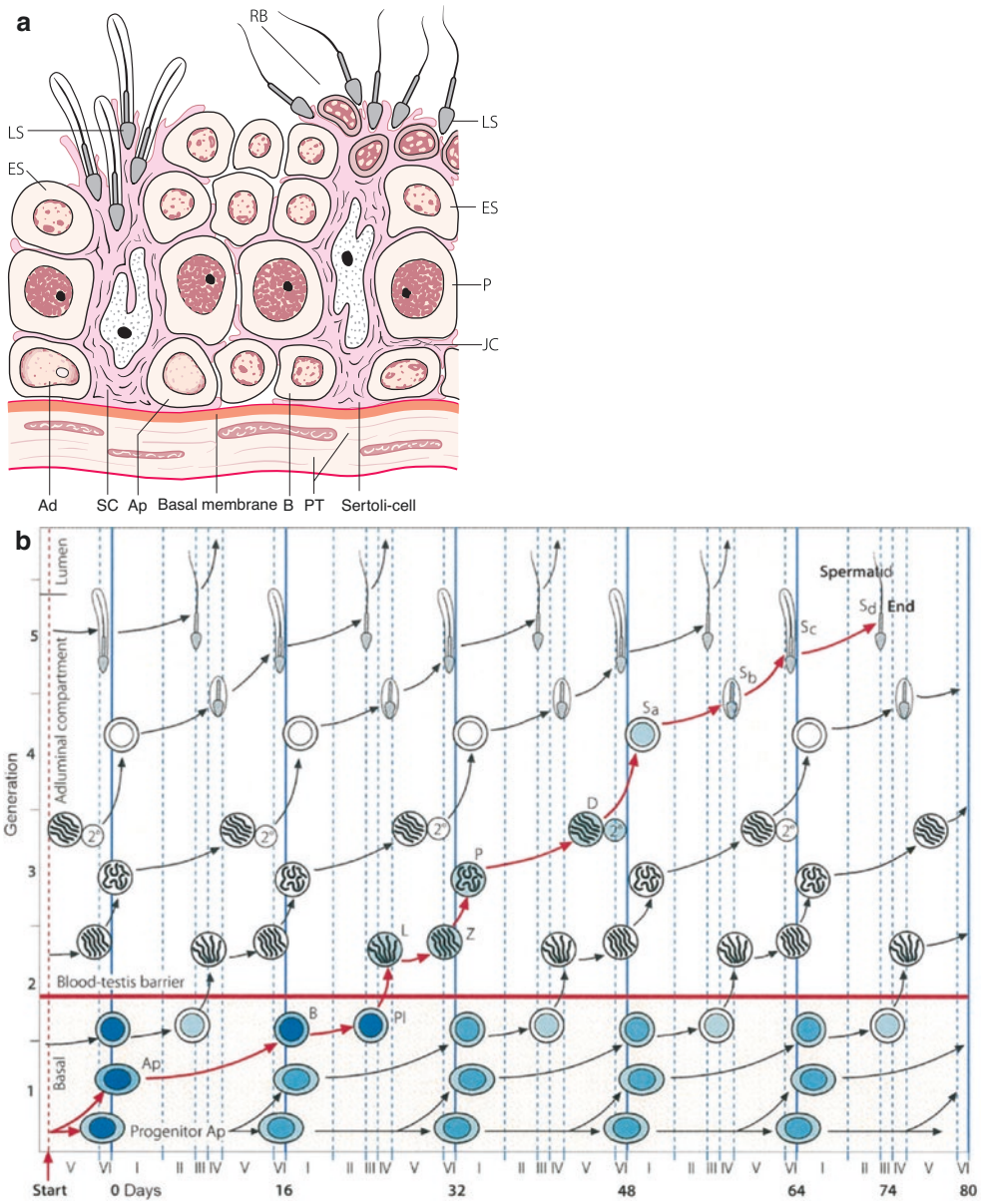


Fig. 2.2 (**a**) Schematic representation of the architecture of the human seminiferous epithelium. Note that the tubular wall is composed of several layers of peritubular cells (PT) and a basal lamina (BL). RB = residual body, LS = late/elongating and elongated spermatids, ES = early/round spermatids, P = spermatocytes, Ad = A-dark spermatogonia (testicular stem cells), Ap = A-pale spermatogonia, B = type B spermatogonia, SZ = Sertoli cells, JC = junctional complexes forming the blood–testis barrier built by interconnected Sertoli cells (SC). (**b**) Depicts the kinetics of the human seminiferous epithelium. Ap spermatogonia are the progenitor stem cells that enter the spermatogenic cycle (color-coded). All descendents of this progenitor cell represent a single clone of germ cells. It takes 4–4.6 cycles ("generation" on the y-axis, denoted as Start and End) until a sperm (Sd) has developed from a progenitor cell (modified from Amann 2008)

outer layers differ slightly. While the inner layers are desmin-positive and thus displaying smooth muscular character, the outer layers express vimentin more strongly and thus more closely resemble connective tissue (Mayerhofer 2013). The human testis differs from the majority of other mammalian testis whose seminiferous tubules are surrounded by only 2–4 myofibroblast layers.

Peritubular cells produce a number of factors that enable cell contractility: **panactin**, **desmin, gelsolin**, **smooth muscle fiber** myosin, and smooth muscle fiber actin (Holstein et al. 1996; Mayerhofer 2013). At the same time, these cells secrete extracellular matrix and form molecules typical for connective tissue: **collagen, laminin**, **vimentin**, **fibronectin**, **growth factors,** and **adhesion molecules** (Albrecht et al. 2006; Schell et al. 2008). In a cell culture system with human peritubular cells, it could be shown that nerve growth factor and proinflammatory factors (e.g., interleukin-1beta and cyclooxygenase-2) are secreted under the influence of TNF-alpha (Schell et al. 2008). Myofibroblasts are moderately differentiated myocytes, but are capable of contraction. By these contractions, mature sperm are transported through the testicular ducts to enter the epididymis finally. **Oxytocin, prostaglandins**, androgenic steroids, **endothelins**, endothelin-converting enzymes, and endothelin receptors mediate this process. Endothelin is an important regulator of cellular contractility and its efficacy is modulated by adrenomedullin derived from Sertoli cells (Romano et al. 2005). Genetic defects associated with cell contractility have been identified in mice with selective androgen receptor deficiency in Sertoli cells, i.e., defects of endothelin-1, endothelin receptor A and B, adrenomedullin receptor, and **vasopressin** receptor (Zhang et al. 2006). Recent studies in transgenic mice have demonstrated important para-

crine functions of this testicular cell type. The anatomical position of the peritubular cells in the transition between the tubular and interstitial compartments seems to predestine them to mediate these signals (Mayerhofer 2013). For example, the Leydig cells are under partial functional control of the peritubular cells. By selectively switching off the androgen receptor in the peritubular cells, Leydig cells reduce the secretion of growth factors such as IGF and IGF3 (Welsh et al. 2012).

There seems to be another, more direct relationship to the Leydig cells. There is evidence that stem cells of adult Leydig cells are recruited from the outer layers of the tubule walls, i.e., from those layers more closely resembling connective tissue characteristics. Such cells could at least be stimulated to show steroidogenic activity in cell culture experiments and expressed typical Leydig cell markers (Landreh et al. 2014).

Disorders of testicular function and reduced or absent spermatogenetic activity may be associated with a thickening of the collagen fiber layer and of the fibrillary material between the peritubular cells. This is called fibrosis or — depending on the microscopic appearance — hyalinization of the tubular wall. Testicular involution leads to a pronounced thickening of the tubular wall. Under these conditions — due to the reduction in size of the testes — the wall structures unfold in the longitudinal direction of the tubules, resulting in a thickening of the tubular wall. Injection of fluid into involuted seminiferous tubules with a thickened tubular wall rescues the tubule diameter and the tubular wall thickness normalizes (Schlatt et al. 1999). On the other hand, active fibrosis through interaction between mast cells and peritubular cells is also discussed: This might provoke inflammatory reactions and restricted testicular function (Albrecht et al. 2006; Mayerhofer 2013; Mayerhofer et al. 2018). For example, the secretion of decorin, a protein that cross-links in the collagenous extracellular matrix, is increased in infertile men. It is suspected that decorin inhibits the activity of certain growth factors and thus disrupts the function of peritubular cells (Mayerhofer 2013). In addition, in a cell culture with human peritubular cells, it could also be shown that peritubular cells secrete androgen-dependent GDNF, a factor that is essential for the incorporation of spermatogonial stem cells into the germinal epithelium. Thus, these cells also contribute to germline differentiation and ultimately to fertility. So far, this function has been insufficiently researched (Mayerhofer 2020).

2.1.1.2.2 Sertoli Cells

Sertoli cells are somatic, nondividing cells in the terminally differentiated adult state and are located in the germinal epithelium. They are named after Enrico Sertoli (1842–1910), who first described them, calling the cells "cellule ramificate" in 1865 because of their cytoplasmic extensions and ramifications. These cells are located on the basal membrane as the supporting framework of the germinal epithelium and extend to the lumen. Along the cell bodies, which extend over the entire height of the seminiferous epithelium, the morphological and physiological differentiation and maturation of the germ cells into the testicular spermatozoa take place (Griswold 2018). Special ectoplasmic structures serve to align and direct the sperm during differentiation (Li et al. 2018). About 35–40% of the volume of the germinal epithelium is occupied by Sertoli cells. With intact spermatogenesis there are 800–1200 × 10^6 Sertoli cells per human testis (Zhengwei et al. 1998) or about 25 × 10^6 Sertoli cells per gram of testicular parenchyma (Raleigh et al. 2004).

Sertoli cells produce and secrete a variety of factors, including proteins, cytokines, growth factors, opioids, steroids, prostaglandins, and modulators of cell division. The morphology of Sertoli cells corresponds to their multiple physiological functions. The cytoplasm contains endoplasmic reticulum of the smooth (steroid synthesis) and rough type (protein synthesis), a prominent Golgi apparatus (packaging and transport of secretory products), lysosomal granules (phagocytosis) as well as microtubules and intermediate filaments (adaptation of cell shape during the different phases of germ cell development (Li et al. 2018)). Sertoli cells are in mutual regulatory exchange with germ cells. On the one hand, they control the course of spermatogenesis topographically and functionally. On the other hand, there are findings that the germ cells influence the secretory activity of Sertoli cells. Experiments with heterologous germ cell transplantation (Nagano et al. 2001) underline the autonomy of the germ cells at least with regard to the temporal sequence of gametogenesis: the cycle of spermatogenesis lasts about 8 days in mice and about 12–13 days in rats. The cycle duration of rat germ cells transplanted into mouse testes continued to be 12–13 days while that of mouse germ cells was 8 days (Franca et al. 1998).

Sertoli cells produce and secrete fluid and thereby form the tubular lumen, into which more than 90% of the fluid is released. Special structural elements of the **blood–testis barrier** prevent backflow of the secreted fluid. The resulting fluid-induced pressure maintains the lumen. The spermatozoa, once released from the nursing Sertoli cells are also transported to the epididymis within this tubular fluid. The composition of the tubular fluid is known in detail only in rats (Setchell 1999). Compared to blood, tubular fluid contains considerably more potassium and correspondingly less sodium ions. Other components are carbonate, magnesium, and chloride ions, inositol, glucose, carnitine, glycerylphosphorylcholine, amino acids, androgens, and various proteins. Thus, the germ cells are embedded in a unique liquid environment.

Proliferation of Sertoli cells in the adult state is prevented by terminal differentiation and each Sertoli cell can only supply a certain number of germ cells, thus the number of the polarized, terminally differentiated adult Sertoli cells determine **testis size** and the extent of possible sperm production. Each individual Sertoli cell is in morphological and functional

contact with a certain number of germ cells; in men this is about **ten germ cells per Sertoli cell** (Zhengwei et al. 1998). This number is species-specific, as shown by flow cytometry and stereological analysis (Wistuba et al. 2007). Nevertheless, comparative studies between different primate species showed that testicular cell counts per gram of testicular weight are similar and that testicular size ultimately determines the total number of germ cells produced (Luetjens et al. 2005). In rats, prolongation of the division phase of Sertoli cells — caused by a change in the balance of thyroid hormones — causes an 80% increase in testicular weight and sperm production. Conversely, halving the Sertoli cell count after administration of an antimitotic substance resulted in a reduction of testicular size and sperm production. Patients with Laron's dwarfism syndrome suffer from thyroid dysfunction and growth hormone/IGF-I deficiency and often have testicles of above-average size.

The proliferation of Sertoli cells during the first postnatal months of life leads to an approximately fourfold increase in their number and is associated with growth of the testicular tubules to about the same extent. The main signal that mediates this increase is the gonadotropin **FSH**, but **LH** is also involved in this regulation (Mäkelä et al. 2019). GnRH controls this, as experiments have shown in which GnRH was antagonized and thus testicular growth was significantly reduced by impaired Sertoli cell proliferation (Sharpe et al. 2000). Sertoli cells already express the FSH receptor during postnatal life. The **anti-Müller hormone (AMH)**, on the other hand, is only produced by immature, not yet terminally differentiated Sertoli cells and is therefore an excellent marker for the maturity of these cells. AMH secretion is lost with the end of cell divisions and the beginning of cell differentiation during puberty. Instead, in response to the pubertal increase in intratesticular testosterone concentration and the onset of meiosis of the germ cells, the cells begin to express the **androgen receptor** and produce **inhibin** that acts on the hypothalamic-pituitary-gonadal axis (Mäkelä et al. 2019).

At the onset of puberty, Sertoli cells have built up close connections among themselves, allowing transport of substances and molecules only through cell membranes and thus in a controlled manner. These connections, i.e., desmosomes, **tight and gap junctions**, form the so-called **blood–testis barrier**, making the testis an immune-privileged organ (Mruk and Cheng 2015; see Chap. 28). Two main functions are postulated for the blood–testicle barrier: The physical isolation of the haploid and thus antigenic germ cells to prevent their recognition by the immune system (prevention of autoimmune orchitis; see Chap. 28) and the provision of a special milieu for the course of meiosis and sperm development. In seasonally reproducing animals, the breakdown and reconstruction of the blood–testis barrier does not depend on the developmental phase of the germ cells, but rather on the activity of the Sertoli cells.

Connexin-43 is a gap-junction protein that essentially controls the maturation and function of Sertoli cells. In transgenic mice with connexin 43 deficiency, spermatogenesis arrests at the level of the spermatogonia, which can no longer switch between mitosis and meiosis (Gerber et al. 2016; Rode et al. 2018; Hilbold et al. 2020).

Androgens control the expression of numerous indicators of Sertoli cell function, e.g., transferrin, androgen-binding protein, cadherins, connexin-43, gelsolin, laminin-gamma3, occludin, testin, nectin, cyxin, and vinculin (Mruk and Cheng 2015). These factors are involved in the formation of the blood–testicle barrier, spermatogenesis, and the formation and remodeling of Sertoli cell-cell connections, among others (Yan et al. 2008).

In the basolateral area of adjacent Sertoli cells, there are "occluding tight junctions" that close the intercellular gap. The physiological function of the blood–testis barrier has been demonstrated in experiments in which applied dyes or lanthanum only reached the tight junctions and not the lumen of the spermatic tubules. The blood–testis barrier divides the germinal epithelium into two regions that are anatomically and functionally completely different. The basal region contains the early germ cells and the adluminal region contains advanced and mature germ cells. During development of the germ cells, they are literally passed through the blood–testis barrier (Mruk and Cheng 2015).

The formation of the **blood–testis barrier** and its selectivity in the exclusion of molecules means that the cells located in the adluminal compartment have no direct access to metabolites from the periphery or interstitium. Therefore, these germ cells are dependent on supply by Sertoli cells. This nutritional function can probably be achieved by different mechanisms: selective transport and transcytosis as well as synthesis and vector secretion.

However, at least as important as the nutritional function of Sertoli cells is their ability to provide the undifferentiated germ cells in the seminiferous epithelium with a niche that enables their double function of self-renewal and provision of differentiating daughter cells. Sertoli cells secrete GDNF, a neurotrophic factor to which the undifferentiated spermatogonia bind and which is essential for the regulation of germ cell homeostasis (Parekh et al. 2019). The communication between Sertoli cells and undifferentiated germ cells, which enable their incorporation, takes place via the so-called C-X-C system, which is composed of chemotactic cytokines (chemokines) and specific ligands and receptors. This system enables the migrating germ cells to find and colonize the niches in the epithelium provided by the Sertoli cells (Heckmann et al. 2018).

2.1.1.2.3 Germ Cells

The process of spermatogenesis begins with the division of stem cells and culminates in the formation of spermatozoa (Figs. 2.3 and 2.4). The different germ cells are arranged within the seminiferous tubules in characteristic cell associations called stages of spermatogenesis (Fig. 2.5). In

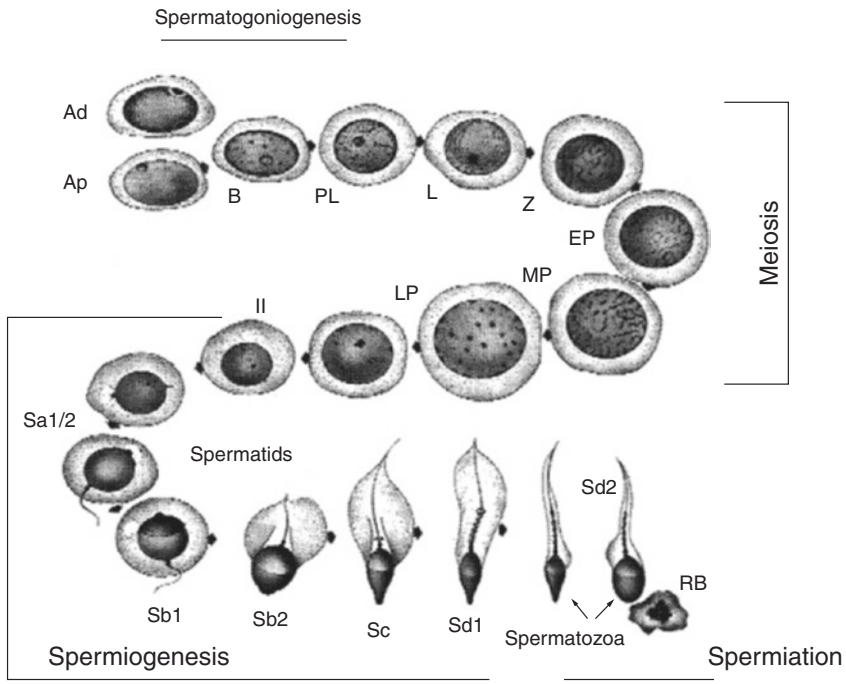


Fig. 2.3 Schematic representation of all germ cell types that occur in the human seminiferous epithelium. Ap spermatogonia enter the spermatogenic process (arrow on the cell indicates direction of germ cell development). Ad spermatogonia are the inactivated reserve stem cells and likely cover the germ line stem cell population. Ad = A-dark spermatogonium, Ap = A-pale spermatogonium, B = B spermatogonium, Pl = preleptotene spermatocytes, L = leptotene spermatocytes, EP = early pachytene spermatocytes, MP = mid pachytene spermatocytes, LP = late pachytene spermatocytes, II = second meiotic division, RB = residual body, Sa1 – Sd2 = developmental stages of spermatid maturation

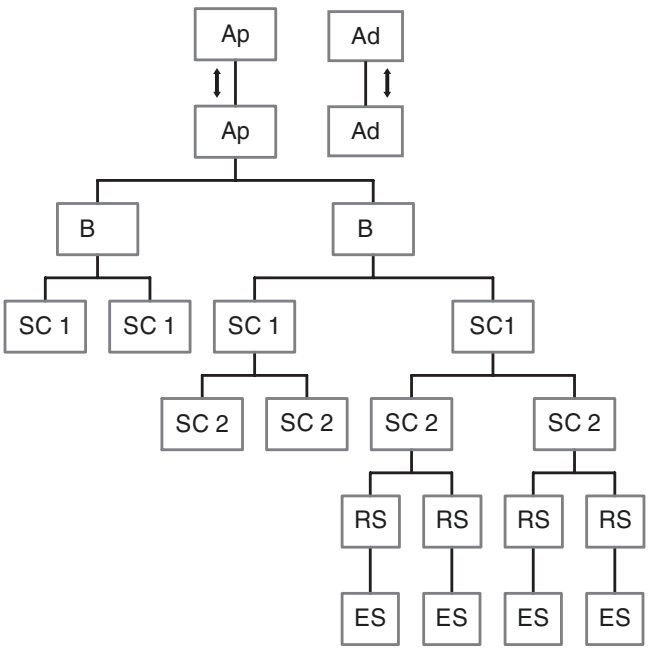


Fig. 2.4 Schematic representation of the proliferative kinetics of human gametogenesis. For the sake of clarity, complete development of only one spermatogonium is shown. The human testis contains about 1 billion sperm and releases around 25,000 sperm every minute (Amann 2008). The two types of A spermatogonia represent a dynamic system. Ap spermatogonia can derive from Ad (inactive reserve) or Ap (active) spermatogonia. Ap cells can also become Ad spermatogonia, e.g. after recolonization processes which might be induced by radiation therapy. One Ap spermatogonium can be the progenitor for 16 elongated spermatids. Since the human seminiferous epithelium contains only one generation of B-type spermatogonia, the final germ cell number produced is lower than in species with multiple spermatogonial divisions. Ad = A-dark spermatogonium (testicular reserve stem cells, divide rarely, giving rise to Ad or Ap), Ap = A-pale spermatogonium (self-renewing and progenitor cell for spermatogenesis), B = B spermatogonium, SC1 = primary spermatocyte, SC2 = secondary spermatocyte, RS = round spermatid, ES = elongated spermatid

principle, four phases of germ cell maturation can be distinguished:

1. **Mitotic proliferation** and differentiation of diploid germ cells (spermatogonia)
2. **Meiotic maturation** division of tetraploid germ cells (spermatocytes)
3. Transformation of **haploid germ cells** (spermatids) into spermatozoa (spermiogenesis)
4. **Release of sperm** from the seminiferous epithelium into the tubular lumen (spermatozoa)

Traditionally, three criteria are used for morphological identification of the different germ cell types in tissue sections. In addition to the localization of the cells within the sperm tubules, the characteristic morphology of the respective cells and cell nuclei is also taken into account. A comprehensive molecular analysis of the different cell types at the RNA level has become possible by technical advances in single-cell RNA sequencing. Using high-throughput approaches, the transcriptional profiles of thousands of unselected cells from testicular tissue cells can be analyzed. Based on the comprehensive sequencing data, the analyzed cells are assigned an identity only afterwards and based on their respective expression profile. Corresponding studies consistently show that, from a transcriptional perspective, germ cell maturation is a continuum in which one germ cell type passes into the next (Guo et al. 2018; Sohni et al. 2019). Furthermore, these analyses have enabled a more differentiated characterization of spermatogonia, which exhibit a surprising transcriptional heterogeneity and can be differentiated into at least five subpopulations based on the transcriptional profiles (Guo et al. 2018; Sohni et al. 2019).

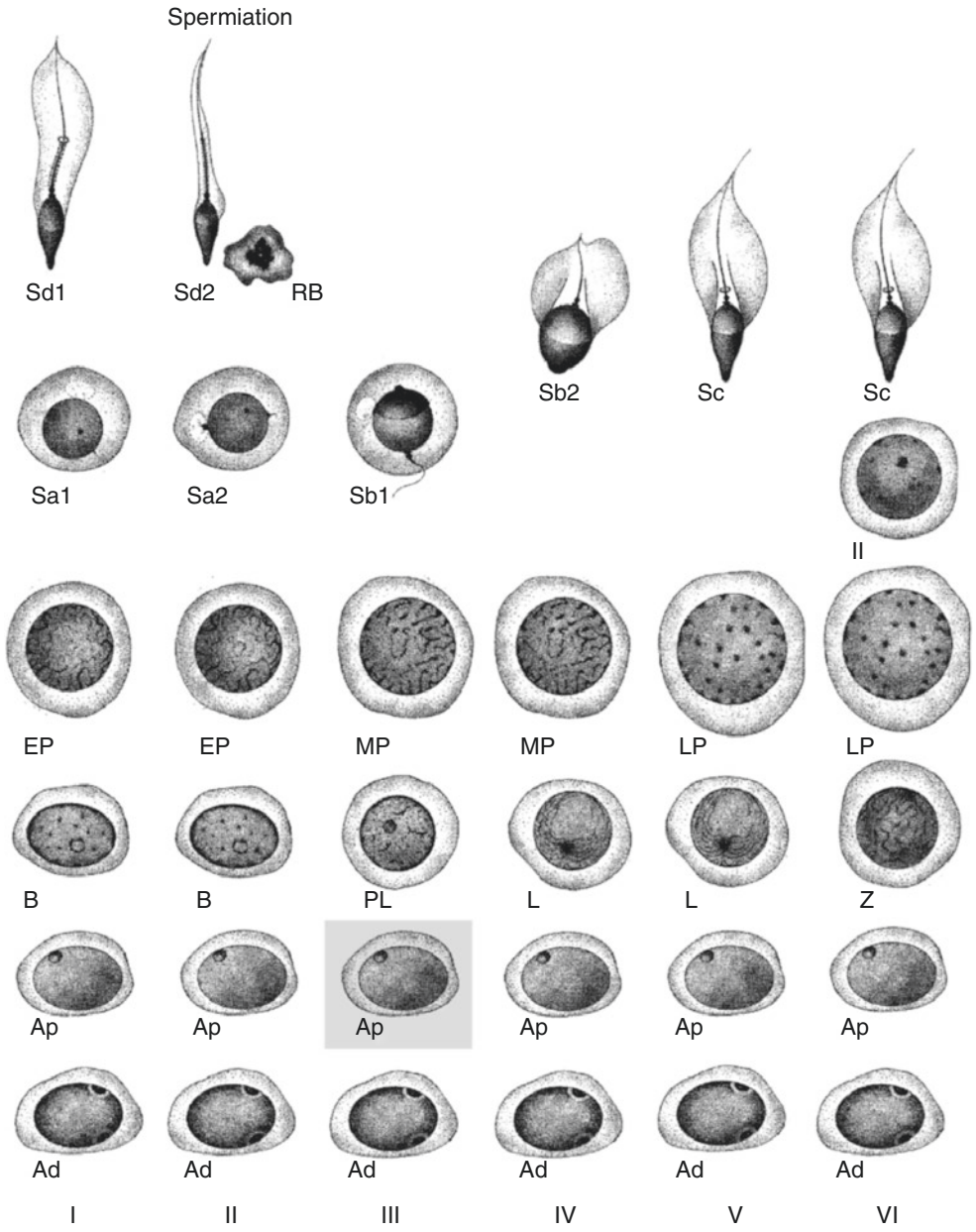


Fig. 2.5 Representation of the specific stages of spermatogenesis of the human testis using the 6-stage system. A tubular cross section contains typical germ cell associations that are denoted as stages of spermatogenesis. The six stages (I–VI) in the human last altogether 16 days. Since a spermatogonium has to pass through at least four cell layers (red line), the complete duration of spermatogenesis in men is 72 days. The complete duration of the human spermatogenic process is still not entirely clear (Amann 2008). Ad = A-dark spermatogonium (reserve stem cells, divide rarely, giving rise to Ad or Ap), Ap = A-pale spermatogonium (self-renewing and progenitor cell for spermatogenesis), B = B spermatogonium, Pl = preleptotene spermatocytes, L = leptotene spermatocytes, EP = early pachytene spermatocytes, MP = mid pachytene spermatocytes, LP = late pachytene spermatocytes, II = second meiotic division, RB = residual body, Sa1–Sd2 = developmental stages of spermatid maturation

The existence of multiple spermatogonial subpopulations is consistent with the model of a heterogeneous stem cell pool. Based on this model, the individual spermatogonial subpopulations can react differently to stimuli of the microenvironment depending on their current marker profile. However, since the potential of individual stem cells is not defined by a fixed marker profile, it is conceivable that stem cells with different marker profiles will behave similarly in the long term (Krieger and Simons 2015).

Apart from the transcriptional profile, the localization of the respective spermatogonia in relation to other germ cells and somatic cells is also relevant. Relevant factors such as GDNF and FGF2 are secreted by cells of the microenvironment, especially by Sertoli cells, and influence proliferation and differentiation of spermatogonia (Oatley and Brinster 2012; Sharma et al. 2019). Furthermore, the position of the spermatogonia relative to the blood vessels is of crucial relevance for the differentiation behavior of the spermatogonia (Yoshida et al. 2007). Another aspect of this model of a heterogeneous stem cell pool is the assumption that spermatogonia do not follow linear, hierarchical differentiation but reversibly transfer between individual spermatogonial subpopulations. In addition to tissue homeostasis, this could promote a rapid recovery of the spermatogonial population, as observed in the case of spermatogonial depletion after gonadotoxic treatment.

The model of a heterogeneous stem cell pool is in contrast to the traditional stem cell model, which assumes a unidirec-

tional differentiation process of undifferentiated spermatogonia. In the course of this process, the potential for long-term cellular self-renewal is increasingly lost. This traditional model is consistent with the morphological classification into Type A and Type B cells of spermatogonia based on their location and nuclear chromatin structure.

Two forms of A spermatogonia are distinguished based on cytology and physiology: The Ad(ark) spermatogonia and the Ap(ale) spermatogonia. Ad spermatogonia show no or very low proliferation activity under normal circumstances and can be regarded as testicular reserve stem cells (Sharma et al. 2019; Caldeira-Brant et al. 2020). These germ cells begin to divide actively when the population of the remaining spermatogonia types has been drastically reduced, e.g., by irradiation (Sharma et al. 2019). Also with increasing age, the Ad spermatogonia become apparently activated and they begin to divide, probably to maintain the efficiency of spermatogenesis when the pool of active Ap-spermatogonia becomes increasingly depleted (Pohl et al. 2019). Both types of A-spermatogonia can be transformed into each other, i.e., Ap-spermatogonia can be inactivated to Ad-spermatogonia and the reserve cells can be activated to Ap-spermatogonia. Ap-spermatogonia represent the active part of the spermatogonial stem cell population, which self-renew maintaining the stem cell pool and give rise to daughter cells that differentiate into B-spermatogonia (Sharma et al. 2019).

From the B-spermatogonia, preleptotene spermatocytes form immediately before the beginning of meiotic divisions. The latter germ cells initiate DNA synthesis. The mother cell and the resulting daughter cells remain in contact with each other via intercellular bridges. This clonal mode of germ cell development is the basis and prerequisite for the coordinated maturation of gametes in the germinal epithelium (Sharma et al. 2019).

Double diploid (meiotic) germ cells are called **spermatocyte**s and pass through the various phases of meiosis (preleptotene-zygotene). The pachytene phase is characterized by a pronounced RNA synthesis. Meiosis finally results in **haploid germ cells, i.e., spermatid**s. The meiotic process is a critical event during gametogenesis, during which the recombination of genetic material, reduction of the chromosome number from a diploid into a haploid set and differentiation of spermatids must be successfully completed. Secondary spermatocytes are formed from the first meiotic division. These germ cells contain a haploid set of chromosomes but in duplicate. During the second meiotic division, the secondary spermatocytes divide into the haploid spermatids. The prophase of meiosis I lasts 1–3 weeks while the remaining phases of meiosis I and complete meiosis II take place within 1–2 days.

The **spermatids** resulting from the second meiotic division are round and nondividing cells and undergo a striking and complicated transformation from which differentiated, elongated spermatids (testicular spermatozoa) emerge. These processes include the condensation and structural formation of the cell nucleus, the formation of a **flagellum,** and the reabsorption of large cytoplasmic portions. The entire process is called spermatogenesis and is qualitatively similar amongst most species. **Spermiogenesis** can appropriately be separated into four phases: the Golgi, cap formation, acrosome, and maturation phases. In the Golgi phase, the acrosome vesicle and craniocaudal symmetry are formed. In the cap formation phase, the acrosome forms and covers the cranial half to two-thirds of the elongated spermatids. The acrosome is loaded with enzymes that enable the spermatozoon to penetrate the eggshells during the fertilization process (see Chap. 3). In the **acrosome phase**, the cell nucleus condenses and elongation of the germ cell continues. In the course of nucleus condensation, most of the histones are lost and gene transcription is suspended. The DNA is now in the "packaging form." Transcription of the RNA, which is necessary for the protein biosynthesis that is now still taking place, must therefore have taken place first. These mRNAs are therefore comparatively long-lived. In the human testis, this applies, for example, to the mRNA encoding the transition proteins and protamines that play a role in chromatin condensation. The flagellum is now well developed.

The main event of the **maturation phase** of spermatids is the extrusion of the remaining cytoplasm in the form of so-called residual bodies. These are phagocytosed by Sertoli cells and have regulatory significance as elongated spermatids or their residual bodies influence the secretory function of Sertoli cells (production of tubule fluid, inhibin, androgen-binding protein, interleukin-1, and interleukin-6). In parallel to degradation of the residual bodies, a new cycle of spermatogenesis begins.

With the release of testicular sperm into the tubular lumen (**spermiation**), the process of spermatogenesis ends. The plasminogen activator and possibly Thimet oligopeptidases are involved in this process. This process is particularly sensitive to hormonal changes, temperature changes, and toxins. Unreleased sperm are absorbed by the Sertoli cells through phagocytosis. Haploid testicular germ cells, i.e., round and elongated spermatids taken from testicular tissue, can be used for assisted reproduction to induce pregnancies by intracytoplasmic injection (see Chap. 3 for details).

2.1.1.2.4 Kinetics of Spermatogenesis

The complex processes of division and differentiation of the germ cells follow a precisely defined pattern. These differentiation sequences over several stages lead to the appearance of typical cell associations ("cellular arrangements"), the so-called spermatogenic stages. Certain spermatogenesis events, such as acrosome development, are stage-dependent. The number of **spermatogenesis stages** are species-specific to a certain extent. In rats, 14 (I–XIV) stages are distinguished

and in macaques 12 (I–XII), as also described in the older literature for humans. In the meantime, however, for the comparative study of **human spermatogenesis,** a separation of **six stages** has become established (I–VI) (Nihi et al. 2017; Fig. 2.5). This simplified system is not only sufficiently precise, but has the advantage of allowing comparative studies of the organization of seminiferous epithelia during the spermatogenic cycle, for example between different primate species (Wistuba et al. 2003; Luetjens et al. 2005). The spermatogenic cycle signifies the sequence of all stages.

The duration of the spermatogenic cycle in mammals varies between 8 and 17 days depending on the species. A spermatogenic cycle in men lasts 16 days.

During the development and differentiation of a single spermatogonium into a mature spermatozoon, at least four spermatogenetic cycles are completed. The calculations of the total **duration of spermatogenesis** are based on this information. The values determined are approximately 50 days in rats, 37–43 days in various monkey species, and 74 days for the entire process including the maintenance divisions of Ap-spermatogonia in males (Amann 2008). In primates, the temporal sequence of spermatogenesis is independent of hormonal influences (Aslam et al. 1999), but the first cycle of spermatogenesis during puberty is more rapid than that which follows in the adult state. The fact that the duration of germ cell maturation can be manipulated by exogenous factors has been shown in rats.

The clonality of germ cell differentiation and the temporal sequence of spermatogenesis stages also determine a spatial sequence of germ cell arrangements. In serial cross sections of seminiferous tubules of rats, stage II was always found adjacent to stage I; stage IV adjacent to stage III, etc. This arrangement of stages represents the spermatogenic wave. Since each tubule cross section presented with only one specific stage, it was suggested that the topography of the stage arrangement is based on a longitudinal pattern. However, in the human testis and in testes of various monkey species, several stages were identified per tubular cross section (Fig. 2.6). By quantitative analysis of the germ cell populations, it could be shown that the distribution of the stages does not follow an irregular pattern as originally suspected. The distribution of stages becomes plausible when assuming a helical arrangement (Schulze and Rehder 1984; Zannini et al. 1999; Wistuba et al. 2007; Amann 2008). Other investigators were able to confirm the principle of helical arrangement, but not the occurrence of full helices (Johnson et al.

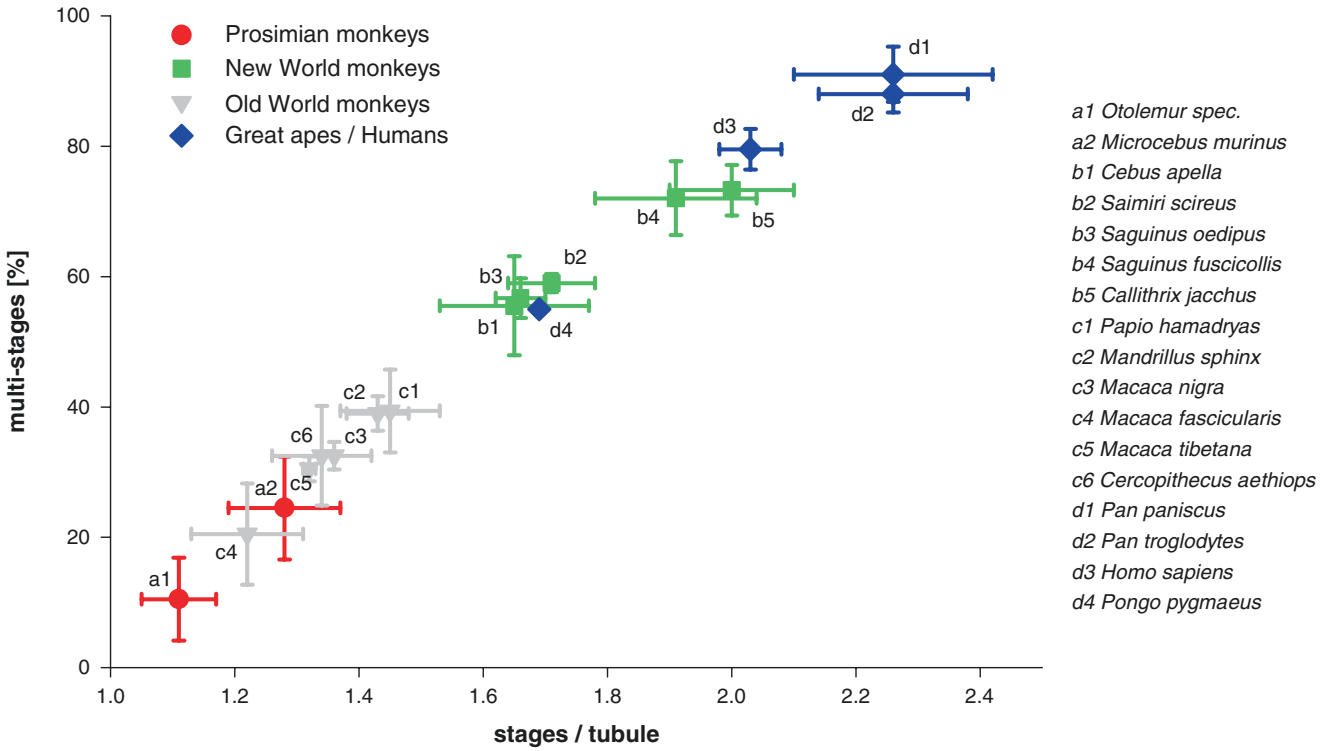


Fig. 2.6 Frequency (%) of seminiferous tubules containing more than one spermatogenic stage versus the number of stages per tubular cross section across the primate order. a1–a2: prosimians, b1–b5: new world monkeys, c1–c6: old world monkeys, d1, d2, and d4: great apes, d3: men. Note the clustering of multistage distribution and the increased number of stages in New world monkeys, great apes, and humans. The incidence of multistage versus single-stage tubules was not related to germ cell production (Luetjens et al. 2005 and Fig. 2.7)

1996). A maximum of 2–4 successive stages could be detected in serial sections. The observed distribution pattern of the stages could also be reproduced by distributing random numbers over the six stages. Therfore, it was concluded that the topography of spermatogenesic stages in the human testis corresponds more to a random distribution and one stage of spermatogenesis corresponds to a single cell clone (Nagano et al. 2001). Thus, the occurrence of one or more stages per tubule cross section could be determined by the respective size of the synchronously differentiating cell clones (Wistuba et al. 2003). A comparative and quantitative analysis of the distribution of spermatogenesic stages in 17 primate species showed that the seminiferous tubules in new world monkeys, great apes, and humans contain more than one stage, whereas in prosimians and old world monkeys there is predominantly one stage per tubule cross section (Luetjens et al. 2005; Fig. 2.6).

Quantitative studies using stereological methods as well as comparative flow cytometric studies in a larger number of primate species confirmed that the efficiency of spermatogenesis between primates is comparable and similar to that of rodents (Wistuba et al. 2007; Luetjens et al. 2005, Fig. 2.7). There is a difference in the number of spermatogonial stem cells, of which rats and mice apparently require fewer, and different numbers of mitotic spermatogonial divisions. For example, in men, only one generation of Ap-spermatogonia exists before differentiation into B-spermatogonia (Fig. 2.4), while in macaques four generations, and in mice numerous divisions of A-spermatogonia occur (Sharma et al. 2019).

Sperm production in primates is controlled by the number of spermatogonia entering meiosis.

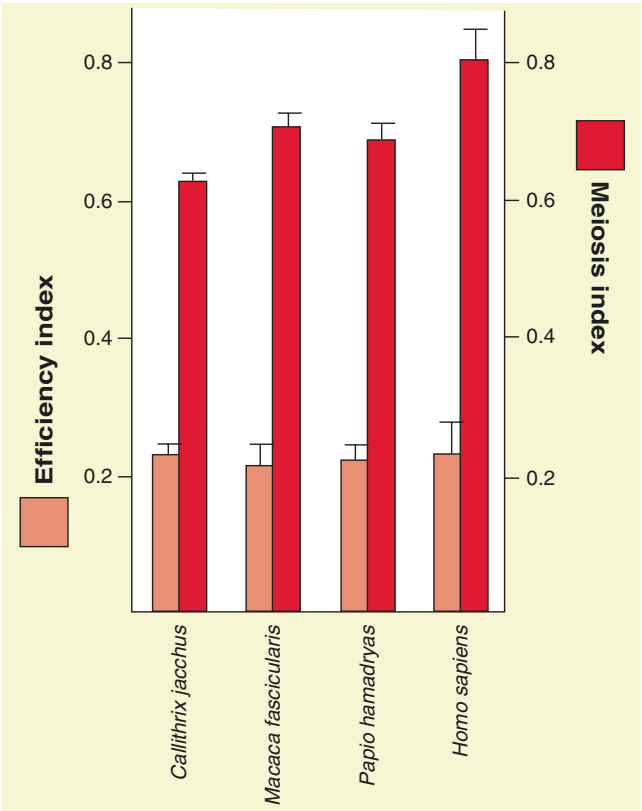


Fig. 2.7 Spermatogenic efficiency index (mean ± SEM) and meiosis indices for new world monkeys (*Callithrix jacchus* = marmoset, $n = 4$), old world monkeys (*Macaca fascicularis* = cynomolgus monkey, $n = 5$; *Papio hamadryas* = *Hamadryas* baboon, $n = 6$), and man (*Homo sapiens*, $n = 9$) based upon flow cytometric analyses of testicular tissue (mean ± SEM). The efficiency index is defined as the number of elongated cells divided by total cell number. The meiosis index is defined as the number of haploid cells divided by total cell number. Note that efficiency and meiosis indices are comparable between human and other primates based on Wistuba et al. (2003) and Luetjens et al. (2005)

2.1.1.2.5 Apoptosis and Spermatogenesis

Apoptosis refers to **programmed cell death**, which is characterized by the fact that a controlled sequence of signaling cascades leads to the "suicide" of the cell. In contrast to necrosis, this type of cell death occurs under physiological conditions (spontaneous apoptosis) but can also be induced by exposure to toxicants or disturbances of the endocrine milieu. Apoptotic spermatogonia, spermatocytes, and round spermatids occur regularly during normal spermatogenesis. **Apoptosis is a physiological process necessary for spermatogenesis.**

Animal experimental studies show that apoptosis of spermatogonia is a prerequisite for the spermatogenic processes, since, when apoptosis is experimentally induced, only spermatogonia are present in the seminiferous tubules. Heat treatment of the testes or hormonal disturbances induce apoptosis of germ cells in Cynomolgous macaques (*Macaca fascícularis*) via intrinsic and extrinsic mechanisms (Jia et al. 2007). The differentiation of Ap-spermatogonia into B-spermatogonia is gonadotropin-dependent in primates (Marshall et al. 2005). Gonadotropin deficiency leads to apoptotic spermatogonial loss (Ruwanpura et al. 2008). Gonadotropins can apparently act as stimulators of spermatogonial proliferation or as survival factors.

2.1.2 Hormonal Control of Testicular Functions

The endocrine regulation of testicular function (production of sperm and androgenic steroids) is well studied. The understanding of hormonal interactions has led to a number of clinical applications, which are described in the following sections. Figure 2.8 gives an overview of the organ systems involved, the endocrine factors, and their physiological effects.

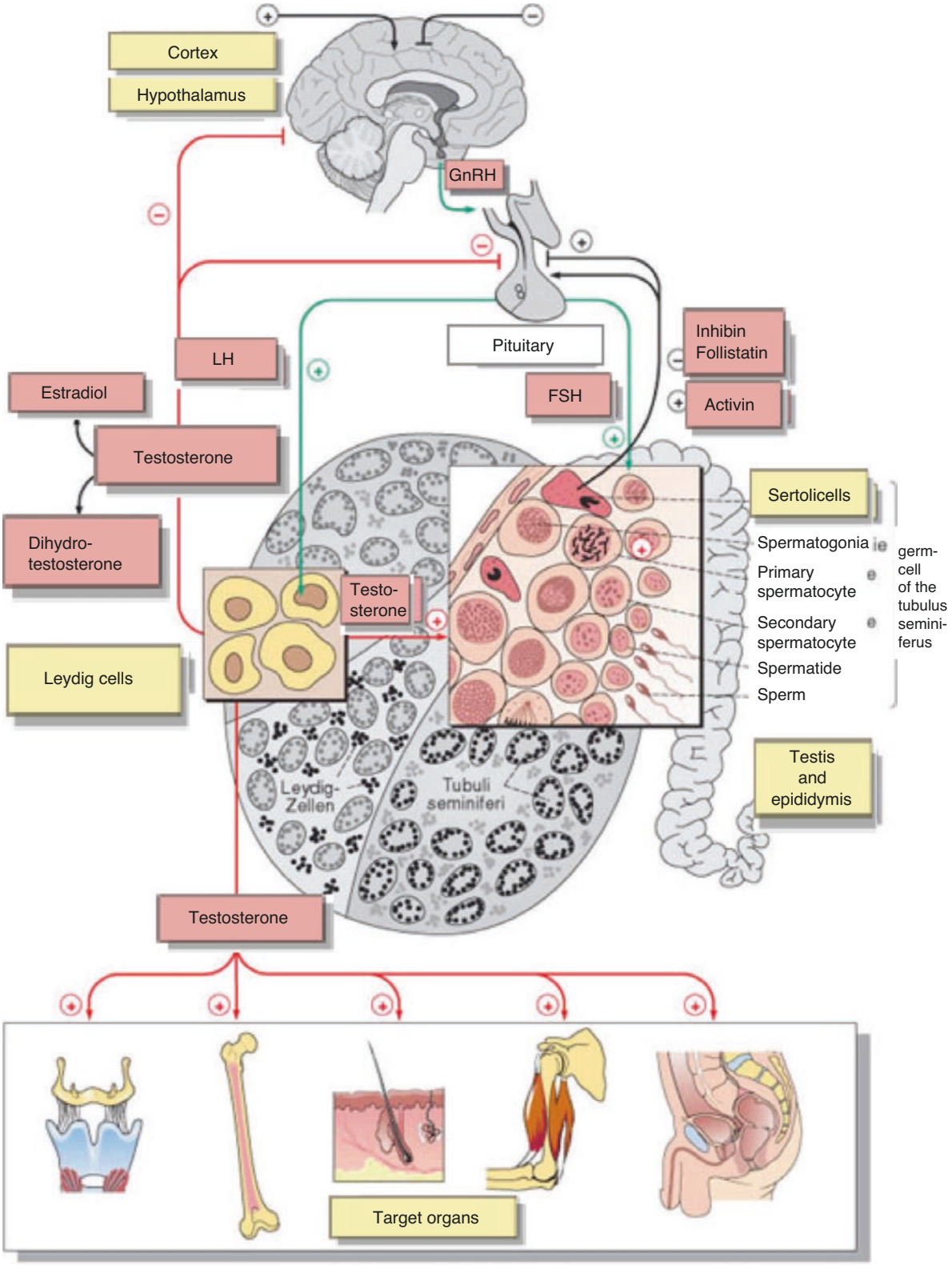


Fig. 2.8 Hormonal regulation of the testicular function and effects of androgens. Key hormones are luteinizing hormone (LH) and follicle-stimulating hormone (FSH), synthesized and secreted under hypothalamic control of gonadotropin-releasing hormone (GnRH). Leydig cells are located between the seminiferous tubules, synthesize, and secrete testosterone under the control of LH. Testosterone stimulates the maturation of germ cells in seminiferous tubules. FSH acts directly on the seminiferous tubules. In the germinal epithelium, only Sertoli cells possess receptors for testosterone and FSH. It is therefore believed that the trophic effects of testosterone/FSH on gametogenesis are mediated via somatic Sertoli cells. The testis and the hypothalamic-pituitary system communicate through steroids and protein hormones. Testosterone inhibits the secretion of GnRH and gonadotropins. Inhibin B and follistatin suppress selectively the release of FSH from the pituitary gland, while activin stimulates this process. Beside the effects on spermatogenesis, testosterone plays an important role in hair growth, bone metabolism, muscle mass and distribution, secondary sexual characteristics, and function of the male reproductive organs (adapted from Weinbauer et al. 2019)

2.1.2.1 Functional Organization of the Hypothalamic-Pituitary System

The gonadotropins luteinizing hormone **(LH**) and follicle-stimulating hormone **(FSH)** are synthesized and secreted in the gonadotropic cells of the **adenohypophysis**. They are named according to their function in the female sex, as historically these were identified earlier. In the male organism, they are involved in the control of testicular steroid synthesis and gametogenesis (Wistuba et al. 2007; Kaprara and Huhtaniemi 2018). The hypothalamus regulates the release of gonadotropins by the pituitary gland through pulsatile secretion of gonadotropin-releasing hormone (GnRH) under the control of the kisspeptin-GPR54 system (see Sect. 2.1.2.2). The pulsatile nature of GnRH release provokes also pulsatile gonadotropin secretion. The pulsatility of LH is more pronounced than that of FSH due to the shorter half-life of the hormone in serum. Both the synthesis and the release of GnRH, LH, and FSH are influenced by testicular factors (feedback mechanism; Kaprara and Huhtaniemi 2018). Due to the anatomical and regulatory relationships of the hypothalamus and pituitary gland, both are also considered as a functional unit.

Anatomically, the **hypothalamus** is the rostral extension of the reticular brain stem formation. It contains the perykaryon parts of the neurons that project the axon protrusions into the eminentia mediana, a special area at the bottom of the third ventricle from which the pituitary stem emerges. The hypothalamus is divided into three longitudinal areas, the periventricular, median, and lateral zones. The lateral zone acts as the connecting link between the limbic and brainstem regions, whereas the periventricular and median zones contain a variety of nuclei involved in the control of neuroendocrine and visceral functions. In the eminentia mediana, the protrusions of the hypothalamic axons reach a portal vein system. This is derived from a capillary plexus formed by the upper pituitary arteries and establishes the humoral connection to the adenohypophysis (neurosecretion of GnRH). Thus, the eminentia mediana and the portal vein system represent the essential link for functional communication between the pituitary gland and the hypothalamus and thus for the control of testicular function. The eminentia mediana is located outside of the blood–brain barrier and is therefore exposed to hormones and substances from the circulation.

The **pituitary gland** is embedded in the sella turcica, is located below the hypothalamus and the optic chiasm (crossing of the optic nerves) and is covered by a diaphragm. The **adenohypophysis** consists of the pars intermedia, pars distalis, and pars tuberalis. The pars distalis is of utmost importance for pituitary function. The gonadotropin-producing cells make up about 15% of the cell population of the adenohypophysis. These basophilic and PAS-positive cells are distributed over the postmedial region of the pars distalis. Although LH and FSH can be secreted separately, their synthesis occurs predominantly in the same cell type (Kaprara and Huhtaniemi 2018). The total content of FSH in the pituitary gland in healthy men is about 200 IU, of LH about 700 IU. About 80% of gonadotropic cells contain both FSH and LH. These cells have a very rough endoplasmic reticulum, a large Golgi complex and many secretory granules. They are often associated with prolactin-producing cells, suggesting a paracrine interaction between both cell types.

2.1.2.2 The Kisspeptin-GPR54 System

GnRH release is controlled by the **kisspeptin GPR54 system**. Kisspeptin is encoded by the gene KISS1, which is located on chromosome 1q32.1. Discovered and described in Hershey, Pennsylvania, in 1996, kisspeptin owes its name to the most famous product in the region, the "Kisses" of the Hershey Foods Company, a chocolate specialty (Dhillo 2013). The gene first became known as a tumor suppressor gene in melanoma and breast cancer ("metastatin," Harms et al. 2003). Kisspeptin is the ligand of the GPR54 orphan receptor, which plays an important role in the initiation of pubertal GnRH secretion.

The *KISS1* gene encodes a 145 amino acid peptide, which is cleaved into four shorter peptides: Kisspeptin-54, e.g., −10, −13, and −14, each with the corresponding number of amino acids. They all have a common C-terminal domain and an RF-amidated motif (Dhillo 2013). Kisspeptin-expressing neurons have been described in the anteroventral periventricular nucleus, periventricular nucleus, anterodorsal nucleus, and arcuatus nucleus. Outside the nervous system, expression of the KISS1 gene was detected in placenta, testis, pancreas, liver, and intestine (Irfan et al. 2016; Dudek et al. 2018).

Kisspeptin stimulates LH secretion by binding to the GPR54 receptor (synonymous with Kiss1 receptor; Ruohonen et al. 2020), which is located on the surface of GnRH neurons. This is a G-protein-coupled orphan receptor. The GPR54 gene is located on chromosome 19p13.3. Loss-of-function mutations of the gene lead to failure of puberty onset and hypogonadotropic hypogonadism (HH) (Feng et al. 2019). Thus, the kisspeptin-GPR54 system is essential for initiating gonadotropin release during puberty and for maintaining androgenization in adults. Between the kisspeptin-producing neurons in the anteroventral periventricular nucleus and in the nucleus arcuatus, there are projections to the preoptic region, where numerous GnRH neurons are located.

GPR54 signals are mediated by a Gq protein. Under experimental conditions, kisspeptin stimulates the turnover of phosphatidylinositol, the mobilization of cellular calcium, and the release of arachidic acid from GPR54-expressing cells and induces the phosphorylation of kinases activated by mitogenic protein. During continuous infusion, kisspeptin

stimulates LH secretion within 2 h followed by a decrease after 12 h as the GPR54 is desensitized. The pulsatile secretion of kisspeptin seems to directly induce the pulsatile release of GnRH and LH (Plant 2019).

Androgens, estrogens, and gestagens act via specific receptors and inhibit gonadotropin secretion — but not by direct action at the GnRH neurons. Kisspeptin mediates the negative and positive feedback effects of peripheral steroids on gonadotropin secretion. The steroid hormones act directly on the kisspeptin-producing neurons in the nucleus arcuatus and thus represent the site of negative feedback control of GnRH production. The positive feedback effects are mediated by kisspeptin, which acts in the anteroventral periventricular nucleus. The anteroventral periventricular nucleus is a sexually dimorphic nucleus under steroid influence (Plant 2019). Kisspeptin appears to mediate the effect of metabolic signals, e.g., of leptin, on reproduction, for example, the inhibition of hormone secretion in states of hunger. Connections between obesity, KISS1 expression, and GnRH release have also been described. Ghrelin, which mediates the release of growth hormones, is also thought to have an influence on kisspeptin synthesis (Tena-Sempere 2008). The interactions between kisspeptin and GnRH are shown schematically in Fig. 2.9.

2.1.2.3 Gonadotropin-Releasing Hormone

2.1.2.3.1 Structure of the GnRH

Two variants of GnRH, GnRH-I or GnRH, and GnRH-II are described. Different genes encode both variants. Although structurally very similar, there are significant differences in tissue distribution and control of gene expression (Cheng and Leung 2005). **GnRH-I** controls the gonadotropins. **GnRH-II** acts as a neuromodulator-stimulating sexual behavior. GnRH-I is a decapeptide that controls gonadotropin secretion. GnRH-I is synthesized in the neurons of the hypothalamus. These are derived from olfactory neurons and migrate toward the basal forebrain along the branches of the terminal and vomeronasal nerves during embryonic development. This migration of the different GnRH neurons along the olfactory bulb is influenced by various factors (Tobet and Schwarting 2006). The importance of these factors is reflected in the mutations of the coding genes in patients with Kallmann syndrome. In about 10% of patients with Kallmann syndrome (hypogonadotropic hypogonadism and anosmia caused by hypoplasia of the olfactory bulb), mutations or deletions in the KALL-1 gene on the X chromosome are detected (Topaloğlu 2017). The product of the **KALL-1 gene**, **anosmin-1**, is transiently expressed in the olfactory bulb and in various other tissues as a protein of the extracellular matrix and basement membrane during organogenesis. Further gene involvement in the migration of GnRH neurons and thus the etiology of Kallmann syndrome has been described: **fibroblast-growth-factor receptor 1 (FGFR1),** its ligand fibroblast growth factor 8 (FGF8), prokineticin 2 (PK2), and its receptor (PKR2) (Falardeau et al. 2008; Kim et al. 2008).

In primates, GnRH neurons are located in the mediobasal hypothalamus and in the nucleus arcuatus. In addition, GnRH neurons are also present in the anterior hypothalamus and other areas of the forebrain. The GnRH neurons are linked synaptically to nerve endings in which POMC-(proopiomelanocorticotropin)related peptides and enzymes involved in catecholamine and GABA (gamma-aminobutyric acid) metabolism occur. Furthermore, GnRH-positive neurons of the nuleus arcuatus are directly connected to neuropeptide Y (NPY)-positive neurons in the area preoptica and in the eminentia mediana. All these substances can influence GnRH secretion (Evans 1999; Smedlund and Hill 2020).

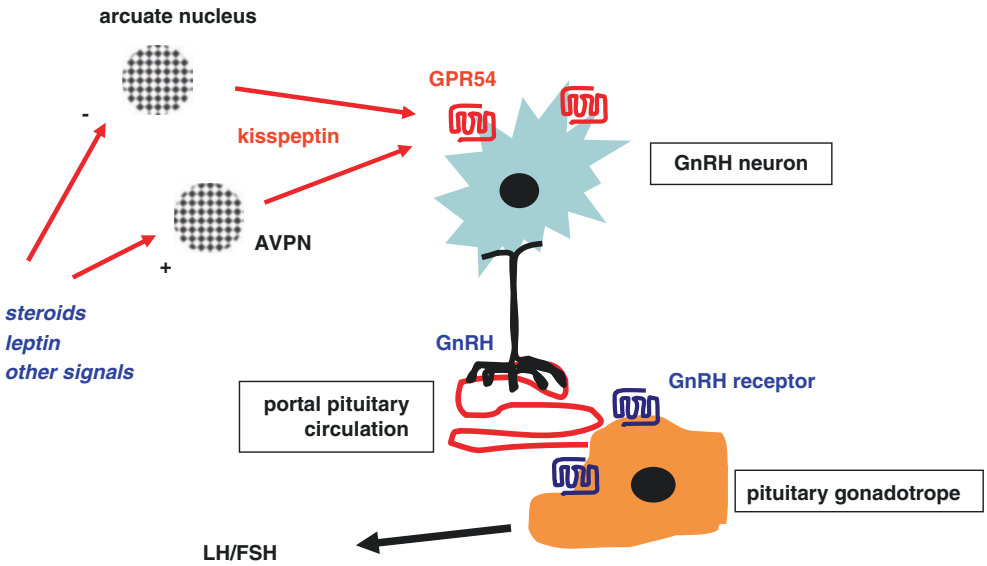


Fig. 2.9 Model of the regulation of gonadotropin secretion. Kisspeptin produced by the arcuate nucleus and in the anteroventral periventricular nucleus (AVPV) stimulates GnRH release by acting on the G protein-coupled receptor GPR54. GnRH release results in increased gonadotropin secretion from the pituitary gland, which stimulates LH and FSH production. Peripheral sex steroids (e.g., androgens, estrogens, and progesterone) as well as metabolic signals (e.g., leptin) regulate Kiss1 expression and signaling to GnRH neurons

The gene coding for **GnRH** has the chromosomal localization 8p21-p11.2. GnRH is produced by successive processing of a precursor protein, prepro-GnRH. Phylogenetically, GnRH is a very old hormone and shows a high homology between species. Thus, the homology between mammals and fish is 80%. The precursor consists of 92 amino acids (AS), preceded by a signal peptide consisting of 24 AS, which is necessary for transport to the cell membrane. 56 AS of the precursor form the so-called GnRH-associated peptide (GAP). The prepro-GnRH is processed in the rough endoplasmic reticulum, the signal peptide is cleaved in the Golgi apparatus, and the N-terminal glutamine (Gln) is cyclized to pyroGln. The sequence glycine-lysine-arginine, which is located at the border between GnRH and GAP, represents a processing signal and is responsible for the C-terminal amidation. The mature GnRH is a single-stranded decapeptide with cyclic conformation at the N-terminus, amidation at the C-terminus is folded (Millar et al. 2008; Stamatiades et al. 2019).

GnRH has a very short half-life of less than 10 min. It is largely degraded by peptidases immediately after secretion in the pituitary gland. By deciphering the amino acid sequence of GnRH, the importance of the different amino acids for the function of the GnRH molecule could be elucidated. Substitution experiments with amino acids showed that in positions 1–3, the biological activity is determined; in positions 6 and 10, the receptor binding; and in positions 5–6 and 9–10, the enzymatic degradation of the GnRH molecule. The discovery of the amino acid sequence of GnRH by A. Schally, who won the Nobel Prize in 1977, enabled the development of GnRH analogs with agonistic or antagonistic effects.

2.1.2.3.2 Secretion of GnRH

GnRH is secreted in **pulsatile fashion** into the portal vein system, whereby a GnRH pulse (Plant 2019) precedes each LH pulse. Frequency and amplitude of GnRH secretion determine the pattern of LH and FSH release from the pituitary gland (Fig. 2.10). Thus, GnRH is the decisive factor for the synthesis and release of gonadotropic hormones (Stamatiades et al. 2019; Plant 2019). By changing the pulse rate, it is possible to release LH or FSH preferentially. High-pulse frequencies or continuous GnRH application lead to an inhibition of gonadotropin release.

The causes of GnRH pulsatility have not yet been clearly clarified. Isolated, immortalized GnRH neurons show spontaneous pulsatility in vitro. Under in vivo conditions, GnRH release is controlled by the kisspeptin/GPR54 system, which mediates the effects of peripheral steroid hormones on the pulsatile release of GnRH. Furthermore, the pulse generator is subject to in vivo influences of noradrenergic neurons, galanin, and nitric oxide. Gonadectomy leads to an immediate increase in frequency and amplitude of the pulse generator. This observation points to a tonic suppression of GnRH secretion by peripheral steroids. In the absence of these steroids, the pulsatility of GnRH release is maintained, but there is no synchronization with the effector organs (Stamatiades et al. 2019; Feng et al. 2019; Smedlund and Hill 2020; Plant 2019).

Testosterone regulates GnRH secretion in men, with inhibition of gonadotropin secretion at both the hypothalamic and pituitary levels via negative feedback (Fig. 2.8). Effectors can be either testosterone or its metabolite dihydrotestosterone (DHT) or estradiol. Testosterone and DHT

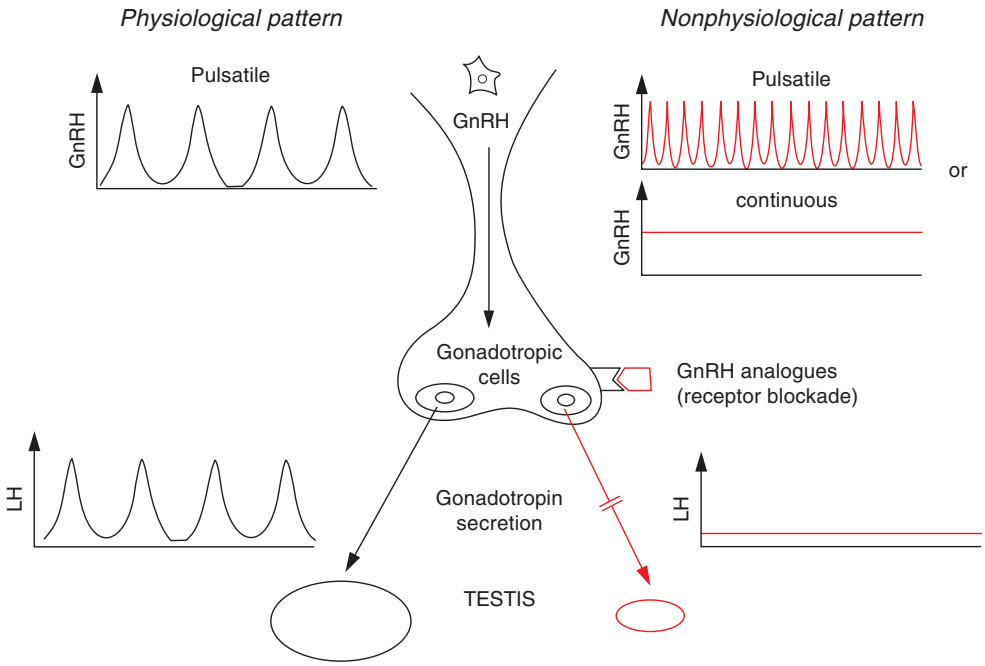


Fig. 2.10 Importance of the pulsatile pattern of GnRH secretion for gonadotropin secretion and testicular function: GnRH pulse frequencies above physiological levels or continuous administration of GnRH inhibit gonadotropin secretion and testicular function (red). Similarly, blockade of GnRH receptors by GnRH analogs results in suppression of testicular function

act on the hypothalamus by lowering the frequency of GnRH pulsatility. Estrogens suppress the secretion of gonadotropins by reducing the amplitude of LH and FSH secretion directly in the pituitary gland. Progesterone partially inhibits gonadotropin release via dopaminergic neurons and NPY neuron in the nucleus arcuatus (Dufourny et al. 2005). The negative feedback effect of androgenic and gestagenic steroid hormones is of great importance for the development of hormonal male contraception (see Chap. 48). Among the multitude of neurotransmitters and neuromodulators that can influence GnRH secretion, the noradrenergic system and the neuropeptide Y have a stimulatory effect. Interleukin-1, the dopaminergic, serotoninergic, and GABAergic systems, on the other hand, have inhibitory effects on GnRH secretion. Furthermore, leptin levels influence gonadotropin secretion. This effect is probably due to a direct hypothalamic influence via NPY- and POMC-positive neurons and especially kisspeptin-containing neurons with numerous leptin receptors (Popa et al. 2008; Malik et al. 2019).

2.1.2.3.3 Mechanism of GnRH Action

The effect of GnRH on the pituitary gland is mediated by a specific receptor. The **GnRH receptor (GnRHR)** belongs to the group of G-protein-coupled receptors, all of which have a typical structure of seven membrane-bound domains. Three different GnRHRs are expressed in most vertebrates (GnRHR-1, -2, -3), but only two of these are found in mammals (GnRHR-1 and -2; Stamatiades et al. 2019). The 7-transmembrane G-protein-coupled receptor family also includes the receptors for LH, FSH, and TSH (thyroid-stimulating hormone). The GnRHR-1 (GnRHR) is predominant. Both GnRH-I and GnRH-II bind to the GnRHR-1. With 328 amino acids, the GnRHR is the smallest member of the receptor family (Fig. 2.11). Characteristic for the GnRH receptor is the small extracellular domain and the absence of the intracellular C-terminal domain. Signal transduction occurs by interaction of the intracellular loops with the G-proteins.

The gene encoding the GnRH receptor contains three exons and two introns. The promoter contains several transcription start sites and some binding sites for transcription factors. It has been shown that cAMP, glucocorticoid, progesterone, thyroxin, PEA-3, AP-1, AP-2, and Pit-1 sensitive sequences are present. In the pituitary gland, the GnRH receptor gene is specifically expressed in the gonadotropic cells. The orphan receptor steroidogenic factor-1 (SF-1) is important for the specific expression of the GnRH receptor (Ngan et al. 1999; McDonald et al. 2016). In general, the transcription factors SF-1, Pit-1, and Prop-Pit-1 are very important for the formation and development of the hypothalamic-pituitary-gonadal axis. Both SF-1-deficient mice and patients with mutations of the Prop-Pit-1 gene show marked disturbances in gonadotropin secretion.

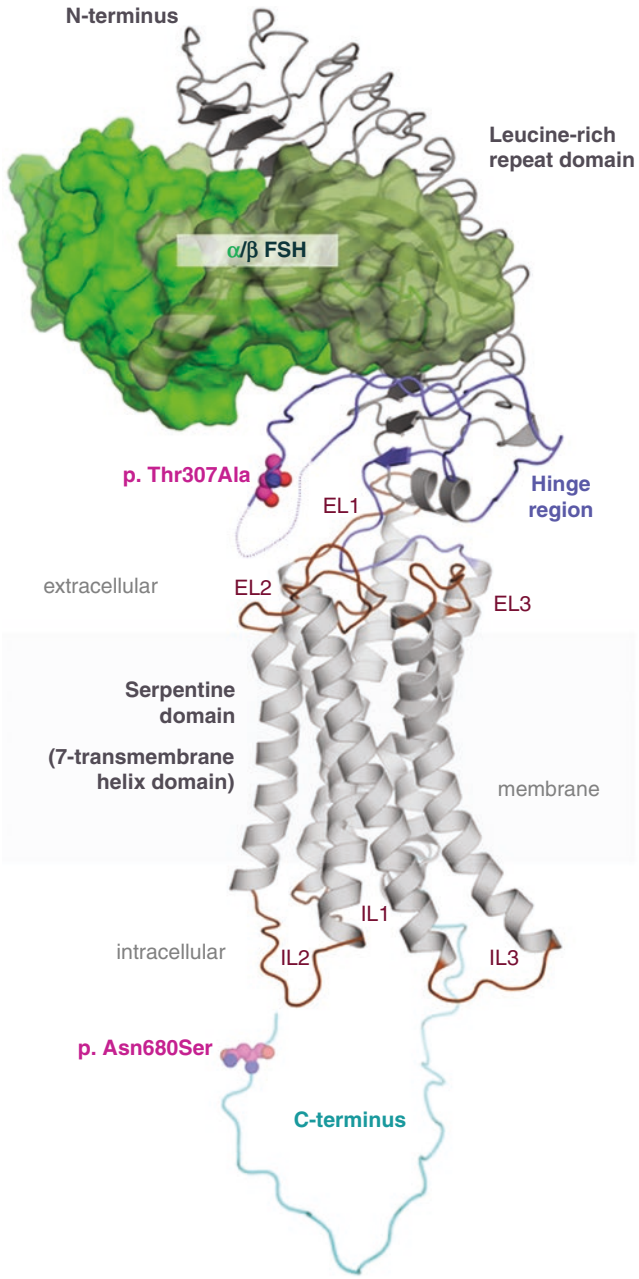


Fig. 2.11 Protein structure of the FSH receptor as an example for the family of G-coupled receptors. These receptors consist of several domains: one extracellular, seven transmembrane (serpentine domain), and one intracellular. The gonadotropin receptors show an extended N-terminal extracelluar domain which is necessary for the specificity of hormone binding and a C-terminal domain responsible for the signal transduction. The hinge region links the leucin-rich repeat domain to the transmembrane serpentine domain. The FSHR polymorphism is marked by p.Thr307Ala and p.Asn680S (from Schubert et al. 2019)

After binding of GnRH to the receptor, a **hormone-receptor complex** is formed. The hormone-receptor complex acts on a G protein (Gq), which in turn leads to activation of the inositol-3-phosphate signal transduction pathway, i.e.,

the formation of diacylglycerol and inositol phosphates, which induce a release of calcium from intracellular calcium stores. Furthermore, there is also an increasing calcium influx into the cell. Diacylglycerin and calcium activate protein kinase C (PKC), which vice versa is responsible for protein phosphorylation and further activation of the calcium influx. The increased intracellular calcium level then leads to a release of the gonadotropins by exocytosis and, as a longer-lasting effect, to a stimulation of gonadotropin synthesis (Stamatiades et al. 2019). Thereafter the hormone-receptor complex is internalized by endocytotic processes and degraded in the lysosomes.

GnRH can directly perform regulation of the **GnRH receptor**. Thus, receptor expression is high when GnRH is administered in a pulsatile fashion and low when the receptor is subject to continuous activation by GnRH. The process of receptor downregulation is called desensitization and is of therapeutic benefit. Although the use of a GnRH agonist leads to a short-term increase in gonadotropin secretion, continued stimulation results in slow receptor desensitization and thus in decreased gonadotropin secretion. The molecular mechanism of receptor sensitization is not yet fully understood. Due to the absence of the intracellular domain in the GnRH receptor, phosphorylation and rapid desensitization by GnRH agonists are impossible.

2.1.2.4 Gonadotropins

2.1.2.4.1 Structure of Gonadotropins

LH and **FSH** are both **glycoprotein hormones** that are structurally closely related to TSH and human chorionic gonadotropin (hCG). This group of hormones consists of two polypeptide chains each, a **α subunit** and a **β subunit**, and contains carbohydrate groups coupled to the AS asparagine. The α subunit occurs in all of these hormones, whereas the different β subunits for each hormone are responsible for biological specificity and effect.

Although the different subunits are structurally very similar, the genes for the subunits are located on different chromosomes. The gene for the α subunit consists of four exons and three introns each, whereas the gene for the β subunit consists of three exons and two introns each. The FSH-β gene is located on chromosome 11 and has an unusually long untranslated region at the 3′ end. This probably leads to a stabilization of the mRNA. The LH-β gene belongs to a complex group of genes including seven nonallelic hCG-like genes, all of which are located on chromosome 19. The regulation of gene expression for LH and FSH has been investigated intensively and includes complex interactions between hypothalamic GnRH, gonadal steroids, and peptides acting at the pituitary and hypothalamic levels.

The common α subunit contains two glycosylation sites, namely at positions 52 and 78. The glycosylation sites for the β subunit for FSH are located at positions 7 and 24 and those for the β subunit of LH are located at position 30. In mammals, the α subunit for hCG is synthesized in the placenta. Very small amounts of hCG are also produced by the pituitary gland and can be detected in men's blood.

The β subunits of LH and hCG are structurally very closely related and both LH and hCG bind to the same receptor. A special feature of hCG, however, is a C-terminal extension of four sugar residues, which leads to reduced hormone metabolism and thus to a longer half-life. This structural peculiarity has been used to produce therapeutic and experimental gonadotropin analogs containing a C-terminus similar to hCG. By this modification, the half-life of FSH and LH could be significantly extended.

These glycoprotein hormones contain a central mannose molecule, which is bound to the amino acid asparagine (Asn) via two N-acetyl-glucosamine residues and to which tetrasaccharide branches are attached, ending in sialic acids in the case of FSH and in sulfate groups in the case of LH. Changes in the length or composition of these carbohydrate structures lead to the heterogeneity of the gonadotropins, which manifests itself in separable isoforms of different half-lives. LH, which has a high percentage of N-acetyl-glucosamine sulfates, is rapidly removed from the blood via specific sulfate receptors in the liver, resulting in the short half-life of about 20 min for LH, which in turn explains the pulsatility of LH levels in the blood. FSH, on the other hand, is predominantly sialized and is therefore more resistant to degradation by the liver. The half-life for FSH is therefore considerably longer, at 2 h. Although both gonadotropins, LH and FSH, are stimulated by GnRH, only LH shows pronounced pulsatility, while FSH shows low pulsatility (Stamatiades and Kaiser 2018).

> The glycosylation of gonadotropins is of crucial importance for their secretion: Nonglycosylated hormones are not secreted and, moreover, are not bioactive under in vivo conditions as they are rapidly degraded in peripheral blood.

The importance of glycosylation for the biological activity of gonadotropins could be shown experimentally by means of nonglycosylated hormone molecules. Isoforms without carbohydrate residues cannot be secreted. It could be shown in vitro that glycosylation is not necessary for receptor binding, but for its activation. Recombinant variants of hCG and FSH, which had defects at the sialization sites or mannose branching sites, proved that glycosylation at position 52 is necessary for triggering the cAMP response and steroidogenesis. Glycosylation is therefore a fundamental process for gonadotropin secretion and — as it affects half-life — also for bioactivity.

LH occurs in two different polymorphic variants in the normal population, one of which is characterized by the exchange of two amino acids at positions 8 and 15 of the β LH chain. This leads to the introduction of a second glycosylation site at position 13, an allelic variant found in approximately 12% of the central European population. The two amino acid changes give the gonadotropin increased in vitro bioactivity and shorter half-life (Huhtaniemi et al. 2010; Punab et al. 2015).

FSH is also polymorphic. Interestingly, the hormone variability is balanced by an equally polymorphic receptor, so that overall there are correlations with influences on fertility, such as lowered free testosterone concentrations, reduced testicular size, and larger ejaculate volumes as well as increased concentrations of testosterone, estradiol, and SHBG. These findings have recently led to therapeutic proposals to use this variability of the FSHß/FSHR system for the treatment of infertility by means of individually tailored FSH administration (Tüttelmann et al. 2012; Busch et al. 2015; Schubert et al. 2019).

2.1.2.4.2 Gonadotropin Secretion

After synthesis, LH and FSH are mainly stored in different granules, which leave the cells via exocytosis in response to a GnRH stimulus. However, some of the molecules are not stored, but are directly and constitutively secreted. However, storage is the main reason why the release of gonadotropins occurs preferentially in response to a GnRH stimulus. A low GnRH pulse frequency mainly induces FSH release, which is partly due to the different expression of the GnRH receptors (Ferris and Shupnik 2006).

During fetal development, LH and FSH can be detected in the pituitary gland from week 10 of gestation and in blood from week 12. At the fetal stage and in early childhood, FSH levels are higher than those of LH; the FSH/LH ratio is higher in women than in men. The relative amounts of the two gonadotropins change during development.

Fetal LH and maternal chorionic gonadotropin (CG) represent the gonadotropic signal that induces the production of testosterone in the fetal testes from week 10 of gestation. This **testosterone release** is essential for the **initial phase of testicular migration** and the **development of the external genital organs**. Maternal CG alone can also induce a sufficient increase in testosterone, as can be deduced from experiments with mutated and biologically inactive LH. Despite its ineffectiveness, sexual differentiation was normal. However, if the LHCG receptor (**LHCGR, 2.2.4.3**) fails due to inactivating mutations or is reduced in its binding affinity, different phenotypes associated with disturbances in sexual development develop, up to complete androgen resistance with a female phenotype of the external genitals (Troppmann et al. 2013).

In the first years of life, serum levels of gonadotropins are very low; pulsatile secretion of gonadotropins does not begin until **puberty**. The pulsatile release of gonadotropins first occurs during nightly sleep. Before puberty, gonadotropin levels are low and secretion of GnRH is not detectable, although small amounts of steroids are produced in the gonads. It can be assumed that the hypothalamus is suppressed by the negative feedback regulation mechanism of the gonadal steroids and thus GnRH secretion is absent. In addition, factors such as physical maturity and nutritional status — probably mediated by leptin levels — and central nervous control also play a role.

The regulation of gonadotropin gene expression by steroids is complex and is influenced by a variety of factors. Steroids act mainly in the hypothalamus via the kisspeptin/GPR54 system, where they inhibit the release of GnRH. The effects on the pituitary gland have not yet been fully elucidated, but estrogens may be involved in the inhibition of GnRH release and gonadotropin synthesis.

Other factors are involved in the regulation of FSH secretion. The glycoprotein **inhibin B** also plays an important role in **feedback control of FSH secretion**. Serum levels of physiologically relevant inhibin B are inversely correlated with FSH levels, testicular volume, and sperm concentration. Intact spermatogenesis and functional Sertoli cells are crucial factors in the regulation of inhibin B secretion (Iliadou et al. 2015).

2.1.2.4.3 Mechanism of Gonadotropin Action

The **effect of LH and FSH** is mediated by **specific receptors**. Like the GnRH receptor, the receptors for LH and FSH belong to the family of G-protein-coupled receptors. A special characteristic of glycoprotein hormone receptors is the large extracellular domain involved in hormone binding (Simoni et al. 1997, Fig. 2.11).

The genes for the LH and FSH receptor are located on chromosome 2 and consist of 11 exons for the LH receptor and ten exons for the FSH receptor. The last exon codes for the transmembrane and the intracellular domain, respectively. The extracellular domain contains a high-affinity hormone-binding site and consists of many leucine-rich regions. The promoter of the two genes has different transcription start sites and does not have classical binding sites for transcription factors. Since the LH receptor also interacts with CG, the term **LH/CG receptor (LHCGR)** is increasingly used in recent literature. Different from the classical receptor, a variant occurs in primates and humans in which a cryptic exon called exon 6A is present. In rare cases, this exon can lead to LH resistance (Troppmann et al. 2013). A characteristic of the gonadotropin receptors is the occurrence of **isoforms** which are formed by alternative splicing of the primary transcript, but it is not clear whether these RNA isoforms are translated into proteins with biological function. Allele variants with different biological activities are also known. **Activating and inactivating**

mutations have been described for both gonadotropin receptors (Piersma et al. 2007).

The binding specificity of **gonadotropin receptors** is mediated by the generally present α subunit and the hormone-specific β subunit. After binding of the ligand to the receptor, a dimer is formed, the FSH-FSH receptor complex, which is crucial for signal transduction. This results in a conformational change with activation of a G-protein. Signal transduction is achieved by the synthesis of cyclic adenosine monophosphate (cAMP) and activation of protein kinase A. This leads to an activation of proteins by phosphorylation. The signal transduction pathway for gonadotropins works primarily via cAMP and intracellular calcium. This activation of the calcium signal transduction pathway seems to be dependent on gonadotropin concentration (Casarini and Crépieux 2019; Casarini et al. 2020).

2.1.2.5 Endocrine Feedback Mechanism and Relative Importance of LH and FSH for Spermatogenesis

The two functions of the testes, androgen production and gamete formation, are generally regulated by a **negative feedback mechanism** of the hypothalamus and the pituitary, whereby this system can be modulated in a species-specific fashion. Testosterone inhibits the release of LH and FSH. There is also another regulator for FSH, the proteohormone inhibin. **Inhibin B** shows a pronounced inverse correlation to FSH concentrations in men and, in conjunction with FSH, is an indicator of spermatogenesis activity (Schlatt et al. 2016).

When interpreting hormone effects on spermatogenesis, the following terms must be distinguished:

- **Initiation**: Hormonal induction of the first complete gametogenesis during puberty.
- **Maintenance**: Hormone requirements for maintaining intact spermatogenesis in the adult.
- **Reinitiation**: Hormone requirement for the restimulation of gametogenesis after interruption.
- **Qualitatively normal spermatogenesis**: All germ cells present, but possibly in reduced numbers.
- **Quantitatively normal spermatogenesis**: All germ cells present in normal numbers.

The relative importance of testosterone/LH and FSH for the initiation, maintenance, and reinitiation of qualitatively and quantitatively normal spermatogenesis has been the subject of numerous studies (Fig. 2.12). It is generally believed that both testosterone/LH and FSH can induce the initiation, maintenance, and reinitiation of qualitatively normal spermatogenesis (Schlatt et al. 2016). For quantitative effects on spermatogenesis under physiological conditions, the action of both hormone systems is necessary (Schlatt and Ehmcke 2014).

The sensitivity of the regulation of hormonal regulation can be extensively illustrated by numerous preclinical studies in nonhuman primates as well as controlled clinical studies and various case reports. For example, foci of complete spermatogenesis are occasionally found in childhood in the immediate vicinity of testosterone-producing Leydig cell tumors and in patients with activating mutations of the LH receptor; high local testosterone concentrations or increased sensitivity to androgen action may thus induce sperm formation (Fig. 2.11). Conversely, in patients with incomplete spermatogenesis, an attempt can be made to produce sufficiently high intratesticular testosterone concentrations to provide a remedy. However, exogenous administration of such testosterone doses is not advisable because it shifts the feedback mechanism in such a way that intratesticular concentrations would be reduced. Therefore, in hypogonadotropic hypogonadism, testicular functions are stimulated with hCG and FSH. The hCG used as LH surrogate has a higher activity at LH/CGR and thus achieves high intratesticular testosterone production, which, together with FSH, stimulates spermatogenesis.

Patients with defective FSH subunit and azoospermia have also been described (Lindstedt et al. 1998; Phillip et al. 1998). These data suggest that FSH is normally necessary for the initiation of spermatogenesis. Since patients with selective LH deficiency (Pasqualini syndrome) have qualitatively normal spermatogenesis, this suggests that FSH can also initiate complete spermatogenesis. The exogenous administration of high amounts of testosterone and simultaneous treatment with gestagens leads to an inhibition of gonadotropin release and subsequently to a decrease of sperm concentrations in the ejaculate. For complete suppression of sperm production, complete inhibition of FSH secretion is necessary in primates (Weinbauer et al. 2001). Even with complete suppression of LH bioactivity, a short episode of FSH secretion led to a partial recovery of spermatogenesis (Weinbauer et al. 2001).

Thus, even with experimentally suppressed gonadotropin secretion, both FSH and LH can maintain spermatogenesis at least qualitatively (Matthiesson et al. 2006). This is of importance for hormonal male contraception, where both hormones have to be suppressed. Although both LH and FSH have the potential to stimulate spermatogenesis on their own, this is not always the case in patients under therapy with androgens/hCG. However, the combination with FSH can lead to reinitiation of spermatogenesis (Bouloux et al. 2003).

The interaction of LH and FSH in connection with spermatogenesis is species-specific. In some species, such as the Djungarian hamster, FSH is the key hormone for spermatogenesis, while testosterone/LH are necessary for the stimula-

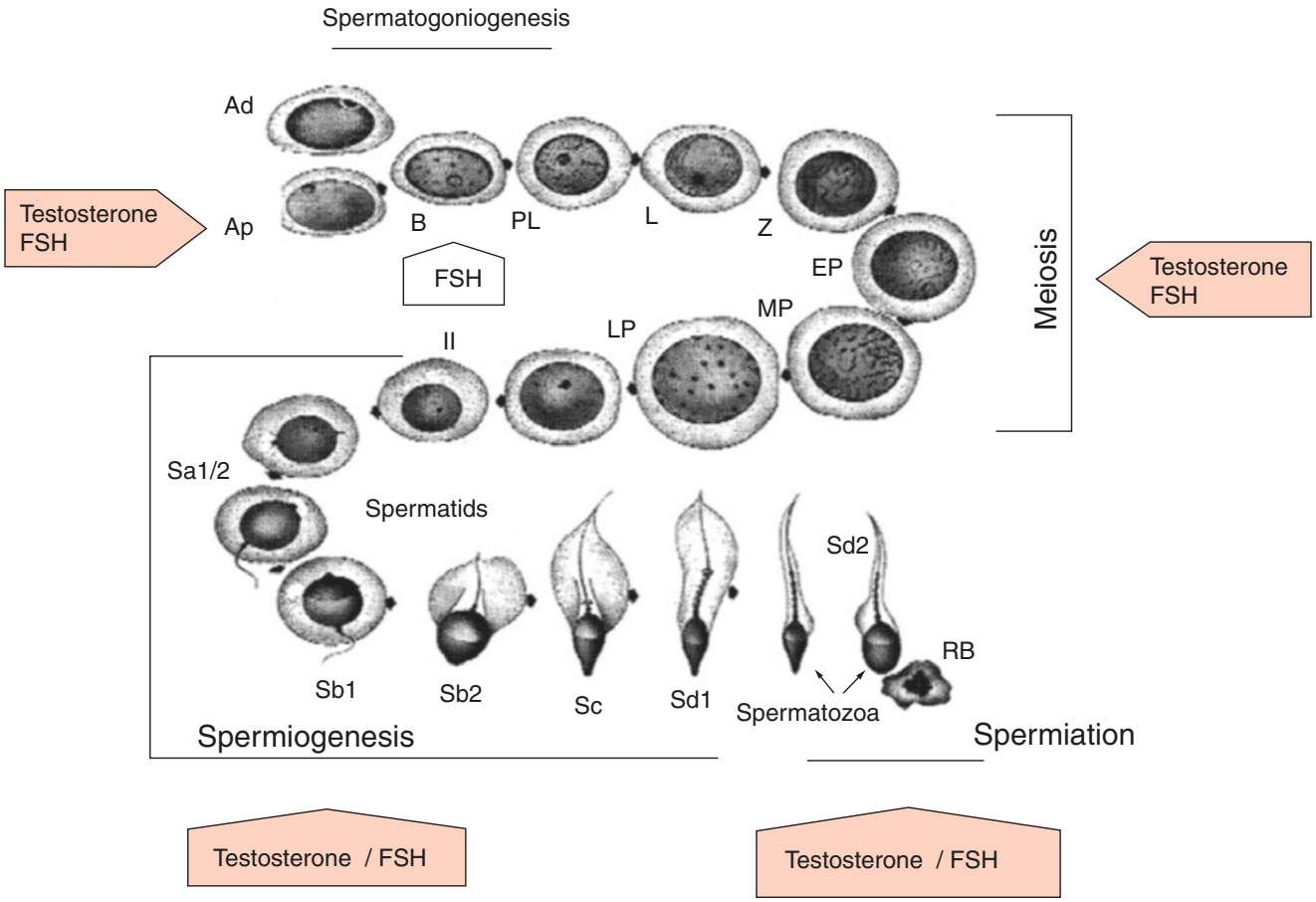


Fig. 2.12 Sites of action of testosterone and FSH on the spermatogenic process in primates. Ap spermatogonia enter the spermatogenic process (arrow on the cell indicates direction of germ cell development). Ad spermatogonia are believed to constitute the testicular stem cells. The majority of available data indicate that testosterone and FSH act on spermatogenesis via increasing numbers of type A-pale spermatogonia followed by an increase of subsequent germ cell populations. These endocrine factors might act via stimulation of proliferation and/or prevention of cell death. Meiotic transitions appear to be independent of testosterone/FSH action. FSH has been reported to play a role in chromatin condensation during spermiogenesis. Whether testosterone is needed for spermiogenesis still remians under debate. Spermiation is affected by gonadotropin sufficiency but it is currently unclear whether this is related to diminished actions of testosterone or FSH or both. Ad = A-dark spermatogonium (reserve stem cells, divide rarely, giving rise to Ad or Ap), Ap = A-pale spermatogonium (self-renewing and giving rise to differentiating daughter cells), B = B spermatogonium, Pl = preleptotene spermatocytes, L = leptotene spermatocytes, EP = early pachytene spermatocytes, MP = mid pachytene spermatocytes, LP = late pachytene spermatocytes, II = second meiotic division, RB = residual body, Sa1–Sd2 = developmental stages of spermatid maturation

tion of androgen-dependent hormones and sexual behavior. In contrast, in primates, LH and FSH are necessary for spermatogenesis. In marmosets, however, testosterone plays a subordinate role in maintaining spermatogenesis compared to Old World monkeys and great apes, where gonadotropin action is crucial. The biological relevance of this **dual control of spermatogenesis** has not yet been conclusively clarified (Wistuba et al. 2013; Schlatt and Ehmcke 2014):

For all practical clinical concerns, it is suggested that the **synergism of LH/testosterone and FSH** is necessary for the initiation, maintenance, and the reinitiation of normal spermatogenesis.

2.1.2.6 Local Regulation of Testicular Function

The complexity of cell types and testicular architecture requires a variety of local control mechanisms and interactions. Local regulatory processes can be divided into several categories: The term "**paracrine"**refers to the communication and interaction between adjacent cells (mainly by diffusion) and is used between the different compartments of the testis. "**Autocrine"** comprises those factors that, after release from the cell, have a retroactive effect on it. "**Intracrine"**refers to factors and substances whose production and site of action are in the same cell and which do not leave this cell.

Endocrine mechanisms certainly have priority in the regulation of testicular function. Presumably, the importance of **locally produced factors** lies in the fact that they modulate the action of endocrine factors, mediate **hormone effects,**

and serve **intercellular communication**. From this point of view, both gametogenesis and endocrine function of the testes are also under local control. For example, by the observation that the androgen receptor in the human testis is expressed differently in the different spermatogenesic stages supports this (Suarez-Quian et al. 1999).

The view that only Sertoli cells coordinate and regulate germ cell differentiation solely has given way to the insight that these processes are rather an interplay between germ cells and Sertoli cells, for example during epithelial implantation and mitotic and meiotic processes. This assumption is plausible because spermatogonia also compete with each other and influence Sertoli cells via certain factors (such as the CXC system and growth factors). These processes require different support from Sertoli cells depending on the degree of differentiation ((Griswold 2018).

Among the **local testicular factors** involved in this interplay are growth factors (e.g., fibroblast growth factor (FGF2)), stem cell factors, immunological factors, opioids, oxytocin and vasopressin, peritubular-cell-modifying-substance, renin and angiotensin, GHRH, CRH, ACTH, GnRH, calmodulin, ceruloplasmin, transport proteins, glycoproteins, plasminogen activator, dynorphin, PACAP, and metalloproteases. It can also be assumed that other, yet unidentified molecules exist that mediate communication between the interstitial and tubular compartments, between Sertoli cells and germ cells, and between the germ cells themselves.

2.1.2.6.1 Steroid Hormones

Among the secretion products of the testicles, such as 5α-dihydrotestosterone (DHT), androsterone, androstenedione, 17-hydroxyprogesterone, progesterone, and pregnenolone, **testosterone** is the most important. The importance of androsterone, 17-hydroxyprogesterone, and progesterone for spermatogenesis is not fully understood. However, progesterone receptors have been detected in peritubular cells and on sperm (Luetjens et al. 2006; Modi et al. 2007). Interestingly, the calcium ion channel CatSper, which plays a central role in fertilization processes, reacts to progesterone concentrations in the female tract. At least the expression of progesterone receptors in germ cells could thus be plausibly explained (Strünker et al. 2011).

In addition to its classical endocrine function, testosterone is also important as a **local regulator of spermatogenesis**. Testosterone is produced in the interstitium of the testes in the Leydig cells and acts directly on the sperm tubules. This **local effect of testosterone** is believed to be essential for fully functioning spermatogenesis. Exogenous doses of testosterone increase serum levels, thus lead to suppression of gonadotropins, lower intratesticular testosterone levels, and arrest germ cell differentiation — due to the local lowering of testosterone. However, studies in transgenic mice in which the LH receptor has been functionally depleted show that exogenous testosterone doses can also drive spermatogenesis, even when normal intratesticular levels are not reached. This led to the hypothesis that possibly high intratesticular testosterone levels are not always necessary. However, since these are purely animal studies, further research will be necessary to clarify these mechanisms (Shiraishi and Matsuyama 2017; Huhtaniemi 2018). The fact that body builders who consume the highest doses of testosterone develop azoospermia, supports the suggestion that the situation is different in men (see Chap. 37).

Although there is no question that testosterone is an essential regulator of spermatogenesis, there is no direct quantitative relationship between the extent of germ cell production and intratesticular testosterone levels.

In men, testicular testosterone concentrations are about 200 times higher than those for binding proteins SHBG and ABP, and compared to serum levels, testicular concentrations are about 150 times higher (Shiraishi and Matsuyama 2017). In tissue, testosterone is metabolized by 5α reductase to DHT and by aromatase activity to estradiol. To what extent these metabolites are important for spermatogenesis has not been completelyclarified. Treatments with 5α reductase inhibitors (finasteride/dutasteride) either had no influence on spermatogenesis or only led to a slight decrease in sperm counts (Kinniburgh et al. 2001; Overstreet et al. 1999; Amory et al. 2007).

Estradiol may play a role in extratesticular fluid transport, as is suspected from experiments on transgenic mice. Aromatase activity and estrogen β receptors were identified on Sertoli cells and germ cells in males. Local testicular estrogen concentrations may be partially higher than those in female serum. Although many mechanistic details are still unclear, numerous testicular physiological processes have recently been linked to direct and indirect estrogen activity. Among other actions, estradiol influences the proliferation of gonocytes, spermatogonial Leydig and Sertoli cells, the apoptosis of spermatocytes, spermatids and Sertoli cells, androgen metabolism, and spermatogenesis (Dostalova et al. 2017).

In addition to its effect on germ cell differentiation, testosterone has other physiological functions within the testes. It induces the formation of **smooth muscle actin** in the peritubular cells expressing the **androgen receptor** during prepubertal testicular development. This effect of testosterone is significantly enhanced by FSH, so it must be influenced by the Sertoli cells, since only these cells show **receptors for FSH**. Thus, a local effect in the testis can also be attributed to FSH (Mäkelä et al. 2019).

Testosterone mediates both endocrine and local effects (paracrine and autocrine) in the testes.

2.1.2.6.2 Insulin-Like Factor 3

Insulin-like factor 3 (INSL3) is a relaxin-like proteohormone produced in Leydig cells (Sansone et al. 2019). The promoter region of the gene has binding sites for steroid-dependent modulators, so that it can be assumed that testosterone and estrogens are involved in the regulation of INSL3. INSL3 acts via a G-protein-coupled receptor (RXFP2) which is expressed in Leydig cells and germ cells (Anand-Ivell et al. 2006). INSL3 is also involved in testicular descent and gubernacular cells express the RXFP2 receptor, which is a local regulator of Leydig cell differentiation. Although the hypothalamic-pituitary-gonadal axis is not directly involved in the regulation of INSL3, it should be noted that an LH stimulus is necessary to initiate formation of significant amounts of INSL3, which is consitutively formed after puberty. Mutations of INSL3 and its receptor RXFP2 provoke cryptorchidism, but are only responsible for a very small fraction of these pathologies. Since the receptors for INSL3 have also been found on meiotic and postmeiotic germ cells, a role in germ cell maturation is likely, although the exact mechanisms are not yet understood (Sansone et al. 2019).

2.1.2.6.3 Growth Factors

Growth factors bind to membrane receptors and induce cell-specific differentiation processes via signal transduction. Particularly **transforming growth factor-α and -β** (TGF-α and -β), **inhibin** and **activin, nerve growth factor** (NGF), **insulin-like growth-factor-I** (IGF-I), fibroblast-growth-factor (FGF), **epidermal growth factor** (EGF) are involved in local regulation of spermatogenesis. In primates, inhibin and activin are found in Sertoli and Leydig cells. Inhibins and activins are structurally related proteins, whereby inhibin is formed as a heterodimer from an α subunit and a βA — or βB subunit. Activins are homodimers (ββA and B). Activins stimulate, whereas inhibins suppress spermatogonial proliferation. Clinically, inhibin B is of particular interest because serum levels correlate well with testicular size and spermatogenesis status. This growth factor can be used as an endocrine indicator of spermatogenesis disorders (Iliadou et al. 2015).

From in vitro studies, it can be concluded that the local effect of inhibin and activin is a modulation of the steroidogenic activity of Leydig cells. Activin A induces species-specific testosterone production in Leydig cells either as a stimulator or as a repressor. IGF-I and TGFα have a general stimulating effect in the testes while TGF-ß shows inhibitory effects. In rats, the development of Leydig cells is controlled by an interplay between TGF-α and TGF-β. The steroidogenic activity of human Leydig cells is also stimulated by IGF. This growth factor directly influences human spermatogenesis: testicular IGF-I concentrations are positively correlated with the number of pachytene spermatocytes. IGF-1 is most strongly expressed in pachytene spermatotytes and stimulates DNA synthesis of mitotic germ cells. NGF localized in peritubular cells apparently plays an important role in the structural organization of human spermatozoa, as they can only be maintained in culture in the presence of this factor. For FGF, mitotic effects and effects on Sertoli cell-germ cell interaction are suspected.

2.1.2.6.4 Factors of the Immune System

The blood–testicle barrier and immunocompetent cells (macrophages) ensure that the germ cells are not recognized by the immune system as foreign to the body (see Chap. 28). There is evidence that factors of the immune system are involved in the control of steroidogenesis and gametogenesis and play a role in testicular dysfunction (Albrecht et al. 2005; Fijak and Meinhardt 2006; Hedger 2002).

These factors include secretory products of leukocytes, macrophages, and mast cells, for example **cytokines** (e.g., **interferon, tumor necrosis factor** [TNF], interleukins, leukemia inhibiting factor [LIF]**, stem cell factor** [SCF], **macrophage migration inhibitory factor** [MIF]) that bind to membrane-bound receptors and cause proliferative and differentiating reactions in the target cells (Hedger and Meinhardt 2003). TNF and LIF play a role in **Sertoli cell-germ cell interaction** and the autocrine control of Sertoli cell proliferation. MIF is specifically produced by the Leydig cells. If Leydig cells are eliminated experimentally, expression is also found in Sertoli cells, low level differentiated germ cells, and peritubular cells; i.e., compensatory mechanisms exist in the testis to ensure cytokine production. In contrast, SCF and its receptor (c-kit) are essential for the colonization of the testis by germ cells and their incorporation into the epithelium during individual development. Sertoli cells synthesize and secrete SCF, the receptor is expressed on spermatogonia. Thus, the SCF/c-kit system is exemplary for the relevance of local interactions within the germinal epithelium.

2.1.3 Testicular Descent

Abnormalities of testicular position are among the most common congenital abnormalities with an incidence of 1.8–8.4% at birth and are associated with impaired spermatogenesis and an increased risk of testicular tumors (Rodprasert et al. 2020, Fig. 2.13).

Descent of the male gonads is divided into two phases, the androgen-independent and the androgen-induced phase. First, the testis descends from the vicinity of the urinary tract into the pelvic cavity due to swelling and shortening of the gubernaculum (**transabdominal phase of descent**). This is controlled by the nervus genitofemoralis. The gubernaculum deforms, assuming a conical shape, pulling the gonad behind it and excretes an extracellular matrix rich in glycosamino-

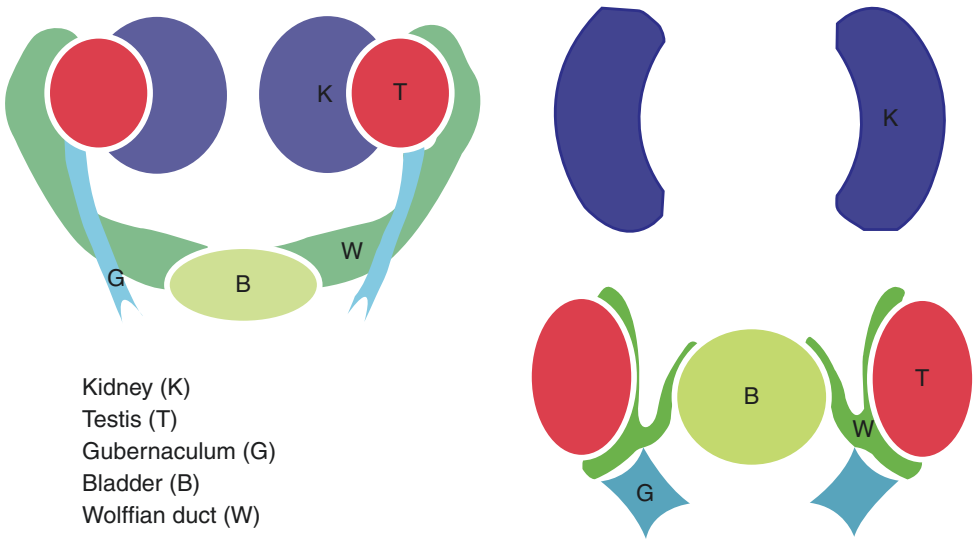


Fig. 2.13 Testicular descent. At an early stage, the gonad precursor organ is located next to the kidney (K). During the first testicular descendent phase between week 8 (left image) and week 17 (right image), the testis is anchored by the swollen gubernaculum close to the inguinal region. The limb bud-like growth of the gubernaculum is regulated by INSL-3 and androgens

glycans and hyaluronic acid. As the fetus continues to grow, the developing testes move into the scrotum (**inguinoscrotal phase of descent**), a process induced by androgens (phase 2 of descent) (Rodprasert et al. 2020). Gubernaculum outgrowth and penetration of the inguinal canal occurs between the 26th and 35th week of pregnancy. The gubernaculum partially degenerates and becomes a scrotal ligament. Reduction of the gubernaculum and intra-abdominal pressure contribute to displacement of the testes. At birth, the testis reach the bottom of the scrotum and in 97% of the boys, testicular descent is completed 12 weeks later (Fig. 2.13).

The physiological and endocrinological mechanisms of descent involve **androgens, calcitonin-like peptide, epidermal, and fibroblast growth factors** (Nightingale et al. 2008; Rodprasert et al. 2020). Furthermore**, Hoxa-10** and **INSL3** are involved in this regulation, since — in cases of experimental elimination of these factors — the testes cannot move out of the pelvic cavity. INSL3 is produced under androgenic induction by Leydig cells and stimulates the growth of the gubernaculum (Sansone et al. 2019; Rodprasert et al. 2020), which expresses the INSL3 receptor RXFP2 and thus normally "pulls" the testis behind it. In Hoxa-10 knockout mice, the testes descend but do not reach the scrotum, so that this gene product apparently regulates later processes that only take effect after the INSL3-induced descent. Endocrine disruptors such as xeno- or phytoestrogens are suspected to lower the expression of INSL3 and thus interfere with testicular descent (Fénichel et al. 2019).

2.1.4 Vascularization, Temperature Regulation, and Spermatogenesis

Vascularization of the testis has two tasks: transport of endocrine factors and the regulation of **testicular temperature**. The arterial supply of the testicular parenchyma follows the lobular arrangement of the seminal tubules. Each lobule is supplied by an artery from which segmental arteries emerge at intervals of about 300 µm and supply parts of the lobules with blood (Ergün et al. 1994a, b). The segmental arteries end in capillary vessels that branch out between the Leydig cells and finally enter the venous system.

In the adult male, the testicular temperature is 3–4 °C below body core temperature and 1.5–2.5 °C below the temperature of scrotal skin. Two **thermoregulatory systems** are available to the testis to maintain the physiologically low temperatures. First, the scrotal skin is very thin, without subcutaneous fatty tissue and has a large surface area, so that heat can be easily dissipated to the outside via this mechanism.

The second regulatory system, the **plexus pampiniformis**, is located in the spermatic cord. Here, the highly tortuous testicular artery is surrounded by several draining veins and the incoming arterial blood is cooled according to the countercurrent principle. Both cooling systems are involved with downregulation of testicular temperature, which prevents thermally induced spermatogenesis disorders such as those occurring in cryptorchidism in connection with an increased risk of germ cell tumors. In the event of excessively low external temperatures, the scrotum can contract very effectively and the testicle can be retracted into the abdominal cavity with the help of the cremasteric muscle. Some mammals, e.g., in whales, seals, and elephants, have internal testicles (which indicates that modern elephants had aquatic ancestors (West 2001)). Here there is evidence (albeit incomplete) that another system exists to cool the testicles by building up and melting body fat.

In **varicocele,** a varicose disorder of venous outflow, there is an increase in scrotal temperature (Hassanin et al. 2018). The prevalence of clinically relevant cases in different male populations can range from 5% to 20% and of these patients

many have primary or secondary infertility and reduced sperm quality (Zavattaro et al. 2018). The venous outflow and the associated increase in temperature (hyperthermia) contribute to spermatogenesis disorders. An induced increase in testicular temperature can reversibly block sperm production (Jung and Schuppe 2007). However, significant increases in temperature must be achieved. Warming of the scrotum by 0.8–1 °C in healthy volunteers over a period of 52 weeks showed no negative effects on the number and quality of the seminal filaments in the ejaculate (Wang et al. 1997).

Such sustained heat therapy induces mitogen-activated protein kinases (MAPK13 and MAPK14). Activation of these kinases in testicular lysates is accompanied by an increase of the B-cell leukemia/lymphoma (BCL) 2 protein in the cytosolic and mitochondrial compartments. This protein is active in the regulation of other cell proteins involved in cell apoptosis, including those in male germ cells (Jia et al. 2007). Under the influence of heat, BCL2 can be inactivated by phosphorylation of a serine residue. This disrupts the balance between apoptotic and antiapoptotic processes in the germ cells.

2.1.5 Testicular Androgens

Androgens are essential for the development of the male phenotype. The formation and function of the gonads and secondary sexual characteristics, formation of the musculoskeletal system, libido, and stimulation of spermatogenesis all depend on a regular concentration of androgens. The physiological effect of androgens depends on various factors: the number of **androgen molecules** in the cell, the degree of conversion to other **metabolite**s within the cell, interaction with the **androgen receptor**, polymorphisms of the receptor molecule, and actual receptor activation (Palazzolo et al. 2008). The concentration of androgens in the organism, in turn, depends on the rate of synthesis, which must be in balance with the metabolic rate of excretion.

Androgens can show their steroidal effects via both, a genomic and a nongenomic pathway, whereby their effects may in part overlap (Lorigo et al. 2020). **Genomic effects of androgens** usually occur after hours, whereas **nongenomic effects** often start after seconds or minutes. This mode of action cannot be reversed by common transcription or translation inhibitors, since it is presumably mediated by interaction with membrane-bound factors. Nevertheless, androgens modulate the transcriptional activity of the androgen receptor (AR) and other factors via this rapid pathway, leading to overlapping effects (Lorigo et al. 2020).

In men, **testosterone** is the most important serum androgen. More than 95% of the secreted androgens originate from the testes, which synthesize approximately 6–7 mg testosterone per day; the remaining androgens are mainly produced in the adrenal gland. Serum testosterone has a lifetime of about 30 min in the body and is mostly converted into **17β-estradiol** and **5α-dihydrotestosterone (DHT**). The site of testicular androgen synthesis is the Leydig cell. Both synthesis and secretion are regulated by pituitary LH and paracrine factors (Lei et al. 2001; Sriraman et al. 2005).

Androgen biosynthesis occurs always de novo, as Leydig cells are unable to store them. The starting point for androgen synthesis is cholesterol, a basic molecule for many metabolic processes in the organism, which displays the typical steroid-ring system and can be converted into androgens. In most cells, cholesterol is mainly needed for the formation and maintenance of the cell membrane. In Leydig cells, however, it is necessary to absorb more cholesterol molecules beyond this basic requirement in order to generate steroids from these precursors. LH controls the cholesterol balance and steroidogenesis in Leydig cells. By receptor-mediated endocytosis of low-density lipoproteins (LDL), either cholesterol is taken up directly by the Leydig cell or a de novo synthesis of acetyl coenzyme A takes place inside the cell. Cholesterol is stored in the Leydig cell within lipid droplets in the cytoplasm. The main product of the Leydig cell, testosterone, in turn regulates the balance of lipid droplets in the Leydig cell in a feedback mechanism. This mechanism also affects the synthesis rate of cholesterol by controlling enzyme expression (Eacker et al. 2008). The number of lipid droplets correlate with the synthesis performance of the Leydig cell, i.e., high synthesis performance leads to lower content of lipid droplets and vice versa.

2.1.5.1 Biosynthesis of Androgens

Androgen synthesis requires the **conversion of cholesterol to testosterone** (Fig. 2.14). Five different enzymatic processing steps are required for this conversion, during which the side chain of cholesterol is shortened through oxidation from 27 °C atoms to 19 °C atoms in two steps. During this process, the A-ring of the steroid in position 3 is converted into a keto configuration. The starting point for the conversion of cholesterol into testosterone is the side chain shortening by C22 and C20 hydroxylases followed by cleavage of the carbon bridge between C20 and C22, leading to the formation of pregnenolone. From pregnenolone, the endoplasmic reticulum follows either the Δ4 or Δ5 synthesis pathway.

The term Δ4 or Δ5 refers to the localization of the double bond of the involved metabolites. In men, the **Δ5 synthesis pathway** predominates in the buildup of testosterone. In the **Δ4 synthesis pathway**, pregnenolone is then dehydrated to progesterone, the most important biological key substance. The Δ4 synthesis pathway proceeds to the intermediate 17-hydroxyprogesterone. If the side chain is cleaved off, androstene-3,17-dione is formed and, through further reduction at the C-17 atom, testosterone results. In the Δ5 synthe-

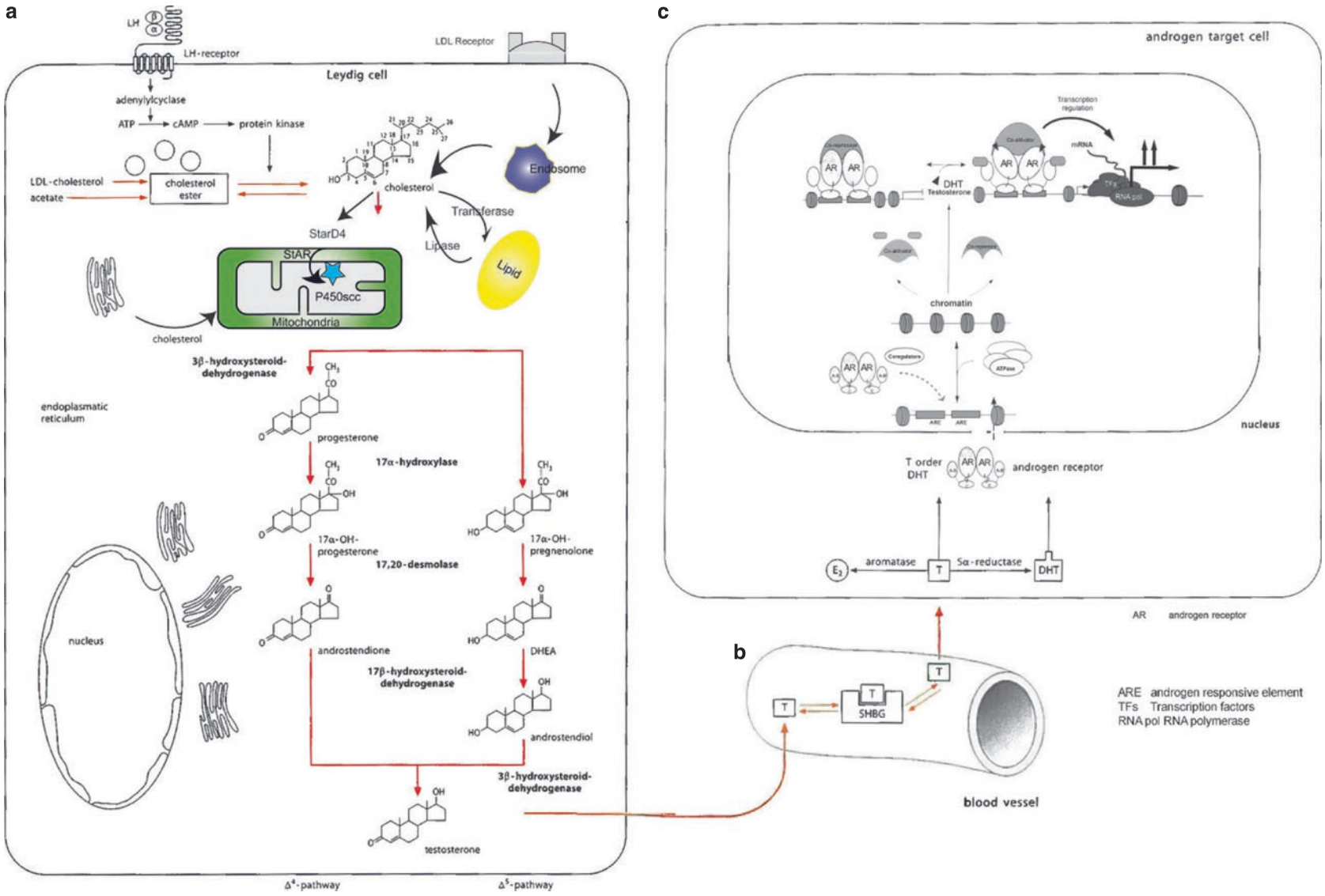


Fig. 2.14 (**a**) Steroid biosynthesis in the Leydig cell. Steroid biosynthesis from cholesterol or acetate is induced by LH through activation of adenylyl cyclase. The C atoms of cholesterol are numbered in order to follow the different enzymatic modifications and their localization. StAR (Steroidogenic acute regulatory protein) plays a key role during steroidogenesis. StAR is localized to the inner mitochondrial membrane and governs cholesterol transport. (**b**) Cholesterol uptake and transport to the inner mitochondrial membrane. Normally human steroidic cells take up circulating low-density lipoproteins (LDL) through receptor-mediated endocytosis, directing the cholesterol to endosomes. It may be esterfied by acyl-CoA cholesterol transferase and stored in lipid droplets as cholesterol esters. Free cholesterol, set free by the hormone-sensitive lipase, is probably bound by StarD4 for transcytoplasmic transport to the mitochondrial membrane. StAR is responsible for the rapid movement of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane, where it is converted by P450scc to pregnenolone.

sis pathway, testosterone is synthesized via the intermediates 17-hydroxypregnenolone and dehydroepiandrosterone (DHEA).

Cholesterol is of eminent importance for the biosynthesis of many groups of substances, such as the already mentioned steroids, oxysterols, and bile acids. In the cell, cholesterol is esterified and stored in the lipid droplet compartment until consumption of this initial metabolite in various syntheses shifts the balance. The cholesterol esters are hydrolyzed in the Leydig cell by stimulation of the LH receptor to reintegrate them into the biosynthetic cycle. The resulting cholesterol can only be further metabolized in the mitochondria and must therefore first be transported across the mitochondrial membranes.

The **Steroidogenic Acute Regulatory (StAR)** protein (regulatory mitochondrial protein) is 30 kDa in size and is responsible for the transport of sterols such as cholesterol from the outer to the inner mitochondrial membrane. StAR mRNA expression is rapidly triggered in many steroidogenic tissues (e.g., in the adrenal gland or corpus luteum) under endocrine stimulation. Cholesterol is likely to be taken up at the mitochondrial outer membrane by StAR mediation. A cholesterol-binding pocket is formed by a conformational change (Miller 2007). By phosphorylation of the StAR protein, the receptor-ligand complex is associated with a voltage-dependent anion channel 1 (VDAC1) and reduced to a smaller phospo-StAR, which can be passed through the membranes. If the phospho-StAR cannot bind its partner VDAC1, it is rapidly degraded by a cysteine protease (Bose et al. 2008). The essential role of StAR for normal steroidogenesis is illustrated by the phenotype in patients with an inactivating mutation in the StAR gene. Patients with such a mutation suffer from life-threatening congenital adrenal hyperplasia, which is provoked by their inability to synthesize sufficient amounts of steroids.

At the inner membrane of the mitochondria, cholesterol is converted from cytochrome P450 SSC (SSC = side chain cleavage) to pregnenolone. As other enzymes involved in steroid biosynthesis, cytochrome P450 belongs to the group of monooxygenases that contain the prosthetic heme group. The reaction sequence consists of three sequential monooxygenations with two electrons to activate molecular oxygen, C22 hydroxylation, C20 hydroxylation, and a cleavage of the C20–C22 bond. The pregnenolone formed in this process can then diffuse freely from the mitochondria into the cytoplasm and subsequently be converted into testosterone in the endoplasmic reticulum by the enzyme cytochrome P450 C17, which also belongs to the group of monooxygenases. However, not all pregnenolone molecules are converted into testosterone and intermediates may occur.

In addition to testosterone, the testes secrete **5α-dihydrotestosterone**, androsterone, androstendione, 17-hydroxyprogesterone, progesterone, and pregnenolone. The conversion of testosterone to DHT occurs in the target tissues, e.g., the prostate. Androstendione serves as a precursor for the extratesticular production of **estrogens**. Bioactive estradiol can be produced by extratesticular aromatization of androstendione to estrone and subsequent reduction in the peripheral organs. Only a very small portion of testosterone is stored in the testes, the greatest part is secreted into the blood.

The testosterone concentrations in the testicular lymph vessels and in venous blood are approximately the same, but there are significant differences in the flow rate of the two systems. For this reason, to a large extent the transport of testosterone into the general blood circulation takes place via the vena spermatica. Androgens diffuse through the interstitial tissue into testicular vein capillaries or are secreted directly into the veins by Leydig cells located at blood capillaries. The transport mechanism from the Leydig cell to the blood or lymph is not completely known. Probably, lipophilic steroids are distributed within cells or smaller cell assemblies predominantly by passive diffusion.

2.1.5.2 Transport of Testosterone in Blood

In plasma, testosterone is transported predominantly in a bound state. Two proteins are of particular importance here**, albumin** and **sex hormone-binding globulin (SHBG)**, a β globulin consisting of nonidentical protein subunits. The synthesis site is the testis and the liver. Plasma SHBG has a molecular weight of 95 kDa, has a high proportion (30%) of carbohydrates and has one androgen-binding site per molecule. A similar protein, namely androgen-binding protein (ABP), is secreted in the testis and was first described in rats and rabbits, in which it serves as the only binding partner for androgens as SHBG is missing in these species. Germ cells also express SHBG in the testis, but this is about 4–5 kDa smaller than the plasma SHBG. This second SHBG isoform is characterized by strongly reduced androgen-binding capacity (Selva et al. 2005). Plasma SHBG is expressed by Sertoli cells and is mainly taken up via the tubules in the caput epididymis by epithelial cells, which regulate maturation of the spermatozoa that pass through the isoform in an androgen-dependent manner. During this maturation, the sperm contain testicular SHBG, which is released afterwards during the capacitation reaction.

In men, 2% of testosterone is normally present in the blood unbound **(free testosterone),** while 44% is bound to SHBG, and 54% to albumin. Interestingly, the binding affinity of albumin is about 100 times lower than that of SHBG. However, due to the high albumin concentration in the plasma, the absolute binding capacities for both proteins are about the same. The ratio of SHBG-bound testosterone to free SHBG is proportional to the SHBG concentration. As direct determination of the free testosterone content in serum is difficult, this value is calculated from the total testosterone value.

The dissociation of testosterone from the binding protein occurs in capillaries. There, an interaction of the binding proteins with the endothelial glycocalyx occurs, which leads to a conformational change of the proteins in the hormone-binding site and thus to a change in affinity. Testosterone is subsequently released and can diffuse into the target cell. Thus, SHBG has a central function in the regulation of free testosterone. Steroids can reach their destinations in different ways. Either directly through the membrane as free steroid or bound to a steroid carrier molecule via a membrane-bound receptor. The entire complex can be actively taken up by endocytosis at the LDL molecule, as in the case of the **megalin protein**, or introduced into the cell via a steroid channel (Hammes et al. 2005). Megalin is expressed in the target organs of sexual steroids and is also a member of the LDL superfamily of endocytotic proteins. In serum 98–99.5% of the sex hormones are bound to protein. Compared to the free diffusion of biologically active hormones, endocytotic uptake plays a far more important role in the transport to the target organs.

SHBG is also capable of binding estradiol, hence the alternate name "**Testosterone-Estradiol-Binding Globulin (TeBG)**." However, this binding is influenced by various isoforms of SHBG and testosterone binds approximately three times more strongly than estradiol. Post-translational changes in the carbohydrate composition of SHBG can modulate the protein's binding affinities to testosterone and estradiol. The concentration of SHBG in serum is subject to hormonal regulation mainly by opposite effects of sexual steroids on hepatocytes — estrogens activate, while androgens inhibit. Other hormones, such as thyroid hormones, are also strong activators of SHBG production.

In men, the SHBG concentration is 30–50% of the female concentration. In healthy men with an intact hypothalamic-pituitary-gonadal axis, an increase in SHBG plasma levels leads to a short-term reduction in free testosterone while stimulating testosterone synthesis until normal levels are restored. Both an increase and a decrease in SHBG levels can be regulated by this feedback mechanism.

2.1.5.3 Extratesticular Metabolism of Testosterone

Testosterone serves as a prohormone for two other important hormones: Through 5α-reduction of testosterone, the biologically highly active **5α-dihydrotestosterone (DHT)** is formed, while aromatization of testosterone produces **estradiol**. Continuous conversion of testosterone causes a half-life of testosterone in plasma of only about 12 min. Estrogens can influence the testosterone effect synergistically or antagonistically. Low concentrations of estradiol in women and of testosterone in men are associated with a strong increase in bone turnover and can apparently reduce bone density while increasing **osteoporotic bone fractures** (Vanderschueren et al. 2014). In addition, estrogens have other specific effects. Estrogen receptor-inactivating mutations or aromatase deficiency prevent closure of the epiphyseal fissure during bone growth and may contribute to continuous linear bone growth and giant growth.

A synergistic effect of testosterone and estradiol is evident in transgenic mouse lines: in aromatase or estradiol receptor knockout mice, testosterone levels are elevated, but estradiol levels are lowered — resulting in impaired glucose tolerance (Takeda et al. 2003). The absence of aromatase is also associated with **increased insulin resistance** and diabetes mellitus type 2 in male patients and probably also with a (partial) loss of neuroprotective effects on the brain. The change in the testosterone-estradiol balance is mainly responsible for glucose intolerance and insulin resistance, which may also lead to gynecomastia in men.

The reduction of testosterone to DHT by the **enzyme 5α-reductase** takes place in the endoplasmic reticulum. Two isoforms of 5α-reductase exist in men, both of which are NADP-dependent enzymes that reduce the double bond from the fourth to the fifth carbon ring chain and metabolize a C19 into a C21 steroid. The **5α-reductase type 1 gene** is located on chromosome 5 and translated into a protein of 259 amino acids, whereas the gene for the slightly shorter, 254 amino acid **5α-reductase type 2** is located on chromosome 2. Both isoforms show a high degree of homology, but have different biochemical properties. Type 1 has its optimum in the basic range, while the optimum for type 2 is in the acidic range. The distribution of these two forms in tissue is also different. Type 1 5α-reductase is localized in the skin (with the exception of scrotal skin), liver, and brain, while type 2 5α-reductase is active in the classical androgen-dependent tissues such as epididymis, testes, scrotal skin, seminal vesicles, and prostate. Furthermore, it is also active in hair follicles and the liver as well as in the breast, uterus, and placenta of women. Changes in the activity of Type 2 5α-reductase can lead to a **partial androgen insensitivity syndrome** (PAIS).

Both androgens, testosterone and its derivative, DHT, bind intracellularly to the same gene-regulating androgen receptor (AR). However, the different biological mechanisms of action of both hormones mediated by the AR are still not fully understood. DHT has a differentiating and growth-promoting effect at the cellular level and is eminently important for undisturbed male sexual development and virilization. But it also has a positive effect on muscle mass, and the deeper vocal range. DHT transactivates the AR and causes a growth spurt via expression in the prostate and other organs. DHT is metabolized by **3α-HSD (aldo-keto-reductase (AKR) 1C2)**, which produces **3α-androstandiol**. This reaction can be reversed in the prostate by the NADH-dependent enzyme RoDH (HSD17B6), thus contributing to prostate growth (Penning et al. 2000). Five different isoforms of ASR are expressed in different human tissues, which in

turn exert their effects at the 3-, 17-, and 20-ketosteroid positions. However, mainly isoform AKR1C12 is encountered with the reduction of 5α-DHT to 3α-androstandiol. Inactivated metabolites of DHT are excreted via urine.

Some **androgen metabolites** are excreted freely, others are **coupled to glucuronic acids** before excretion in the liver. In doping tests, potential abuse of anabolic steroids is investigated on the basis of these excreted metabolites (Saudan et al. 2006). A glucuronidase response at C17 of the DHT metabolite androstane-3α,17ß-diol has been associated with some risk factors for men as the ratio of this metabolite to DHT is associated with fat distribution and therefore allows the evaluation of liver fat content, lipid droplet composition, insulin resistance, and diabetes (Vandenput et al. 2007).

The actual testosterone effect always consists of the interaction of testosterone and its metabolites, estradiol, and DHT.

2.1.5.4 Mechanism of Androgen Action

At the target organs, testosterone molecules dissociate from the SHBG and diffuse through the membranes into the target cells. Whether conversion from testosterone to DHT occurs depends on the organ. This is the case in the prostate, for example, where DHT is the biologically active androgen. Here, hydroxysteroid dehydrogenases in combination with the enzyme RoDH mediate binding of the ligand DHT to the cytosolic AR (Fig. 2.15). Steroid-binding hormone receptors take up their ligand in the cytosol through a confirmation change of the α-helix exposure. At the same time, chaperones are released and dimerization of the receptor enables transport into the nucleus, where the characteristic mechanism of action of these receptors begins: binding to specific sequences of genomic DNA and inducing expressiveness of the target genes. The mineral corticoid, glucocorticoid, thyroid hormone, retinoid, estrogen, and progesterone receptors also belong to this family of receptors. All receptors of the family show a strong homology throughout and probably originate from a common origin, which must have emerged very early in evolution due to great functional diversity (Bertrand et al. 2004). The members of this receptor family have several functional subunits; they have an N-terminal domain, a DNA-binding domain, a joint region, and a hormone-binding domain (Fig. 2.16.). Compared to other nuclear receptors, the homology of the N-terminal domain in steroid receptors is only small. The function of this domain is DNA-binding, transactivation, and transrepression of ARs (Klocker et al. 2004).

Nuclear receptors can be divided into two classes depending on their ligands. The first class forms homodimers with one bound ligand per monomer, e.g., the AR and other steroid receptors. The second class forms heterodimers with only one bound ligand, e.g., the thyroid hormone receptor. Most nuclear receptors have 12 α-helices at the C-terminal end. The binding of the ligand leads to an angular change of one of the 12 α-helices and to a transactivation of the receptor so that a pocket is formed in the hormone-binding protein sequence in the tertiary structure. This pocket allows the uptake of lipophilic molecules, because they completely enclose the ligand.

An important feature of the N-terminal domain is the occurrence of **polymorphic repetitions of CAG triplets**, which code for polyglutamine chains of various lengths, and of polymorphic GGC triplets, which code for polyglycine chains of various length. In healthy men, 17–29 glutamine and 13–17 glycine amino acid repeats are present, while 37–72 such CAG repeats can occur in patients with Kennedy's syndrome, a disease of the motor neurons. The strong prolongation of both amino acid repeats is probably related to a reduction of the **transactivation possibilities**. **The longer these chains are, the less the receptor activates gene expression**. Conversely, if the CAG repeats are particularly low (<19), activity of the androgen receptor increases. These short chains have been associated with increased risk of prostate cancer, early onset of physiopathological symptoms in Kennedy's syndrome (and thus advanced disease progression at diagnosis) and increased aggressiveness (Shukla et al. 2016).

With about 90 kilobase pairs, the **AR gene** is localized on the X chromosome and (Fig. 2.16) encodes two different isoforms. The coding sequence consists of eight exons and comprises 2757 base pairs. The N-terminal domain is completely encoded by exon 1, the DNA-binding domain by exons 2 and 3, the steroid-binding domain by exons 4 to 8. The localization of the AR gene is conserved and can also be found in phylogenetically less evolved mammalian taxa such as monotremes and marsupials. Such conserved localizations of genes may indicate that the encoded gene products are developmentally relevant and therefore under high selective pressure.

As in all steroid receptors, the DNA-binding domain encoded by exons 2 and 3 of the AR gene, which makes up about 10% of the AR, consists of two zinc fingers. The domain comprises about 70 AS and is located between the N-terminal and androgen-binding domain. In this sequence section, eight cysteines with their sulfur atoms are aligned so that four of them bind one zinc atom each, resulting in the typical structure of two overlapping helices. While the first zinc finger, encoded by exon 2, is mainly required for the DNA, the second zinc finger, encoded by exon 3, stabilizes the receptor-DNA interaction with the negatively charged phosphate groups of the DNA and the dimerization of two receptor molecules to a homodimer due to its basic nature. In the immedi-

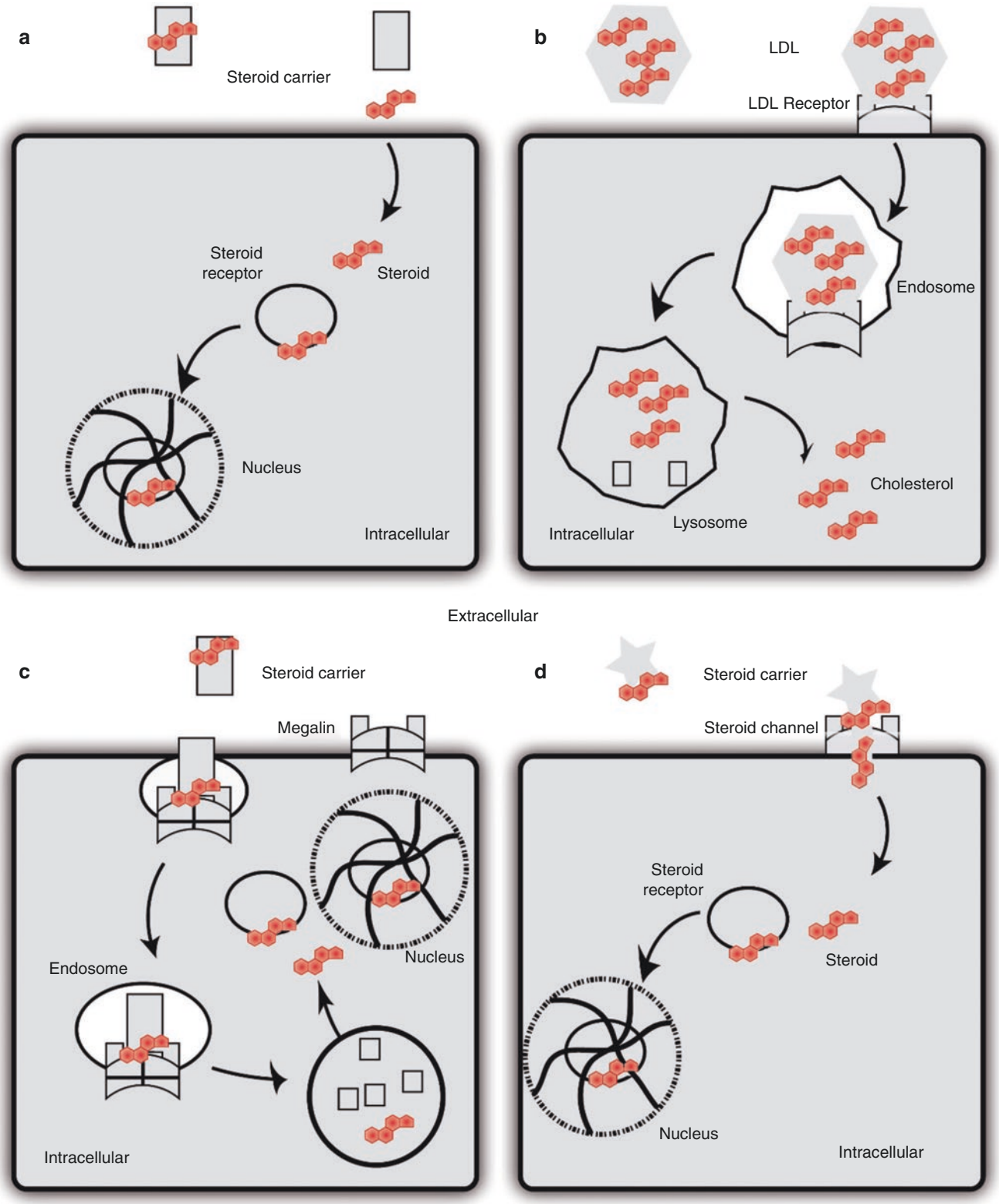


Fig. 2.15 Mechanisms by which steroid hormones can enter cells. (**a**) The hormone diffuses freely across the cell membrane, binds to an intracellular receptor, and enters the nucleus to regulate gene expression. (**b**) Receptor-mediated endocytosis of steroid containing lipophilic molecules. The lipoprotein LDL binds its receptor and is taken up, degraded in lysosomes and the steroid cholesterol can enter different metabolic pathways. (**c**) Receptor-mediated endocytosis of steroids. The entire hormone-carrier is endocytotically bound after binding to a carrier protein. Following intracellular, the degradation of the carrier, the ligand hormone is released into the free cytoplasm. (**d**) Transport-mediated uptake of molecules through the membrane. The steroid-carrier is recognized by a membrane receptor and the ligand is transported into the cell

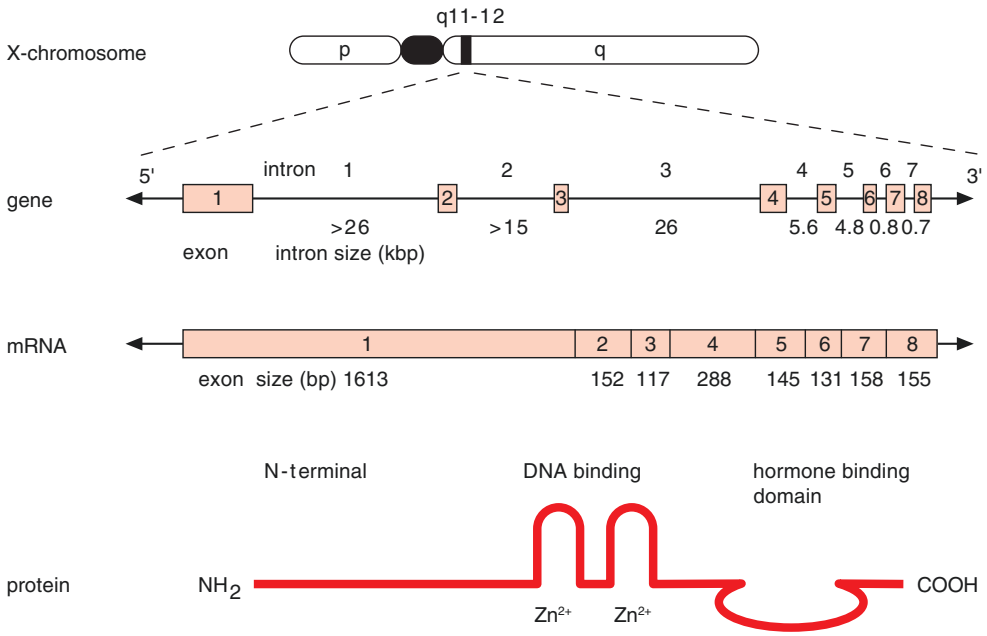


Fig. 2.16 Organization of the androgen receptor and chromosomal localization of the androgen receptor and structure of the androgen receptor gene and protein. Upper figure: the gene for the androgen receptor is localized in the pericentromeric region of chromosome X, at the site Xq11-12. Middle figure: the entire gene encompasses a region of about 90 kilobase pairs (kbp) on genomic DNA. Different exons are represented by boxes 1–8, with corresponding intron sizes reported below. Exon 1 encodes the N-terminal region, exons 2 and 3 the DNA-binding domain, and exons 4–8 the steroid-binding domain. Lower figure: schematic representation of the androgen receptor protein with the different functional domains. The genome spans more than 80 kb of chromosome X with eight exons. In exon 1, short tandem repeat regions (Gln and Gly). The protein structure consists of a transcription regulation domain, a DNA-binding domain, a hinge region, and the androgen-binding region

ate vicinity of the DNA-binding domain, there is a short amino acid sequence that represents a recognition signal that marks the receptor for transport into the cell nucleus.

The **androgen-binding domain of the AR** comprises about 30% of the total AR. The domain forms a lipophilic pocket built by 24 amino acids, which enables binding of testosterone or other androgens (Fig. 2.16). The experimental removal of this domain led to increased gene transcription in in vitro studies. Obviously, this part of the receptor is required for inducible and regulated gene transcription. The AR is regulated by many modulators as well as by phosphorylations at different protein positions. AR antagonists modulate the activity differently, depending on phosphorylation extent and position, whereas agonistic effects are not influenced by the degree of phosphorylation. Phosphorylation itself is regulated by steroid hormones but also by forskolin and phorbol esters at different amino acid residues. DHT binds to the receptor with a higher affinity than testosterone, but mainly because testosterone dissociates from the receptor faster than DHT. Other steroids such as androstendione, estradiol or progesterone bind to the AR with a much lower affinity than testosterone. Phosphorylation processes also have a crucial influence on the activity of the receptor in the joint region of the receptor and are involved in signal transduction of cyclic AMP and protein kinase C.

For inactivation, the AR forms complexes with chaperones such as heat-shock protein 90 (HSP90). When this complex is dissolved, HSP90 can join with other homologous or heterologous ligands in the cytoplasm and thus counteract apoptotic processes. In transgenic mice without functional HSP90, it became clear that this protein is also necessary for the transport of AR into the nucleus, as well as of special import proteins. In the cytoplasm, the androgen receptor ligand complex binds to importin-α, which, together with the complex, binds to importin-β. This importin-α-importin-β-androgen receptor complex can in turn be actively transported — with the aid of GTPases — through the nuclear pores into the nucleus. There the association dissolves and the released AR-ligand complex can bind to the DNA.

The two zinc fingers in the **DNA-binding domain** of the receptor enable attachment to DNA. It transforms into the homodimeric conformation, i.e., it consists of two identical hormone receptor complexes. The dimer binds to DNA sequences, called androgen responsive elements, which typically contain palindrome sequences (nucleotide sequences that are independent of the reading direction). These typical palindromes precede the androgen target genes and serve as recognition sequences for the AR. The homodimer can only bind to the DNA after modifications of the covalent bonds in their histones have taken place. Actylation, phosphorylation,

ubiquitination or methylation of the N-terminal end that regulate the DNA-binding properties of the histones are regulated, so that gene expression is either suppressed or supported. The methylation of arginine or lysine residues of histone proteins is associated with transcriptional activation or inhibition of certain steroid hormone-regulated genes. The lysine residues can be monomethylated, dimethylated or trimethylated. These differential methylations in turn serve as docking sites for control proteins that act as corepressors or coactivators.

If no nuclear receptor is present, the enzyme lysine-specific demethylase 1 (LSD1) counteracts gene transcription. If a steroid-specific receptor is present, LSD1 has the function to demethylate lysine residues and thus promote expression of the gene. This activation occurs through the attachment of the enzyme to the chromatin-bound AR complex. Actual gene expression only takes place when another coactivator, the demethlyase JHDM2A, binds to this complex. Two different demethylase mechanisms are therefore necessary for the AR to release its target genes for transcription (Metzger et al. 2006). The complete complex then induces transcription of the target genes by mRNA synthesis.

Receptor-associated proteins, **coactivators or corepressors**, age, specific cell type, and specific tissue modulate the activity of the transcription complex. There are also molecules called **selective androgen receptor modulators (SARMs)** that have regulatory activity. These SARMs are cofactors that exert nonsteroidal effects on AR and can cause different receptor activities in different tissues. They are therefore also used pharmacologically.

The SARMs described so far include arylpropionides, bicyclic hydantoin, quinoline, and tetrahydroquinoline analogs. What they have in common is that they are not substrates for aromatase or 5α-reductase. As AR ligands, they only mediate anabolic effects on muscles and bones, for example, but not on other male organs such as the prostate or seminal vesicles. The tissue-specific molecular effects of these SARMs include ligand-dependent receptor-conformant changes, differential interactions with DNA promoter sequences, and the incorporation of AR-dependent coregulators. These modulators are expected to provide improved treatment options for primary or secondary hypogonadism, anemia, osteopenia or osteoporosis. They could also be helpful in the development of a hormone-based contraception method for men.

More than 1000 mutations have been found for the **AR gene** so far (http://www.androgendb.mcgill.ca). Of these, about 10–15% are associated with metastatic prostate cancer. Other malignant tumors are apparently less frequently affected by AR mutations, such as testicular, liver, and breast cancer.

Inactivating mutations of ARs have dramatic effects, as they have no androgen effect at all (Fig. 2.17). Males with a 46,XY karyotype with such mutations show a feminized phenotype, but no uterus is formed and the vagina is only partially developed and postpubertal body hair is often barely present. This syndrome is called **Complete Androgen Insensitivity Syndrome (CAIS)** (see Chap. 31).

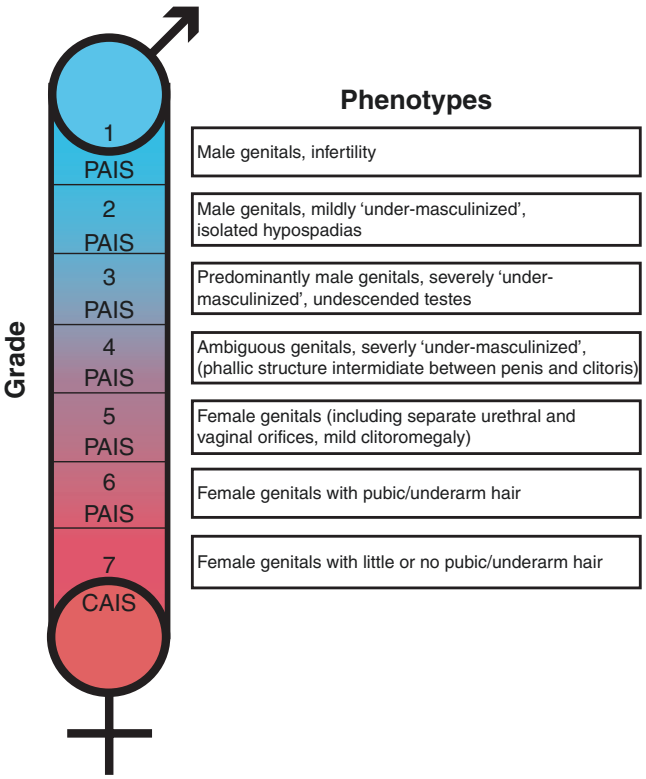


Fig. 2.17 Grading of the different severities of androgen insensitivity. The highest grade (7) is named CAIS (complete) while the lowest form is PAIS grade 1 (partial), adapted from Rajender et al. (2007)

Clinical consequences also include mutations in the DNA-binding or androgen-binding domain, which severely impair the function of the AR. Furthermore, mutations are also described that can affect the coactivators or corepressors and lead to varying degrees of AIS. Mutations in the N-terminal domain, which limit the function of the AR, rarely seem to provoke idiopathic male infertility (Zuccarello et al. 2008).

The CAG repeats in the AR gene appear to be associated with AR activity. Reproductively healthy men with shorter CAG repeats have higher sperm concentrations than those with longer ones (von Eckardstein et al. 2001; Hiort et al. 2012; Zitzmann 2020). Although there is general agreement that the repeats affect almost all AR-mediated effects, the mechanism and exact effectiveness is still unclear. While ethnic differences have been confirmed, apparently related to population-specific inheritance, the actual effects on peripheral sites of action are disputed, due to incomplete data. For example, no clear links to infertility have been found (Tirabassi et al. 2015).

2.1.5.5 Biological Effects of Androgens

In primates, AR can be found in the classically androgen-dependent organs such as muscle, prostate, seminal vesicle, epididymis, and testes, and in almost any other tissue such as hypothalamus, pituitary gland, kidney, spleen, heart, and salivary glands. Testosterone induces effects in all these organs (overview in Nieschlag and Behre 2012) (Fig. 2.18).

In the testis, the Sertoli, peritubular, and Leydig cells express the AR, while germ cells do not. Using transgenic mice, it was possible to reproduce effects caused by androgen regulation. The Sertoli cells need androgens to maintain spermatogenesis. Maturation of spermatocytes and spermatids is directly dependent on androgen action, as is the passive transport of testicular sperm, since the contractility of peritubular cells, which is crucial for this peristalsis, is also linked to the presence of androgens. Leydig cells need a functional AR to maintain the production of testosterone and thus the glandular function of the testes.

Androgens remain permanently important during the various phases of a man's life. In the **embryonic phase**, testosterone determines **sexual differentiation** of the reproductive organs; in **puberty,** the development into a man; and in **adults,** the male phenotype and important anabolic functions.

For **epididymis, vas deferens, seminal vesicle, and prostate**, DHT is the main active androgen. Correct function

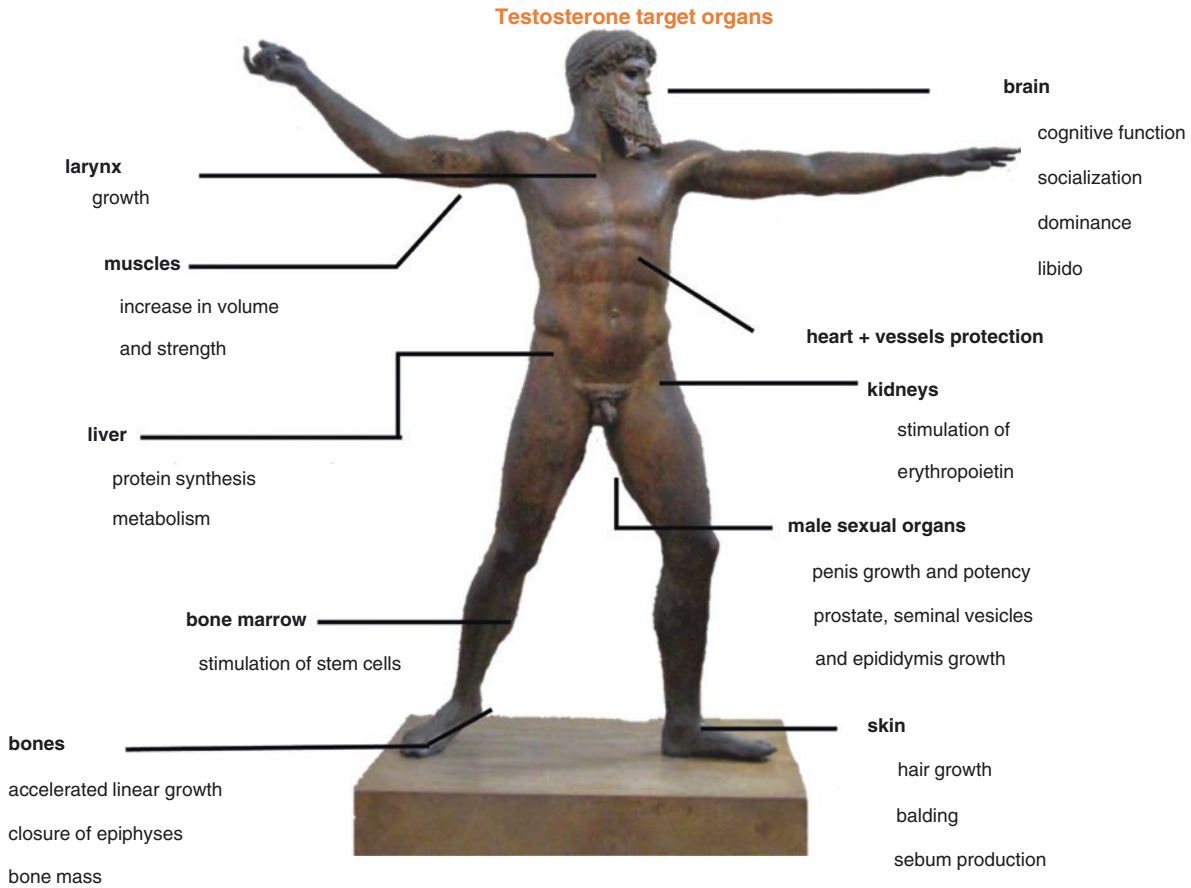


Fig. 2.18 Targets of testosterone action in the male

of these tissues is particularly dependent on continuous androgen action. In addition, aromatization of testosterone to estrogens plays an important role in prostate growth. The estradiol concentration in the prostate stroma increases significantly in benign prostatic hyperplasia (BPH). Estrogens exert their effect in combination with androgens and the estrogen receptor and are necessary for controlling prostate growth and differentiation. In the epididymis, seminal vesicles and vas deficiency of testosterone lead to a regression of the secretory epithelium, which can cause aspermia (lack of ejaculate production). Testosterone effects in the epididymis, seminal vesicles, and vas deferens are mediated not only by DHT but also by testosterone and estradiol.

Both testosterone and DHT are necessary for normal **penis growth** during puberty; penis growth positively correlated with increasing testosterone concentrations. However, after puberty, the androgen receptors in the penis are lost, so postpubertal testosterone deficiency leads to only a slight reduction in penis size. For this reason, testosterone doses in adults do not lead to a further increase in penis size. The maturation of the Wolffian duct is mainly due to testosterone; development of the external genitals, the urethra, and the prostate are mainly induced by DHT.

In **muscles —** with low 5α-reductase activity **—** testosterone is the crucial androgen (Bhasin et al. 2012). The skeletal muscles can convert circulating dehydroepiandrosterone (DHEA) into testosterone and estrogen. Skeletal muscle cells can probably also synthesize DHT to drive glucose metabolism. Mediated by the androgen receptor, testosterone leads to hypertrophy of the muscle fibers and thereby to an increase in muscle mass in both smooth and striated muscles. The number of muscle fibers does not change. Testosterone deficiency leads to atrophy of the muscles. Testosterone increases the synthesis of mRNA and glycogen in striated muscles. Testosterone also has an anabolic effect on the heart muscle with increased mRNA synthesis (Kirby et al. 2019).

Both androgens and estrogens lead to an increase in **bone density** by increased mineralization, while a deficiency can lead to the development of osteoporosis. In both female and male bodies, skeletal growth takes place at the periosteal membranes. With puberty, different gender-specific physiology develops, also leading to different **skeletal development**. In men, the rising testosterone concentrations at the beginning of puberty induce increased linear bone growth. Gender differences related to bone density and bone formation (mainly bone diameter) are regulated by the fine-tuning of the two hormones, testosterone and estradiol. At the end of puberty, depending on testosterone levels, closure of the epiphyseal fissures occurs with a significant delay at low testosterone concentrations. Low testosterone concentrations correlate with an increased risk of fracture in men, with hip and bones of the extremities being particularly at risk (Rochira et al. 2018). The androgen effect on bone metabolism is mediated by estradiol. Serum estradiol and testosterone contribute to bone formation, with testosterone enhancing bone formation and, conversely, estradiol inducing bone formation and resorption. High doses of testosterone can therapeutically induce closure of the epiphyseal fissures in cases of suspected excessive height. In addition to the two sex hormones, SHBG can apparently also stimulate bone formation independently (Valimaki et al. 2004).

Testosterone leads to increased growth of the sebaceous glands and increased **sebum production**, especially in the face, upper back, and breast area. Testosterone can also be partly responsible for the development of acne vulgaris, while estrogens can lower the sebum concentration. DHT and testosterone affect hair development through the androgen sensitivity of the hair follicles. DHT induces the synthesis of NO in human skin cells, which acts as a signal transduction molecule and regulates hair follicle activity. While even low androgen concentrations may stimulate **growth of hair** on arms and legs and the lower pubic hair, higher androgen concentrations are necessary for the growth of beard, upper pubic, and chest hair.

The **hair line** is determined by genetic factors and the individual distribution of androgen receptors and depends on circulating androgen levels. In men with a high hairline or bald head, high 5α-reductase activities were found in the affected scalp areas, while in patients with AR defects, 5α-reductase deficiency, but also in hypogonadal patients, no frontal and temporal hair line regression ("receding hair line") was observed. Since the growth of scalp hair is associated with increased 5α-reductase activity, associated premature hair loss could indicate premature aging of hair follicles. Androgens are not only involved in hair growth; they also influence hair color and diameter (Randall 2012) (see Chap. 33).

Depending on testosterone, the **larynx** grows by about one centimeter during puberty. This increase in size and the increase in length and mass of the vocal cords lead to a lowering of vocal pitch. The deeper voice is directly mediated by androgens, so that a deeper voice can also be induced in women by testosterone (Deuster et al. 2016; Glaser et al. 2016). The depth of male vocal pitch correlates with the duration of puberty, after which the androgen receptors on the target organ are lost. Once reached, vocal pitch is retained, so that even in postpubertal hypogonadal patients, no change in vocal pitch occurs. The gender-specific change in vocal pitch correlates with the degree of ossification of the laryngeal cartilage. This change is associated with the expression of alkaline phosphatase. Only a few of the chondrocytes involved express AR along with alkaline phosphatase, sug-

gesting a relationship between these two bone grafting components (Claassen et al. 2006). When boys are castrated before puberty, their voices remain high. This effect was exploited in earlier centuries to use high volume soprano voices in operas and church choirs (Nieschlag and Nieschlag 2019).

In the **central nervous system (CNS)**, testosterone is aromatized and also converted into DHT. The individual activities of the different enzyme systems and distribution of receptors are not homogeneously distributed throughout the CNS, but vary depending on the brain area. During pregnancy, androgens influence the development of the CNS in a gender-specific way, i.e., the brains of boys develop in the male direction through testosterone action. In girls, the androgen is absent and the CNS can develop as a female. Gender identity, sexual orientation, and other CNS-controlled behaviors are therefore already induced during intrauterine life. Sexual steroids control gender-specific behavior throughout life and play a major role in ensuring that female and male behavior is adapted to the biologically different functions of the sexes. In men, normal physical and mental performance as well as good mood are closely related to normal androgen levels (Zitzmann 2020).

The frequency or presence of **sexual fantasies**, **morning erections**, **masturbation** and **coitus frequency**, and **sexual activity** correlates closely with testosterone levels. An androgen deficiency, on the other hand, is often associated with listlessness, lethargy, depressive moods, loss of libido, and sexual inactivity. It is also believed that testosterone has neuroprotective properties in the adult male. How testosterone works in the CNS is not yet fully understood, but it is believed to be involved in neuronal regeneration in cognitive disorders such as dementia. It appears to activate the cortical network and treatment with testosterone can improve spatial awareness in both younger and older hypogonadal men. Conversely, low testosterone levels may be associated with depressive disorders and may also depend on the AR genotype (CAG repeats) (Tirabassi et al. 2015; Zitzmann 2020).

The functions of the **liver** are also influenced by steroid hormones. Sexual dimorphism is known to occur both in proteins synthesized by the liver and in many hepatic enzyme systems. This is reflected in gender-specific standard values for the various hepatic enzyme systems. With regard to protein production, estrogen and testosterone/DHT often act as antagonists (e.g., SHBG synthesis). Testosterone and estrogens can also have synergistic effects, e.g., in α-1-antitrypsin.

Androgens influence the **hematopoietic system** in two ways. On the one hand, androgens lead to increased erythrocyte formation via receptor-controlled erythropoietin synthesis. On the other hand, androgens also act directly on the hematopoietic stem cells and lead to an increased synthesis of heme and globin. These effects can also be demonstrated in vitro on granulopoietic and thrombopoietic stem cells.

The dependence of gender-specific **blood pressure** on androgens is already well established. Since coronary heart sclerosis is one of the most common causes of death in Western industrialized countries and the risk of disease in men is twice as high as in women, gender-specific differences in hormone secretion patterns are suspected as one cause. The more frequent presence of androgen receptors in the post-trematic and preoptic regions suggests that gender-specific blood pressure and heart rate are regulated there as a function of androgen levels. The differences in blood pressure between men and women may be a consequence of gender-specific differences in brain development, since the CNS ultimately regulates blood pressure.

Suppression of testosterone levels leads to a slightly delayed increase in the concentration of **high-density lipoprotein (HDL)**. The influence of testosterone and estradiol on fibrinolysis is also discussed. Under testosterone deficient conditions, the levels of the plasminogen activator type 1 are increased. This can lead to reduced fibrinolysis. On the other hand, treatment with testosterone may lead to a reduction in vascular pressure, but the long-term effects seem to be vasoconstriction. Thromboxane A2 receptors, angiotensin II, and endothelin-1 are expressed and norepinephrine is synthesized. In this context, androgens enhance arteriosclerosis, vascular remodeling, and induce renal prehypertensive processes of the renin-angiotensin-aldosterone system. On the other hand, effects of sex hormones on different parts of the renal-vascular system are often debated or not fully understood. A link between androgens and blood pressure and an androgenic effect on cardiovascular diseases are undisputed.

In Western industrialized countries, the incidence of the **metabolic syndrome** has increased significantly, affecting about one-fifth of the population, with this proportion increasing with age. Men who suffer from the underlying disease, type II diabetes, are also hypogonadal, overweight, and often show elevated blood pressure. The disease is associated with altered body fat content, impaired glucose metabolism, and a reduction in antioxidant substances in the body (Pérez-Martínez et al. 2017). The polymorphism of ARs seems able to influence the syndrome as well. In fact, men who have diabetes and obesity are also at increased risk of developing hypogonadism, either by lowering SHBG serum levels or by reducing androgen production.

2.1.5.6 Androgen Secretion and Sexual Differentiation

The development of the gonads follows the **sex determination**, which leads to sexual dimorphism. Sex determination is under genetic control and is mainly determined by a transcription factor, the "**sex determining gene on the Y-chromosome" (SRY**), which then induces the activity of other transcription factors (Fig. 2.19). Together with other

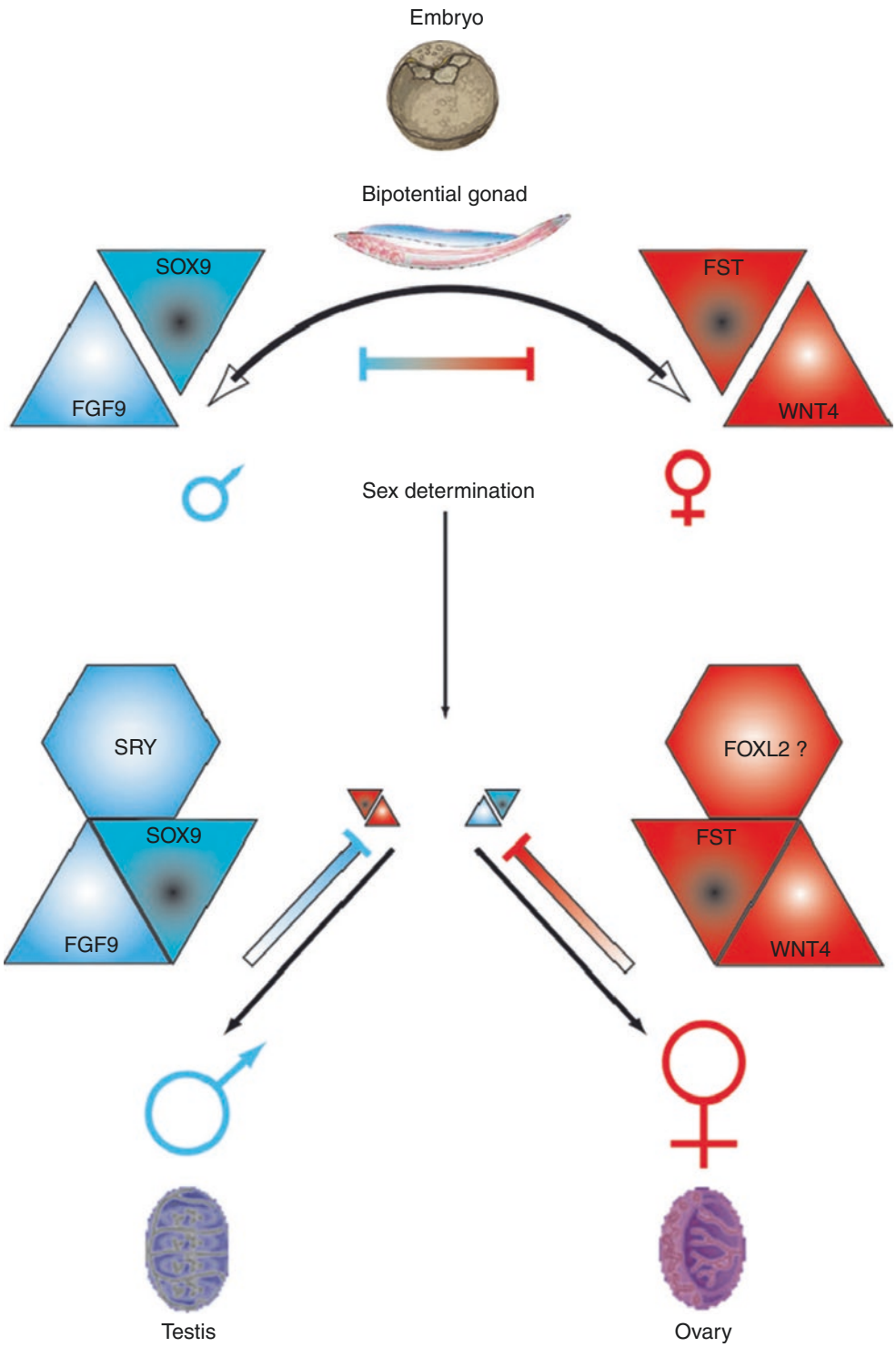


Fig. 2.19 Mammalian sex determination. The male-promoting (SOX9 and FGF9) and female-promoting (WNT4 and possibly FST) genes keep the gonad in a bipotential state. In the presence of SRY expression, the balance changes to the male pathway and outcompetes the female signals. In the absence of SRY and possibly the presence of FOXL2, the female-promoting signals shut down these signals and ovarian differentiation is induced

transcription factors, the SRY gene is already expressed in the zygote and remains active until the blastocyst stage. Men with Camptomel syndrome carrying an **SOX-9 (SRY HMG-box-related gene 9)** mutation show partial sex reversal in their gonads. In contrast, fetuses with 46,XX, i.e., a female karyotype, which carry a duplication of the SOX-9 gene, show masculinization. SOX-9 has the task of regulating the expression of FGF9, which drives male development and suppresses the female. In the embryo, the gonad is bipotent, i.e., it is so undifferentiated that it can differentiate in either the male or female direction. Only under the influence of testosterone is the male differentiation path taken. If this hormonal stimulus is absent, the female sex is induced — independent of the karyotype.

In the second stage of **sex differentiation,** the development and maturation of the secondary sexual organs take place under endocrine regulation. During the first weeks of gestation, the external genitals of both sexes are also identical and can develop in the female or male direction. Under the influence of DHT, from the eighth week of gestation onwards, the differentiation of the bipotent disposition to the male phenotype takes place, i.e., the induction of growth of the genital hump and the fusion of the genital folds and bulges. Anti-Müller hormone (AMH), derived from Sertoli cells, suppresses the development of the internal female sexual organs (tubes, uterus, upper part of the vagina). Testosterone, on the other hand, product of the embryonic Leydig cells, stabilizes the Wollf's ductt and induces further development of the inner male sexual organs. The external male sex organs, as well as the prostate, require DHT produced by 5α-reductase type 2. The 5α-reductase type 2 is mainly produced by genital skin and the urogenital sinus, so that DHT is produced locally. After completion of differentiation, around the 14th week of gestation, there is continuous growth of the external sexual organs, especially the penis, under the influence of DHT. Fetal testosterone production reaches its maximum between the 9th and 14th week of gestation at the time of differentiation of the external genitals and the urogenital sinus, and then decreases again. This provides sufficient testosterone for enzymatic conversion to DHT. The stimulation of testicular testosterone production seems to be predominantly by hCG at this time. In the second half of pregnancy, fetal LH then takes over stimulation of the Leydig cells.

In the male newborn, the serum testosterone concentration reaches almost adult levels, drops until the end of the first week of life, then peaks again until the second month of life and drops to levels similar to those of female infants until the sixth month. The androgen serum concentration remains low until the age of 7 years, but the free, nonprotein-bound testosterone concentration increases noticeably. During this phase, the Leydig cells can be stimulated to produce testosterone by exogenous administration of hCG.

From the age of 7 years onwards, the adrenal cortex starts to produce more androgen — especially dehydroepiandrosterone (adrenarche). At the age of 10, the first nocturnal LH pulses can be measured, which in the course of puberty become part of the regular pulsatile LH secretion pattern of the adult male. After an initially weak increase in FSH, the basal LH and testosterone concentration in the serum also increase. In parallel, the various pubertal characteristics appear in characteristic order.

The normal concentration of testosterone in peripheral blood serum of the adult male is 12–30 nmol/l. The concentration of testosterone in blood is subject to a circadian rhythm. High levels are registered in the morning hours, in the evening the levels are about 25% lower. In the senescence phase, there is a decrease in free testosterone with a simultaneous increase in SHBG concentration. In some men, this decrease may be so severe that symptoms of late-onset hypogonadism occur with clinical consequences such as frailty, changes in body fat, cardiovascular disease, functional sexual disorders, and osteoporosis.

Key Points

- The testes perform two central functions, the production of male gametes, the sperm, and of the androgens that determine the male phenotype.
- The male gonad develops when the SRY gene is expressed embryonically and a testosterone signal occurs.
- Testicular tissue can be divided into two compartments, the seminiferous tubules, which house the germinal epithelium, and the interstitium, which contains the Leydig cells that produce androgens.
- Sertoli cells form the blood–testis barrier that immunologically protects the more differentiated germ cells and the niche of spermagogonial stem cells.
- Endocrine function is part of the hypothalamic-pituitary-gonadal axis along which hormones and receptors act in a balanced feedback mechanism.
- Kisspeptin and GnRH drive the secretion of the pituitary gonadotropins LH and FSH.
- LH stimulates the production of testosterone; FSH acts on Sertoli cells and provokes an inhibitory response.
- Testosterone and inhibin regulate gonadotropin secretion in negative feedback. FSH and androgen action are essential for quantitatively and qualitatively complete spermatogenesis.
- Testosterone and the metabolites dihydrotestosterone and estradiol are necessary for normal male development in many target organs, including bone metabolism, muscles, hair and beard growth, voice pitch, fat distribution, and certain brain functions.
- Disorders of testicular function lead to pathological consequences such as infertility or hypogonadism.

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Physiology of Sperm Maturation and Fertilization

3

Verena Nordhoff and Joachim Wistuba

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Abstract

Successful fertilization of an egg is the ultimate goal of a mature sperm. For this process to occur correctly in vivo, the natural fertilization process follows a time- and location-dependent sequence of complex steps, beginning with production in the testis, maturation in the epididymis, and finally the fertilization of the egg. Specific proteins and surface components in and on the spermatozoa play an important role in this process. In fertile men, natural fertilization follows the logically determined sequence of events, but in infertile men, these pathways can be partially shortened therapeutically to ultimately achieve paternity. Thus, not only ejaculated spermatozoa, e.g., in vivo after insemination in the female genital tract, but also spermatozoa from the testis and epididymis, after injection into the egg, can perform predetermined fertilization steps in vitro and thus lead to an intact pregnancy. This chapter follows the path of spermatozoa from the site of production to the fertilizable egg and describes important processes that spermatozoa undergo to ultimately reach their destination.

3.1 Introduction

During coitus, spermatozoa pass from the distal cauda epididymis into the ejaculate and are deposited in the female cervix. In order to fertilize the freshly ovulated egg (see Chap. 9), the spermatozoa must pass from the cervix into the uterus, and from there into the fallopian tubes. Once there, it is the sperm's task to penetrate the egg shell (**zona pellucida**) and fuse with the egg's oolemma before the

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chromatin is finally released into the cytoplasm of the egg. Here, decondensation of chromatin and the formation of a **male and female pronucleus** occur, now called a **zygote.** These events are the starting point for subsequent mitotic cell divisions and thus the formation of an intact **embryo**. The preparatory processes for fertilization are synchronized by parallel events, such as capacitation of the spermatozoon during passage through the female genital tract. The spermatozoon has the important task of activating the egg and releasing it from the meiotic block (the egg is arrested in metaphase II) so that the second meiotic division can continue, the second polar body is extruded, and the female pronucleus is finally formed. After fusion with the egg, the sperm provides the centrioles, which are important for subsequent mitotic divisions, and which, with the forming asters, attract the resulting male pronucleus to the female pronucleus and subsequently coordinate the first mitotic division (Fig. 3.1).

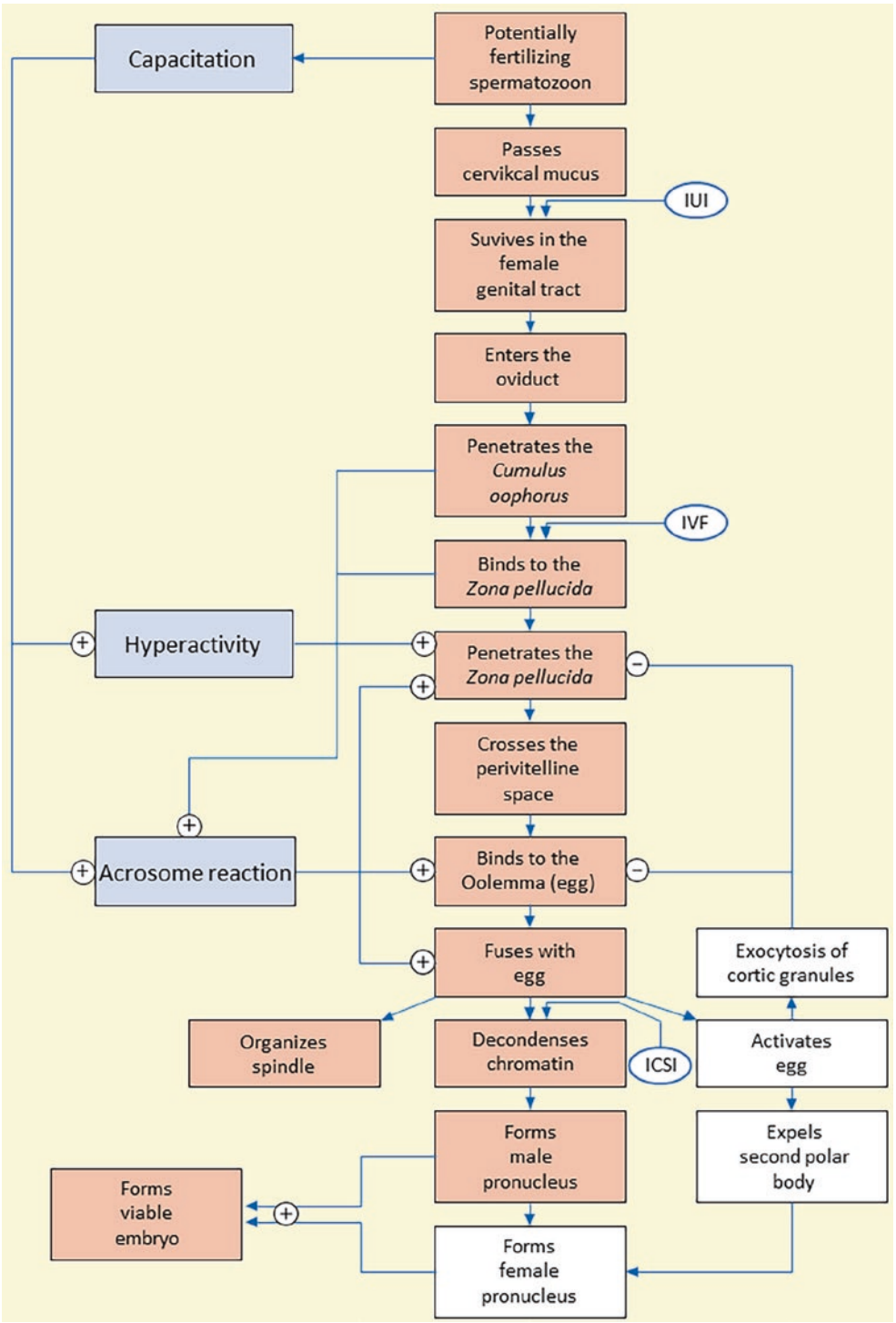


Fig. 3.1 Scheme of events taken by the naturally fertilizing spermatozoon post coitum. *Pink rectangular boxes*: sequential events; *grey rectangular boxes*: concomitant events, *IUI, IVF,ICSI*, entry points of sperm provided by intrauterine insemination, in vitro fertilization, intracytoplasmic sperm injection, respectively. *White boxes*: events undergone by the egg. (+), positive influence (−), negative influence

During fertilization, the two haploid gametes fuse, and a new individual can emerge.

The spermatozoa acquire their functionality, which is necessary for the complex fertilization processes, during their passage through the epididymis.

This chapter describes the relevant **sperm functions** acquired during maturation within the epididymis and the process of fertilization that is possible only after optimal maturation of the spermatozoa. In some pathologies of male infertility, spermatozoa can be obtained for assisted fertilization from the proximal regions of the epididymis, or even from the testis itself. However, this also means that numerous natural fertilization processes and potentially related biological reproductive barriers are therapeutically bypassed.

3.2 Maturation of Spermatozoa in the Epididymis

3.2.1 Anatomy of the Epididymis and Sperm Transport

Together with secretions from the Sertoli cells, spermatozoa are flushed from the **seminiferous tubules** through **the recti tubuli** into the cavities of the **rete testis** and enter the 12–18 efferent ducts by absorption pressure. Each efferent duct is 0.2–0.5 m long, and together they form the globus major of the **epididymis** (caput epididymidis). The ductuli efferentes finally unite to form the tubule of the epididymis (Sullivan and Mieusset 2016).

The cells of the **tubules efferentes** can be classified into at least 5 different types, depending on their shape and height (Breton et al. 2019; Sullivan et al. 2019). They form a resorptive epithelium that concentrates spermatozoa entering the tubules from the testes by mediating the transfer of ions and water from the lumen to the interstitium; the tubules also have secretory functions (Breton et al. 2019; Sullivan and Mieusset 2016).

The duct forming the epididymis is coiled into lobules, which constitute the epididymis proper. Unlike other species, the caput, midpiece, and caudal region of the epididymis are not clearly distinguishable in humans (Sullivan et al. 2019). In humans, the epididymal duct measures **5–6 m in total length.** The epididymis itself has a pseudo-stratified epithelium consisting of principal cells of various sizes (usually distally shorter) and basal cells that do not extend to the lumen, as well as a population of migrating lymphocytes. The stationary cells have both resorptive and secretory functions and maintain a specific milieu in which spermatozoa can remain alive for up to 2 weeks. Basal cells also have similarities to macrophages (Rinaldi et al. 2020). In the epididymal epithelium, **"tight junctions"**that control the transport of substances across the epithelial tissue are found in all regions (Breton et al. 2019).

Depending on the testicular production rate, sperm remain in the epididymis for 2–7 days where they mature and are stored until ejaculation (Johnson and Varner 1988).

3.2.2 Epidydimal Secretion and Absorption

One function of the epididymal epithelium is the estrogen-dependent **reabsorption of water** in the ductuli efferentes, which is driven by the absorption of Na^+ ions (Hess and Cooke 2018). A second function is the androgen-dependent **secretion of components** that prepare epididymal sperm for fertilization and keep mature sperm inactive before ejaculation. During the course of **epididymal passage**, sperm and fluids are further concentrated in the lumen of the epididymis. The secretion products lead to some important changes in the maturing sperm, some of which are also related to their respective storage duration (Björkgren and Sipilä 2019).

Three secretion products of low molecular weight occur in high concentrations, in particular: **L-carnitine**, which is not synthesized in the epididymis but is concentrated in the epididymis after being imported from the bloodstream; **myo-inositol**, which is both synthesized and transported by the epididymal epithelium; and **glycerophosphocholine (GPC),** which is synthesized from circulating lipoproteins in the spermatozoa themselves.

Molecular biology techniques were used to characterize the epididymal proteins. Table 3.1 provides an overview of proteins of the epididymis in humans that play an essential role in fertilization (Cooper 2007b; Björkgren and Sipilä 2019).

Gene expression of these proteins is regulated by luminal secretions (Dubé et al. 2008; Reyes-Moreno et al. 2008). After **vasectomy,** a local shift of expression in the epithelium into a proximal direction has been shown for some proteins, and a general decrease in protein expression is found in patients with **cryptorchid testes** (Légaré et al. 2004). These changes are also found in other pathologically occurring obstructions and lead to a higher concentration of secretion products, which may have an impact on the fertility of sperm collected from these regions. In principle, there appear to be correlations of fertility disorders and the transcriptome and proteome of seminal plasma (Cannarella et al. 2020). For example, it has long been known that the neutral form of **α-glucosidase** is an important secretory product of the human epididymis. If the concentration of α-glucosidase in

Table 3.1 Selection of potential epididymal proteins that play a role in fertilization (modified from Björkgren and Sipilä 2019)

Protein	Function or potential role
Proteins of the calcium signalling cascade	
Ca^{2+}-ATPase isoform 4 (PMCA4a)	• Maintenance of low intracellular Ca^{2+} levels required for proper sperm activation.
CRISP1	• Decapacitating factor. • Transiently bound to sperm and released during capacitation.
Beta-defensins, e.g., SPAG11E (Bin1b) or DEFB15	• Antimicrobial peptide, "host-defense" function. • Bind to sperm head during epididymal passage and induce progressive sperm motility (e.g., rat). • Possible role in Ca^{2+} channel regulation.
Lipocalins (LCNs), e.g., LCN6	• Extracellular proteins capable of transporting small hydrophobic molecules such as steroids and lipids. • Expression in the proximal epididymis leads to binding of the protein to the postacrosomal region in human spermatozoa. • Potential role in sperm maturation or Ca^{2+} influx.
Proteins that modify sperm proteins	
IZUMO1	• Required for sperm-ovum fusion. • Phosphorylation re-localizes the protein in the sperm membrane or during sperm-ovum fusion.
ADAM3 (cyritestin)	• Gamete interaction. • Is modified during epididymal passage.
Inositol polyphosphat 5-phosphatase (INPP5b)	• Regulates proteases for ADAM2 and ADMA3 cleavage in the epididymis. • Potential role in motility and binding to the ZP and in fusion with the egg.
Wnt	• Regulates glycogen synthase kinase-3 (GSK3), regulation of motility. • Wnt ligands are expressed in the epididymis and act through two receptors: Frizzled (FZD) and low-density lipoprotein receptor-related 6 (LRP6).
Serine peptidase inhibitor, Kazal type 13 (SPINK13)	• Protease inhibitor. • Required for sperm maturation. • Secreted into the epididymal lumen and localized to the acrosome of maturing spermatozoa. • Additional function in sperm-ovum interaction?
Endoplasmic reticulum protein 29 (Erp29)	• Belongs to the protein disulphide isomerase (PDI) family. • Causes conformational changes in proteins and promotes cell-cell interaction. • Relocated to the equatorial segment after sperm-ovum interaction, the initial site of gamete fusion.
Proteins that influence the lipid composition of the sperm membrane	
Incorporation of unsaturated lipids and gradual degradation of cholesterol	• More fluid membrane, but epididymal environment „immobilizes" sperm, including through binding of so-called decapacitation factors, localized at sperm surface.
Binder of SPerm Protein Homolog 1 (BSPH1)	• During passage, BSPH1 is bound to the sperm and prevents the movement of lipids and protects sperm from premature capacitation.
Carboxylesterase CES5A (previously CES7)	• Secreted into the lumen of corpus and cauda. • Potential role in sperm capacitation.
SERPINA16	• Decapacitating factor. • Potential role in sperm capacitation.
Colipase-like 2 (CLPSL2)	• Secreted by the caput epithelium and binds to spermatozoa. • Localized in the acrosomal region and in the caput. • Integrity of the acrosome and progressive motility. • Probable function in remodelling the liquid profile of the sperm membrane.
Glutathione peroxidase (GPX5)	• Secreted into the lumen and protects sperm from lipid oxidation.
Proteins involved in cell-cell interaction	
CRISP1	• In addition to the calcium signalling cascade, also required for gamete interaction. • After sperm binding to the *Zona pellucida* (ZP) and acrosome reaction, CRISP1 is translocated from the dorsal part of the acrosome to the equatorial region destined for gamete fusion.
Dicarbonyl/L-xylulose reductase (DCXR) or sperm surface protein P34H	• Catalyzes the reduction of various aromatic dicarbonyl and sugar compounds. • Acts non-catalytically by interacting with proteins such as cadherins and catenins, thereby affecting cell-cell adhesion. • Higher expression in the corpus, increase in the acrosome during epididymal passage. • Molecular mechanism during sperm-ZP binding unknown.
Sperm oocyte-binding antigen 2 (SOB2) and FLB1	• Expressed in caput and corpus. • SOB2 shows diffuse localization in the sperm head. • Later restricted to the post-acrosomal and neck area in the corpus.
Other	
Ubiquitins and epididymal sperm binding protein 1 (ELSPBP1)	• Hypothesized to bind to defective or dead spermatozoa during transport.

the spermiogram is severely decreased or even absent in azoospermia, this is a clinical sign of **obstruction.** A similar relationship between impaired fertility and seminal plasma proteins exists for the concentration of P34H in sperm, which is correlated with IVF success rates (Sullivan et al. 2006).

3.2.3 Sperm Maturation in the Epididymis

In caput and midpiece of the epididymis, sperm undergo a maturation process associated with a series of physiological, biochemical, and morphological changes, and sperm acquire the ability to fertilize an egg (Sullivan and Mieusset 2016). Animal experiments have shown that ejaculated sperm and sperm from the cauda epididymis can fertilize eggs, but that spermatozoa from the testis, neither in insemination, nor in in vitro fertilization, are able to do so (Cooper 2007a). Although sperm maturation in humans can hardly be morphologically mapped (Bedford 1994), there are nevertheless findings on the interaction between eggs and spermatozoa obtained from different regions of the epididymis.

3.2.3.1 Findings from Surgical Anastomoses

Refertilization surgeries provide the opportunity to observe the **fertilization capacity** of sperm from different regions of the epididymis. In epididymovasostomy, the ductus deferens is anastomosed with a portion of the epididymis above the blockage (Schwarzer and Steinfatt 2013) (Fig. 3.2). Pregnancies can occur naturally during a period of 6 months to 2 years after surgery, depending on whether the connection was made to the cauda, midpiece, or caput of the epididymis. The longer the portion of the epididymis through which sperm passage occurs, the greater the success (Cooper 2007b). This suggests that epididymal passage in principle positively influences sperm function (Sullivan and Mieusset 2016).

3.2.3.2 Findings from Assisted Reproduction

Another way to determine the "fertility profile" for spermatozoa from the epididymis is provided by using **assisted reproduction techniques (ART)** (see Chap. 23). Under these artificial conditions, where sperm are exposed to quite different challenges than in vivo, various stages of fertilization can occur in vitro even by sperm that are actually "immature," i.e., not ejaculated into the female tract. Unlike in epididymovasostomies, where sperm are naturally deposited in the female tract through sexual intercourse and must survive there, here all reproductive barriers that ejaculated sperm naturally would have to overcome are bypassed (Cooper 2007b; Tournaye 2012).

Assisted reproduction, more specifically **intracytoplasmic sperm injection (ICSI),** involves the retrieval of sperm from different regions of the blocked duct, for example in men with congenital bilateral aplasia of the vas deferens **(CBAVD)** or cystic fibrosis, which provokes the absence of vasal structures and surgical correction is not possible. Thus, pregnancies can be induced by fertilization of eggs with sperm obtained from the caput, corpus, or cauda by **microepididymal sperm aspiration (MESA)**, from the ductuli efferentes, from epididymal cysts and tubules recti, or from testicular biopsies (**testicular sperm extraction, TESE)** (Oseguera-López et al. 2019). Sperm from the more proximal region are less competent to achieve pregnancies (Cooper 2011) (Fig. 3.3h) and also provoke higher embryonic losses.

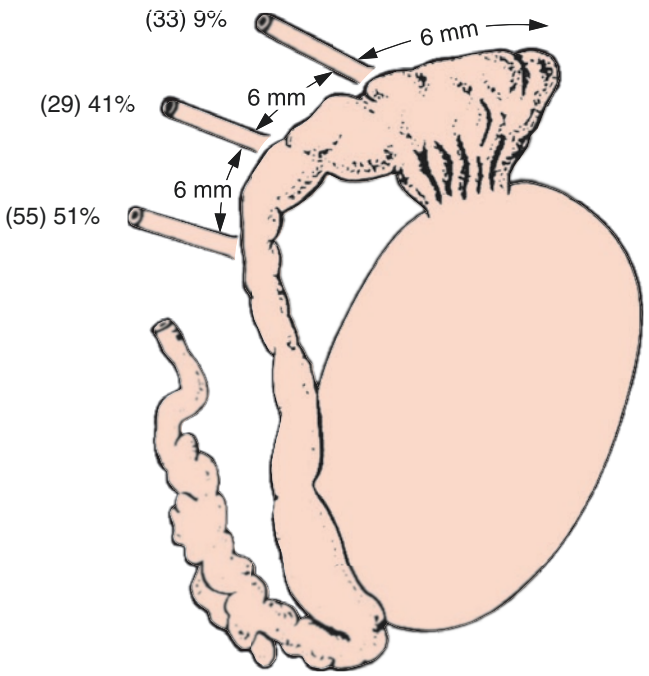


Fig. 3.2 Scheme indicating how epididymal occlusion can be overcome surgically by epididymovasostomy, in which the remaining functionally patent vas deferens is anastomosed to an unobstructed region of the epididymis upstream of the block. The length of the epididymis through which spermatozoa pass naturally varies with the site of attachment. The pregnancy rates (%) increase the more distal the connection is (number of patients in parenthesis). (From Bedford 1988, with permission)

Regions of the epididymis: *1* efferent ducts; *2–3* proximal corpus; *4* mid-corpus; *5–6* distal corpus; *7–8* cauda epididymidis.

By using **ICSI** (see Chap. 23), (human) testicular sperm can successfully achieve a pregnancy (Cissen et al. 2016; van Wely et al. 2015). Even immature germ cells from ejaculate or testicular biopsies can initiate pregnancies by intracytoplasmic injection techniques (**ELSI** = elongated spermatid injection, **ROSI** = round spermatid injection, or **ROS(N)I** = injection (of the nuclei) of round spermatids), but the efficiency of these techniques in humans is rather limited.

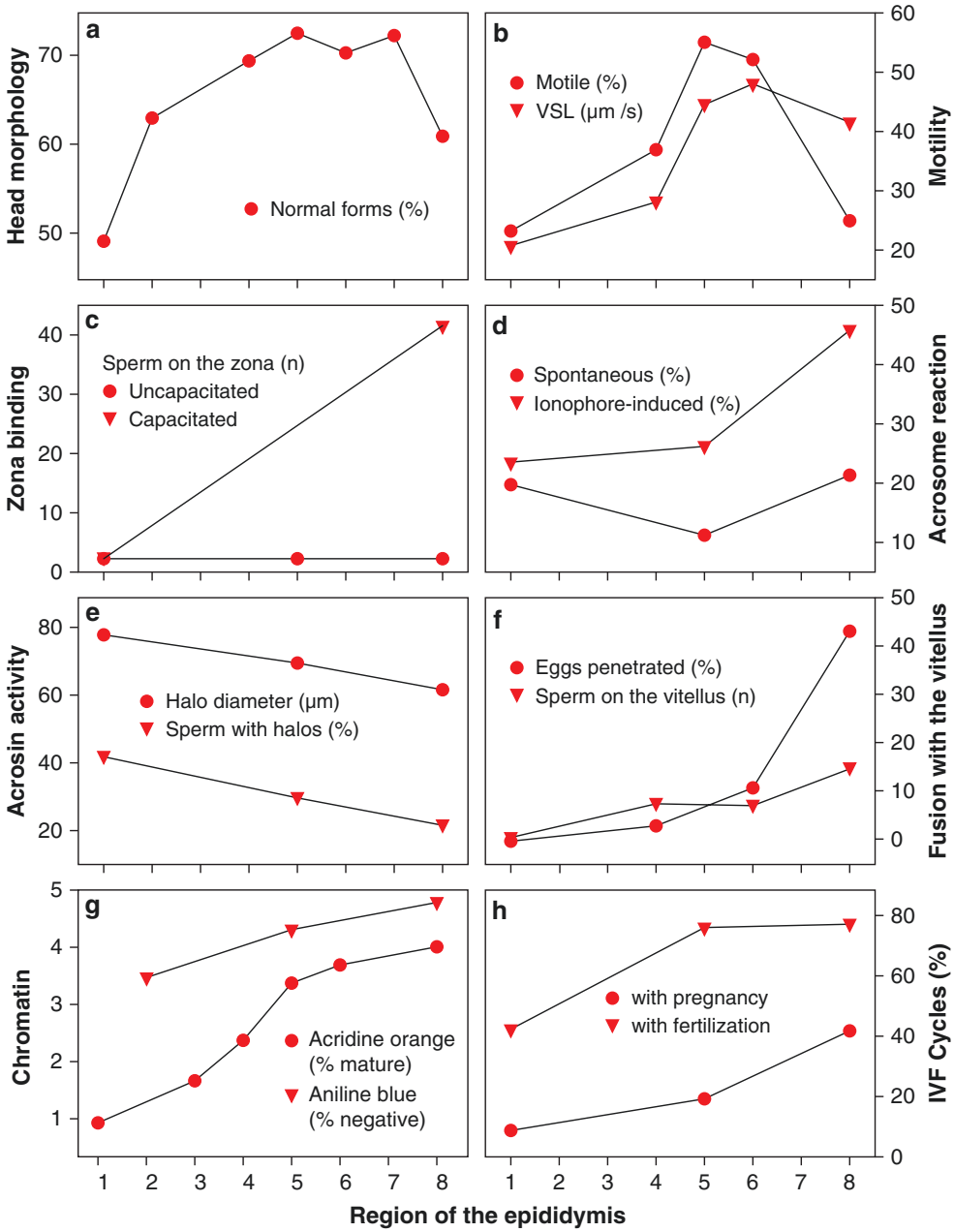


Fig. 3.3 Morphological and physiological changes of human spermatozoa removed from different regions of the epididymis. (**a**) normal sperm head morphology (uniform head shape); (**b**) motility (percentage and straight-line velocity); (**c**) binding ability of uncapacitated cells is low but only mature capacitated bind to the zona; (**d**) spontaneous acrosomal loss is constant in all epididymal regions, but ionophore-induced acrosome reactions occur only to spermatozoa obtained from corpus and cauda; (**e**) acrosin activity as detected on substrate declines or is less accessible upon maturation; (**f**) penetration of zona-free hamster eggs by capacitated, acrosome-reacted spermatozoa increases as they mature; (**g**) chromatin assumes the mature compacted form of packaging as assessed by aniline blue binding to nucleoprotein and acridine orange binding to nucleic acids; (**h**) fertilization and pregnancy rate are higher when distal epididymal spermatozoa are used in IVF

Spermatids induce the fertilization cascade in the egg using **phospholipase C (PLC) ζ**, which is released during gamete fusion and triggers Ca^{2+} oscillations in the egg (Yeste et al. 2017). PLCζ deficiency in spermatozoa leads to complete fertilization failure (Yelumalai et al. 2015). This deficiency seems to particularly affect patients with globozoospermia (Chansel-Debordeaux et al. 2015). To compensate for the deficiency, eggs can artificially be activated by chemical compounds, such as calcium ionophores, to induce fertilization (Amdani et al. 2016). However, how the mode of action of PLCζ is terminated remains poorly understood (Parrington et al. 2019).

3.2.4 Sperm Morphology and Motility

3.2.4.1 Changes in Sperm Morphology

The percentage of spermatozoa with normal head morphology increases from 50% to approximately 70% along the **epididymal passage** (Soler et al. 2000). However, epididymal normal forms are not directly comparable with the normal forms of ejaculated spermatozoa (definition according to the WHO manual 2010), because those are already epididymally matured (Soler and Cooper 2016). For example, the finding of a high proportion of macrocephalic forms with distended post-acrosomal regions can be explained by air-

drying during analysis and therefore should not be considered representative of the situation in the epididymal tubule (Soler et al. 2000; Cooper 2011).

3.2.4.2 Development of Sperm Motility

Testicular sperm have all the morphological requirements for motility, but are largely immobile and are therefore transferred to the epididymis by peristalsis. In patients with **congenital bilateral aplasia of the ductus deferens (CBAVD)** or **obstructive azoospermia**, and less commonly in patients with non-obstructive azoospermia, sperm with minor motility can be obtained from the testis (Wu et al. 2005; Hosseini and Khalili 2017). Motility induction is a time-dependent phenomenon, which is why testicular sperm are incubated in vitro before being used for ICSI (Hosseini and Khalili 2017). Alternatively, the addition of methylxanthine derivatives, such as **pentoxyfylline**, can be considered to induce sperm motility (Archer and Roudebush 2013; Nordhoff 2015). However, this effect appears to be short-lived, as sperm metabolic resources may be rapidly depleted (Angelopoulos et al. 1999).

During passage through the epididymis, spermatozoa acquire the basic capacity for independent motility, develop their typical pattern of movement, and show a general trend toward an increase in kinetic properties. The percentage of motile spermatozoa, the velocity of progression (VSL = straight-line velocity: **"progressive velocity"**(µm/s)**, CASA** see Chap. 9), and the straightness along the distance travelled increase (Fig. 3.3b). The increase in VSL is due to a better coordination of the **flagellar movement**, accompanied on one hand, by a parallel increase in the linearity of the progression, and on the other hand, by an increase in the **beating force**, which can be followed by the change in the curvilinear velocity (VCL = curvilinear-velocity: "track velocity" (µm/s), see Chap. 9). The maturation-induced change in sperm kinematics occurs in the proximal half of the epididymis and is completed in the middle section of the midpiece. In contrast, the amplitude of head excursion undergoes only minor changes distally.

The different maturation of spermatozoa with respect to their motility leads to a **heterogeneity of the sperm population** in the different regions of the epididymis. The reason for this is probably the anatomy of the **caput** epididymis, into which some spermatozoa enter by an end-to-end connection from the efferent ducts into the epididymis, but others overtake them by a more distal entry (Yeung et al. 1991). The highest homogeneity is found in the middle part of the **corpus** epididymis, where freshly matured spermatozoa have the best kinetic values. In various species, especially humans, either the percentage of motile spermatozoa, or their progression property, or both, decrease in the **cauda**, which then often results in an inhomogeneity of the sperm population and can also be directly correlated with sexual abstinence; this indicates a direct relationship with aging of mature spermatozoa (Yeung et al. 1993; Bedford 1994).

3.2.4.3 Regulation of Sperm Motility

The **axonemal apparatus** of testicular spermatozoa is in principle already functional, as proven by the inducability of the flagellar beat of testicular spermatozoa after experimental administration of ATP; however, the full maturation process occurs in the epididymis. Mature spermatozoa from the cauda epididymis swim better than immature ones from the caput because several motility-promoting factors are taken up during passage, and factors inhibiting motility are degraded, so that signal transduction is initiated. Such factors include changes in intracellular pH, Ca^{2+}, cAMP, and protein phosphatase PP1γ2. Epididymal spermatozoa possess adenyl cyclase and can thus be stimulated by bicarbonate produced by epididymal epithelial cells containing carbonic anhydrase (Björkgren and Sipilä 2019; Sullivan and Mieusset 2016).

3.2.4.4 Effects of Increased Activity of Mature Spermatozoa

The increased activity of mature spermatozoa, measured as VCL levels, confers properties that younger spermatozoa in the proximal epididymis still lack. This relates to the abilities to **penetrate cervical mucus**, to travel within the female genital tract, and to **penetrate the egg membrane (oolemma)**. Epididymal sperm brought into direct contact with the eggs during IVF in vitro are insufficiently equipped to penetrate the egg oolemma.

However, once a sperm has penetrated and fused with the egg, the sperm's movements stop abruptly (Florman and Fissore 2015).

For **SUZI (subzonal insemination)**, a procedure no longer practiced today, the zona was mechanically opened to bring the sperm into the perivitelline space (Halvaei et al. 2018). For this procedure, sperm do not need motility as long as they have the capacity to fuse with the egg, for example, in pathologies such as **Kartagener syndrome** or other ciliary dysfunctions.

When an egg is fertilized by **ICSI**, a hit onto the flagellum disrupts the integrity of the plasma membrane. This activation is necessary, even when using immotile sperm, to initiate fusion with the egg and all subsequent fertilization steps (O'Neill et al. 2018).

3.2.5 Interaction with the Egg

3.2.5.1 Requirements for Sperm-zona Pellucida Binding

The fact that capacitated sperm from the epididymal caput region bind weaker to the **zona pellucida** than sperm from

the ejaculate indicates that the binding process is dependent on sperm maturation in the epididymis (Fig. 3.3c). Several sperm-zona receptors have been identified for gamete interaction in different species; some are already present on testicular spermatozoa and are modified in size or localization on the sperm head during passage through the epididymis; others are secretory products of the epididymis (Table 3.1).

Infertility may result from a lack of sperm binding to the zona pellucida, e.g., deficiency of epididymal P34H, a glycoprotein secreted by the epididymis (Moskovtsev et al. 2007).

3.2.5.2 Development of the Acrosome Reaction

When epididymal spermatozoa are incubated under normal **capacitational conditions**, spermatozoa from the caput undergo the spontaneously occurring **acrosome reaction** rather less frequently than those from the corpus or cauda (Fig. 3.3d). Experimental triggering of the acrosome reaction of epididymal spermatozoa with a calcium ionophore (Fig. 3.3d) or by prolonged incubation at 4 °C resulted in a higher rate of acrosome reactions in mature spermatozoa. This indicates that more mature spermatozoa exhibit a higher responsiveness to external stimuli. A prerequisite for the acrosome response is the fluidity and organization of the cell membrane so that plasma and the outer, especially the equatorial, acrosomal membrane can fuse with the oolemma. If still immature sperm show the acrosome reaction, they should already contain sufficient **acrosin** for zona penetration. This is certainly the case for spermatozoa from the testis and epididymal caput (Ito and Toshimori 2016) (Fig. 3.3e), because only acrosome-reacted sperm have the ability to fuse with egg microvilli (Florman and Fissore 2015).

Infertility can be caused by an insufficient acrosome response (Ito and Toshimori 2016).

3.2.5.3 Sperm Chromatin Condensation and Pronucleus Formation

In the epididymis disulfide, bridges accumulate in the **chromatin** of maturing sperm due to the oxidation of SH groups of DNA-binding proteins, i.e., protamines; associated with this, **sperm chromatin** condensates. The increasing condensation of chromatin can be visualized by a steady decrease in the staining intensity of aniline blue, which interacts with histones, or acridine orange, which binds to nucleic acid, as sperm maturation progresses (Fig. 3.3g). In the egg, sufficient reduction potential generally exists in the cytoplasm so that decondensation of mature sperm nuclei is unproblematic. Since immature sperm nuclei contain fewer S-S bonds, they can be decondensed even more easily in the egg's plasma.

3.2.5.4 Contribution of Sperm to the Development of a Healthy Embryo

Despite the ability of epididymal sperm to fertilize in vitro, not all generated and subsequently transferred embryos result in pregnancies. Comparison of treatment cycles in which a pregnancy was achieved shows that sperm from the distal end of the epididymis produce more embryo(s) capable of implantation and further development than those obtained from more proximal parts (Cooper 2011) (Fig. 3.3h). Sperm from testicular tissue, regardless if fresh or cryopreserved, result in similar pregnancy rates after **ICSI**. The rates are nonetheless equal if sperm from testicular tissue or the epididymis are used. However, this has a limitation in cases of nonobstructive azoospermia as fertilization rates with these TESE sperm are lower compared to testicular or epidydimal sperm from obstructive azoospermic patients (Prudencio et al. 2010).

3.2.6 Sperm Storage in the Epididymis

3.2.6.1 Storage Capacity

The capacity of the human epididymis to **store sperm** is low and transport through the epididymis occurs within only 2 days. Sperm do not undergo long-term storage in the epididymis (Breton et al. 2019; James et al. 2020). The size and storage capacity of the epididymis (but not the testis) is reduced by pathological retention in the abdomen at **maldescensus**. Sperm appear in the urine approximately 2 weeks after the last ejaculation. The sperm reserve in the cauda epididymis is not completely emptied by a single ejaculation. "Old sperm" ejaculated from the epididymis by short-term multiple ejaculations after 2 weeks of **abstinence** are still intact (Cooper et al. 1993, see Chap. 9). With the aid of prolonged periods of **abstinence** followed by multiple ejaculations, an increase in motile and normally formed spermatozoa can be achieved. This procedure can lead to higher total sperm counts in patients with **oligozoospermia**, for example, on the day of ICSI. However, even in the absence of epididymal emptying and in the presence of isolated ejaculations, spermatozoa do not lose their ability to fertilize (De Jonge et al. 2004; Levitas et al. 2005). Even after death, spermatozoa in the epididymis retain their ability to fertilize for at least 30 h (Shefi et al. 2006).

3.2.6.2 Resting Stage and Protection of Spermatozoa

Within the epididymis, spermatozoa are immotile. This is caused in part by lower sodium and higher potassium concentrations in the epididymal fluid compared to serum concentrations. Low sodium concentrations prevent the sodium-dependent increase in intracellular pH that normally promotes motility when spermatozoa have left the epididymis by ejaculation or withdrawal. Decreases in intracellular pH due to penetrating acids (e.g., lactate) may result in inhibition of motility initiation within the epididymis. High potassium concentrations depolarize the membrane potential and result in a decrease in ion transport. During passage through the epididymis, spermatozoa are protected from lipid oxidation by various secretion products such as superoxidodismutase and glutathione peroxidase; an acrosin inhibitor preserves them from damage by effluent acrosomal enzymes (Kirchhoff 2007; Breton et al. 2019).

Impaired storage of spermatozoa in the epididymis can cause **necrozoospermia** (Correa-Perez et al. 2004).

3.2.6.3 Mechanisms Preventing an Autoimmune Reaction Against Spermatozoa

Sperm production does not begin until the immune system has matured and gained the ability to immunologically discriminate between "endogenous" and "exogenous". And unlike diploid somatic cells, sperm cells are haploid. Therefore, spermatozoa are perceived as foreign by the **immune system**. However, the organism has developed various mechanisms to protect spermatozoa from an **immunological reaction**. First, sperm are shielded in the epididymal duct from the rest of the organism. If the epididymal duct is not damaged, for example through vasectomy, injury, disease, or a missing or malformed ductus deferens, the sperm are largely separated from the circulating immunocompetent cells. However, the "**tight junctions**"of the epididymis that control transport through the cell assemblies are not as impermeable as in the testis, and antigenic material may be able to enter the interstitium despite these "tight junctions" or even overcome this barrier by transcytosis (Breton et al. 2019).

Moreover, under physiological conditions, another protective mechanism exists; although lymphocytes are present in the epididymis epithelium, **no** "**antisperm reaction**" occurs. Accordingly, the epididymis has an immunosuppressive activity (Pöllänen and Cooper 1994). Macrophages, which could present autoantigens against spermatozoa to the immune system, are found only occasionally in the normal epididymal epithelium, but are abundant in the interstitium. Since spermatozoa have neither MHC-I nor MHC-I antigen, which CD4- or CD8-positive lymphocytes require to mediate an antigenic response, this type of immune response against spermatozoa is probably not present in the epididymis.

Complement-mediated **degradation of spermatozoa**, i.e., their immunologically induced lysis, occurs only to a limited extent because of low complement concentrations in the lumen, the absence of co-stimulators, and the secretion of high concentrations of complement inhibitors. The latter are present in the developing germ cells in the testis (e.g., CD46, CD55, and CD59) and are secreted by the epididymal epithelium into the epididymal fluid (e.g., SGP-2) (see Fig. 3.4). Interferon-dependent chemokines have been detected in the human epididymis (Linge et al. 2008).

Antisperm antibodies are found in the serum of men with epididymal obstruction, both of obstructive and congenital origin. Because their abundance is higher in a distal occlusion, it suggests that the duct is able to suppress antigenic responses, which is why fewer antibodies are present when the duct remains in place for a longer time. Basal cells are possibly involved in the elimination of sperm autoantigens in the human epididymis, as suggested by their macrophage-like expression pattern (Yeung et al. 1994; de Kretser et al. 1998).

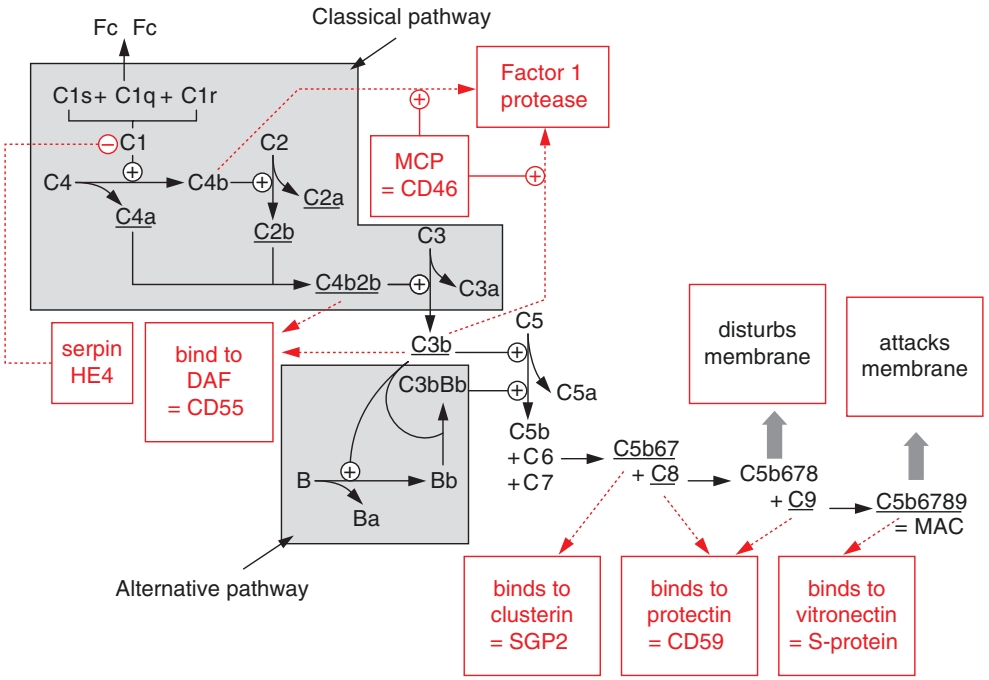


Fig. 3.4 A scheme outlining putative complement regulators in the male reproductive tract. *Black text*, sequential events; *grey boxes*, classical and alternative pathways; *red text*, immunoprotective pathways. In the *classical pathway*, the C1 component interacts with appropriately situated Fc fragments of immunoglobulins and stimulates production of a cascade of proteases (C4b, C2b) that combine to yield C4b2b which cleaves C3 to C3b. In the *alternative pathway*, C3b is produced spontaneously or by conversion of Factor B to Bb and production of the protease C3bBb. *Both pathways* generate C3b which converts C5 to C5b, and which in turn combines with other complement components C6, C7m, C8, and C9 to yield a membrane attack protein (MAC). Protection from attack (*red text*) is at the level of (i) inhibition of initial C1 protease by serine protease inhibitors (e.g., serpins); (ii) destruction of the proteases C4b and C3b by Factor 1 protease, involving a sperm membrane cofactor protein (MCP, CD46); (iii) binding of the proteases C4b2b and C3b by decay accelerating factor (FAF, CD55); (iv) binding of components of the membrane attack complex by clusterin (SGP-2), protectin (CD59), and vitronectin (S-protein) on sperm. CD46, CD55, and CD59 are present on testicular spermatozoa and the epididymis secretes SGP-2 and a serpin

3.3 Natural Fertilization

During natural fertilization, mature spermatozoa stored in the epididymis come into contact with secretory products of the **accessory sex glands** during ejaculation, this before entering the female genital tract. Accessory glands belong to the male reproductive tract and activate spermatozoa to become motile; they also provide inhibitors that delay fertilization-related processes.

3.3.1 Erection and Ejaculation

Ejaculation is composed of two sequential processes and is controlled by the autonomic nervous system. Psychological, visual, and auditory stimuli induce parasympathetic impulses via the nervi erigentes, resulting in acetylcholine release that causes vasodilation of the pudendal arteries. This increases the blood flow to the corpora cavernosa and corpus spongiosum and prevents venous outflow, resulting in an erection due to the increasing swelling of the penis (for an overview of the sex organs, see Fig. 3.5). Parasympathetic impulses provoke secretion from the urethral and bulbourethral glands.

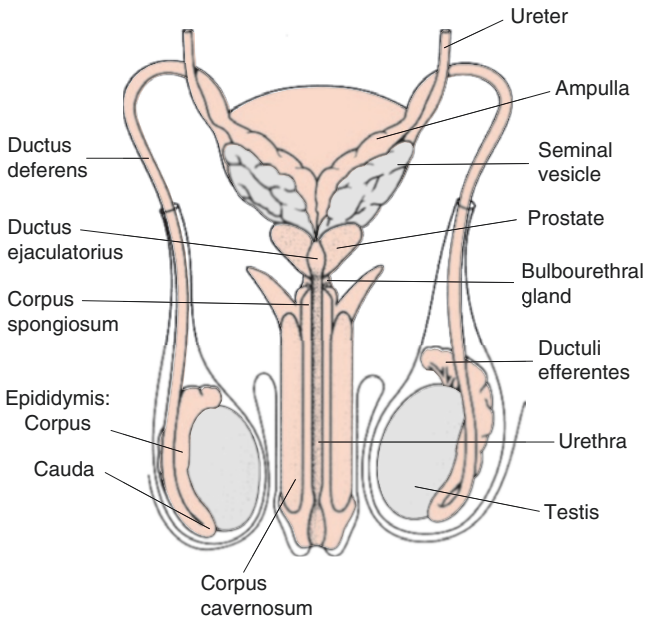


Fig. 3.5 Schematic overview of the male reproductive tract

The next phase, the **emission**, is under the control of the sympathetic nervous system. Irritation of the rami communicantes and hypogastric nerves releases adrenaline, which causes contraction of the smooth muscle around the ampulla, the ductus deferentes, and the caudae epididymides, transporting the spermatozoa into the urethra.

During **ejaculation** proper, parasympathetic fibers of the lower lumbar and upper sacral centers initiate contractions of the bulbocavernous muscles via the pudendal nerves, resulting in the expulsion **(ejection)** of the seminal fluid containing the spermatozoa from the urethra. At the same time, ascending nerve impulses convey the sensation of orgasm. Finally, release of norepinephrine results in dilation of the penile vessels and thus **detumescence** (swelling down), outflow of blood, and flaccidity of the penis.

3.3.2 The Ejaculate

The **ejaculate** is only formed upon ejaculation and only exists outside of the body. The analysis of the ejaculate fractions ("split ejaculates") shows the **sequence of secretions** of the accessory glands and can identify pathological disorders in the natural pattern, which in the fertile male is as follows: the bulborcavernous glands secrete an alkaline solution containing glycoprotein to neutralize the environment in the urinary tract and to lubricate it. The **prostate, epididymis, and deferent ducts** contract simultaneously so that prostatic secretions and spermatozoa meet; finally, the seminal vesicles contract and carry the pellet to the urethra, whereupon semen is ejected from the penis (for an overview of sex organs, see Fig. 3.5).

Coagulation of the ejaculate is a result of the interaction of a zinc-binding protein from the seminal vesicles (seminogelin I), which first binds to the spermatozoa and then to zinc from the prostate, and then fixes the spermatozoa in a semisolid coagulum (Yoshida et al. 2008). CD 52, which is a secretion product of the epididymis bound to spermatozoa by GPI, is a receptor for seminogelin I and thus integrates the spermatozoa into the coagulum (Flori et al. 2008). From this, spermatozoa are released by a complex process promoted by the proteolytic action of another zinc-binding protein, **prostate-specific antigen (PSA).** PSA is inhibited by zinc, but this inhibition is abolished by seminogelin (Jonsson et al. 2005). During and after liquefication, which occurs within 20–30 min, the osmolality of seminal plasma increases (Cooper et al. 2005).

An ejaculate collected in the laboratory differs from that produced during coitus in that the various fractions are combined in a collection vessel and thus cannot be examined until after they have liquefied. Although the **seminal vesicles** produce the largest fraction of ejaculate, precise cooperation of all organs involved is required to provide sufficient fluid and to deliver an optimal composition of normal ejaculate. Sperm-free seminal plasma can be examined to verify the function of the accessory glands. The main secretory products in seminal plasma are: **fructose** from seminal vesicles, **zinc, acid phosphatase, citric acid, and prostate-specific antigen (PSA)** from the **prostate, L-carnitine, glycerophosphocholine, and neutral α-glucosidase** from the **epididymis**. These substances can be used in infertility diagnostics, with concentrations providing clues about the functions of the various organs and possible occlusions in the efferent organs (see Chap. 9).

3.3.3 Sperm Motility

3.3.3.1 Structure of the Axoneme and Flagellar Movement

The morphological prerequisite for sperm motility is the **flagellum ("sperm tail")**, which consists of a middle, main, and terminal segment, with its central axoneme and the outer nine dense longitudinal fibers associated with it. The central part of the flagellum is surrounded by **mitochondria** (Fig. 3.6). The **axoneme** (Fig. 3.7) consists of nine circularly arranged double microtubules connected to each other by nexin and to the central microtubule pair by radial spokes ("9 + 2 structure," Pereira et al. 2017).

The motor of flagellar movement is a series of outer and inner **dynein arms** arising from each microtubule pair (**doublet**). When ATPase is activated in doublet 1–4 of the dynein on one long side of the axoneme, all arms of the adjacent doublet 2–5 pull with repeated formation and disruption of the dynein bridge, resulting in a sliding movement of the doublet and translating it into bending of the flagellum. When this active sliding movement occurs on the other side, bending in the opposite direction occurs (Lindemann and Lesich 2016; Pereira et al. 2017). The coordinated gliding movements of the microtubules that lead to the rotational oscillatory movements of the flagellum drive the rheotactically oriented swimming motion of the sperm (Schiffer et al. 2020).

In contrast to normal forward motility, the pattern of tail movement changes during **hyperactivation** (more vigorous flagellar beat but less progression of the sperm), which is observed in capacitated spermatozoa. Hyperactive motility is usually examined by computer analysis of sperm head movements, recording different quantifying parameters according to the measurement conditions (e.g., 3D movements: "Thrashing, Star-spin, Erratic, Circular or Helical" movement).

Various axoneme defects have been identified as causes of impaired or absent sperm motility: failure of dynein arms, the central pair, the connecting elements, or the outer dense longitudinal fibers, as well as disorganization of the axonemal apparatus (Pereira et al. 2017).

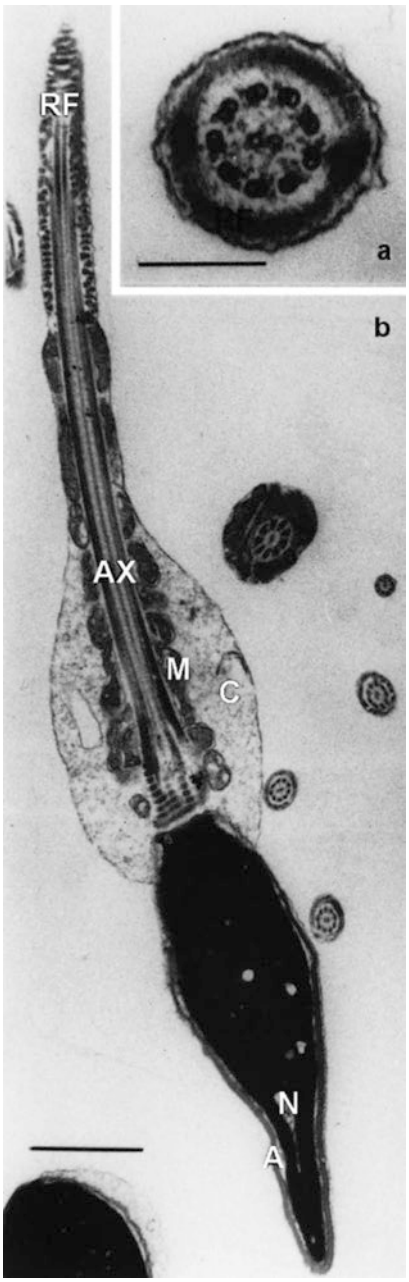


Fig. 3.6 Electron micrographs of human spermatozoa. (**a**) Longitudinal section of a normal sperm: sperm head with nucleus (N) and the acrosome (A). The midpiece is composed of the axoneme (AX) and numerous supporting fibrils, mitochondria (M), and cytoplasm (C). The main piece is composed of the axoneme and the ring fibers (RF). Bar = 1 µm. (**b**) the cross-section through the main piece of a sperm shows the axoneme consisting of two central microtubules surrounded by 9 double tubules connected to each other and to the sheath around the central tubules (dynein arms). Bar = 0.2 µm (from Neugebauer et al. 1990)

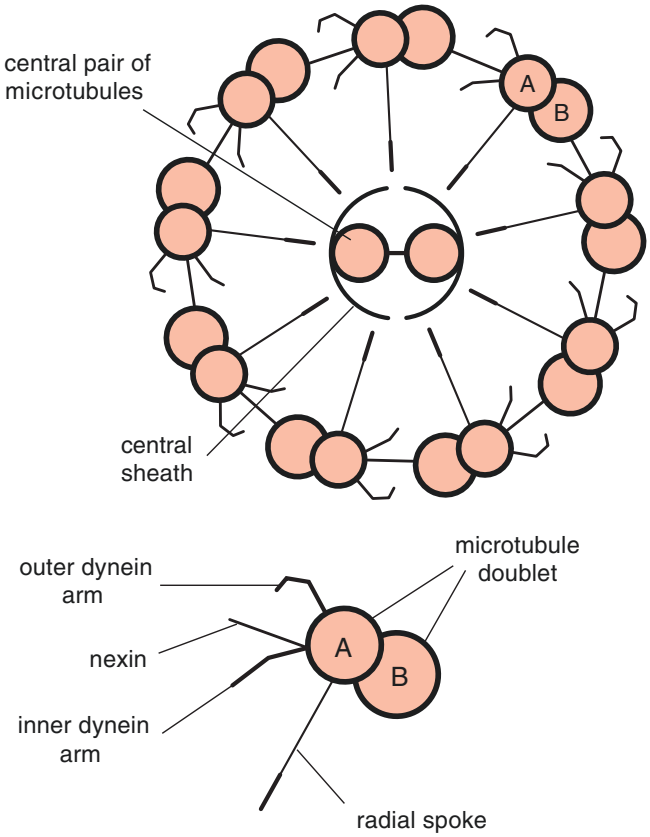


Fig. 3.7 Components of the axoneme are represented schematically. The axonemal complex comprises 9 microtubule doublets interlinked by nexin and connected to the central sheath of the central pair by radial spokes

Axonemal abnormalities result in complete sperm **immotility**. Absence of the outer or inner dynein arms leads to dysfunction; the inner arms are responsible for initiating bending and maintaining the degree of bending. The outer arms are rather not important for the generation of the rowing stroke, but they develop the impact force to overcome the resistance that arises with increasing bending as well as the increasing stroke frequency (Lindemann and Lesich 2016).

Motility is an essential requirement for sperm migration to the site of fertilization and important for passing through the cumulus and the cellular and acellular barriers of the egg.

3.3.3.2 Energy Source for Flagellar Movement

The **mitochondria** of the midpiece generate the energy required for flagellum movement by **oxidative phosphorylation** via the respiratory chain. A correlation between mitochondrial membrane potential and progressive motility and success rates in IVF has been documented (Marchetti et al. 2004). However, additional energy is also generated by gly-

colysis: causative are glycolytic enzymes along the ring fiber (Kim et al. 2007), which provide a scaffold for the molecules of signal transduction and thus sperm function (Carr and Newell 2007; Freitas et al. 2017: Pereira et al. 2017).

Lack of motility may be due to dysfunction of mitochondria, as too little energy is provided. Dysfunctional axonemata may also be responsible if they cannot be activated by ATP and other factors.

3.3.4 Movement of Sperm Through the Female Genital Tract

3.3.4.1 Movement of Uncapacitated Spermatozoa Through the Cervical Mucus

During coitus, together with the seminal plasma, spermatozoa are deposited in the vagina. The seminal plasma neutralizes the acidic vaginal environment and immunologically protects the spermatozoa. During **liquefacation**, spermatozoa migrate from the coagulum to the cervix, whose crypts periovulatory secrete a fluid mucus. The first, sperm-rich fraction may immediately come into contact with the cervical mucus extending into the vagina; moreover, the glycoproteins in the **mucus** favor sperm passage.

However, the mucus can also be an obstacle for sperm passage, namely if sperm antibodies are present. Sperm with antibodies are fixed in the tail region and then exhibit the "shaking phenomenon", i.e., they adhere to the mucus but cannot penetrate it and therefore only move place-bound. This observation has clinical relevance in sperm-mucus interaction diagnostics (Kremer test) (see Chap. 10).

Not only spermatozoa with impaired motility but also those with abnormal morphology have difficulties penetrating the mucus; however, this is mostly related to the hydrodynamics of abnormal head shapes, which present an obstacle for passing through the mucus. Similarly, sperm that cannot regulate their volume have difficulties penetrating the mucus.

In addition to their self-motion, sperm are moved toward the ipsilateral tube by mass movement stimulated by **uterine peristalsis**, estrogens from the dominant follicle, and oxytocin (Kunz et al. 2007).

3.3.4.2 Capacitation and Ascent of Spermatozoa to the Oviduct

On their way to the egg, sperm must cross the uterotubal isthmus to reach the oviduct (Suarez and Pacey 2006). The composition of the tubal fluid causes the spermatozoa to be arrested at the site of subsequent fertilization and to become attached to the tubal epithelium. As the spermatozoa travel through the female genital tract, they undergo **capacitation**, the indispensable prerequisite for fertilization. Prior to capacitation, which can be asynchronous and highly variable in time (e.g., 1 h in the mouse or 6 h in the primate; Yanagimachi 1994), sperm cannot fertilize an egg, either in the female genital tract or in in vitro fertilization (Yanagimachi 1994; Florman and Fissore 2015). Capacitation alters the functional status of spermatozoa and is a type of functional reprogramming. Capacitation is a prerequisite for hyperactivated motility and the ability of spermatozoa to penetrate the egg sheaths as well as induce the acrosome response on the zona surface (Fig. 3.8). Uncapacitated spermatozoa presumably attach to the endosalpinx in vivo prior to ovulation, thereby maintaining viability.

3.3.4.2.1 Mechanism of Capacitation

During capacitation, surface proteins (called decapacitation factors) previously secreted from the epididymis and seminal vesicles during ejaculation are removed. This process is initiated by bicarbonate-dependent changes through cAMP protein kinase A (PKA) signalling pathways in sperm membrane lipids. These changes start in the seminal fluid and continue into the female genital tract (Harrison and Gadella 2005). Sterols and their sulfates are present in spermatozoa and sterol-binding proteins and sterol sulfatases are found in the female genital tract and follicular fluid (Zarintosh and Cross 1996). Enzymatic cleavage of polar esters to nonpolar sterols alters membrane rigidity, and removal of cholesterol by sterol-binding proteins alters the status of membrane lipid microdomains, also known as "**lipid rafts**." These rafts are the sites where signal transduction recognizes and binds to the zona pellucida (Shadan et al. 2004: Freitas et al. 2017). These microdomains are rich in cholesterol, sphingomyelin, glycosphingolides, glycosylphosphatidylinosytol (GPI), other proteins, and molecules and play a role in signal transduction. Removal of cholesterol from sperm membranes is an important step to increase membrane fluidity and accomplish membrane fusion, which is required for the acrosome reaction and sperm-ovum fusion (Cross 2003). Fundamental to this is the alteration of sperm protein localization and their cAMP- and protein kinase A-dependent phosphorylation (Mitchell et al. 2008). However, it should be remembered that these molecular changes and mechanisms of sperm capacitation can only be studied in vitro and thus the physiological processes in vivo are only derived from extrapolation.

3.3.4.2.2 Hyperactivation and Migration to the Site of Fertilization

The first consequence of capacitation is **hyperactivation**. Microscopically, a motility pattern consisting of powerful beats of the sperm tail, large deflections with rapid but not progressive movements, is observed. The trigger of the

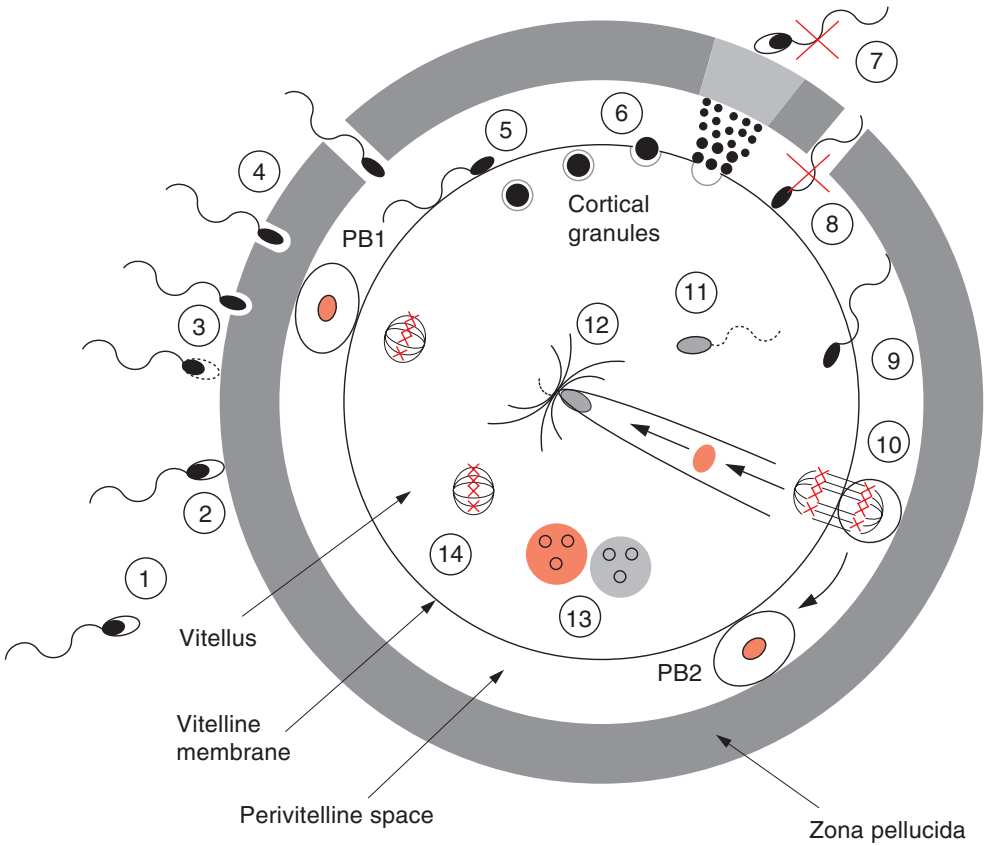


Fig. 3.8 The sequence of events leading to fertilization. After the intact, capacitated spermatozoon penetrates the *cumulus oophorus* (1), it makes contact with the zona pellucida (2), the acrosome reaction is induced in the cumulus layer, at the latest on the surface of the zona (3). The acrosome-reacted spermatozoon drives its way through the zona substance (4) into and across the perivitelline space (PVS). Binding to and fusion of the acrosome-reacted spermatozoon with the oolemma of the egg (5) initiates membrane-mediated calcium-dependent electrical spikes and introduces sperm-associated an egg-activating factor (e.g., PLC ζ) that promotes migration of the cortical granules to the egg surface where their contents are liberated into the PVS (6) and diffuse across it to alter the zona substance such that subsequent spermatozoa cannot bind to the zona, the "zona block to polyspermy" (7). Fusion of further acrosome-reacted spermatozoa that may simultaneously be in the PVS is blocked (8). Penetration of the fertilizing spermatozoon *(9)* further activates the egg, overcoming the metaphase-promoting factors, so that the second meiotic division ensues, leading to extrusion of the second polar body (PB2) and the formation of the female pronucleus (10). Meanwhile, the sperm chromatin decondenses in the cytoplasm (11), its protamines are replaced by histones and the male pronucleus forms. The sperm centrosome organizes the microtubules (12), which brings the two pronuclei together (13). The membranes of the pronuclei dissolve and the parental chromosomes are aligned in a common metaphase plate in preparation for the first mitotic division (14)

hyperactivity is unclear, but the molecular basis is provided by Ca^{2+} current through **CatSper ion channels** (Strünker et al. 2011; Brenker et al. 2012) and cAMP-dependent tyrosine phosphorylation of sperm proteins. This type of movement, which is quite different from the high-frequency, low-amplitude motility of uncapacitated, freshly ejaculated sperm, gives the sperm a higher penetration force.

Such hyperactivated movements lead to sperm detachment from the tubal epithelium and increase the chance of meeting the **cumulus**. In addition, they provide the sperm with the force to penetrate through the viscous luminal fluids to the **cumulus oophorus** and ultimately to the **zona pellucida** surrounding the egg. In humans, hyperactivation is not as clearly defined as in laboratory animals, and probably only a small fraction of all spermatozoa are hyperactivated at the same time. The extent of hyperactivated motility of a sperm population correlates positively with the extent of ability to bind to the zona, as well as with acrosome response, egg penetration, and fertility in vitro, e.g., after IVF (Garrett et al. 2007).

It is postulated that **sperm migration to the egg** in the ampulla tubae uterinae is induced by various stimuli; these could be processes such as **rheotaxis**, **thermotaxis,** and also **chemotaxis**. Experimentally, it has been shown that rheotactic targeting of sperm in a countercurrent appears to be potentially important for the pathway toward the oviduct; thermotaxis is thought to be responsible for sperm migration along a temperature gradient en route to the egg; and ultimately chemotaxis, the recognition of molecules such as progesterone or chemokines produced from the cumulus

cells, is thought to be responsible for finding the egg (Yan et al. 2020). However, only capacitated spermatozoa appear to respond to thermotaxis and chemotaxis.

3.3.5 Sperm Penetration Through the Egg Envelopes

Once spermatozoa have reached the site of fertilization in the ampulla of the oviduct, there are still two hurdles to overcome: (1) first, the **cumulus oophorus** in which the egg is embedded must be overcome, and (2) the **zona pellucida** which surrounds the egg must be penetrated; only then can the sperm bind to the egg membrane and fertilize it.

3.3.5.1 Penetration of the Cumulus Oophorus

The **cumulus oophorus** consists of specialized granulosa cells. The cells immediately surrounding the egg are also called **corona radiata**. Cumulus cells are embedded in a viscous-elastic matrix composed primarily of hyaluronic acid. Although lytic enzymes on the sperm surface, such as hyaluronidases, facilitate sperm passage through the cumulus, it is clear that sperm hyperactivation and the resulting physical deformation of the cumulus matrix are necessary (Dandekar et al. 1992). It is possible that the sperm is directed to the egg within the cumulus by chemotaxis, which propels the sperm to regions of higher concentration (Yan et al. 2020). The cumulus cells, particularly those of the corona radiata, secrete substances that could possibly induce sperm chemotaxis (Sun et al. 2005). Progesterone is one of the identified substances that stimulates sperm motility through Ca^{2+} signalling at the CatSper ion channel (Strünker et al. 2011; Lishko et al. 2011).

3.3.5.2 Sperm Interaction with the Zona Pellucida

The noncellular **zona pellucida** consists of a network of glycoproteins: ZP1, ZP2, ZP3, and ZP4/ZPB (Lefievre et al. 2004). ZP1, ZP3, and ZP4 are important for sperm-zona binding, and ZP2 is responsible for acrosome-responsive sperm binding (Gupta 2018). ZP1 provides structural strength to the zona pellucida through cross-linked filaments. Binding to the zona occurs in two stages: primary sperm binding appears to be mediated by multiple receptors (e.g., zona adhesin, β1,4-galactosyltransferase, SED1, or ADMA3; Gupta 2014; Yeste et al. 2017); subsequently, secondary binding is mediated by proacrosin (Honda et al. 2002).

How proacrosin, or acrosine as a mature form, mediates its function is not known exactly. It is thought that binding occurs first to the acrosome, then to the zona, which is then enzymatically softened (Honda et al. 2002). Complete hydrolysis of the zona appears to be unnecessary (Mao and Yang 2013).

3.3.5.3 The Acrosome Reaction

The **acrosome response** is thought to be triggered by the interaction of sperm and zona glycoproteins, but probably binding alone is not sufficient for the final induction of the acrosome response (Yeste et al. 2017). Although it has been suggested that sperm contact with cumulus cells can trigger the acrosomal reaction, it has been shown in animal models that acrosome exocytosis already occurs in the upper isthmus of the tubules and a few sperm still have an intact acrosome when they reach the ampulla (La Spina et al. 2016). Undoubtedly, sperm must be acrosome-reactive before they can penetrate the zona pellucida to then fuse with the egg. However, whether this occurs in the cumulus or only on the surface of the zona pellucida, and which cellular and molecular mechanisms play a role, remains controversial (Florman and Fissore 2015). It appears that there is no predetermined sequence of events, but rather that it may be diverse (Clark et al. 2006), thus better explaining the complex fertilization events in mammals.

A prerequisite for the acrosome response is a change in the composition and organization of membrane lipids to increase fluidity as a requirement for fusion ability (Gadella and Harrison 2002; Jones et al. 2007). Another prerequisite is the reorganization and distribution of cytoskeletal actin and a subsequent depolymerization of F-actin so that the plasma membrane above the acrosome and the underlying outer acrosomal membrane are brought into contact (Liu et al. 2005; Breitbart et al. 2005). This leads to the formation of hybrid vesicles that are released when the sperm passes through the zona pellucida (Figs. 3.9 and 3.10).

Infertility may be a consequence of various **sperm-ovum binding** disorders, resulting from the inability of the sperm to undergo the acrosome reaction or the inability of sperm to enter, or fully penetrate, the zona.

3.3.6 Fusion of the Sperm with the Oolemma and Activation of the Egg

3.3.6.1 Membrane Fusion

In addition to releasing hydrolytic enzymes for zona penetration, the acrosome induces changes in the equatorial region of the acrosome and in the post-acrosomal region that are necessary for subsequent sperm-ovum fusion (Fig.3.10). In contrast to the proximal region of the acrosome, the equatorial region lacks vesicles and is considered, along with the post-acrosomal region, to be the binding site for initial sperm-ovum contact (Primakoff and Myles 2007). A number of candidate proteins have been identified for specific egg-sperm binding and fusion, but their specific roles are still not

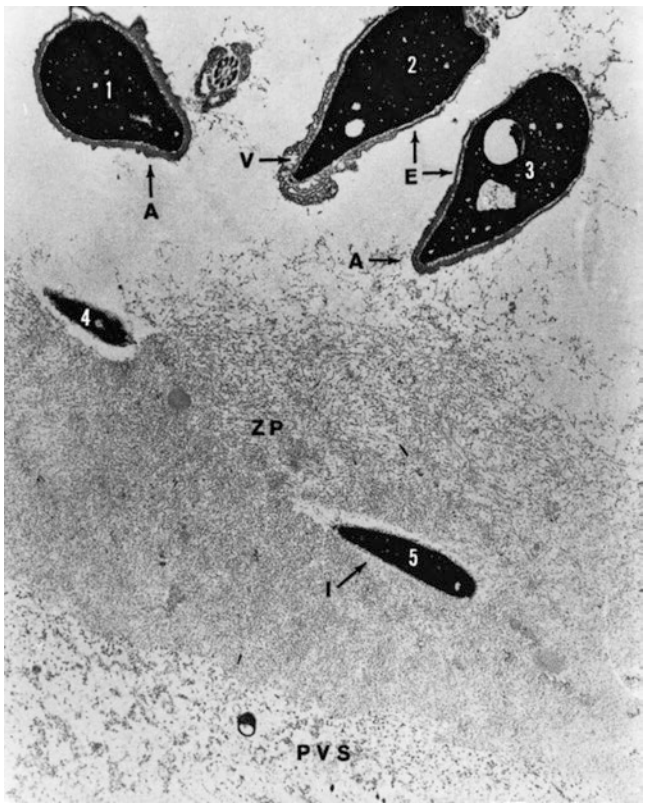


Fig. 3.9 Sperm inside and outside the zona pellucida 8 h after in vitro fertilization. Sperm 1 and 3 show no acrosome reaction and intact membranes over the acrosomal cap (A) and equatorial segment (E). Sperm 2 shows acrosomal membrane changes and vesiculation (V). Sperm 4 and 5 within the zona (ZP) have lost their plasma membrane and outer acrosomal membrane. The inner acrosomal membrane (I) is now in contact with the zona substance. (PVS) perivitelline cleft (from Overstreet and Hembree 1976)

well validated (see Table 3.1; Vjugina and Evans 2008; Florman and Fissore 2015).

Once the sperm has entered the perivitelline space, the acrosome reaction has already taken place, so the necessary changes in the post-acrosomal equatorial region have also already occurred. The equatorial and post-equatorial regions of the sperm head bind to the oolemma in a region rich in microvilli.

Information on sperm-ovum fusion at the molecular level comes mainly from studies in mice, and the exact mechanism is still not fully understood. Some current candidates have been identified by in vivo and in vitro studies (see also Table 3.1): for example members of the ADAM family (A Disintegrin And Metalloprotease domain or also referred to as Fertilin). The interaction of integrins on the oolemma with sperm disintegrins (ADAMs) appears to play an important role in fusion (Reiss and Saftig 2009). Besides the ADAMs proteins, **Izumo**, which is formed by human spermatozoa, is considered a second promising candidate. Izumo is testis-specific and belongs to the immunoglobulin family and expressed in the acrosomal membrane before acrosome reaction. After completion of the acrosome reaction, Izumo is relocalized from the anterior sperm head to the equatorial region of the head, the site where sperm-ovum fusion occurs (Inoue et al. 2005, 2011). The binding counterpart on the oolemma is **Juno** (folate receptor 4) and was discovered in 2014 (Bianchi et al. 2014); both proteins are highly conserved in the animal kingdom and are important not only for egg-sperm membrane fusion, but also for the formation of the polyspermy block (Bianchi et al. 2014; Gupta 2014).

If acrosome-responsive spermatozoa have defects in fusion capacity, infertility can be the consequence.

3.3.6.2 Egg Activation

Although the oocyte developing in the follicle is intended to deliver the **maternal haploid genome**, meiosis is not complete at the time of ovulation; oocytes are arrested in the **metaphase of meiosis II**. This arrest is controlled by the maturation-promoting factor (MPF), whose two components are cyclin-dependent kinase 1 (CDK1) and cyclin B (Florman and Fissore 2015). Upon sperm penetration, this arrest is released, allowing meiosis to progress. Sperm-ovum fusion causes the release of calcium ions from the intracellular stores of the egg, which is followed by characteristic Ca^{2+} oscillations that can last for several hours. The factor responsible for egg activation has been identified as **phospholipase C (PLC) ζ** (Amdani et al. 2016). The release of PLCζ into the ooplasm stimulates Ca^{2+} release from the endoplasmic reticulum, which in turn activates a protein kinase (CaMKII) leading to proteolysis of EMI2 and subsequent destruction of cycline B and a loss of MFP activity (Florman and Fissore 2015). The degradation of these inhibitors leads to the progression of meiosis II and the egg can enter anaphase II (Kishimoto 2005; von Stetina and Orr-Weaver 2011). The subsequent ejection of the second polar body is the sign of meiotic completion (Fig. 3.8). Simultaneously, the Ca^{2+} oscillations lead to the formation of pronuclei, and the resulting pronucleus stage forms and represents the starting point of the **preimplantation development** which finally results in an implantable blastocyst.

Although in **ICSI** sperm-ovum fusion does not occur by active binding of the sperm to the egg, egg activation can occur. The slower release of PLCζ from the perinuclear theca of acrosome-intact injected sperm may delay this process. Compared to natural fertilization processes, this delay in degradation of the perinuclear theca and exposure of sperm chromatin may result in a slowing of chromatin decondensation (Williams 2002).

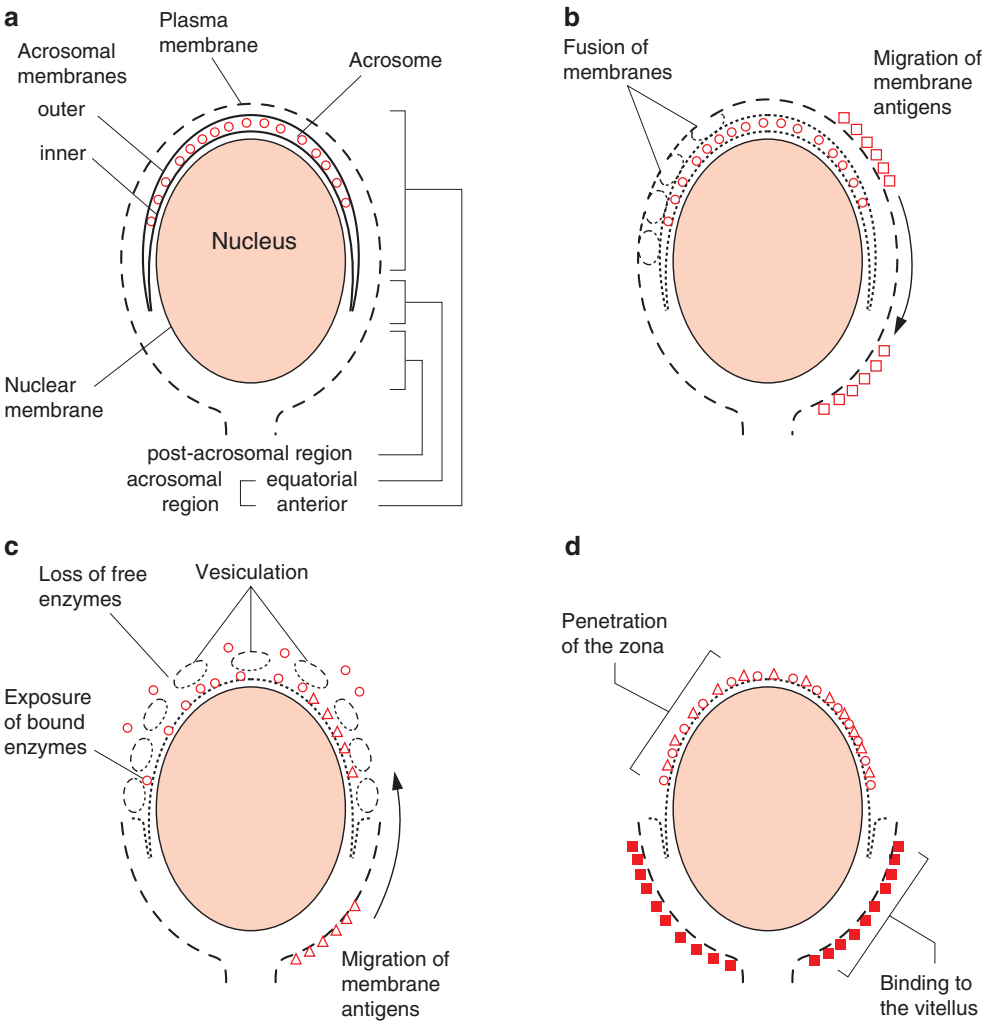


Fig. 3.10 Scheme of events occurring during the acrosome reaction. (**a**) the membranes of the sperm head; (**b**) the fusion of the outer acrosomal membrane and overlying plasma membrane and migration of components to the posterior sperm head; (**c**) formation and loss of acrosomal vesicles with attendant exposure of bound, and loss of soluble, lytic enzymes and migration of components to the anterior head; (**d**) the final state with membrane domains exposed for further sperm-egg interaction

3.3.7 Processes After Fusion

3.3.7.1 Polyspermy Blockade

In terms of timing, an early aspect (approximately 15 min after sperm "entry") induced by Ca^{2+}-signalled egg activation is the exocytosis of cortical granules localized near the surface of unfertilized eggs (Fig. 3.8). The **function of these granules is to prevent polyspermy** by blocking the **entry of a second sperm** at the level of the egg (egg block) and the zona (zona block). The composition of the egg membrane appears to be altered by fusion of the granules with the oolemma, leading to an egg block; however, the exact mechanism is unknown (Florman and Fissore 2015). The zona block emerges when ovastacin, one of the enzymes of the granules, alters (or cleaves) ZP2 by proteolytic modification, leading to hardening of the zona and preventing further sperm penetration. In addition, modification of ZP3 by yet unknown mechanisms appears to prevent further sperm from binding to the zona (Florman and Fissore 2015). The antagonist of ovastacin is fetuin-B (FETUB), which is present in follicular fluid and prevents premature hardening of the zona by inhibiting egg-specific ovastacin (Dietzel et al. 2013). Several factors appear to be involved in the regulation of "zonal blockade against polyspermy" (Fig. 3.8).

> The lack of egg activation due to PLCζ deficiency in sperm can lead to infertility. The induction of artificial egg activation by diverse stimuli (mechanical, electrical, or chemical) can compensate for the deficiency of these sperm (Sun and Yeh 2020).

3.3.7.2 Formation of the Male Pronucleus

Sperm chromatin is extremely condensed as the arginine-rich histones are replaced by the more basic protamines in so-called toroids during spermiogenesis. To form a pronucleus from the sperm head, the sperm nucleus must be reprogrammed in several steps. An initial phase of **chromatin**

dispersion is followed by a short **recondensation**, and then the final and complete decondensation into the male pronucleus. The disulfide bonds of the nucleoprotein protamine are now reduced by glutathione in the egg cytoplasm and the protamines are exchanged by histones (Florman and Fissore 2015). After protamine-histone exchange, DNA methylations are removed, this probably earlier and by active demethylation in the paternal genome (Carrell 2012; Laurentino et al. 2016). The sperm tail, as well as the sperm paternal mitochondrial DNA, is degraded as only the maternal mitochondria provide energy in the later **embryo**.

The fusion of sperm and egg also inactivates the "metaphase-promoting factor", which leads to the extrusion of the second polar body from the egg. Now the female pronucleus forms from the haploid chromosome set of the egg. At the same time, the **centrosomes** from the entering spermatozoon are activated, as they are responsible for the subsequent organization of the microtubal spindle. After replication of the centrosome, two microtubule-organizing centers are available to form the spindle apparatus for the first embryonic division (Navarra et al. 1995; Sathananthan et al. 1996).

The male pronucleus establishes contact with **the female pronucleus** and both pronuclei are pulled to the center for the first mitotic divisions to start. The chromosomes condense during the prophase of **syngamy** before the nuclear membranes dissolve and a **zygote** is formed. Interestingly, the paternal and maternal chromosomes do not mix prior to the first mitotic division, but remain where they were located after dissolution of the nuclear membranes. In addition, not only a single spindle but a bipolar spindle seems to be responsible for the independent segregation of the parental genomes; at least in mice (Reichmann et al. 2018).

There are sperm that cannot be "activated" in the ooplasm. This can lead to infertility if the entering sperm does not form the structures (e.g., the centrosomes) necessary for the mitotic spindle.

3.3.7.3 Early Embryonic Development

After formation of the **zygote**, the first mitotic cell divisions begin in the oviduct and a two-cell organism is formed. Further divisions then result in an eight-cell organism on the third day after fertilization, a point at which the newly recombined genome is activated for the first time. As the embryo migrates through the Fallopian tube toward the uterus, it continues to divide and then forms a **morula** 4 days after fertilization, consisting of about 16 totipotent cells which now begin to compact. After 4.5–5 days, the preimplantation embryo enters the uterus as an early **blastocyst.** The cells continue to divide steadily, forming **"gap**" and **"tight junctions**"and epithelial differentiation begins. The outer cells isolate the embryo from the environment, and ion transport leads to fluid influx into the blastocyst cavity (blastocoel) as well as differential ion concentration within the preimplantation embryo. At this stage, the blastocyst consists of up to 180 cells: on the one hand, outer cells that form the trophectoderm, and on the other hand, inner cells that form the inner cell mass, from which, e.g., the later embryo arises. During migration of the preimplantation embryo towards the uterus, it undergoes morphological and biochemical changes in dependence on ovarian progestogens and estrogens: the endometrium changes from the proliferative to the secretory phase and thus acquires the ability to accommodate a blastocyst.

3.3.7.4 Implantation of the Embryo

The **blastocyst** hatches from the zona pellucida approximately 6–7 days after fertilization and is now ready to **implant into the endometrium. Hatching** is a necessary condition for implantation and is controlled by steroid-dependent lytic factors. The free blastocyst now binds to the endometrium, ruptures the luminal epithelium, and passes into the tissue. The endometrial cells surrounding the embryo transform and decidualize, forming a **hemochoreal placenta** (**placentation**). The **decidua** is transformed endometrium that undergoes vascular remodelling (Ashary et al. 2018) and protects the embryo from the mother's immune system. The **implantation process** is now complete and further development occurs in a timely manner over the next weeks, ending with the birth of a child.

Key Points

- Successful fertilization of an egg is the ultimate goal for a mature sperm. For this process to occur correctly in vivo, the natural fertilization process follows a time- and location-dependent sequence of complex steps, beginning with production in the testis, maturation in the epididymis, and finally fertilization of the egg.
- At fertilization, the two haploid gametes fuse, and a new individual can emerge. If the sperm cannot bind to the zona pellucida, for example due to insufficient acrosome reaction, this results in infertility.
- Spermatozoa remain in the epididymis for 2–7 days, where they mature and are stored until ejaculation. If this storage of spermatozoa in the epididymis is disturbed, sperm dying may occur.
- Various **axoneme defects**, such as the failure of dynein arms, have been identified as causes of impaired or absent sperm motility, which is an

essential prerequisite for sperm migration to the site of fertilization and to overcome the cumulus and the cellular and acellular envelopes of the egg.

- Lack of activation of the egg due to a deficiency of PLCζ can lead to infertility.
- **Infertility** can also result from sperm that are not "activated" in the ooplasm and then fail to form certain structures (e.g., the centrosomes) after penetrating the egg.
- In infertile men, not only ejaculated spermatozoa but also spermatozoa from the testis and epididymis can be introduced into an egg by ICSI and lead to an intact pregnancy.
- In medically assisted reproduction (MAR), the natural barriers are therapeutically bypassed.

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Part II

Classification and Diagnosis of Andrological Disorders

Classification of Andrological Disorders

4

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Abstract

Male infertility, hypogonadism, and sexual dysfunction are classified according to localization of the disease as the first ordering principle and cause as the second. The corresponding ICD digits are listed. An overview of diagnoses and an additional classification according to therapeutic options and references to the relevant chapters of this book complete this classification of andrological disorders.

4.1 Classification by Localization and Causality

Infertility, hypogonadism, and **erectile dysfunction** are symptoms or diseases characterizing a plethora of clinical disorders which will be discussed in the following chapters. The physician must be able to make the correct diagnosis by nuancing these symptoms and using appropriate additional procedures. As in all fields of medicine, in andrology the most accurate diagnosis possible is a prerequisite for optimal therapy. Correct diagnosis and therapy in turn require pathophysiological knowledge of the cause of the clinical picture.

The **causes of disturbed male reproductive function** can be localized at various somatic levels. The **testes** themselves can be affected, the causes can lie in the **excurrent seminal ducts** or the **accessory sex glands**, there can be disturbances of **penile function** and/or of **semen deposition**, but also central structures such as **hypothalamus** and **pituitary gland** or the **androgen target organs** can be affected.

In order to apply a systematic approach to the clinic of disturbed male reproductive functions, a classification of the clinical pictures according to the **topographic localization of the cause** is the **first organizing principle.** As a **second organizing principle**, the **type of cause**, e.g., endocrine, genetic, inflammatory, etc., may be applied.

Such classifications are the basis of this volume. An overview is provided by Table 4.1, which also serves as a rough diagnostic guide to whether signs of androgen deficiency, infertility, erectile dysfunction, or a combination of all symptoms are present in the individual clinical pictures. The **International Classification of Diseases (ICD)** is also oriented to the localization and causality of infertility and hypogonadism as well as disturbed sexual function. In Table 4.1, the relevant ICD digits have been included.

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Table 4.1 Classification of disorders of testicular function based on localization of cause

Localization of disorder	Disorder	ICD-10	Cause	Androgen deficiency	Infer-tility
Hypothalamus/ pituitary	Isolated lack of GnRH, Congenital hypogonadotropic hypogonadism (CHH) Kallmann syndrome	E23.0	Genetic disturbances of GnRH secretion due to mutations of the *ANOS1* (*KAL1)*-, *FGFR1*- (*KAL2*), *PROK2*-, *PROKR2*-, *KISS1R*-genes	+	+
	Prader-Labhart-Willi syndrome	Q87.1	Genetic disturbances of GnRH secretion	+	+
	Constitutionally delayed puberty	E30.0	Delayed biological clock	+	(+)
	Secondary disturbance of GnRH secretion	E23.0	Tumors, infiltrations, trauma, irradiation, disturbed circulation, malnutrition, systemic disease	+	+
	Hypopituitarism	E23.0	Tumors, infiltrations, trauma, irradiation, ischemia, surgery	+	+
	Pasqualini syndrome FSHB-polymorphism	E23.0	Isolated LH deficiency Isolated FSH deficiency	+ –	(+) +
	Hyperprolactinemia	E22.1 (D35.2)	Adenomas, medications, drugs	+	+
Testes	Congenital anorchia	Q55.0	Fetal loss of testes	+	+
	Acquired anorchia	E89.5	Trauma, torsion, tumor, infection, surgery	+	+
	Maldescended testes	Q53.9	Testosterone, AMH deficiency, congenital, anatomical hindrance	(+)	+
	Varicocele	I86.1	Venous insufficiency (?)	(–)	+
	Orchitis	N45.-	Infection with destruction of germinal epithelium	(–)	+
	Spermatogenetic defects (Sertoli-cell-only syndrome,	N46	Congenital, acquired	–	+
	Spermatogenic arrest)				
	Globozoospermia	N46	Absence of acrosome formation	–	+
	Immotile cilia syndrome Sperm ion channels defects	N46 N46	Lack of dynein arms Lack of Ca-channel	–	+
	DSD (Differences of sex development)		Genetic disturbance in gonadal differentiation		
	Klinefelter syndrome, 47,XXY	Q98.0	Meiotic nondisjunction in meiosis	+	+
	46,XX-Männer	Q98.2	Translocation of part of Y-chromosome	+	+
	Gonadal dysgenesis	Q96.9	Varying genetic disturbances	+	+
	Persistent oviduct	–	AMH receptor mutation	–	(–)
	Leydig cell hypoplasia	E29.1	LH receptor mutation	+	(+)
	Disorders of steroid synthesis	E29.1	Enzymatic defects in testosterone synthesis	+	+
	47,XYY-Männer	Q98.5	Meiotic nondisjunction	(+)	(+)
	Noonan syndrome	Q87.1	Mutations of the *PTPN11*-, *KRAS*-, *SOS1*-, and *RAF1*-gene	+	+
	Structural chromosomal anomalies	Q99.-	Deletions, translocations, etc.	–	+
	Testicular tumors Germ cell tumors Stroma tumors	C62.-	Congenital/acquired?	+	+
	Disorders caused by exogenous factors or systemic disease	div.	Medication, irradiation, heat, environmental and recreational toxins, liver cirrhosis, renal failure	+	+
	Idiopathic infertility	N46	Unclear causality	–	+
Mixed hypothalamus/ pituitary/testes	Late-onset hypogonadism (LOH)	–	Primary and secondary hypogonadism	+	+
Excurrent seminal ducts and accessory sex glands	Infections	N49.-	Bacteria, viruses, Chlamydia	–	+
	Obstructions	N50.8	Congenital anomalies, infections, vasectomy, appendectomy, herniotomy, kidney transplantation	–	+
	Cystic fibrosis	E84.-	Mutations of the *CFTR*-gene	–	+
	Congenital bilateral aplasia of the vas deferens (CBAVD)	Q55.3	Mutations of the *CFTR*- or *ADGRG2*-gene	–	+
	Disturbance of liquefaction	–	?	–	+
	Immunologic infertility	N46	Autoimmunity	–	+

(continued)

Table 4.1 (continued)

Localization of disorder	Disorder	ICD-10	Cause	Androgen deficiency	Infer-tility
Disturbed semen deposition	Ectopic urethra	Q54.-Q64.0	Congenital	–	(+)
	Penis deformation	N48.8	Congenital/acquired	–	(+)
	Induratio penis plastica	Q55.6 N48–6	Acquired	–	–
	Erectile dysfunction	F52.2 N48.4	Multifactorial origin	(+)	(+)
	Disturbed ejaculation	F52.3/4	Congenital/acquired	–	+
	Phimosis	N47	Congenital	–	(+)
Androgen target organs	Complete Androgen Insensitivity Syndrome (CAIS)	E34.51	Androgen receptor defect	+	+
	Partial Androgen Insensitivity Syndrome (PAIS)	E34.50	Mild androgen receptor defect	+	+
	Bulbospinal-muscular atrophy	G12.1	Androgen receptor defect	(+)	–
	Perineoscrotal hypospadias with pseudovagina	E29.1	5α-reductase deficiency	+	+
	Estrogen resistance	E28.0	Estrogen receptor defect	(–)	(–)
	Estrogen deficiency	–	Aromatase deficiency	(–)	(–)
	Gynecomastia	N62	Multiple causes	(+)	(–)
	Androgenic alopecia	L64.9	Genetic	–	–

Currently, ICD-10 is valid; a revised ICD-11 was adopted by the WHO in 2019, but will only be implemented in 2022 (in Germany). As an essential improvement, the revised version eliminates the distinction between organically caused disturbed sexual function and that of nonorganic origin; a summary appears in the chapter dealing with conditions related to sexual health (Chaps. 30, 43 and 44). These will be divided into four main categories. A sexual problem only turns into sexual dysfunction when additional qualifications of morbidity are also present, such as persistence, severity of symptoms, and suffering; these will also be included in ICD-11. However, in large part the ICD is too imprecise with regard to the classification of andrological clinical pictures and especially for scientific evaluations, so that an extension by sub-digits and/or the combination of several diagnoses has proven useful for the complete description of a patient.

In recent years, the term **"functional hypogonadism"**has been introduced to complement **"organic hypogonadism"**in addition to the classic classification into primary and secondary hypogonadism (Grossmann and Matsumoto 2017; Bhasin et al. 2018; Corona et al. 2020; Dohle et al. 2018). While "organic hypogonadism" denotes congenital or destructive disorders resulting in permanent dysfunction of the hypothalamus, pituitary, or testes, "**functional hypogonadism**" considers as a subset those disorders caused by temporary suppression of gonadotropins and testosterone that are fundamentally reversible once the underlying cause has been treated. These include hypogonadism caused by obesity, diabetes, kidney, heart and liver disease, medication, and drug abuse as well as athletic overtraining. Moreover, as aging men acquire numerous diseases, **late-onset hypogonadism (LOH)** is also interpreted as functional hypogonadism. In addition, however, it should not be forgotten that some of the affected individuals have a genuine, i.e., organic, hypogonadism, since the hypothalamus, pituitary gland, and testes may show irreversible dysfunction (Nieschlag 2020). Special aspects of the reproductive functions of the aging male and genetically caused diseases in the offspring are addressed separately in Chap. 25. Moreover, genetic diagnoses have given rise to causes on the hypophyseal level which may in turn induce isolated gonadotropic deficiency, supplementing the classic form of hypophyseal hypogonadism. This in turn may lead to pharmacokinetic consequences for therapy of the afflicted person. The relevant chapters deal with these cases (Chaps. 12, 13, 14, 15, and 38).

General and systemic diseases can affect gonadal function, fertility, and sexuality at all above-mentioned levels and often at several simultaneously; therefore, general diseases are dealt with separately (Chap. 34). **Toxins and environmental factors**, which are discussed in a separate chapter (Chap. 35), have hardly been definitively assessed in terms of their significance for fertility and are characterized by a great need for research. **Psychological factors** play an important role in all the disorders listed, even if they may not be the cause of infertility themselves. For this reason, separate chapters are devoted to the psychology of male fertility disorders and to sexual medicine, respectively (Chaps. 43 and 44).

In addition, there is a relatively large, heterogeneous group of patients whose fertility disorders cannot be clearly identified. So-called **idiopathic infertility** may have a wide variety of causes. Investigating these causes is a current task of andrology. In these patients, the ejaculate parameters may

be in the normal range or subnormal values may be found. These can be symptomatically described using terms composed of oligo-, astheno- or terato-zoospermia. Since a wide variety of therapeutic approaches are pursued even without knowledge of the causes, idiopathic infertility is dealt with in the context of andrological therapy (Chap. 39).

Genetic aspects are becoming increasingly important in the causal classification of spermatogenetic disorders. In recent years, new genes have been described in which mutations lead to qualitative and/or quantitative failure of spermatogenesis (Tüttelmann et al. 2018). The corresponding investigation possibilities have changed rapidly in a short time due to so-called next-generation sequencing and are presented in Chap. 8. The genetic syndromes are described throughout the book in the respective chapters, e.g., hypothalamic-conditioned hypogonadotropic hypogonadism and Kallmann syndrome in Chap. 12, rare forms of secondary hypogonadism in Chap. 13, pituitary-related hypogonadotropic hypogonadism in Chap. 15, Klinefelter syndrome in Chap. 21, XX male in Chap. 22, structural chromosomal abnormalities in Chap. 23, cystic fibrosis in Chap. 27, and differences of sex development (DSD) in Chap. 31.

The term DSD (**Differences [formerly Disorders] of Sex Development = DSD**) is also used as a systematic and correct term for disorders of sexual development in German-speaking countries (German Medical Association, Statement on DSD 2015). The terms intersexuality, hermaphroditism, and pseudohermaphroditism are no longer in use for various reasons. On the one hand, they are not pathophysiologically distinct entities and, on the other hand, for psychological reasons of patient management, it seems better to refrain from using these terms, which are sometimes perceived as discriminatory. Furthermore, since the transitions from the completely normal to the intersexual phenotype are fluid, we have assigned the differentiation disorders to the levels according to their cause. The different clinical pictures (as far as they refer to the male) are therefore found in a separate chapter (Chap. 31). Because of its frequency and importance in andrology, a special chapter is devoted to Klinefelter syndrome (Chap. 21).

Increasingly, **gender diversity** and **sexual dysphoria** are dealt with by andrologists in addition to testosterone treatment and fertility protection. For these reasons, relevant chapters have been included as well (Chap. 44).

4.2 Classification According to Therapeutic Options

In contrast to the systematics of andrological disorders described so far, which are based on localization and causality, a purely pragmatic classification can also be made according to treatment options. In this case, pathophysiological aspects are largely neglected and the answer to the patient's primary question of whether and how he can be treated is in the foreground. Since such a classification according to treatment options can also be helpful in discussions with the patient or couple, such an overview is presented in Table 4.2.

Table 4.2 Classification of male infertility according to therapeutic possibilities

Disorder	Therapy	Discussed in chapter of this volume
Rational therapy		
CHH and Kallmann syndrome	GnRH or gonadotropins	12, 13, 38
Pituitary insufficiency	Gonadotropins	15, 38
Prolactinoma	Dopamine agonists	15
Infections	Antibiotics	26
Chronic general disease (e.g., renal insufficiency, diabetes mellitus)	Treatment of underlying disease	34
Medication, drugs, toxins	Elimination	35
Obstructive azoospermia	Vasovasostomy, vasotubolo-stomy, TESE, MESA	15
Erectile dysfunction Anejaculation	PDE-5 inhibitors, SKAT, penile prosthesis psychosexual counseling, Rectal stimulation, vibrator stimulation, TESE/ICSI	30
Retrograde ejaculation	Imipramine, Midodrine	30
Preventive therapy		
Maldescended testes	Orchidopexy (GnRH/hCG)	17
Delayed puberty	Testosterone/GnRH/hCG	14
Infections	Timely use of antibiotics	26
Exogenous factors (X-rays, drugs, toxins)	Elimination	34/35
Malignant disease	Gonadal protection/ cryopreservation of sperm or germ cells	42
Symptomatic therapy		
Bilateral anorchia	Testosterone substitution, donor insemination, adoption	16/36
Complete SCO syndrome	Donor insemination, adoption	20
Gonadal dysgenesis Spermatogenetic defects Testicular tumors Couple infertility	Testosterone substitution, donor insemination. Adoption TESE Ablatio testis, tumor enucleation Assisted reproduction (IUI, IVF)	31 11 24
Severe male infertility	ICSI / TESE.ICSI	42
Empirical therapy		
Idiopathic infertility	Various medications	39
Immunologic infertility	Assisted reproduction	28
Varicocele	Various treatments of varicocele, optimization of female reproductive function	18

As will be dealt with in the following chapters, there are a number of fertility disorders whose pathophysiological causes are known and which can be treated rationally. However, for some disorders with known causes, there is no **rational therapy** (yet). For others, such as positional abnormalities of the testes and infections, potential infertility can be treated by timely **preventive therapy** or, e.g., by cryoconservaton of germ cells in the event of gonadotoxic therapy. Procedures of **assisted reproduction** (intrauterine insemination, in vitro fertilization, and intracytoplasmic sperm injection) are effective means available for the treatment of couple infertility, as well as for male infertility but these must be regarded as **symptomatic therapy**, since they do not causally remedy the disorder of procreative ability (Chap. 41). Accordingly, they are also used independent of diagnosis and solely on the basis of ejaculate values or the extractability of sperm from the testis or epididymis.

Before the introduction of assisted reproduction (especially before ICSI and TESE/ICSI), and even today, numerous **empirical therapies** were and are used for male infertility. These procedures are often used with little discrimination for a wide variety of diagnoses. Most affected are varicocele, immunological infertility, and especially idiopathic infertility, which account for about 30–60% of patients visiting andrology centers (Tüttelmann and Nieschlag 2009; Punab et al. 2017). Because of the dimension of the problem, separate chapters are devoted to the empirical treatment of **idiopathic infertility** (Chap. 39), to **varicocele** (Chap. 18) and to **immunological infertility** (Chap. 28).

Key Points

- Male infertility and hypogonadism are classified according to localization of the disease as the first ordering principle and cause as the second.
- The corresponding ICD digits are listed.
- An overview of diagnoses and an additional classification according to therapeutic options and references to the relevant chapters of this book complete this classification of andrological disorders.

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Anamnesis and Physical Examination

5

Eberhard Nieschlag and Hermann M. Behre

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Abstract

Anamnesis and physical examination are the beginning of every medical intervention. Especially in the field of andrology and reproductive medicine, they are particularly important for establishing a relationship of trust between doctor and patient, without which the physician cannot obtain information necessary for dealing with conditions which are sometimes considered taboo. Anamnesis and thorough physical examination provide signposts for further diagnostics and therapy.

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5.1 Anamnesis

Anamnesis and physical examination are the basis of all medical procedures. Especially in the field of andrology and reproductive medicine, they are vital for establishing a relationship of trust between physician and patient, without which the physician will not receive required information in an area that continues to be characterized by strong taboos. Anamnesis and a thorough physical examination provide the signals for further diagnostics and therapy.

The **anamnesis** provides important information for the assessment of testicular function. Impairment of general performance, a diminution of beard growth and a decrease in shaving frequency, a decrease in erection frequency, particularly spontaneous nocturnal and morning erections, and a lessening of sexual desire and phantasies provide important information on **possible androgen deficiency**. Because of a concomitant decrease in libido, patients' complaints may be muted in contrast to those generated by erectile dysfunction not caused by testosterone deficiency.

Important aspects emerge from suspicions aroused by particular clinical pictures. In the case of a suspected pitu-

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itary tumor, for example, the field of vision should be checked for impairment; in the case of a suspected Kallmann syndrome with hypergonadism of hypothalamic origin, disturbances of the olfactory sense have to be considered.

As regards **medical history**, age of onset of puberty, voice mutation, and the beginning of beard growth have to be recorded. Any testicular maldescent and the age at which medical therapy (hCG or GnRH) or surgery (orchidopexy) were carried out are of importance. Herniotomy, possibly with subsequent testicular damage, is to be recorded. Since general diseases (diabetes mellitus, liver or kidney diseases, metabolic syndrome, oncological diseases, etc.) can lead to hypogonadism and/or infertility, the relevant symptoms must be noted (see Chap. 34). Changes in weight should be noted. Recurrent bronchitis or sinusitis in childhood or adulthood indicates diseases of the respiratory system which may, e.g., in cases of ciliary diskinesia, Kartagener syndrome, or cystic fibrosis, be associated with infertility. Mumps, with or without testicular involvement, must be registered. Infectious diseases with or without clinically manifesting orchitis or epididymitis can lead to androgen deficiency and/or infertility. Sexually transmitted diseases (syphilis, gonorrhoe, AIDS) and their respective treatments must be recorded. Any infection with a zika virus or extended stays in risk areas may be of relevance (Weberschock et al. 2018). Malignancies and type of treatment (chemotherapy, radiation) must be taken note of.

The **medical family history**, including data on the fertility status of parents, siblings, and other relatives, provides essential information for a possible genetic cause of hypogonadism and infertility. Is there involuntary childlessness in the family? Are or were there genetic diseases among relatives or carcinoma, especially prostate, breast, or testicular cancer? Often, establishing a pedigree is indispensable to obtain clues to the inheritance of certain conditions and syndromes.

An exact **medication and drug history** is important, since a multitude of substances can lead to side effects of androgen deficiency and infertility (e.g., sulfasalazine, antihypertensive drugs, antibiotics, cytostatic agents, anabolic hormones) (overview in Semet et al. 2017; Duca et al. 2019; Ajayi and Akhigbe 2020) (see Chap. 34).

Lifestyle medication, e.g., anabolics (Nieschlag and Vorona 2015) and finasteride as well as drugs like marijuana (Zufferey et al. 2020), opioids, and cocaine, can have a negative influence on fertility and hormone balance. Occupational exposure to heat and chemicals can lead to infertility. In addition, exposure to **exogenous toxins,** which may impair spermatogenesis and testicular testosterone production, should be carefully recorded (see Chap. 37). Athletic activities, particular habits, and nicotine and alcohol abuse are rarely mentioned spontaneously and must be probed insistently by the physician.

Occupational exposure to heat and chemicals can reduce fertility. In addition, a careful anamnesis is required to identify exogenous noxae that may affect spermatogenesis and testosterone production in the testes (see Chap. 35). Sporting activities (overtraining?) and special lifestyle habits, nicotine and alcohol consumption, are recorded. While moderate alcohol consumption has no influence on semen parameters, high consumption can negatively influence ejaculate volume and sperm morphology (Ricci et al. 2017).

Since involuntary childlessness is a problem common to the **couple** (see Sect. 1.4), the medical history should be taken in the presence of both partners. In case of infertility, the duration of childlessness, unprotected intercourse, and intercourse frequency are documented. Periodic separations, e.g., because of shiftwork or frequent travel, are recorded. Indications for dyspareunia and (perhaps temporary) erectile dysfunction should also be pursued. Professional or private stress factors which may lead to conflicts between the partners are explored. Earlier pregnancies with the present partner or in another partnership are also recorded. All previously performed or anticipated infertility examinations of both partners should be noted.

Libido and frequency of sexual intercourse must be inquired after. Is erectile dysfunction occasional or permanent? If so, do morning erections occur or not? Do orgasms occur during intercourse - of both partners? Does ejaculation praecox occur regularly or occasionally?

Special **psychological and sexological problems** are dealt with in Chaps. 43, 44 and 45.

5.2 Physical Examination

A thorough physical examination provides an overview of all organ systems and of diseases which may be associated with hypogonadism and/or infertility. In the following sections, special examinations are mentioned when hypogonadism or infertility is suspected. It should be kept in mind that the clinical presentation of hypogonadism is dependent on the time of manifestation (Table 5.1). If androgen deficiency becomes manifest after puberty, clinical symptoms can be rather discrete.

5.2.1 Body Proportions, Skeletal Structure, Fat Distribution

If androgen deficiency exists at the time of normal onset of puberty, **eunuchoid tall stature** will result because of delayed or absent pubertal development with delayed epiphyseal closure. Consequently, arm span (measured from the tip of one middle finger to the other while arms are stretched out) will exceed body length and the legs become longer than the trunk. Because of these characteristic body proportions, these patients appear short when sitting ("**sitting dwarfs**") and tall while standing ("**standing giants**"). Patients may

Table 5.1 Symptoms of hypogonadism relative to age of manifestation

Affected organ/function	Before completed puberty	After completed puberty
Bones	Eunuchoid tall stature, osteoporosis	Osteoporosis
Larynx	No voice mutation	No change of voice
Hair	Horizontal pubic hairline, straight frontal hairline, diminished beard growth	Diminishing secondary body hair
Skin	Absent sebum production, lack of acne, pallor, skin wrinkling	Decreased sebum production, lack of acne, pallor, skin wrinkling
Bone marrow	Low degree anemia	Low degree anemia
Muscles	Underdeveloped	Atrophy
Penis	Infantile	No change of size
Testes	Possibly maldescended testes, small volume	Decrease of testicular volume
Spermatogenesis	Not initiated	Involuted
Prostate	Underdeveloped	Atrophy
Libido and potency	Not developed	Erectile dysfunction

remain short if other central disorders are present, especially those affecting thyroid function or growth factors. However, bodily proportions will develop similarly to those seen in eunuchoid tall stature. Onset of androgen deficiency after puberty will not result in a change of bodily proportions, although the **musculature can be atrophic** depending on the duration and degree of androgen deficiency (Table 5.1).

Long-standing androgen deficiency leads to **osteoporosis** which can result in severe **lumbago** and pathological spine and **hip fractures**. Androgen deficiency does not directly cause an increase in subcutaneous fatty tissue; however, **fat distribution** will have female characteristics (hips, buttocks, lower abdomen). Exact determination of BMI and **abdominal circumference** (by tape measure) is part of every medical status, as they not only correlate with testosterone levels (Svartberg et al. 2004), but also with life expectancy . There is a good correlation between bodily fat distribution measured by DXA or MRT and the quotient of abdominal circumference and height (Ketel et al. 2007).

5.2.2 Voice

In normal physiology, larynx growth correlates with testicular growth and increase of testosterone during puberty (Harries et al. 1997). In a cohort of Danish boys, voice mutation occurred at the mean age of 13.5 years at testosterone levels of 12 nmol/L and at a testicular volume of 2 × 12 ml (Busch et al. 2019). If hypogonadism is present before normal puberty, **no voice mutation** will occur because of a lack of laryngeal growth. Often patients are addressed as females despite advanced age, especially on the telephone, with negative effects on the patient's self-esteem. When hypogonadism develops after puberty, the voice, already mutated, remains unchanged.

5.2.3 Skin and Hair

When puberty fails to occur, the **frontal hairline** remains straight. **Temporal hair recession** or **balding** will not occur, but remain if androgen loss occurs after puberty, and secondary sexual hair and body hair become sparser (Randall 2012). When evaluating hair distribution, the Hamilton scale (Fig. 5.1) can be consulted (see Chap. 33).

Nonoccurrence or only partial puberty causes lack of or sparseness of **beard growth**; shaving is seldom or never necessary. Even Shakespeare noted, "He that hath a beard is more than a youth, And he that hath no beard is less than a man." (*Much Ado about Nothing*, 1600). The **upper pubic hairline** remains horizontal.

Polymorphism of the androgen receptor (CAG repeats in exon 1) is discussed as a determinant of hair pattern (Ellis et al. 2001). When evaluating hair distribution, ethnic differences must be considered (Santner et al. 1998).

Another typical feature is early, **fine wrinkling of the perioral and periorbital skin**. Additionally, because sebaceous gland stimulation is absent, the skin remains dry. Anemia, along with decreased blood circulation of the skin, causes **pallor**.

5.2.4 Olfactory Sense

The presence of **hyposmia** or **anosmia**, which are important diagnostic indicators of Kallmann syndrome, is recorded following specific questioning and systematic examination. Patients with Kallmann syndrome are unable to smell aromatic substances (e.g., vanilla, lavender). Substances irritating to the trigeminal nerve (e.g., ammonia) are, however, recognizable. Suitable for olfactory testing are the Sniffin Sticks®, which consist of 12 standardized sticks with different scents (peppermint, fish, coffee, banana, orange, rose, lemon, pineapple, cinnamon, cloves, leather, and liquorice) (Ottaviano et al. 2015).

5.2.5 Mammary Gland

Gynecomastia is defined as enlargement of the mammary gland in the male (see Chap. 32). It must be distinguished by palpation or sonography from pure **lipomasti**a. In most cases gynecomastia is bilateral, more rarely unilateral without side preference. The stages of breast development can be distinguished according to the scheme by Tanner usually used for girls (Fig. 5.2) (Marshall and Tanner 1969). In cases

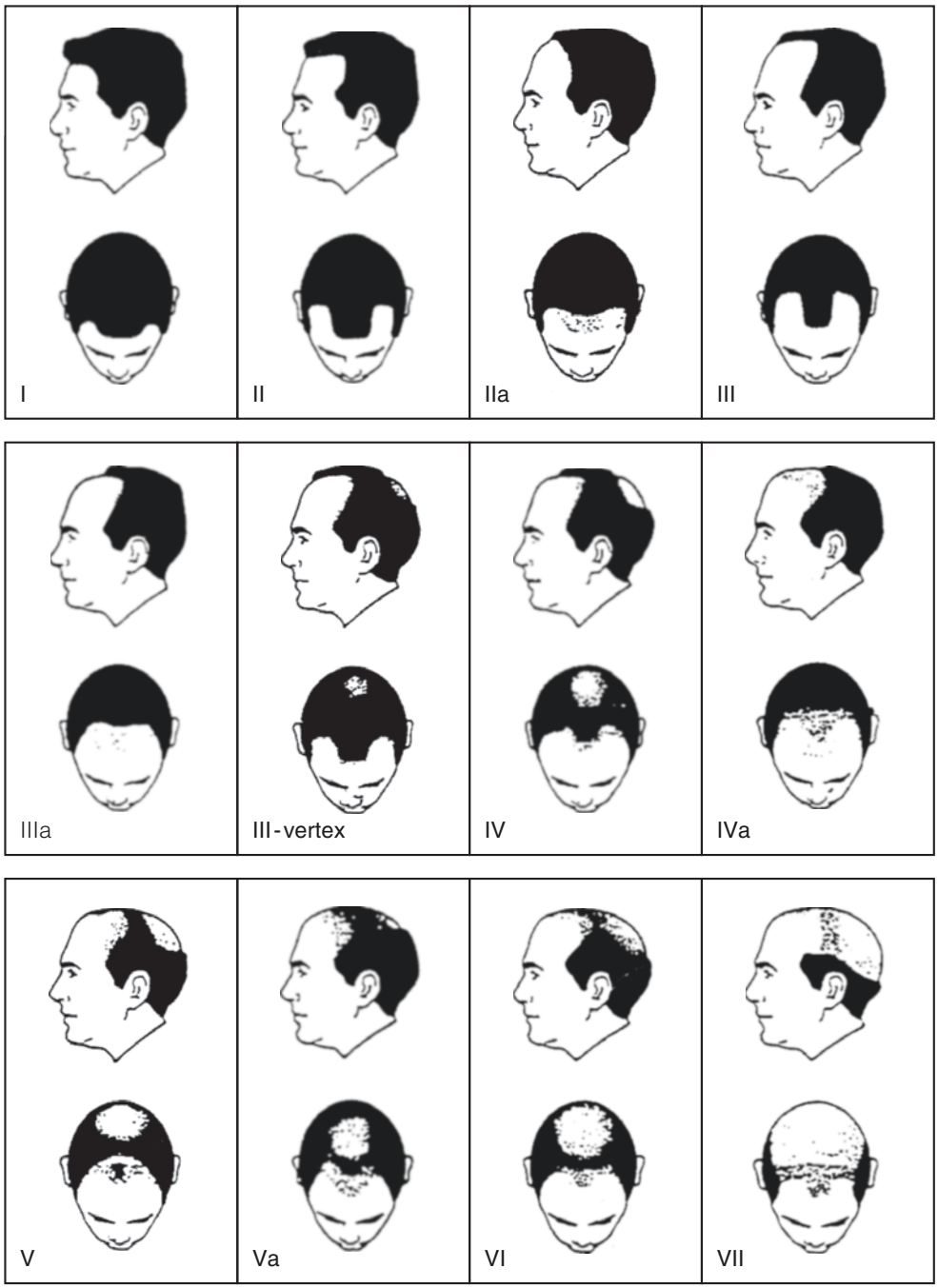


Fig. 5.1 Hamilton classification of the development of male hair patterns and androgenetic alopecia (Norwood 1975)

of marked, especially unilateral enlargement, and suspicious findings at palpation, a **mammography** should be performed for diagnosis of a possible mammary cancer. Gynecomastia can cause breast tension and the mammillae may be sensitive to touch. In most cases, however, gynecomastias is asymptomatic.

Gynecomastia develops frequently in pubescent boys at the age of about 14 years and disappears within 2–3 years. Concomitant obesity augments and prolongs the clinical picture. Gynecomastia can occasionally persist into adulthood without clinical significance. It may again appear in the aging male. Small, firm testes in combination with gynecomastia are typical for **Klinefelter syndrome**. Gynecomastia can also be present in other forms of primary hypogonadism or diseases of androgen target organs. Hyperprolactinemia can lead to gynecomastia which is caused more often by concomitant hypogonadism than by the increased prolactin itself.

Rapidly developing gynecomastia may indicate an **endocrinologically active testicular tumor**. The symptom triad gynecomastia, loss of libido, and testicular tumor is characteristic. **Careful palpation and sonography of the testes is obligatory in all cases of gynecomastia.**

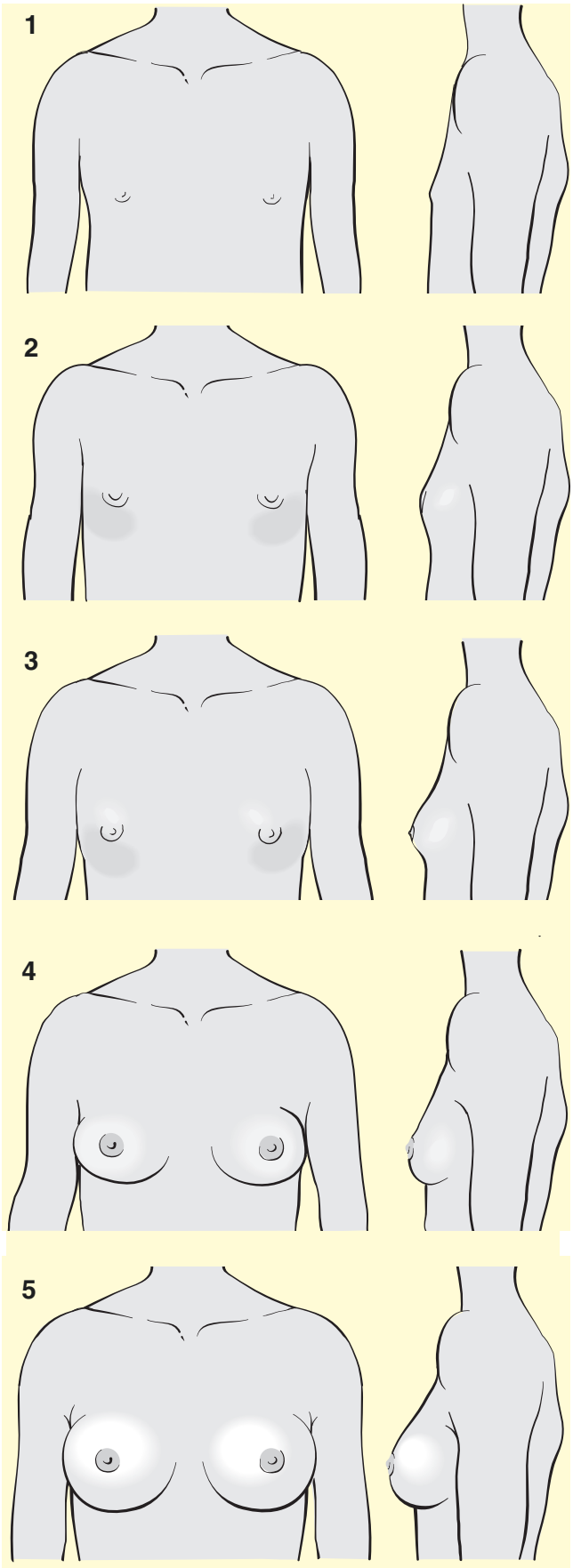


Fig. 5.2 The five Tanner stages of the development of the female breast, which can also be used for the classification of gynecomastia (Marshall and Tanner 1969)

Testicular tumors (Leydig cell tumor; embryonic carcinoma, teratocarcinoma, chorioncarcinoma, combination tumor) lead either directly or via elevated hCG secretion to increased estradiol production by the Leydig cells. Chronic, general illnesses (e.g., liver cirrhosis, terminal renal failure under hemodialysis, hyperthyroidism) can also cause gynecomastia. A large number of **medications and drugs**, with quite different mechanisms of action, may cause or exacerbate gynecomastia.

5.2.6 Testes

The normal testis has a firm **consistency**. When LH and FSH stimulation are absent, the testes are usually soft; small, very firm testes are typical for Klinefelter syndrome (Fig. 21.2). Fluctuating to tightly elastic consistency indicates a hydrocele, which is confirmed through diaphanoscopy or, preferably, through ultrasonography. Differences in testicular consistency between the two sides, a very hard testis, or an uneven surface raise suspicion of a testicular tumor.

A healthy European man has, on average, a **testicular volume** of 18 ml per testis; the normal range lies between 12 and 30 ml. A higher testicular volume is known as megalotestis (macroorchia). A megalotestis should raise suspicion of a fragile X syndrome (Meschede et al. 1995). Although exactness in determining testicular volume increases with the experience of the investigator palpating the testis, the margin of error remains great. Especially in cases of incomplete testicular descent and intrascrotal pathological processes, ultrasound imaging must be carried out (see Chap. 6. As testicular volume is largely correlated with sperm production (Fig. 9.2), normal testicular volume with azoospermia raises suspicion of obstructed seminal ducts or spermatogenic arrest.

The presence of **maldescended testes** or **anorchia** should be recorded.

In the case of **cryptorchidism**, the testis lies intra-abdominally or retroperitoneally above the inguinal canal and cannot be palpated or seen.
The **inguinal testis** is a testis fixed in the inguinal canal.
The **retractile testis** is located at the orifice of the inguinal canal and can be temporarily moved to the scrotum or migrates spontaneously between the scrotum and the inguinal canal, e.g., in response to cold or coitus.
In the case of an **ectopic testis**, the testis lies outside the normal path of descent (e.g., abdominal, femoral, or inguinal).

Palpation is performed with the patient standing or supine. Exposure to cold, stimulation, and excitement of the patient are to be avoided, since they can induce the cremasteric reflex

and thus cause retraction of the testis. Ultrasonography is especially indicated when diagnosing maldescended testes. In cases of bilateral cryptorchidism or ectopic testes, measuring anti-Muellerian hormone (AMH) or performing an hCG-test distinguishes the condition from anorchia (see Chap. 7). When testes are unpalpable, they can be localized by **magnetic resonance tomography (MRT)** as the imaging method of choice.

5.2.7 Epididymis

The normal epididymis can be palpated as a soft organ in a cranio-dorsal position relative to the testis. Smooth cystic distensions indicate a distal **obstruction;** indurations indicate an obstruction caused by diseases such as gonorrhoe or epididymitis. **Spermatoceles** appear as tense-elastic spherical formations, mainly in the area of the head of the epididymis. Painful swelling of the epididymis indicates acute or chronic **inflammation**; soft tumorous swelling of the epididymis can be found in rare cases of a tuberculoma.

5.2.8 Pampiniform Plexus

A **varicocele**, a distension of the venous pampiniform plexus, usually appearing on the left side, will be diagnosed by careful palpation of the standing patient. During the Valsalva maneuver, with increasing abdominal pressure, the veins distend. Depending on the results of palpation, the varicocele is assigned to one of the following grades.

- **I° varicocele** can be palpated only during the Valsalva maneuver.
- **II° varicocele** can be palpated without a Valsalva maneuver.
- **III° varicocele** is a visible distension of the pampiniform plexus.

While III° varicoceles can be easily diagnosed, diagnosis of smaller varicoceles depends largely on the experience of the investigator. In addition, palpation can be complicated by previous surgery, hydroceles, or maldescended testes. Here, ultrasonography offers the best methods for diagnosis (see Chap. 18).

5.2.9 Deferent Ducts

Using thumb and index finger, the deferent duct can be palpated between the vessels of the spermatic chord in the upright, standing patient. Absence of the deferent duct leads to obstructive azoospermia. Obstructive azoospermia caused by congenital malformation of the epididymis and/or deferent duct (unilateral or **bilateral congenital aplasia of the vas deferens = CBAVD**) is found in approximately 2% of infertile patients attending infertility clinics (see Chap. 26). Partial obliterations or aplasias of the deferent duct can escape palpation. In such cases, surgical exploration of the scrotal content is indicated.

5.2.10 Penis

According to Marshall and Tanner (1970), regular growth of the penis during puberty is divided into five stages (Fig. 5.3) The non-erect penis has an average length of 9.16 cm (from 8.6 to 10.7 cm) and an average circumference of 9.3 cm. In the erect state, the penis length measures 13.1 cm (from 12.9 to 16.0 cm) and the circumference averages 11.7 cm (Veale et al. 2015). A penis length in an adult man <7.5 cm is called a micropenis. If hypogonadism manifests itself before normal puberty, the penis remains infantile. If hypogonadism manifests itself after puberty, there is hardly any reduction in size.

When examining the penis, the urethra orifice must be precisely located, since even mild forms of **hypospadias** can lead to fertility disorders. **Phimosis** can be diagnosed by attempting to pull back the prepuce. **Deviations** of the penis in an erect state and resulting cohabitation difficulties should be described by the patient and documented by auto-photography.

5.2.11 Prostate and Seminal Vesicles

Rectal examination reveals the normal prostate gland to have a smooth surface and the size of a horse chestnut. In cases of hypogonadism prostate, volume remains small and the normal age-dependent increase in volume is not seen. A doughy, soft consistency points to prostatitis, general enlargement to benign prostatic hyperplasia (BPH), knobby surface, and hard consistency to a carcinoma. Significantly more information can be obtained from simultaneous transrectal sonography of the prostate and seminal vesicles (see Chap. 6).

Key Points

- Anamnesis and physical examination are the beginning of every medical intervention.
- Especially in the field of andrology and reproductive medicine, they are particularly important for establishing a relationship of trust between doctor and patient, without which the physician cannot obtain information necessary for dealing with conditions which are sometimes considered taboo.
- Anamnesis and thorough physical examination provide signposts for further diagnostics and therapy.

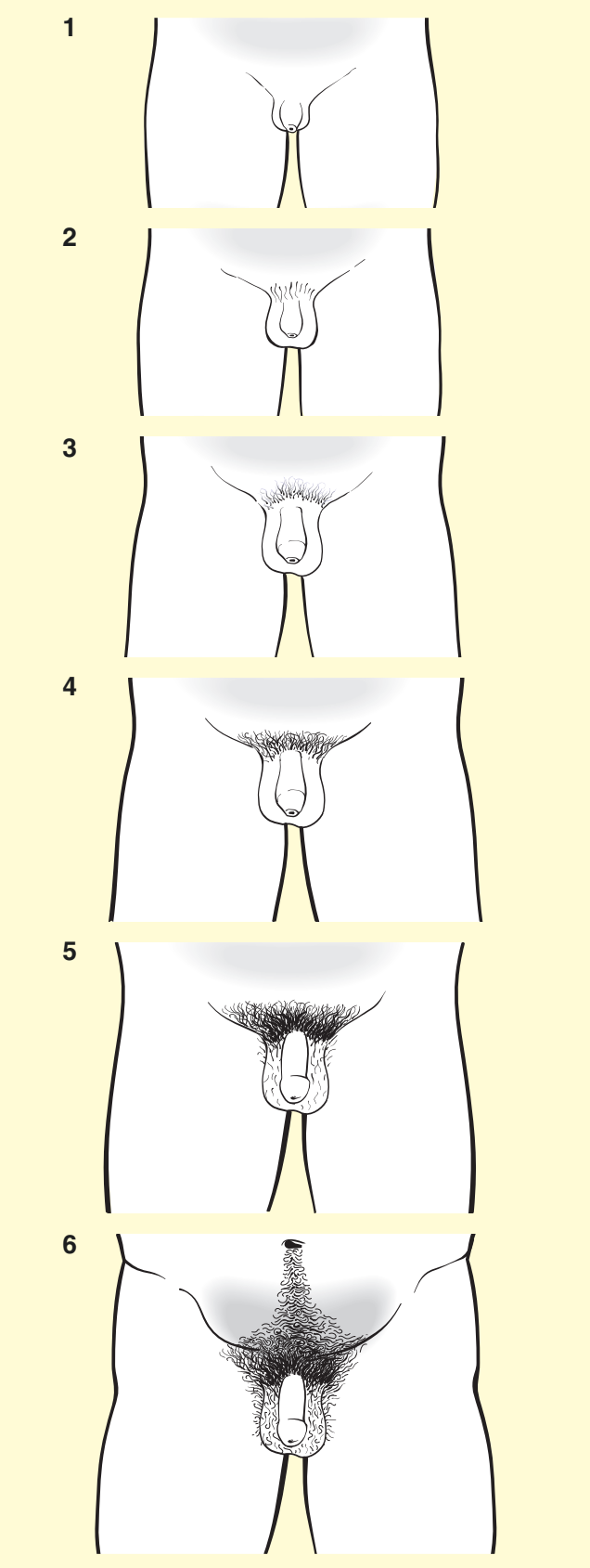


Fig. 5.3 Stages of genital pubertal development in boys (Marshall and Tanner 1970)

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Ultrasound Imaging in Andrology

6

Francesco Lotti, Michael Zitzmann,
and Hermann M. Behre

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Abstract

Male genital tract ultrasound (MGT-US) represents an essential diagnostic tool in andrology. Scrotal US has shown a relevant impact on reproductive and general male health, assessing features related to reproductive health, scrotal pain, masses, and trauma. Transrectal US has assumed growing relevance in infertility and chronic pelvic pain assessment. Penile US is widely used to investigate erectile dysfunction, structural penile abnormalities, and priapism. Finally, US can assess male breast abnormalities such as gynecomastia, lipomastia, and lesions.

Until very recent years, MGT-US lacked standardization. Recently, thanks to international societies including the European Academy of Andrology, several standards in US have been achieved. We report here normal MGT-US anatomy and analyze MGT-US abnormalities in relation to male reproductive and general health. In addition, we

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report here the standards in andrological US as derived from recent guidelines and evidence-based studies.

6.1 Introduction

To date, **imaging of the male genital tract** (MGT) represents an essential diagnostic tool in andrology, allowing physicians to complete the diagnostic workup of the andrologic patient, especially when anamnesis, physical, and biochemical examinations do not provide sufficient information for adequate patient management. In particular, **ultrasound (US)** represents the **gold standard method for scrotal investigation**, and a useful tool to evaluate the prostate–vesicular region (Lotti and Maggi 2015; Lotti et al. 2021a), and the vascular and structural characteristics of the penis and male breast tissue. Using high-frequency sound waves, US is a simple, rapid, and harmless diagnostic tool able to provide live images of the MGT organs and, among imaging techniques, is the least expensive. The high-resolution gray scale mode associated with **color- and power Doppler examination** allows sonographers to investigate size, echotexture, and vascular features of the scrotal and prostate–vesicular organs, as well as penile characteristics and male breast tissue, and to detect their abnormalities (Lotti and Maggi 2015). More recently, the use of **contrast-enhanced US (CEUS)** and **sonoelastography (SE)** have led to further improvements especially in the differential diagnosis of scrotal diseases (Sidhu et al. 2018; Săftoiu et al. 2019). However, in specific cases, other imaging techniques, such as **magnetic resonance imaging (MRI)**, can help to better characterize some equivocal findings at US, as in the case of ambiguous testicular lesions or suspected prostate cancer.

So far, scrotal US has shown a relevant impact both on **reproductive and on general male health** (Lotti and Maggi 2015; Sidhu et al. 2018; Săftoiu et al. 2019; Lotti et al. 2021a), assessing scrotal features related to reproductive health, scrotal pain, masses, and trauma. In addition, **transrectal US (TRUS)** application has assumed a growing relevance especially in infertility and chronic pelvic pain assessment. Furthermore, **penile color Doppler US (PCDU)** is widely used to investigate erectile dysfunction, structural penile abnormalities (including fibrosis, trauma, and tumors), dorsal vein thrombosis, and priapism. Finally, US can be used to assess **male breast abnormalities**, including gynecomastia, lipomastia, and male breast lesions.

Although US has been widely used to explore the MGT organs, until very recently the method used to assess several qualitative and quantitative US parameters had not been standardized, and normative parameters and thresholds to distinguish normal and pathologic features were often not evidence-based (Lotti and Maggi 2015). Recently, thanks to the efforts of different radiological, urological, and andrological societies, including the European Academy of Andrology (EAA) (Lotti et al. 2020; Lotti et al. 2021b), several **standards in US** have been achieved. We report here concepts of normal US anatomy of the MGT and critically analyze MGT-US abnormalities in relation to male reproductive and general health. In addition, we report here the standards in andrological US as derived from recent guidelines and evidence-based studies.

6.2 Scrotal US

6.2.1 Indications

The indications for scrotal US are reported in Table 6.1.

Table 6.1 Current indications for andrological US. Indications for andrological US are adapted from AIUM (2015, 2021), ESUR (Richenberg et al. 2015; Freeman et al. 2020; Ramanathan et al. 2021), and EFSUMB guidelines (Sidhu et al. 2018; Săftoiu et al. 2019)

A. Scrotal US
Gray Scale and Color Doppler US (CDUS)
Reproductive Health
Evaluation of male infertility
Evaluation of TV (especially when physical examination is unreliable[a])
Evaluation of TI (and TML)
Evaluation of testicular nodules
Localization (when possible) and evaluation of nonpalpable testes
Evaluation of cryptorchid testis after orchiopexy and contralateral descended testes
Evaluation of epididymal dilation (and echotexture) suggesting proximal or distal subobstruction
Evaluation of vas deferens presence or absence
Detection/evaluation of varicoceles
Scrotal Pain or Discomfort
Evaluation of infectious or inflammatory scrotal disease (e.g., orchitis, epididymitis), testicular ischemia/torsion, or trauma
Evaluation of palpable testicular, intrascrotal, or inguinal masses
Evaluation of scrotal asymmetry, swelling, enlargement, or potential intrascrotal hernias
Detection/evaluation of varicoceles
Exclusion of scrotal causes for acute scrotal pain
Scrotal Masses/Oncologic Risk
Evaluation of palpable testicular, intrascrotal, or inguinal masses
Evaluation and follow-up of small nonpalpable testicular nodules
Evaluation of scrotal asymmetry, swelling, enlargement, or potential intrascrotal hernias
Localization and evaluation of nonpalpable testes
Evaluation and follow-up of cryptorchid testis after orchiopexy and contralateral descended testes
Follow-up of testicular TML
Follow-up of prior indeterminate scrotal US findings
Detection of occult primary tumors in patients with metastatic germ cell tumors or unexplained retroperitoneal adenopathy
Follow-up of patients with prior primary testicular neoplasms, leukemia, or lymphoma

Table 6.1 (continued)

Evaluation of abnormalities noted on other imaging studies (including but not limited to computed tomography, magnetic resonance imaging, and positron emission tomography).
Scrotal Trauma
Assessment of tunica albuginea integrity
Assessment of testicular parenchyma viability
Identification and follow-up of hematomas and fluid collections
Detection of the testis in post-traumatic dislocation
Evaluation of a Disorder of Sexual Development
Contrast-Enhanced US (CEUS)[b]
Differentiation between truly avascular (likely benign) testicular lesions and poorly vascularized tumors
Identification of segmental infarction
Discrimination of nonviable regions in testicular trauma
Identification of abscess formation and infarction in severe epididymo-orchitis
Evaluation of the integrity of the tunica albuginea
Evaluation of parenchymal testicular vascularization
Sonoelastography (SE)[b]
Helps in differentiating neoplastic and non-neoplastic lesions
B. Transrectal US (TRUS)
Evaluation of obstructive oligospermia/azoospermia (assessment of: midline prostatic cyst; EDs dilation; SV dilation; reduced SV ejection fraction; bilateral absence of vas deferens ± SV)
Evaluation of prostate volume (increased: benign prostatic enlargement [BPE]/benign prostatic hyperplasia [BPH] and adenoma, especially in patients with LUTS [especially obstructive]; reduced: hypogonadal men)
Evaluation of prostate inflammation (especially in patients with: pelvic pain; LUTS [especially irritative], hemospermia; premature ejaculation)
Evaluation of hemospermia (prostate inflammation; midline prostatic cyst; EDs microcalcifications)
Evaluation of seminal vesicles (presence/absence; abnormalities)
Evaluation of distal vas deferens/deferential ampullas (presence/absence; abnormalities)
C. Penile US
Evaluation of erectile dysfunction
Evaluation of penile fibrosis and penile curvature (Peyronie's disease)
Evaluation of dorsal vein thrombosis (penile Mondor's disease)
Evaluation of priapism
Evaluation of penile trauma
Evaluation of penile tumors
Evaluation of abnormal findings on physical examination of the phallus or urethra
Evaluation of urethral stricture, diverticulum, or cyst
Evaluation of a calculus or foreign body of the phallus or urethra
D. Male Breast US
Evaluation of gynecomastia
Evaluation of pseudogynecomastia/lipomastia
Evaluation of breast lesions (including cancer)

[a]TV evaluation at physical examination may be unreliable in case of large hydrocele or large varicocele, inguinal testis, epididymis enlargement or fibrosis, thickened scrotal skin, obesity.
[b]CEUS and SE are used in equivocal cases at CDUS.
TV: testicular volume; *TI*: testicular inhomogeneity; *TML*: testicular microlithiasis; *LUTS*: lower urinary tract symptoms; *EDs*: ejaculatory ducts

6.2.2 Methodological Standards

6.2.2.1 Scrotal Color Doppler Ultrasonography

The standardization of the methodology used to perform **scrotal color Doppler ultrasonography (CDUS)** is relatively new. Practical recommendations for performing scrotal CDUS have been reported by the SIU/SIEUN collaboration in 2014 (Martino et al. 2014) and in the AIUM Practice Guideline in 2015 (AIUM Practice Guideline for the Performance of Scrotal Ultrasound Examinations 2015). In 2015, Lotti and Maggi published a systematic review dealing with the measurement and assessment (as well as clinical significance) of MGT quantitative and qualitative parameters, respectively. In particular, the authors reported how each organ/segment (e.g., testis, epididymal head, body, tail, vas deferens pampiniform plexus) of the scrotal sac had been measured, and the classifications used to stratify (e.g., testis inhomogeneity) severity. In addition, the authors reported the thresholds suggested in previous studies to distinguish normal from pathologic features, in an effort to align them. However, the authors concluded that, for several parameters, sonographic imaging of male genital tract was suffering from a lack of standardization, often leading to subjective and vague diagnoses. For this reason, the EAA promoted an international multicenter study (see at https://www.andrologyacademy.net/eaa-studies) aimed at defining the **male genital tract-CDUS reference ranges** and characteristics as derived from a cohort of healthy, fertile men. The development and methodology of the "EAA US study" were reported in a 2020 study (Lotti et al. 2020). A detailed description of the **Standard Operating Procedures** (SOPs) to evaluate scrotal quantitative and qualitative parameters, and assessment of the CDUS intra- and inter-operator comparability, has been reported in a further study (Lotti et al. 2021b). In our opinion, following the CDUS SOPs proposed by the EAA US consortium in clinical practice will help to reduce operator-dependent differences among sonographers. The EAA-proposed SOPs to assess scrotal CDUS have been reported elsewhere (Lotti et al. 2021a, 2021b) (see https://www.andrologyacademy.net/eaa-studies).

6.2.2.2 Contrasted-Enhanced US

The methodological standards for the clinical practice of **contrasted-enhanced US (CEUS)** in nonhepatic applications, including scrotum investigation, have been reported by the EFSUMB Guidelines and in the 2017 updated version (Sidhu et al. 2018). As a result, the assessment of some pathological conditions using CEUS has improved (Sidhu et al. 2018). Using time–intensity curves, evaluating the wash-in and wash-out curves may help to distinguish malignant from benign tumors, although CEUS analyses still overlap between different histological types. In addition, CEUS can

discriminate nonviable regions in testicular trauma and can identify segmental testicular infarction (Sidhu et al. 2018).

6.2.2.3 Sonoelastography

The methodological standards for the clinical practice of **sonoelastography (SE)** in nonhepatic applications, including testicular investigation, have been reported by the EFSUMB Guidelines and Recommendations in the updated version (Săftoiu et al. 2019). So far, strain elastography and shear wave elastography, which includes acoustic radiation force impulse-based techniques, and transient elastography are available. The basic principles of SE have been extensively described in previous EFSUMB guidelines (Bamber et al. 2013), while methodological standardization for different organs, including the testis, is reported in the updated EFSUMB guidelines (Săftoiu et al. 2019). From a methodological point of view, the use of SE to investigate focal testicular lesions can only be recommended in conjunction with other US techniques, as there is overlap between benign and malignant neoplasms (Săftoiu et al. 2019). SE assessing overall background parenchyma has been used to investigate infertility, TML, and undescended testis (Săftoiu et al. 2019). However, currently, these specific applications are restricted to research.

6.2.3 US Anatomy, Normal and Abnormal Patterns, Clinical Utility, and US Standards

Clinical utility and standards in scrotal US, including CDUS, CEUS, and SE, are discussed below. Table 6.2 shows **normal values** and cutoff of the main scrotal US parameters as well as US classifications used previously and currently in evaluating scrotal organs at gray scale and color Doppler US. Table 6.3 shows scrotal US clinical utility and impact on male reproductive health management. Figure 6.1 shows a schematic representation of the **normal and pathologic features of the scrotal organs** in relation to male reproductive health. Figure 6.2 shows some examples of **normal and abnormal US features of the scrotal organs**. Normal and abnormal scrotal US patterns are discussed below.

Table 6.2 Previous and current normal values, cutoff, and classifications of the main CDUS parameters/characteristics of the scrotal organs

	Previous normal values, cutoff, and classifications at CDUS	Current[a] normal values, cutoff, and classifications at CDUS
Testis		
Mean TV (ellipsoid)	14–19 ml[b]	17 ml
Right TV hypotrophy	<12 ml[c]	<12 ml
Left TV hypotrophy	<12 ml[c]	<11 ml
TI classification	Lenz et al. 1993	***EAA US study*** (Lotti et al. 2021b)
	1. Very uniform pattern	0. Homogeneity
	2. Slightly irregular pattern	1. Mild inhomogeneity (presence of small hypoechoic foci/thin hypoechoic striae)
	3. Moderately irregular pattern or small echogenic points	2. Moderate inhomogeneity (presence of thick hypoechoic striae)
	4. Very irregular pattern or bright echogenic spots	3. Severe inhomogeneity (diffuse TI with "netting"/"geographical map" appearance)
	5. Tumor suspected (demarcated area)	
	Westlander et al. 2001	
	1. Homogeneous	
	2. Homogeneous with some hyperechogenic foci	
	3. Heterogeneous with spread hyperechogenicity	
	4. Heterogeneous with both hyperechogenic and cystic (hypoechogenic) parenchyma	
	5. Postoperative intratesticular lesion	
TML *(most used definitions)*	≥ 5 microcalcifications per field of view	≥ 5 microcalcifications per field of view [d]
	≥ 5 microcalcifications in the whole testis	
Vascularization	Normal, reduced or enhanced	Testicular artery (TA) PSV[e]: 3–11 cm/s

Table 6.2 (continued)

	Previous normal values, cutoff, and classifications at CDUS	Current[a] normal values, cutoff, and classifications at CDUS
		Intratesticular arteries PSV[e]: 3.7–7 cm/s
Epididymis and vas deferens		
Head diameter	≤12 mm[b]	≤11.5 mm
Body diameter	≤4 mm[b]	≤5 mm
Tail diameter	≤6 mm[b]	≤6 mm
Vas deferens diameter	Not reported	≤4.5 mm
Inhomogeneity	Homogeneous or inhomogeneous	*EAA US study (*Lotti et al. 2021b*)*
		0. Homogeneity
		1. Inhomogeneity
Echogenicity	Normoechoic, hypoechoic, hyperechoic	*EAA US study* (Lotti et al. 2021b)
		0. Normal echogenicity
		1. Mainly hypoechoic
		2. Mainly hyperechoic
Vascularization	Normal or enhanced	Head artery (TA branch) PSV[e]: 3.1–4.6 cm/s
		Tail artery (deferential) PSV[e]: 1.8–8.0 cm/s
Varicocele	Several classifications[b]	Venous vessels >3 mm *at rest*[f], irrespective of location, with retrograde venous flow detected at least during Valsalva maneuver, with grading according to Sarteschi et al./Liguori et al.[d] (Freeman et al. 2020; Lotti et al. 2021b)
Prostate		
Volume	Normal: 20–25 ml (Lotti and Maggi 2015) Enlarged: >30 ml (Lotti and Maggi 2015) Severe enlargement: >60 ml (Lotti and Maggi 2015)	Normal: reference range 15–35 ml (Lotti et al. 2020)
Inflammation	Hyperemia: qualitative Hyperemia: semiquantitative (Lotti and Maggi 2015)	Arterial prostatic PSV >11 cm/s (young adults) (Lotti et al. 2014b) or 15 cm/s (elderly/BPH men) (Lotti and Maggi 2015)
Midline prostatic cyst	No cutoff for EDs obstruction	Volume >0.117 and 0.250 ml suggestive of partial and complete obstruction, respectively (Lotti et al. 2018)
Ejaculatory ducts (EDs)	Dilated: >2 mm (Lotti and Maggi 2015)	Dilated: >2 mm (Lotti and Maggi 2015)
Periprostatic venous plexus	Dilated: >3 or 4 mm (not evidence based) (Lotti and Maggi 2015; Salonia et al. 2021)	Normal: reference range 1.6–5 mm
Seminal vesicles		
Diameters	Dilation: anterior-posterior >15 mm (Lotti and Maggi 2015; Salonia et al. 2021) Hypoplasia: anterior-posterior d <5 or <7 mm; SV longitudinal d <25 mm (Lotti and Maggi 2015)	Anterior-posterior d: reference range 5–18 mm (Lotti et al. 2020) Longitudinal d: reference range 36–57 mm (Lotti et al. 2020)
Volume	No cutoff for dilation or hypoplasia	Reference range 2.4–7 ml (Lotti et al. 2020)
SVEF	Not reported	SVEF <21.6% suggestive of distal obstruction (Lotti et al. 2012)
Deferential ampullas		
Diameter	Dilation: anterior-posterior d >6 mm (Lotti and Maggi 2015; Salonia et al. 2021)	Anterior-posterior d: reference range 3.5–5.7 mm (Lotti et al. 2020)

[a] Current normative parameters are mainly derived from the EAA US study (Lotti et al. 2021b), and, in part, from the ESUR guidelines/recommendations ESUR (Richenberg et al. 2015; Freeman et al. 2020; Ramanathan et al. 2021). Of note, we here report the main findings of the EAA US study, which, in the original article (Lotti et al. 2021b), reports extensively normal values/reference ranges of all the scrotal organs CDUS parameters. For a detailed description of "previous normal values," see the main text

[b] See, for review, references Lotti and Maggi 2015 and Lotti et al. 2021a.

[c] A few previous studies defined testicular hypotrophy for a TV < 12 ml, however with no evidence (Condorelli et al. 2013 and Pedersen et al. 2018).

[d] Definitions shared by the EAA and ESUR

[e] Along with peak systolic velocity (PSV) reference range evaluated in testicular and epididymal arteries, the EAA US study reports normal values for acceleration, pulsatility, and resistive index (Lotti et al. 2021b).

[f] EAA reports "at rest," ESUR reports "during Valsalva."

SVEF: seminal vesicles ejection fraction (Lotti et al. 2012); *d*: diameter

Table 6.3 Scrotal and transrectal US clinical utility and impact on male reproductive and general health management

CDUS parameter	Clinical utility	Comments		Impact on male reproductive and general health management
		Pros	Cons	
Scrotal CDUS				
Testis localization	F: Low GH: Moderate	– useful in localizing inguinal testis	– physical examination often informative enough – useless in localizing intraabdominal testis	– debated utility prior orchiopexy planning – no impact on management of nonpalpable testes – useful in follow-up of cryptorchid testis-related malignancy risk and contralateral testis
Testis volume (TV)	F and GH: Low	– US useful when Prader orchidometer is unreliable	– Prader orchidometer-derived TV is strictly related to US-TV; US shows greater accuracy, but Prader orchidometer is informative enough in clinical setting	– positive correlation with sperm and hormonal parameters – poor utility in TESE decision-making – poor utility in spermatogenic arrest
Testis echotexture and calcifications	F: Moderate GH: Moderate	– inhomogeneity suggests testicular function impairment – severe inhomogeneity warns of malignancy – microcalcifications warn of malignancy, suggesting US follow-up and eventually biopsy	– inhomogeneity may be found in several diseases or exposure to harmful causes, hence it is not specific – poor utility in TESE decision-making	– US useful in follow-up for malignancy when severe inhomogeneity or microlithiasis are found, especially when other risk factors are present
Testicular lesions	F: Low GH: Moderate/ High	– detection of small and large lesions and their characteristics (extension, vascularization, echotexture) – useful in follow-up of cryptorchid testis-related malignancy risk or small lesions	– physical examination informative enough to detect large/hard lesions – poor information on the biological behavior of the lesion; histology required	– useful in follow-up of cryptorchid testis-related malignancy risk or small lesions – moderate utility in large/hard lesions decision-making
Testicular vascularization	F: Low GH: High	– useful for torsion, infarction (reduced), orchi-epididymitis, or some tumors (enhanced) – a few reports on positive correlation with sperm parameters and retrieval in TESE	– positive correlation with sperm parameters and retrieval in TESE are still in the research field	– useful for torsion, infarction (reduced), orchitis or some tumors (enhanced) – not standardized for fertility assessment – poor utility for TESE decision-making
Varicocele	F: Moderate GH: Low	US: – confirms clinical diagnosis – useful when physical examination is inconclusive – detects the type of venous reflux – detects subclinical varicocele with persistent reflux – avoids “false” varicocele (dilation without reflux) – detects recurrence/persistence after surgery	– physical examination is considered sufficient for treatment decision – treatment of subclinical varicocele disputable – risk of complications	– poor utility when varicocele is palpable or visible – useful when physical examination is unreliable – useful in detecting recurrence/ persistence after surgery
Epididymal diameters	F: Moderate GH: Low	– US shows greater accuracy compared to physical examination – enlarged epididymis suggestive of past or present inflammation or post-testicular obstruction	– difficult to evaluate after scrotal surgery or in the presence of large varicocele – primary obstruction may only be suggested, but not proven, by US	– epididymal dilation may indicate distal obstruction in men with oligo/azoospermia, eventually leading to extent US to the prostate-vesicular region – epididymal dilation may associate with positive MAR test
Epididymal echotexture	F and GH: Low	– Echotexture abnormalities may associate with past or present inflammation	– very recent standardization	– may associate with reduced sperm count and motility and positive MAR test

Table 6.3 (continued)

CDUS parameter	Clinical utility	Comments		Impact on male reproductive and general health management
		Pros	Cons	
Epididymal vascularization	F: Low GH: Moderate	– Hyperemia indicates inflammation – follow-up after medical treatment	– very recent standardization	– hyperemia indicates inflammation
Epididymal abnormalities	F: Moderate GH: Low	– US detects abnormalities or absence of the epididymis	– epididymal cysts have low utility in infertility assessment	– useful in suggesting absence or downstream obstruction
Vas deferens (VD)	F: High GH: Moderate	– US detects abnormalities or absence of the proximal or distal VD – VD dilation may indicate distal obstruction (see "epididymal diameters")	– the intermediate portion of the VD is not detectable by US	– useful in defining OA cause – positive sperm retrieval by TESE – VD absence suggests CFTR gene and kidneys US evaluation
Transrectal US				
Prostate volume	F: Low GH: Moderate	– US shows greater accuracy than physical examination – useful to assess adenoma and characterizing LUTS	– physical examination may reveal normal, reduced, or enlarged prostate – TRUS is uncomfortable	– not very useful in infertile male management – reduced volume may suggest hypogonadism – useful in BPH/LUTS management
Prostate echotexture	F and GH: Low	– abnormalities suggestive of past or present inflammation (see Table 6.2)	– abnormal echotexture: frequent, poorly specific	– impact on fertility not demonstrated
Prostate vascular parameters	F: Low GH: Moderate	– may indicate current inflammation – correlation with CPPS, PLS and PE	– few available studies – recent standardization	– impact on fertility not demonstrated
Prostate midline prostatic cyst	F: Moderate/ High GH: Low	– large cysts may cause ejaculatory duct obstruction – volumetric cutoff suggesting ED obstruction available – US used in follow-up after cyst aspiration	– recent standardization	– useful in defining OA cause – aspiration in OA subject may lead to semen parameters improvement
Ejaculatory ducts (ED)	F: Moderate/ High GH: Low	– TRUS detects abnormalities (dilation, cysts, calcifications)		– useful in defining OA cause – ED cyst may indicate CFTR gene evaluation
Seminal vesicles (SV) agenesis	F: Moderate/ High GH: Low	– TRUS detects uni- or bilateral absence		– SV absence suggests CFTR gene and kidneys US evaluation
Seminal vesicles volume	F: Moderate GH: Low	– dilation after ejaculation may indicate partial ED obstruction	– dilation or hypoplasia are not fully standardized	– dilation or reduced SVEF suggestive of partial ED obstruction, but no standardization
Seminal vesicles echotexture	F: Low/ Moderate GH: Low	– abnormalities may indicate past or present inflammation or stasis – giant cyst may indicate genitourinary anomalies investigation	– should be evaluated after ejaculation	– giant cyst may indicate genitourinary anomalies investigation – abnormalities may indicate inflammation or stasis

The clinical utility of the parameters investigated by CDUS has been arbitrarily classified as low, moderate, and high, in relation to fertility (F) or general health (GH), according to the main text statements (see, for review, references Lotti and Maggi 2015 and Lotti et al. 2021a).
MGT: male genital tract; *US*: ultrasound; *CDUS*: color Doppler ultrasound; *TRUS*: transrectal ultrasound; *TV*: testis volume; *TESE*: testicular sperm extraction (including micro-TESE); *OA*: obstructive azoospermia; *ED*: ejaculatory duct; *SV*: seminal vesicles; *CFTR*: cystic fibrosis transmembrane conductance regulator; *BPH*: benign prostatic hyperplasia; *LUTS:* lower urinary tract symptoms; *CPPS*: chronic pelvic pain syndrome; *PLS*: prostatitis-like symptoms; *PE*: premature ejaculation; *SVEF*: seminal vesicles ejection fraction

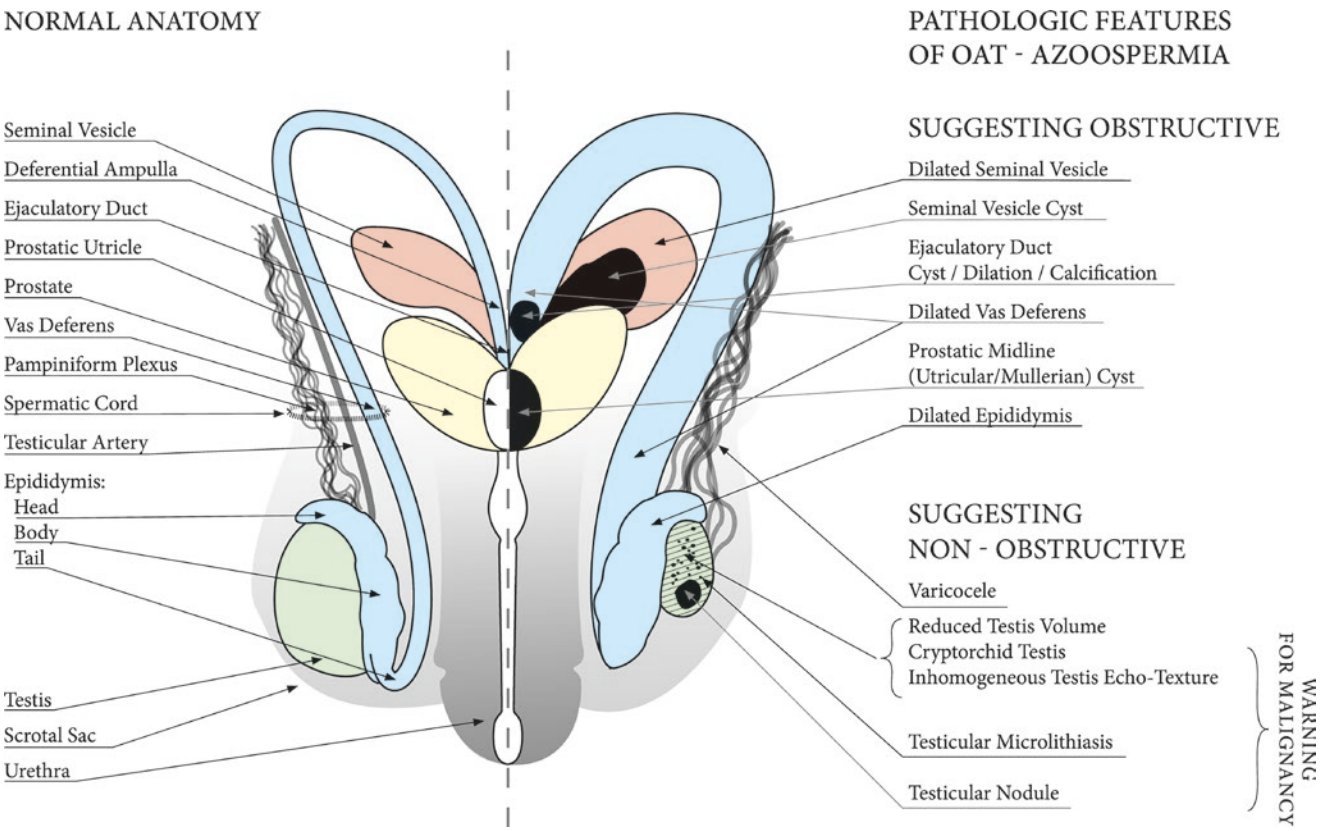
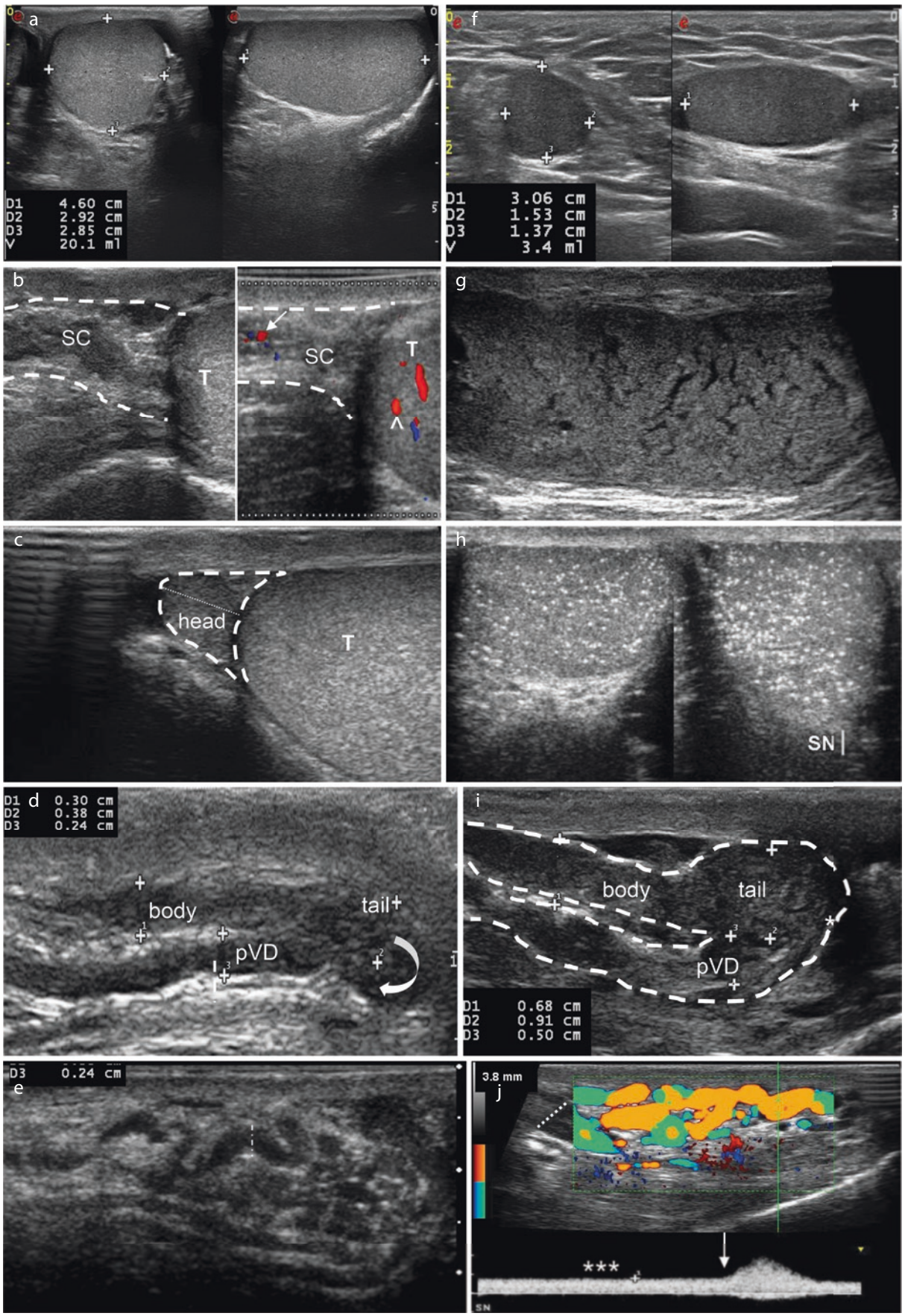


Fig. 6.1 Schematic representation of the normal and pathologic features of the male genital tract (MGT) in relation to male reproductive health. Left side: Normal anatomy of the MGT. Right side: pathologic features of the MGT suggesting obstructive or nonobstructive oligo-astheno-teratozoospermia (OAT)/azoospermia. Warning for malignancy is extensively discussed in the text. Adapted from Lotti and Maggi 2015

So far, **scrotal US** has shown a relevant impact on both **reproductive and general male health**. In fact, US has been used to assess scrotal features related to (i) reproductive health, (ii) scrotal pain, (iii) scrotal masses, and (iv) scrotal trauma (Isidori and Lenzi 2008; Lotti and Maggi 2015; Richenberg et al. 2015; Sidhu et al. 2018; Săftoiu et al. 2019; Freeman et al. 2020; Lotti et al. 2021a). (i) Regarding reproductive health, US can detect alterations in size, echotexture, and vascularization of the testes associated with sperm abnormalities and, eventually, with low testosterone levels. In addition, scrotal US provides information on epididymal and deferential abnormalities, possibly associated with

Fig. 6.2 Normal (left side) and abnormal (right side) color Doppler ultrasound (CDUS) features of the scrotal organs. **Panel** (**a**), **T**estis of normal volume, homogeneity, and echogenicity with ellipsoid shape. Longitudinal (right figure) and transversal (left figure) scans of the testis, with length (D1), width (D2), and height (D3) measurements reported. **Panel** (**b**), Left figure: B-mode appearance of the spermatic cord (SC) and the upper pole of the testis (T) in longitudinal scan. Small, nondilated venous vessels of the pampiniform plexus are difficult to differentiate from the other structures of the SC. Right figure: CDUS detection of the testicular artery (arrow) in the SC and recurrent ramus (^) of an intratesticular centripetal artery. Venous reflux at rest in the venous vessels is not detectable. **Panel** (**c**), normal epididymal *head* with triangular shape (dashed line) in longitudinal scan, homogeneous, with echogenicity comparable to that of the testis (T). Its length is measured from the top to the base of the triangle (dotted line). **Panel** (**d**), homogeneous epididymal *body* and *tail* and proximal vas deferens (pVD) in longitudinal scan. Their echogenicity is slightly hypoechoic compared to the testis and the epididymal head in panel C. Their diameters are reported as dashed lines. Red dashed line indicates the end of the epididymal tail and the beginning of the pVD. The curve arrow indicates the epididymal–deferential handle. **Panel** (**e**)**,** normal pampiniform plexus. Venous vessels are not dilated (<3 mm). **Panel** (**f**), testis with low volume and hypoechoic echotexture, detected in a man with a history of cryptorchidism. **Panel** (**g**)**,** testis with echotexture inhomogeneity in sagittal scan. **Panel** (**h**), testicular microlithiasis with "starry sky" appearance. **Panel** (**i**), dilated, and inhomogeneous epididymal *body* and *tail* and proximal vas deferens (pVD), with irregularly shaped mass (*) in the epididymal tail region, detected in sagittal scan. **Panel** (**j**), CDUS evaluation of dilated veins of the pampiniform plexus with colored signal (left), showing continuous reflux at rest (***), increasing with Valsalva (arrow), identifying a severe, sonographic-defined, varicocele, according to different classifications


a
D1 4.60 cm
D2 2.92 cm
D3 2.85 cm
V 20.1 ml
f
D1 3.06 cm
D2 1.53 cm
D3 1.37 cm
V 3.4 ml
b
SC
T
SC
T
g
c
head
T
h
SN
d
D1 0.30 cm
D2 0.38 cm
D3 0.24 cm
body
tail
pVD
i
body
tail
pVD
D1 0.68 cm
D2 0.91 cm
D3 0.50 cm
D3 0.24 cm
e
3.8 mm
j
***
SN

semen quality impairment, or on their bilateral absence, causing obstructive azoospermia. Finally, scrotal color Doppler US (CDUS) is able to detect and stage varicocele, which may exert a negative role on sperm parameters. (ii) Regarding scrotal pain, CDUS can detect testicular or epididymal size and echopattern abnormalities as well as hypervascularization, suggesting inflammation (i.e., orchitis and epididymitis), or an absent vascularization, indicating spermatic cord/testicular torsion. Furthermore, CDUS can assess severe varicocele or inguinal/scrotal hernias, eventually associated with mild discomfort and even overt pain. (iii) Moreover, US plays a key role in investigating testicular or extratesticular masses, characterizing them as benign or malignant with fair accuracy, although without providing diagnostic certainty. In addition, US can assess testicular microlithiasis (TML), which, when associated additional risk factors (see below), might underlie a coexisting or developing testicular malignancy. (iv) Finally, US plays a crucial role in the evaluation of scrotal trauma.

6.2.3.1 Testis

6.2.3.1.1 US Anatomy

Testicles are pair organs normally located in the scrotal sac. This peculiar position requires the use of a high-spatial resolution transducer dedicated to the study of soft parts (7–15 MHz) (Lotti and Maggi 2015). Usually, the patient lies supine with the penis resting on the suprapubic region and gel applied to the scrotum, supported by a towel placed between the thighs. The testes are examined in transverse, oblique, and longitudinal planes, and images are acquired in both gray scale and color Doppler modes, to assess testicular blood flow. At US, normal adult testicles appear as **ellipsoid organs** of 3–5 cm length, 2–4 cm width, and 3 cm anterior-posterior size (Lotti and Maggi 2015). They are surrounded by an echoic fibrous capsule, the tunica albuginea, which projects into the interior of the testis with fibrous septa. Septa may be seen as delicate linear hypoechoic striae, converging to form the mediastinum testis, which appears in a longitudinal scan as a hyperechoic line, eccentrically located. Septa divide the testis into 200–400 lobules. Each lobule contains interstitial Leydig cells and seminiferous tubules, with differentiating germinal cells and somatic Sertoli cells. It is estimated that seminiferous tubules account for ~85% of the entire testicular volume (Lotti and Maggi 2015), while Leydig cells for ~3%. Hence, changes in testicular volume are mainly related to variations of the testicular parenchyma.

The normal adult testis is characterized at US by a **homogeneous granular echotexture**, made up of uniformly distributed medium-level echoes resembling the echogenicity of the normal thyroid gland (Isidori and Lenzi 2008; Bertolotto and Trombetta 2012; Lotti and Maggi 2015) (see paragraph "Testicular homogeneity and echogenicity"). Scrotal arterial perfusion has been well described and depicted elsewhere (Lotti and Maggi 2015). The testes are mainly perfused by the testicular arteries, which arise from the aorta, distal to the renal arteries, enter the spermatic cord at the deep inguinal ring, and reach the upper testicular pole. Each **testicular artery** lies in the spermatic cord with the ipsilateral cremasteric artery (a branch of the inferior epigastric artery) and the deferential artery (a branch of the vesicular artery). Although there are anastomoses between these vessels, i) the testicular artery primarily supplies the testis, and one of its branches also the epididymal head, ii) the deferential artery perfuses the epididymis (mainly body and tail) and vas deferens, and iii) the cremasteric artery supplies the peritesticular tissues and the scrotal wall. After entering the scrotum, the testicular artery runs along the posterior aspect of the testis and penetrates the tunica albuginea, supplying two sets of arteries, the **capsular** and the **transmediastinal arteries**. The capsular arteries have a superficial course beneath the tunica albuginea in a layer called tunica vasculosa, over the surface of seminiferous tubules. They branch centripetal arteries that enter the testicular parenchyma and flow toward the mediastinum penetrating between the septa separating the seminiferous tubules. As they approach the mediastinum, the centripetal arteries arborize into recurrent rami that branch back in the opposite direction, carrying blood from the mediastinum into the testis. In some men, a large branch of the testicular artery, the centrifugal transmediastinal artery, enters at the mediastinum and runs across the testicular parenchyma with a straight course, to form capsular branches on the opposite side.

6.2.3.1.2 US Normal and Abnormal Patterns

Testicular Volume

Testicular volume (TV) is an essential parameter in clinical practice, reflecting not only the seminal and hormonal male status but also the presence of previous or current testicular or systemic disorders (Lotti and Maggi 2015; Lotti and Maggi 2018). TV is usually assessed clinically by Prader's orchidometer. However, orchidometry overestimates TV when compared to US, and US offers a greater accuracy in TV measurement than physical examination (Behre et al. 1989; Lotti and Maggi 2015; Lotti et al. 2021a). Prader's orchidometer- and US-derived TV are closely related, both in boys and in adult eugonadal or hypogonadal subjects (Lotti and Maggi 2015). Hence, in clinical practice, Prader's orchidometer-derived TV may be considered a reliable surrogate of US-measured TV, easier to perform, and not costly. Nevertheless, US maintains a role in TV assessment when physical examination is unreliable, such in case of large hydrocele or large varicoceles, inguinal testis, epididymis enlargement or fibrosis, thickened scrotal skin, obesity (see Table 6.1).

Previous studies reported that US-estimated TV was positively related to total sperm count, sperm motility, normal sperm morphology, and testosterone levels and negatively with LH and FSH levels (Isidori and Lenzi 2008; Lotti and Maggi 2015; Lotti et al. 2021a, b). A negative correlation between US-TV and nonconventional sperm parameters (sperm DNA fragmentation, percentage of spermatozoa with low mitochondrial membrane potential, phosphatidylserine externalization, or chromatin compactness) has been also reported.

TV US estimation varies according to the mathematical formula applied. In the last decade, no consensus on the best mathematical model was achieved. Previous studies, as discussed in comprehensive articles (Lotti and Maggi 2015), reported US-assessed TV using **ellipsoid formular** (length x height x width x 0.52), Lambert's (length x height x width x 0.71), or Hansen's (length × width × 0.52), making comparisons between different studies complicated. The most commonly applied formula is the ellipsoid formula. However, in studies investigating the difference between US and "real" TV by water displacement, the empirical Lambert's formula was reported to be superior. In 2014, SIU/SIEUN recommendations (Martino et al. 2014) supported the use of the ellipsoid formula, whereas in 2015 AIUM guidelines (AIUM Practice Guideline for the Performance of Scrotal Ultrasound Examinations 2015) reported that, in pediatric patients, TV could be provided using Lambert's or the ellipsoid formula.

Using the ellipsoid formula, healthy German and Danish men showed a median TV of ~14 ml, young Italian and South Korean men a mean TV of ~15 ml and ~ 18 ml, respectively, and fertile Italian men a mean TV of ~19 ml (see Table 6.2) (Lotti and Maggi 2015; Lotti et al. 2021a, b). The relatively wide range of a "normal" average TV could depend on the lack of international US SOPs standardization, on the difference between "mean" and "median" TV values, on the diverse age range of the subjects studied and on differences between populations studied, belonging to different countries. Although TV difference among ethnic groups and TV variations with age in adult men seem to be modest, available studies on these topics are relatively scanty and, in some cases, conflicting, representing possible confounders. A TV <12 ml has been proposed by previous studies to indicate testicular hypotrophy at US, using the ellipsoid formula or irrespective of the mathematical formula used, however, without any evidence-base (Lotti et al. 2021a) (Table 6.2). In addition, a TV <10 ml using Lambert's formula was reported to be associated with testicular dysfunction, however, only in two studies assessing Japanese men with infertility (Lotti et al. 2021a).

Recently, the ESUR guidelines on varicocele (Freeman et al. 2020) supported the use of Lambert's formula to calculate TV at US, considered the most accurate according to previous studies, however, without a "strong" consensus. Most recently, the EAA US consortium, evaluating a cohort of 248 healthy, fertile men (Lotti et al. 2020), reported the US reference range of testicular diameters and TV according to the ellipsoid, Lambert's and Hansen's mathematical formulas, providing evidence-based normative parameters (Lotti et al. 2021b). In the EAA study, the US-TV calculated with the ellipsoid formula showed the most accurate correlation with the Prader orchidometer-assessed TV, while Lambert's formula overestimated orchidometry (Lotti et al. 2021b). Hence, EAA supports the use of the ellipsoid formula, considering that it fits better into clinical (and not experimental) reality (Lotti et al. 2021b). In addition, the ellipsoid formula is easier to use in clinical practice, since it is automatically calculated by most US devices. Using the ellipsoid formula, the EAA US consortium reported a mean TV of ~17 ml in healthy, fertile men (Table 6.2). Since the EAA study is an international multicenter one, the aforementioned value can be considered the "normal" mean TV of the European adult population of reproductive age. The multicenter nature of the study and the limited age range (23–53 years) of the subjects investigated avoid confounders related to nationality/ethnicity and aging. The EAA US-TV lowest **reference limit** for right and left testis is 12 and 11 ml, respectively, defining, in an evidence-based manner, "testicular hypotrophy" as being below these thresholds (Lotti et al. 2021b).

Regarding the evaluation of TV in the pediatric population (Lotti et al. 2021a), reference curves for mean US-TV are available for boys aged 0 to 6 years, 0.5 months to 18 years, and 6 months to 19 years. Of note, the last study also reported the distribution of TV within the Tanner stages of pubic hair development. However, all these studies were performed in the Netherlands; hence, their results might not apply to different ethnic groups. As a corollary, a Korean study also evaluated changes and ranges of pediatric TV in 0- to 10-year-old boys, however, without reporting clear reference curves.

6.2.3.1.3 Testicular Homogeneity and Echogenicity

Since the eighties, the normal adult testis has been described as characterized by a homogeneous granular echotexture, made up of uniformly distributed medium-level echoes (i.e., homogeneous and normoechoic) resembling the echogenicity of the normal thyroid gland (Isidori and Lenzi 2008; Lotti and Maggi 2015). The occurrence of testicular structural abnormalities is associated with an alteration of echo distribution, leading to inhomogenicity and abnormal echogenicity (see below).

The image resolution and clinical significance of **testicular inhomogeneity** (TI) have changed over time. In the past, the US image quality and resolution were significantly lower than today. Hence, at first, severe TI was considered as a warning for the presence of a possible testicular malignancy.

In this setting, the reported association of very irregular testicular patterns and *carcinoma in situ* in cryptorchid men or testicular tumor by Lenz et al. (1993) are paradigmatic. Lenz et al. reported the first classification of TI, proposing a five-point "testicular echotexture score" ranging from 0 (regular pattern) to 5 (tumor suspected) (Table 6.2), which correlated positively with the presence of a *carcinoma in situ*. In addition, the TI score negatively correlated with normal sperm morphology, sperm count, and US-TV. In 1996, the "**striated testis**" was first described, a specific US pattern characterized by the presence of hypoechoic striae within the testicular parenchyma, resembling the black stripes of a zebra's coat (Lotti and Maggi 2015; Lotti et al. 2021a). In the aforementioned study, the striated US pattern was associated with testicular fibrosis and not malignancy. Subsequently, the hypoechoic striae were associated with an exaggeration of the normally inapparent interlobular septa due to seminiferous tubule reduction and interstitial proliferation, and the inhomogeneous, striated testicular pattern was associated with atrophy and fibrosis. Hence, over time, the clinical significance of TI has shifted from a risk factor for the presence of a testicular tumor to an US abnormal pattern associated with other pathological conditions and/or testicular function impairment (see below). In parallel, due to the advancing US technology, "suspected tumors" have been described as "nodular lesions" and no more as "echotexture abnormalities." In agreement, in 2001 Westlander et al. proposed a four-point scale TI classification, a semiqualitative score modified from that of Lenz et al. not considering suspected tumors. The classification was modified soon after in a five-point scale (Table 6.2) introducing a further category, "intratesticular lesions observed after testicular sperm aspiration," which resolved after 6–9 months of follow-up.

Whereas TI is often observed in the elderly and considered normal, in young subjects it has been associated with several pathological conditions, including ischemia, orchitis, trauma, torsion, exposure to physical or chemical agents, chemo- and radiotherapy or alcohol abuse, Klinefelter, and metabolic syndrome (Lotti and Maggi 2015; Lotti et al. 2021a). In addition, TI has been associated with testicular function impairment including hypogonadism, abnormal sperm morphology, impaired sperm quality, and azoospermia. Recently, Pozza et al. (2020) developed, in a cohort of 2230 men, a semiquantitative, multiparametric (including bilateral US-TV, echotexture, echogenicity, and microlithiasis) score, ranging from 0 to 7 and named "testicular ultrasound (TU) score," that has proved significantly more accurate than the Lenz score (Abdulwahed et al. 2013) in predicting impaired spermatogenesis, and able to predict hypogonadism. In the TU score (Pozza et al. 2020), the parameter testicular "homogeneity" has been considered dichotomously (0. homogeneous; 1. inhomogeneous). Since subjects with a homogeneous testis showed better sperm and hormonal parameters compared with the rest of the sample, testis homogeneity can be considered "normal."

In 2021, the EAA US consortium (Lotti et al. 2021b) proposed a new, **four-point scale classification of TI**, easy to use in clinical practice and avoiding the term "suspected tumors" (see Table 6.2). In a cohort of healthy, fertile men, only a very few subjects had TI, always of a mild degree (grade 1). Those subjects showed lower sperm vitality compared with the rest of the sample. Hence, it is possible to define "normal" as the presence of a homogeneous, or at least slightly inhomogeneous (mild TI), testicular pattern, especially from a reproductive point of view.

Regarding **testicular echogenicity**, no standardized classification has been published in the past, although the description of normoechoic, hypoechoic, or hyperechoic testis has been previously reported (Isidori and Lenzi 2008; Bertolotto and Trombetta 2012; Lotti and Maggi 2015). Echogenicity depends on the seminiferous tubules' maturation and germ cell representation. Prepubertal testis has been described as slightly more hypoechoic than the adult one, since seminiferous tubules have not yet developed a lumen. During puberty, as a function of lumen development, testis echogenicity progressively increases, up to average adult level. The normal adult testis appears homogeneous at US (see above). In case of testicular damage (congenital or acquired), a reduction of testicular parenchyma and/or an increase in interstitium occur, often leading to a hypoechoic echotexture. Detection of focal abnormalities may underly several conditions, including tumors, while a diffuse hypoechogenicity may be related to widespread malignancies (see below, in the paragraph "Testicular lesions"). However, more often, diffuse hypoechogenicity is observed in damaged testes (e.g., undescended and/or hypotrophic testes; see below) and can be associated to TI at US, indicating parenchymal reduction and interstitial proliferation. In line with the latter issue, testis hypoechogenicity, as well as TI, have been also associated with increased levels of M540 bodies, round anucleated elements detected by flow cytometry in the semen, considered as markers of testicular apoptosis/spermatogenesis derangement. With reference to the echotexture of the whole testis (and not to focal lesions), the EAA US consortium (Lotti et al. 2021b) has recently classified **testicular echogenicity on a three-point Likert scale** (see Table 6.2).

6.2.3.1.4 Testicular Vascularization

The vascular anatomy of the testis has been described in detail elsewhere (Lotti and Maggi 2015). Pictures of the flow characteristics of the main testicular arteries and their branches have been also reported elsewhere (Bertolotto and Trombetta 2012).

Testis vascularization plays a critical role in the differential diagnosis of orchitis (see below), orchi-epididymitis, or

some malignant conditions (i.e., leukemia, lymphoma (enhanced), testicular torsion (absent), infarction (absent or peripheral)) (Isidori and Lenzi 2008; Bertolotto and Trombetta 2012; Lotti and Maggi 2015; Lotti et al. 2021a). This is also true for other pathological conditions resulting in testicular ischemia, such as tension hydroceles (Lotti et al. 2021a), as well as in the assessment of scrotal trauma, when considered along with other clinical and US features (Ramanathan et al. 2021). In the setting of acute scrotal pain, a normal scrotal content and testis vascularity should prompt extension of the US investigation to the abdomen, to exclude nonscrotal causes of acute pain. In addition, the vascular pattern of intratesticular lesions may suggest their nature as benign or malignant. This is important, in particular, in patients with lymphomas or hematological malignancies, in patients with bilateral synchronous tumors, and in patients with multiple, synchronous lesions in the same testis, in which the therapeutic approach is determined by the most aggressive histotype (Lotti et al. 2021a). However, so far, the assessment of testis vascularization with CDUS is still qualitative, with no clear quantitative cutoff distinguishing the aforementioned conditions. CEUS and SE (see below) have improved the capacity to investigate pathological processes, however, with no diagnostic certainty.

Until 2021, the reference range of testis vascular parameters was lacking. Only one study (Pilatz et al. 2013) reported the reference range of a single parameter, the peak systolic velocity of the testicular artery, evaluating 306 healthy Caucasian men aged 18–88 years. Other available studies investigated testis vascularization to assess different pathological conditions qualitatively (Isidori and Lenzi 2008; Bertolotto and Trombetta 2012; Lotti and Maggi 2015; Lotti et al. 2021a). In addition, some testis vascular parameters have been associated with sperm quality or have been suggested to be useful in discriminating obstructive and nonobstructive azoospermia or residual spermatogenic areas in nonobstructive azoospermia. However, at present, they have been investigated only for research purposes, with no impact on the clinical management of azoospermic men (see below). Finally, CDUS could be used to evaluate possible damage occurring during testicular sperm extraction (Lotti and Maggi 2015).

Recently, the EAA US study (Lotti et al. 2021b) reported a standardization of the measurement of testis vascular parameters (see https://www.andrologyacademy.net/eaa-studies) and their reference ranges in healthy, fertile subjects (see Table 6.2). In addition, a recent meta-analysis demonstrated that CDUS represents an effective imaging modality for diagnosing testicular torsion in adult patients with acute scrotal pain (Lotti et al. 2021a). On the other hand, recently the ESUR published a position statement on imaging in scrotal trauma (Ramanathan et al. 2021), clearly defining the role of CDUS evaluation of testicular/scrotal vascularization in different traumatic conditions, and indicating CEUS and SE as advanced techniques useful as problem-solving modalities in equivocal cases. Brief statements on this topic were also reported in the 2020 AUA urotrauma guidelines (Morey et al. 2021).

6.2.3.1.5 Orchitis

CDUS plays a key role in identifying **orchitis**, detecting enhanced testis vascularization, along with diffuse testicular enlargement, inhomogeneous, mainly hypoechoic, testicular echotexture, and reactive hydrocele (Isidori and Lenzi 2008; Bertolotto and Trombetta 2012; Pilatz et al. 2013; Lotti and Maggi 2015). Post-orchitis testis may present with inhomogeneous echotexture, mainly hypo- or hyperechoic, with normal or reduced vascularization and micro- or macrocalcifications. Although it has been reported that, in the case of testicular involvement, chronic inflammation may result in **testicular atrophy**, a study suggests that, under conservative treatment, no testicular atrophy occurs after acute epididymo-orchitis (Pilatz et al. 2013).

6.2.3.1.6 Testicular Microlithiasis

Testicular microlithiasis (TML) is an US diagnosis. TML is related to the presence of microcalcifications, which are bright echogenic nonshadowing foci less than 3 mm (Lotti and Maggi 2015; Richenberg et al. 2015). They are made of microcalcium deposits with surrounding fibrosis. They do not cause symptoms and are impalpable. **Microcalcifications** must be distinguished from **macrocalcifications**, which have never worried sonographers or clinicians, and which have been associated with a prior testicular insult (trauma, orchitis, infarction, torsion, chemo/radiotherapy), testicular atrophy, or maldescended testis (Isidori and Lenzi 2008; Bertolotto and Trombetta 2012; Lotti and Maggi 2015). However, they can be rarely related to a burnt-out tumor. In these aforementioned cases, the associated pathological condition, and not the presence of the macrocalcification itself, could play a negative role on general and/or reproductive male health.

The first sonographic identification of TML was described in 1987 as "innumerable tiny bright echoes diffusely and uniformly scattered throughout in the substance of testes" (Lotti et al. 2021a). Since then, a large number of varying definitions have been used in the sonographic literature on this topic. However, the two main definitions for TML proposed in the past were as follows: ≥ 5 microcalcifications in the whole testis or ≥ 5 microcalcifications per field of view (Richenberg et al. 2015) (Table 6.2). So far, the ESUR defined TML as the presence of ≥ 5 microcalcifications per field of view, leading to a worldwide accepted definition (Table 6.2).

TML association with testicular tumors and infertility has been widely debated. Recent meta-analyses support a signifi-

cant association between TML and testicular cancer (Lotti et al. 2021a). No meta-analyses have evaluated the association between TML and sperm parameters, while recent reviews reported that the relationship between TML and male infertility is still debated.

According to a previous version (Albers et al. 2013) of the EAU guidelines, the presence of TML with no associated risk factors (see below) did not require scrotal US follow-up or biopsy, while that with associated risk factors (infertility, bilateral TML, atrophic testes, history of cryptorchidism or of testicular cancer) was considered an indication for regular scrotal US follow-up, and, eventually, testicular biopsy. Current EAU guidelines (Salonia et al. 2021; Laguna et al. 2021) on TML management have not changed significantly in the last decade. So far, it is still a critical issue that in men with "TML and additional risk factors" either US follow-up or biopsy is advised, leading to possible different and nonstandardized managements of the patient. In addition, the timing of the testicular US follow-up has not been suggested. However, in 2015, the ESUR published guidelines (Richenberg et al. 2015) on TML imaging and follow-up, suggesting that annual follow-up is advised only in patients with "TML and additional risk factors" (personal/family history of testicular tumor, maldescent testis, orchidopexy, testicular atrophy) up to age 55, and, eventually, in men with no risk factors but diffuse ("starry sky") TML. Annual US follow-up is suggested also for children/adolescents with maldescent testis/post-orchiopexy or with testicular atrophy. In addition, recommendations for men with genetic disorders (including Klinefelter and McCune-Albright syndromes) have been reported and are the same as for the general population. Finally, ESUR indication for biopsy in TML men is very limited. In particular, in men who, at orchiectomy for a germ cell tumor, show TML or atrophy of the contralateral testis, a testicular biopsy may be indicated to look for *carcinoma in* situ.

Regarding the possible relationship between TML and **male infertility**, several studies have reported that the prevalence of TML in infertile men is higher than in fertile men (Lotti et al. 2021a). In addition, in a relatively large cohort of men with fertility intention, those with TML showed worse semen parameters than the rest of the sample. More recently, in a larger cohort of males of infertile couples, men with TML showed lower mean testis volume and sperm concentration and higher FSH levels than those with limited (<5 hyperechogenic spots per sonogram) or no microcalcifications. TML appears linked to infertility as an indicator/part of **testicular dysgenesis syndrome** (Lotti and Maggi 2015; Lotti et al. 2021a). As a corollary, so far, in infertile men TML is not considered a risk factor for the production of antisperm antibodies.

6.2.3.1.7 Testicular Lesions

Since the first application of gray scale US to investigate scrotal content, the main interest of physicians has been to explore **scrotal masses**. Over time, technical US skills have improved, focusing to date on testicular lesions with different approaches, including CDUS, CEUS, and SE (Lotti and Maggi 2015; Bertolotto et al. 2018; Huang et al. 2020). However, US is still not accurate enough to define the origin of several lesions, and **histology** remains the only certain diagnostic tool (Lotti and Maggi 2015; Lotti et al. 2021a).

Until the advent of CEUS, CDUS was the only way to evaluate testicular and extratesticular lesions. Sonologists mainly described the characteristics of the lesion, including size, homogeneity, echogenicity, margins, and vascularization (Lotti et al. 2021a). However, especially for large lesions, surgery was mandatory. Differential diagnosis was difficult, particularly when, at US, severe TI was detected. The difference between anechoic or solid lesions was detectable, allowing clinicians to distinguish intratesticular benign cysts from possible malignant lesions. However, large solid lesions were considered as likely neoplasms. In addition, differential diagnosis between hypoechoic areas, underlying segmental infarction, post-traumatic or post-inflammatory outcomes, intratesticular hematomas, or possible tumors was difficult. The finding of large lesions required compulsory surgery, while that of millimetric lesions was managed with strict follow-up, however requiring surgery in the event of unstable characteristics over time.

So far, with the improvement of US devices' resolution and vascular assessment, gray scale US with power/color Doppler is able to evaluate testicular lesions quite well, suggesting, in some cases, specific diagnosis. Clinical and CDUS patterns of testicular and extratesticular lesions have been described in detail elsewhere (Isidori and Lenzi 2008; Bertolotto and Trombetta 2012). Table 6.4 summarizes the CDUS characteristics of the main **malignant and benign testicular lesions**. In addition, new US imaging techniques, such as CEUS and SE, have improved the characterization of testicular abnormalities, both in adult and in pediatric patients (Bertolotto et al. 2018; Huang et al. 2020). According to EFSUMB recommendations (Sidhu et al. 2018), CEUS can distinguish vascularized from nonvascularized focal testicular lesions, helping to exclude malignancy. In addition, CEUS can discriminate nonviable regions in testicular trauma and can identify segmental infarction. Finally, CEUS can identify abscess formation and infarction in severe epididymo-orchitis. As a corollary, recently ESUR published its position statements on imaging in scrotal trauma (Ramanathan et al. 2021), reporting standardization, methodology, and information derived from CDUS/CEUS/SE application. Regarding SE, according to EFSUMB recommendations (Săftoiu et al. 2019), its use for the evaluation of focal testicular lesions can only be recommended in con-

Table 6.4 Main clinical and US characteristics of malignant and benign lesions of the testis. Data are reported according to references (Isidori and Lenzi 2008; Bertolotto and Trombetta 2012; Lotti et al. 2021a)

Main testis lesions	Age peak incidence and biologic behavior	Most common US characteristics	Associated abnormalities or syndromes
Malignant			
Germ cell tumors (~95%)			
Seminoma (35–55%)	– typical (85%): 20–40/50 year – anaplastic (5–10%): 20–40 year – spermatocytic (5–10%): 50–70 year Good prognosis	– Homogeneous, hypoechoic, solid nodules of various size; round, oval, or with polycyclic lobulated margins; often high-flow, low-resistance vascular arborization; rarely bilateral or in mixed germ cell tumor – Highly vascularized at CEUS, homogeneous enhancement, rapid wash-in, and wash-out – Hard at SE	Cryptorchidism microlithiasis
Nonseminoma – embryonal cell carcinoma (20–25%) – teratoma (5–10%) – choriocarcinoma (0.5%) – yolk sac tumor (<1%) – mixed (20–40%)	20–30 year, aggressive <4 year, benign; 20–30 year, malignant 10–30 year, highly malignant <2 year and 20–30 year, malignant 20–30 year, malignant	– Inhomogeneous, hypoechoic, solid nodules with cystic areas or calcifications within the lesion – Variable vascularization at CEUS, inhomogeneous enhancement – Coexisting stiff and soft areas at SE	
Stromal (~5%)			
Leydig cell tumor (3%)	children and 20–50 year (90% benign, 10% malignant)	– Often small, unilateral, and solitary with circumferential blood flow – Highly vascularized at CEUS, homogeneous enhancement, rapid wash-in, and delayed wash-out – Hard at SE	May secrete estrogens (30%): – gynecomastia, – pseudopubertas precox, – azoospermia, – erectile dysfunction, – loss of libido
Sertoli cell tumor (~1%)	children and 20–30 year ("borderline"; <20% malignant)	Hypo- or hyperechoic nodules with possible calcifications • with dysplastic syndromes: bilateral and multifocal, • without dysplastic syndrome: unilateral and focal, • large-cell calcifying Sertoli cell tumor (LCCSCT): diffusely heterogeneous pattern, hyperechoic, large calcifications. – Highly vascularized at CEUS, homogeneous enhancement, rapid wash-in and delayed wash-out (poorly described, nonapplicable in LCCSCT) – Hard at SE	– Rarely secrete estrogens: gynecomastia. – Klinefelter syndrome – Peutz–Jeghers syndrome – Androgen insensitivity (testicular feminization) syndrome – Carney complex
Others			
Lymphoma	2% or 25% of testicular tumors in subjects with <50 or 50–70 year, respectively. Malignant.	– Homogeneous hypoechoic diffuse testis infiltration or unifocal/multifocal hypoechoic lesions of various size; parallel hypoechoic lines radiating peripherally from the mediastinum (blood vessels), high vascularization; 8–18% bilateral, synchronous, or asynchronous – Highly vascularized at CEUS, homogeneous enhancement, rapid wash-in wash-out, nonbranching linear pattern. – Hard at SE	
Leukemia	Children. Malignant, often recurs	– Unilateral or bilateral, diffuse or focal, hypo- or hyperechoic lesions with longitudinal hypoechoic striae and increased blood flow. – Highly vascularized at CEUS, homogeneous enhancement, rapid wash-in wash-out, nonbranching linear pattern – Hard at SE	

(continued)

Table 6.4 (continued)

Main testis lesions	Age peak incidence and biologic behavior	Most common US characteristics	Associated abnormalities or syndromes
Metastases	50–70 year Aggressive	US findings depend on the primary tumor and necrosis degree	Common primary sites: prostate, lung, bowel, melanoma, kidney
Benign			
Intratesticular cysts	Variable	– Usually solitary, can be multiple; often near the mediastinum; well-defined round anechoic lesions; various size – Avascular at CEUS – SE: triple layout pattern; SWE: signal defect	
Tunica albuginea cysts	40–60 year	– Small round anechoic peripheral lesions of various size – Avascular at CEUS – Palpable, difficult to evaluate at SE	
Dilation of the rete testis	50–70 year	– Multiple micro-or macrotubular fluid-filled structures, often near the mediastinum; no vascularization at CDUS – Single bubbles running in small vessels surrounding the dilated tubules of the rete testis at CEUS – Soft at SE	
Epidermoid cyst	20–40 year; benign	– Variable: 1–3 cm; hyperechoic fibrous or calcified rim; sometimes "onion ring" pattern – Avascular at CEUS – Hard at SE	
Dermoid cyst	Children	Similar to mature teratoma	
Global and segmental ischemia	Variable	– Diffuse or focal, usually segmental, area without blood flow at CDUS in an otherwise normal testicular parenchyma – Avascular at CEUS, perilesional enhancing rim in subacute lesions – Soft at SE, may be harder at the periphery	Results from torsion, epididymal-orchitis, or trauma
Hematoma	Variable	– Hypoechoic nor vascularized lesions – Avascular at CEUS – Soft early, harder when organized at SE	Trauma
Abscess	Variable	– Complex heterogeneous fluid collection, hypo/anechoic, with irregular walls, occasionally with hypervascular margins – Avascular at CEUS, peripheral rim enhancement – Soft at SE	Usually complication of epididymitis and/or orchitis
Adrenal rest	Neonates, untreated adults. Regression with corticosteroids	– Rounded hypoechoic excentric solid masses, which may be bilateral or multifocal. Typically, the vessels' course through the lesion is not deviated. – Variable enhancement at CEUS, – Variable consistency at SE	Congenital adrenal hyperplasia.
Sarcoidosis	20–40 year	– Hypoechoic, irregular or rounded masses, often unilateral – Variable enhancement at CEUS – Soft at SE	
Gummas	20–40 year	– Hypoechoic nodule – Highly vascularized at CEUS, rapid wash-in, and wash-out – Hard at SE. As necrosis progresses, heterogeneous enhancement, coexisting stiff and soft areas at SE	Syphilis

junction with other US techniques, as there is overlap between benign and malignant neoplasms.

Regarding methodology and indications, **palpation** should be the first step of an US investigation of the scrotal content and, in selected cases, can help to identify scrotal lesions that are not immediately seen at US (Isidori and Lenzi 2008; Bertolotto and Trombetta 2012; Lotti and Maggi 2015). According to recent EAU guidelines (Laguna et al. 2021), high-frequency (>10 MHz) testicular US should be used to confirm a testicular tumor even in the presence of a

clinically evident testicular lesion. The use of testicular US can (i) determine whether a mass is intra- or extra-testicular; (ii) determine the volume and anatomical location of the testicular lesion; (iii) be used to characterize the contralateral testicle to exclude other lesions and identify risk factors for **carcinoma in situ** (Laguna et al. 2021). Testicular US is also recommended for all men with retroperitoneal or visceral masses and/or without elevated tumor markers in the absence of a palpable testicular mass and for fertility workup evaluation (Laguna et al. 2021).

According to the EAU guidelines, every subject with a **suspected testicular mass** must undergo **surgical exploration**, with orchiectomy if a malignant tumor is found or testicular biopsy with histological examination if the diagnosis is not clear. Regarding large nodule management, US should be performed even in the presence of a clinically evident testicular mass (Lotti and Maggi 2015; Laguna et al. 2021). In this scenario, US frequently plays an adjuvant role, sometimes allowing differential diagnosis among different clinical conditions (i.e., malignancy, inflammation, cysts) and evaluating the contralateral testis. However, currently, the real challenge is represented by imaging and management of small (millimetric) lesions. Small hypoechoic areas, especially when not vascularized, may be related to spermatoceles, cysts, focal Leydig cell hyperplasia, fibrosis, and focal inhomogeneity due to previous pathologic conditions (Isidori and Lenzi 2008; Bertolotto and Trombetta 2012; Lotti and Maggi 2015). However, they may also indicate small tumors. Hence, they require careful evaluation and follow-up, with periodic US examination, especially if additional risk factors for malignancy are present (i.e., infertility, bilateral TML, cryptorchidism, testicular atrophy, inhomogeneous parenchyma, history of testicular tumor, contralateral tumor, and age <50 years) (Salonia et al. 2021; Laguna et al. 2021). If a small nodule grows, or additional risk factors for malignancy are present, testicular biopsy/surgery should be considered.

Recently, ESUR published recommendations (Rocher et al. 2016) on incidentally **US-detected nonpalpable testicular lesions** in adults. According to the ESUR, characterization of testicular lesions is primarily based on US examination. Most small nonpalpable testicular lesions seen on US are benign simple cysts and require neither follow-up nor surgery. Nonpalpable single sporadic solid nodules <5 mm without any microliths are benign in up to 80% of cases, with Leydig cell tumors being the most frequent. US follow-up can be an alternative to orchiectomy in young and/or infertile men if tumor markers are negative. Large (>1 cm), multiple, mixed cystic, heterogeneous or solid vascularized nodules, irregular margins, associated microliths, or hypoechoic regions may indicate malignancy. CEUS optimizes enhancement in lesions which are apparently avascular at color Doppler. The rate of the wash-in and the wash-out of the contrast agent may help to differentiate malignant from benign tumors. Leydig cell tumors have been reported to demonstrate a prolonged wash-out in one study, and a shorter filling time than germ cell tumors in another. The role of CEUS is evolving; however, only a few studies are available to date, limiting the recommendations for the routine use of CEUS for managing incidental testicular masses. Conversely, the role of SE in differentiating between malignant and benign testicular nodules is still unclear (Săftoiu et al. 2019). Accordingly, a recent study reported that strain ratio measurement offers no improvement over elastographic qualitative assessment of testicular lesions, and that SE may support conventional US in identifying non-neoplastic lesions when findings are controversial, but its added value in clinical practice has yet to be proven (Lotti et al. 2021a). More recently, the same authors prospectively evaluated a large cohort of patients with Leydig cell tumors, reporting a good oncological prognosis when recognized early. The authors indicated that tissue-sparing enucleation is curative and should replace orchiectomy and that conservative surgery or active surveillance in compliant patients through clinical and radiological follow-up can be considered safe options.

6.2.3.1.8 Cryptorchidism

The term *cryptorchidism* is derived from the Greek words *kryptos* and *orchis*, literally meaning "hidden testis." Cryptorchidism, or undescended testis, is the absence of at least one testicle in the scrotum (Lotti and Maggi 2015; Leslie et al. 2021). The **undescended testis** is commonly unilateral, being bilateral in one out of ten cases. It is associated with an increased **risk of infertility and testicular malignancy**. **Cancer** commonly occurs in the undescended testis; however, one out of five tumors occur in the contralateral descended testis. About 80% of undescended testes are located within the inguinal canal, 5–16% high in the abdomen, while rarely the testis can be ectopic from the path of descent or absent/vanishing. The AUA Guidelines report that in pediatric patients, more than 70% of cryptorchid testes are palpable by physical examination and need no imaging. In the remaining 30% of cases with a nonpalpable testis, the challenge is to confirm absence or presence of the testis and to identify its location (Leslie et al. 2021). The role of US in the pediatric preoperative planning before orchiopexy is controversial and has changed over time (Leslie et al. 2021). On the other hand, US is useful in adult men with a history of cryptorchidism/orchiopexy.

Until the last decade, some pediatricians used to require US to locate a nonpalpable undescended testis. In fact, a nonpalpable testis may be present in the inguinal-scrotal region or within the abdominal cavity, or it may be absent (Tasian and Copp 2011; Leslie et al. 2021). **Surgical exploration** is mandatory to localize the testis when present or confirm an absent testis. However, accurate presurgical diag-

nosis of an absent testis would spare a child unnecessary surgery, while the correct localization of the testis could limit the extent of surgery. Observational studies performed in pediatric patients until 2011 revealed conflicting diagnostic performance features and did not rigorously evaluate the clinical utility of US in localizing nonpalpable testes.

In 2011, a systematic review and meta-analysis (Tasian and Copp 2011) reported that US does not reliably localize nonpalpable testes or rule out an intra-abdominal testis in pediatric patients. Hence, all recent guidelines recommend against the use of US in pediatric patients since it does not change management nor add diagnostic accuracy, except in selective cases (e.g., suspicion of sexual development disorders) (Leslie et al. 2021).

On the other hand, guidelines dealing with US in adult men with a history of cryptorchidism are not available. However, it is well recognized that in adult men who have undergone orchiopexy US plays a key role in **cancer detection** or in the follow-up of the cryptorchid and contralateral testis (Lotti and Maggi 2015). Recommendations on the follow-up timing and duration in men with a history of undescended testis/orchiopexy are not available. Considering that cryptorchidism is a risk factor for testicular cancer more relevant than TML, recommendations given by the ESUR for the follow-up of "TML with additional risk factors" (Richenberg et al. 2015) could be suggested in principle, i.e., annual follow-up up to age 55. However, cryptorchid men should be educated on prevention with frequent self-examination of the testes, especially in the age range (15–34 years) (Lotti and Maggi 2015) associated with the highest occurrence of testicular malignancy, to identify a lump possibly underlying a growing tumor early.

As a corollary, some men may present to clinicians with a nonpalpable testis. Since some authors reported that US can reliably identify a cryptorchid testis lying below the level of the internal inguinal ring, US may be suggested to identify the undescended testis at the high scrotal level or in the inguinal canal (Lotti and Maggi 2015). However, if US was unreliable, other imaging investigations or surgical exploration should be suggested.

At US, the cryptorchid testis is often hypotrophic, inhomogeneous, hypoechoic, with or without macro- or microcalcifications and with normal or reduced vascularization (Isidori and Lenzi 2008; Bertolotto and Trombetta 2012; Lotti and Maggi 2015; Lotti et al. 2021a). However, especially in cases of early **orchidopexy**, it could show normal volume and echotexture. Echotexture inhomogeneity can be associated with one or more hypoechoic micronodular lesions, often resulting from cryptorchidism- or postoperative-related damage. Nodular lesions should be managed as reported above (see the paragraph "Testicular lesions") and even more carefully considering the cryptorchidism-related risk of cancer. CEUS can distinguish vascularized from non-vascularized focal testicular lesions, helping to exclude malignancy (Sidhu et al. 2018).

6.2.3.2 Epididymis and Vas Deferens

6.2.3.2.1 US Anatomy

The normal epididymis is a soft organ that lies along the superior margin of the testis and is classically divided into three segments: head, body, and tail. At US, the epididymis is usually detected posterior-laterally to the testis, with the head and tail at the upper and lower pole of the testis, respectively (Lotti and Maggi 2015). The normal **epididymal head** is triangular, with echogenicity comparable to that of the testis and usually slightly more echogenic than the body and tail. Blood flow is detectable by CDUS in discrete vascular spots in all tracts of the epididymis (Isidori and Lenzi 2008; Bertolotto and Trombetta 2012; Lotti and Maggi 2015; Lotti et al. 2021a).

The **vas deferens** is a straight tense cord which extends along the spermatic cord. Vas deferens absence, with or without epididymal agenesis, is often a difficult palpatory diagnosis, to be confirmed by US. The vas deferens appears at US as a straight duct, slightly hypoechoic compared with the epididymis, originating from the epididymal tail and extending, along the spermatic cord, toward the inguinal channel (Isidori and Lenzi 2008; Bertolotto and Trombetta 2012; Lotti and Maggi 2015).

Until 2021, values indicating **epididymal normal and abnormal size** have been only suggested empirically and were lacking for the vas deferens. In addition, normal or enhanced epididymal vascularization was qualitative and operator-dependent concepts. Recently, the EAA US study (Lotti et al. 2021b) reported a standardization of the measurement of the epididymal-deferential parameters and led to the identification of reference ranges and normative thresholds for epididymal segments and vas deferens size and vascular parameters (see Table 6.2). Normal epididymal head, body, tail, and vas deferens size have been defined in an evidence-based way as <11.5, 5, 6, and 4.5 mm, respectively. In addition, normal values of different epididymal (and testicular) vascular parameters have been reported.

6.2.3.2.2 US Abnormal Patterns

CDUS plays a key role in investigating abnormalities of epididymal size, echopattern, and vascularization, which, alone or combined, can suggest different diagnoses.

6.2.3.2.3 Acute and Chronic Epididymitis

In subjects with scrotal pain or prostatitis-like symptoms, the dilation of the whole epididymis or one of its segments (especially head or tail) associated with hypervascularization suggests **inflammation** (Pilatz et al. 2013; Lotti and Maggi 2015; Lotti et al. 2021a). In the acute form, an enlarged hypoechoic

inhomogeneous epididymis with enhanced vascularization is often detected, while in the chronic form the epididymis is often dilated and may appear hyperechoic and vascularization is only slightly increased (see below). A dilated epididymis associated with echopattern abnormalities (including calcifications) may also represent the outcome of a past infection/inflammation, currently asymptomatic. **Acute epididymitis** usually presents with a painful hemiscrotum, epididymal swelling, and fever. CDUS plays a key role in identifying diagnostic features, revealing mild-to-severe hyperemia and epididymal enlargement, mainly of the tail or both the tail and head, along with inhomogeneous echotexture, often hypoechoic with scattered hyperechoic foci, and reactive hydrocele with skin thickening. Concomitant **orchitis**, revealed in almost half of all the cases, is associated with hydrocele, testicular enlargement, hyperperfusion, and pain (Pilatz et al. 2013; Lotti and Maggi 2015). Under conservative treatment, epididymal CDUS parameters normalize (Pilatz et al. 2013). Hence, along with clinical characteristics, CDUS may play a role in the follow-up of acute epididymitis. **Chronic epididymitis** often involves the tail, with coarse calcifications in a variably enlarged hypo- or hyperechoic epididymis. The organ has an irregular profile, inhomogeneous, sometimes with hard irregularly shaped masses or indenting the testicular parenchyma, mimicking a primary testicular mass. Hydrocele and tunica albuginea thickening are commonly associated, the latter sometimes so severe as to be called "fibrous pseudotumor." Acute and chronic epididymitis may result in abnormal sperm parameters and the occurrence of **antisperm antibodies** (Lotti and Maggi 2015; Lotti et al. 2021a). As a corollary, US evaluation of the epididymis after a scrotal trauma shows features mimicking epididymitis (Ramanathan et al. 2021; Morey et al. 2021).

6.2.3.2.4 Epididymal Nodules/Cysts

US allows the assessment of epididymal nodules, often perceived at physical examination, frequently represented by cysts or spermatoceles, but possibly underlying benign (including tuberculosis-related granulomatous masses) or, very rarely, malignant lesions (Isidori and Lenzi 2008; Bertolotto and Trombetta 2012; Lotti and Maggi 2015). At clinical evaluation, cysts and spermatoceles are detected as tense-elastic spherical formations within the epididymis, mainly located in the head. Epididymal *cysts* and **spermatoceles** are detectable in one out of four men and appear at US as anechoic avascular and slightly hypoechoic inhomogeneous formations, respectively. Their clinical significance and association with male infertility have not been yet defined, since their involvement in complete epididymal obstruction and obstructive azoospermia has never been proven. Conversely, epididymal injury secondary to excision surgery, mainly performed for large and painful lesions, may lead to epididymal obstruction. Hence, epididymal cyst surgery is not suggested for restoring fertility.

6.2.3.2.5 Epididymal and Deferential Dilation

In subjects with obstructive azoo- or oligospermia, the detection of **epididymal enlargement** may suggest post-testicular obstruction, which could be (i) at the epididymal level (especially when the downstream vas deferens shows a normal size), (ii) at the vas deferens level, especially in men treated by epididymovasostomy, after vasectomy, or with absence of vas deferens, or (iii) at the prostatic level, the latter to be further investigated extending US to the prostate-vesicular region (Lotti and Maggi 2015; Lotti et al. 2021a).

In subjects with distal obstruction, a dilated, inhomogeneous proximal **vas deferens** may be seen. These characteristics can be seen also in patients with vasectomy as well as chronic inflammation or diabetes which may cause luminal or parietal calcifications, respectively. An epididymal tail >6 mm and/or head >12 mm have been proposed as suggestive of epididymal inflammation, while a head >~11 mm as indicative of obstruction. After **vasectomy**, an epididymal head >15 mm or >2 cm has been reported. To date, according to the EAA US study (Lotti et al. 2021b), epididymal head, body, tail, and vas deferens size may be defined in an evidence-based way as >11.5, 5, 6, and 4.5 mm, respectively (Table 6.2).

6.2.3.2.6 Congenital Uni- or Bilateral Absence of Vas Deferens

Vas deferens, epididymis, and seminal vesicles embryologically originate from the Wolffian duct. Hence, when vas deferens agenesis occurs, it may be associated with agenesis/abnormalities of these structures. Subsequently, if vas deferens agenesis is detected at scrotal US, the examination should be extended to the prostate-vesicular region by TRUS. Interestingly, when complete vas deferens and epididymis agenesis occurs, **epididymal head** persists and is detectable by US (Lotti and Maggi 2015). **Congenital bilateral absence of vas deferens** (**CBAVD**) accounts for 1–2% of infertile men, 4–17% of azoospermic men, and up to 25% of those with obstructive azoospermia (Lotti and Maggi 2015). CBAVD may be isolated or associated with cystic fibrosis. Almost all men with cystic fibrosis also have CBAVD. About 80% of CBAVD subjects have at least one CFTR mutation. CBAVD is associated with bilateral seminal vesicle agenesis in about half of the patients and usually presents with normal kidneys. A few cases of CBAVD may present with kidney agenesis and are usually not related to CFTR gene mutations. Conversely, when kidney agenesis occurs, CFTR gene mutations are rarely involved. In subjects with CBAVD, testes are usually normal in volume and function. Hence, CBAVD investigation by US is essential in the diagnosis of obstructive azoospermia and for clinical

decision-making, since surgical sperm retrieval is virtually always positive.

Approximately 1% of men have **congenital unilateral absence of vas deferens** (**CUAVD** (Lotti and Maggi 2015). CUAVD is associated with ipsilateral and contralateral seminal vesicles agenesis in 90 and 20% of patients, respectively, and with renal agenesis in 80% of cases. Subjects with CUAVD are usually fertile, but at high risk for infertility, having a single patent vas deferens. In addition, those with CUAVD and contralateral SV agenesis may have contralateral deferential ampulla atresia. Therefore, a subset of men with CUAVD may have abnormal semen parameters or azoospermia. Similar problems may be present in subjects with CUAVD and contralateral testis damage.

6.2.3.3 Pampiniform Plexus and Varicocele

6.2.3.3.1 US Anatomy

Normal pampiniform plexus is scarcely appreciable by physical examination, while CDUS is able to examine it with great accuracy (Lotti and Maggi 2015). In normal conditions, the pampiniform plexus appears as a complex network of small vessels converging into the spermatic veins. The right spermatic vein enters obliquely into the inferior vena cava, whereas the left one enters perpendicular into the left renal vein and is therefore burdened by higher blood hydrostatic pressure. When the pressure becomes excessive and/or the venous valvular mechanism is impaired, venous reflux and dilation may occur, leading to varicocele (see below).

6.2.3.3.2 US Abnormal Patterns: Varicocele

An abnormal dilatation of the pampiniform plexus characterized by retrograde venous flow indicates the presence of a varicocele (Lotti and Maggi 2015). According to the aforementioned anatomical considerations (see above), varicocele is mainly (90%) detected on the left side. The prevalence of varicocele, virtually absent in boys aged <11 years, increases with age, up to 15% in the general adult population. It has been estimated that the prevalence of varicocele in adult men with primary infertility is about 35% (Lotti and Maggi 2015). Most studies, but not all, report worse sperm parameters in subjects with varicocele. However, 75% of subjects with varicocele have normal semen parameters. In addition, one out of three fertile men has a varicocele (Lotti et al. 2020). Hence, the impact of varicocele on couple infertility is still under debate.

Varicocele is clinically classified into **three grades**: (1) palpated during Valsalva maneuver, (2) palpated without Valsalva maneuver, and (**3**) visible. While the clinical classification of varicocele has been universally accepted since 1970, the diagnosis and classification of varicocele with CDUS is one of the most debated topics in andrology/urology (Lotti and Maggi 2015; Freeman et al. 2020; Lotti et al. 2021a; b).

Several classifications have been proposed over time, with differences mainly related to the cutoff diameter to indicate a **dilated vein**, the indication or not of the vein's extension in the scrotal sac for grading varicocele, duration of the venous reflux, and the presence or not of testicular hypotrophy in the most severe grade (Lotti and Maggi 2015; Freeman et al. 2020). In a 2015 systematic review (Lotti and Maggi 2015), Lotti and Maggi attempted to align the classifications available until then, supporting for the severe form the presence of a continuous venous reflux at rest, increasing or not during a Valsalva maneuver. In the aforementioned review, previous varicocele classifications have been described in detail. Table 6.5 shows the available CDUS classifications of varicocele severity.

In 2020, the ESUR published guidelines (Freeman et al. 2020) for detection, classification, and grading of varicocele. ESUR reported methodological recommendations, supporting a standardized protocol for varicocele US examination. According to ESUR guidelines, a gray scale and color Doppler examination, with spectral Doppler analysis, should be performed bilaterally with the patient supine and standing, during spontaneous breathing and during the **Valsalva maneuver**. Measurement of the largest vein, irrespective of location, with the patient in the upright position and during the Valsalva maneuver is recommended. A maximum **venous diameter** ≥3 mm can be considered diagnostic for a varicocele, grading varicocele according to Sarteschi classification. A reflux in the testicular veins lasting >2 seconds with the patient standing and during the Valsalva maneuver should be considered to be abnormal. TV should be measured in all cases. In patients with subclinical varicoceles imaging follow-up is recommended. After varicocele repair, US can be used to identify early postoperative complications. Sperm analysis forms the basis of follow-up after varicocele repair, without the routine use of US (Gravas et al. 2021). The recommendation against the routine use of US after varicocele repair did not gain a strong consensus (Gravas et al. 2021). The latter point depends on the fact that some members of the ESUR-Scrotal and Penile Imaging Working Group (SPIWG) involved in the guidelines' production supported the idea that US after varicocele repair is necessary, in order to identify varicocele persistence or recurrence in the short term. This issue is relevant to understand if possible changes in sperm analysis after varicocele repair depends on the treatment itself or on casual oscillation in sperm parameters when there is no evidence of varicocele correction.

More recently, the EAA US study (Lotti et al. 2021b) assessed reference ranges for pampiniform plexus CDUS parameters in healthy, fertile men (Lotti et al. 2020). The EAA US consortium reported SOPs for varicocele evaluation, welcoming most, but not all, ESUR recommendations.

Table 6.5 Proposed classification of varicocele according to clinical and increasing sonographic severity, by different authors

First Author of classification[a]	Varicocele clinical grade				
Dubin and Amelar (1970)	[1] detectable only during Valsalva			[2] palpable	[3] visible
	Varicocele CDUS grade				
Hirsh et al. (1980)	[1] No spontaneous reflux, inducible with Valsalva	[2] Intermittent spontaneous reflux		[3] Continuous spontaneous reflux	
Dhabuwala et al. (1989)	[1] Reflux <2 s	[2] Reflux >2 s		[3] Spontaneous reflux increasing with Valsalva	
Sarteschi et al. (1993) Liguori et al. (2004)	[1] Inguinal reflux only during Valsalva in not enlarged vessels	[2] Supra-testicular reflux only during Valsalva in small posterior varicosities	[3] Peritesticular reflux only during Valsalva in enlarged vessels. Visible but not dilated vessels when supine, enlarged when standing.	[4] Enlarged vessels in supine and standing position, with increasing caliber with Valsalva. Reflux at rest, increasing during Valsalva. Common testicular hypotrophy.	[5] Enlarged vessels in supine and standing position, with caliber not increasing with Valsalva. Reflux at rest, not increasing during Valsalva Intratesticular varices and/or testicular hypotrophy.
Hoekstra and Witt (1995)	[1] Dilated veins <2.5 mm without flow reversal after Valsalva	[2] Dilated veins 2.5–3.5 mm and flow reversal after Valsalva		Dilated veins >3.5 mm and flow reversal after Valsalva	
Cornud et al. (1999)	[1] *Brief* reflux: <1 s [2] *Intermediate* reflux <2 s, decreasing during and stopping prior to the end of Valsalva			[3] *Permanent* reflux: >2 s and with a plateau aspect throughout the abdominal strain.	
Oyen (2002) *B-mode Reflux Doppler*				Diameter of the veins >3 mm increasing during Valsalva and/or in the upright position	
	[1] Slight reflux (<2 s) during Valsalva	[2] Reflux (>2 s) during Valsalva, not continuous		[3] Reflux at rest or during the entire Valsalva	
Isidori and Lenzi (2008) *B-mode Reflux Doppler*	[1] Dilated vessels (>2.5 mm) in inguinal region	[2] Supra-testicular vessel dilation (>3 mm)	[3] Supra- and peritesticular vessel dilation (>3 mm)	Supra[2]- and peri [3]-testicular vessel dilation, [4] increasing with Valsalva. Testicular hypotrophy.	[5]Peritesticular vessel dilation not increasing with Valsalva, or intratesticular vessels and testicular hypotrophy
	[1] Inguinal reflux only during Valsalva (2–3 s)	[2] Supra-testicular reflux only during Valsalva (>3 s)		Peritesticular reflux at rest increasing [3] or not [4] during Valsalva	[5] Peritesticular reflux at rest which increases minimally with Valsalva or dilated intratesticular veins which refill with Valsalva
Pauroso et al. (2011)	[1] Reflux in the inguinal channel only during Valsalva	[2] Small varicosities with reflux only during Valsalva		[3] Enlarged vessels dilating during Valsalva	[4] Enlarged vessels with venous reflux at rest not increasing during Valsalva
Iosa and Lazzarini (2013)	[1] Reflux >1 s only during Valsalva	Spontaneous, discontinuous reflux not increased [2] or increased [3] by Valsalva		[4B] Spontaneous, continuous reflux not increased by Valsalva	[4A] Spontaneous, continuous reflux not increased by Valsalva
Freeman et al. (2020); Bertolotto et al. (2020) (ESUR classification)	[1] venous vessels dilation (>3 mm) during Valsalva maneuver at the funicular region with retrograde venous flow absent at rest and enhanced during Valsalva maneuver.	[2] venous vessels dilation (>3 mm) during Valsalva maneuver at the upper pole of the testis with retrograde venous flow absent at rest and enhanced during Valsalva maneuver.	[3] venous vessels dilation (>3 mm) during Valsalva maneuver at the lower pole of the testis with retrograde venous flow absent at rest and enhanced during Valsalva maneuver.	[4] venous vessels dilation (>3 mm) during Valsalva maneuver (irrespective of location, but usually extending to the peritesticular region) with retrograde venous flow *continuous* (>2 s) at rest and enhanced during Valsalva maneuver. Possible testicular hypotrophy.	[5] venous vessels dilation (>3 mm) during Valsalva maneuver (irrespective of location, but usually extending to the peritesticular region) with retrograde venous flow *continuous* (>2 s) at rest and not increasing during Valsalva maneuver. Possible intratesticular varices and/or testicular hypotrophy.

(continued)

Table 6.5 (continued)

First Author of classification[a]	Varicocele clinical grade				
Lotti et al. (2021b) (EAA classification)	[1] venous vessels dilation (>3 mm) at rest at the funicular region with retrograde venous flow absent/ intermittent at rest and enhanced during Valsalva maneuver.	[2] venous vessels dilation (>3 mm) at rest at the upper pole of the testis with retrograde venous flow absent/ intermittent at rest and enhanced during Valsalva maneuver.	[3] venous vessels dilation (>3 mm) at rest at the lower pole of the testis with retrograde venous flow absent/ intermittent at rest and enhanced during Valsalva maneuver.	[4] venous vessels dilation (>3 mm) at rest (irrespective of location, but usually extending to the peritesticular region) with retrograde venous flow *continuous* at rest and enhanced during Valsalva maneuver. Possible testicular hypotrophy.	[5] venous vessels dilation (>3 mm) at rest (irrespective of location, but usually extending to the peritesticular region) with retrograde venous flow *continuous* at rest and not increasing during Valsalva maneuver. Possible intratesticular varices and/or testicular hypotrophy.

[a]See, for exhaustive references, review by Lotti and Maggi 2015
The grade severity of each classification is reported in brackets. Since the different classifications did not use the same parameter to categorize severity, a strict comparison is not applicable. Extension, size and number of dilated veins, affected side, duration of retrograde flow during Valsalva, presence of spontaneous retrograde flow in the upright position, volume and echotexture of the affected testis and comparison with the contralateral should be reported when varicocele evaluation is performed. Adapted and updated from review (Lotti and Maggi 2015).
CDUS: color Doppler ultrasound; *ESUR*: European Society of Urogenital Radiology; *EAA*: European Academy of Andrology

In particular, the EAA consortium supported the measurement of the largest vein with the patient standing, *at rest* (and not during Valsalva maneuver) in order to avoid the possible confounder of a variable intraabdominal pressure increase with Valsalva, recommending Valsalva maneuver be used for varicocele grading, to be done according to Sarteschi et al./ Liguori et al. classifications (essentially overlapping) (Lotti and Maggi 2015). In addition, the EAA consortium also suggested the evaluation of the maximum diameter of the internal spermatic vein between the inguinal ligament and upper pole of the testis (Lotti and Maggi 2015) besides the assessment of the convoluted vessels below, supporting the 3 cm threshold to define vein dilation. Finally, the evaluation of TV using the ellipsoid instead of Lambert's formula was suggested (Lotti et al. 2021b). Of note, the EAA consortium defined "severe" varicocele as venous vessel dilation (>3 mm) characterized by a continuous (long-lasting, without reporting duration cutoff) venous reflux at rest, increasing, or not during a Valsalva maneuver (Lotti et al. 2021a; Lotti et al. 2021b), consistent with grade 4 and 5 varicocele according to Sarteschi et al./Liguori et al. classifications. Table 6.5 reports the varicocele classification proposed by the EAA US consortium.

The EAA study reported a **varicocele prevalence** of ~37% in fertile men (with a severe form in almost one out of five men), similar to that reported in primary infertile men (Lotti et al. 2021b). These data suggest that varicocele may exert a scanty effect on male fertility and that its surgical correction should be limited to highly selected populations (Lotti et al. 2020; Lotti et al. 2021b). Accordingly, current EAU Guidelines on male infertility (Salonia et al. 2021) support very specific indications for varicocele treatment both in adults and in adolescents.

6.3 Prostate and Seminal Vesicles US

The prostate–vesicular region can be studied by transabdominal or transrectal US (TRUS). Although some authors revealed no significant difference between the two US modalities to measure prostate volume, other authors reported that TRUS is more accurate in predicting adenoma volume in BPH patients (Lotti and Maggi 2015). In addition, in our opinion, TRUS has higher accuracy in detecting echotexture and vascular parameters. Hence, in this chapter we will focus on TRUS. TRUS is performed using a transrectal biplanar probe (linear and convex transducer, 6.5–7.5 MHz) and/or an "end fire" probe (6.5 MHz, field of view 50–200°), with the patient placed in the left lateral decubitus, scanning the organs in transverse, longitudinal, and oblique ways (Lotti and Maggi 2015).

6.3.1 Indications

Although EAU guidelines suggest a very limited value of TRUS in different fields (Gravas et al. 2021; Engeler et al. 2021; Salonia et al. 2021), with clear utility only in evaluating **obstructive azoospermia**, according to other recent publications TRUS can be useful for several aims (see below). Accordingly, possible TRUS indications are reported in Table 6.1.

6.3.2 Methodological Standards

As reported above for scrotal US, the EAA US study (Lotti et al. 2020) recently reported SOPs to assess TRUS according to a multicentric consensus and to previous studies and guidelines. A detailed description of the **SOPs** to evaluate TRUS quantitative and qualitative parameters has been reported on the EAA website (https://www.andrologyacademy.net/eaa-studies).

6.3.3 Anatomy, Normal and Abnormal Patterns, Clinical Utility, and Standards

Table 6.2 shows normal values and cutoff of the main US parameters of the prostate and seminal vesicles. Figure 6.3 shows a schematic representation of the normal and pathologic features of the organs of the prostate–vesicular region in relation to male reproductive health. Figure 6.3 shows some examples of normal and abnormal US features of the prostate-vesicular region. Normal and abnormal TRUS patterns are discussed below.

TRUS **clinical utility** and impact on male reproductive health management are reported in Table 6.3. So far, TRUS has shown a relevant impact on both reproductive and general male health (Lotti and Maggi 2015) (Table 6.1). In fact (i) TRUS shows a key role in obstructive azoospermia; (ii) TRUS is useful in evaluating prostate inflammation and related pelvic pain or premature ejaculation; (iii) TRUS is useful in evaluating prostate volume in relation to LUTS. In addition, (iv) TRUS can offer indirect information on male androgenization evaluating prostate and seminal vesicles volume, which are reduced in hypogonadal subjects. (v) However, TRUS is not useful in assessing prostate cancer.

6.3.3.1 Prostate

6.3.3.1.1 US Anatomy

The prostate is an **exocrine gland**, which surrounds the urethra just below the neck of the bladder. It produces prostatic fluid, an acidic secretion that makes up ~20% of the total ejaculate (Lotti and Maggi 2015). At TRUS, the normal prostate appears different according to age, with a triangular or pear shape in younger and older subjects, respectively. Its base lies at the bladder neck, at the beginning of the urethra, detectable in a longitudinal scan as a hypoechoic duct curving toward the prostatic apex. TRUS identifies a **peripheral zone**, which extends laterally and posteriorly from the apex to the base, and a **transitional zone**, centrally located and slightly hypoechoic. Peripheral and transitional zones show a 3:1 ratio in young men. A central zone has also been described. Prostate volume (PV) is often measured using a planimetric method (Behre et al. 1995; Lotti and Maggi 2015; Lotti et al. 2014a). It is calculated by measuring three diameters (anterior-posterior and transverse in the transversal scan, longitudinal in the sagittal one; using the mathematical formula of the ellipsoid. Transitional zone or adenoma volumes are similarly calculated. A PV of 20–25 ml has been previously proposed as "normal" in young men. However, recently, the EAA US study reported as "normal" a prostate volume between 15 ml and 35 ml (Table 6.2). The normal adult prostate shows thin, densely packed, and homogeneously deployed echoes. Periprostatic venous plexus is detectable as a slightly hypoechoic system of vessels. Intraprostatic arteries are grouped in central/periurethral and peripheral/capsular arteries, supplying the transitional and peripheral zones, respectively. The **ejaculatory ducts** (EDs) appear at TRUS as fine and hypoechoic, with a normal diameter <2 mm. They are detectable in longitudinal scans crossing the prostate up to the urethra (Lotti and Maggi 2015).

6.3.3.1.2 US Normal and Abnormal Patterns

Prostate Volume

Detection of prostate volume (PV) by TRUS is important in subjects with **LUTS**, since it can predict LUTS progression and the risk of complications (Gravas et al. 2021), while it has a low impact in the workup of male infertility (Lotti and Maggi 2015) (Table 6.3). A reduced PV suggests **hypogonadism**, because the prostate is an androgen-dependent gland (Lotti and Maggi 2015). An increased PV is related to **benign prostatic enlargement (BPE)**. A PV >30 ml has been previously suggested as indicative for initial gland enlargement (Lotti and Maggi 2015). However, according to the EAA US study, prostate enlargement should be defined as >35 ml (Table 6.2). A PV >60 ml has been suggested as indicative for a severe increase (Lotti and Maggi 2015). BPE has a continuum spectrum of TRUS abnormalities ranging from larger transitional zone to a well-defined adenoma. The typical TRUS characteristics of BPE are echotexture inhomogeneity, occasional cysts, well- and poorly defined nodules, and calcifications, especially at the "surgical capsule" (Lotti and Maggi 2015). Interestingly, BPE has been recently associated with overweight/obesity and metabolic syndrome (Lotti et al. 2014a).

Prostate Inflammation

Several TRUS features are considered suggestive of **chronic prostate inflammation**, including glandular asymmetry, hypo- or hyperechogenicity with calcifications and periprostatic venous dilation (Lotti and Maggi 2015). However, the aforementioned criteria are observational and not evidence-based, qualitative in some cases (hypo- or hyperechogenicity), lacking thresholds identifying abnormal patterns in others (no cutoff for glandular asymmetry or periprostatic venous dilation), or "static" (the presence of prostate calcifications is long-lasting and detectable lifelong in the same

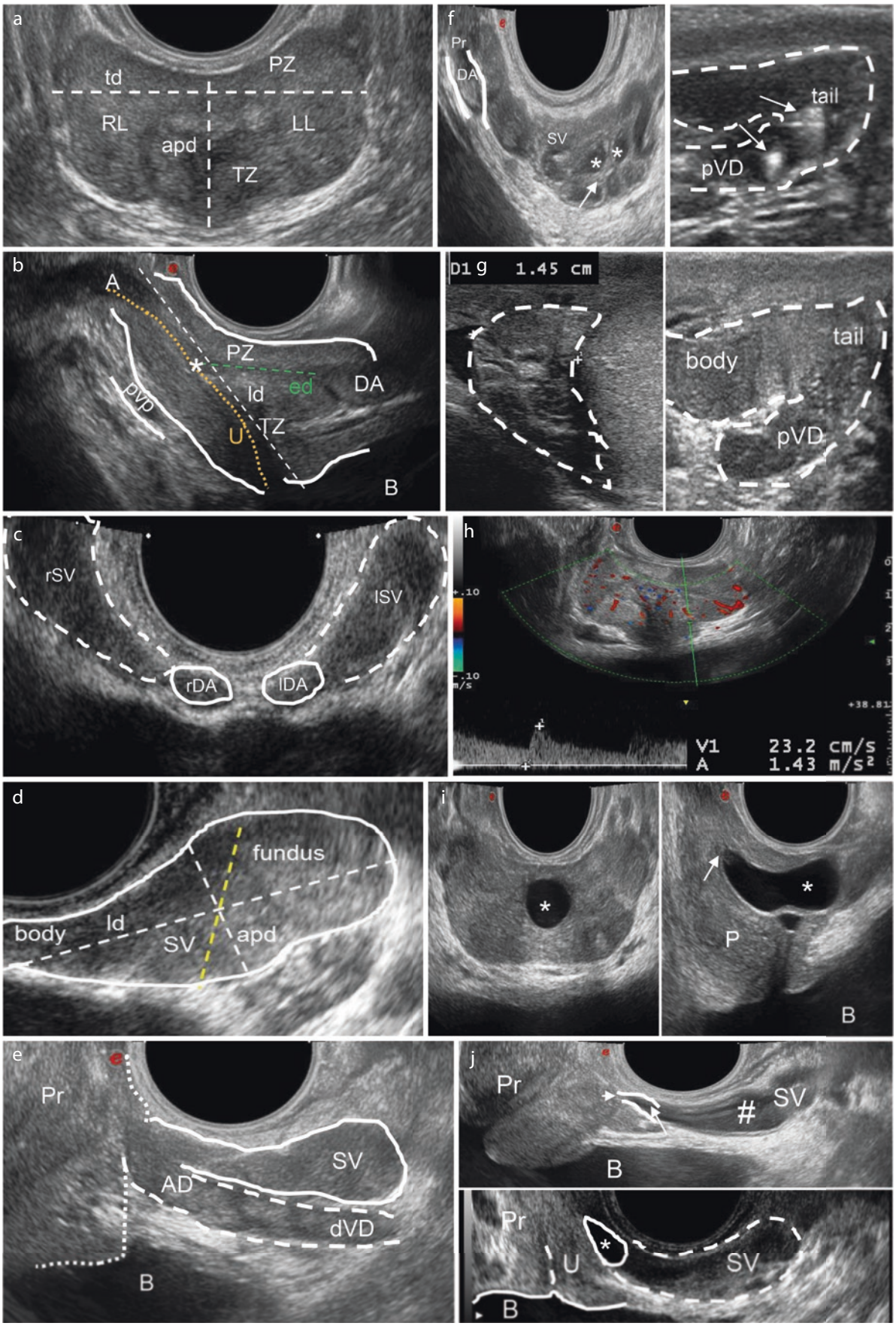

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Fig. 6.3 Normal (left side) and abnormal (right side) color Doppler ultrasound (CDUS) features of the prostate-vesicular region. **Panel** (**a**), prostate of normal volume, homogeneity, and echogenicity in transversal scan. Peripheral and transitional zones (PZ and TZ) show a 3:1 ratio in young men. Right and left lobes (RL and LL, respectively) and periprostatic venous plexus (PVP) are indicated. Anterior-posterior and transverse diameters ("apd" and "td," respectively) are reported. **Panel** (**b**), prostate of normal volume, homogeneity, and echogenicity in sagittal scan evaluated with "end fire" probe. Peripheral and transitional zone (PZ and TZ, respectively) and apex (A) are indicated, as well as bladder (B), urethra (U, yellow dotted line), ejaculatory duct (green dashed line), prostatic utricle (*), deferential ampulla (DA), and periprostatic venous plexus (pvp). The longitudinal diameter ("ld") is reported and represented with a white dashed line. **Panel** (**c**), right and left seminal vesicles (rSV and lSV, respectively) with typical "bow-tie" appearance and, medial to them, right and left deferential ampullas (sDA and lDA, respectively) in transversal scan. **Panel** (**d**), seminal vesicle (SV) assessed by "end fire" probe in sagittal scan. *Fundus* and *body* are reported, as well as longitudinal and anterior-posterior diameters ("ld" and "apd" dashed lines, respectively). A schematic model of SV volume calculation is reported, using the "ellipsoid/prolate spheroid (d1 >d2 = d3) " (red ellipse) mathematical formula (d1 × d2 × d3 × 4/3 × π), with d1 = ld and d2 = apd, and d3 assumed = d2 (red dashed line) (according to Lotti et al. 2012). **Panel** (**e**), distal vas deferens (dVD) and deferential ampulla (DA) beside a section of the seminal vesicle (SV) assessed by "end fire" probe in sagittal scan. Bladder (B) and prostate (Pr) are visible. **Panel** (**f**), left figure: section of a dilated deferential ampulla (DA) beside a dilated seminal vesicle (SV) with areas of endocapsulation (*) and thick septa (arrow) detected by "end fire" probe in sagittal scan. Right figure: dilated, inhomogeneous epididymal *tail* and proximal vas deferens (pVD), with coarse calcifications (arrow). **Panel** (**g**), left figure: dilated (>12 mm), inhomogeneous, hypoechoic epididymal head; right figure: abrupt interruption of the proximal vas deferens (pVD) in a man with congenital bilateral absence of vas deferens. Epididymal *body* and *tail* are also visualized in sagittal scan. **Panel** (**h**), prostate with hyperemia end elevated arterial peak systolic velocity. **Panel** (**i**), midline prostatic cyst (*) in transversal (left) and sagittal (right) scan. The prostatic utricle is indicated with an arrow. P, prostate; B, bladder. **Panel** (**j**), upper figure, ejaculatory duct dilation (arrow) and microcalcification (short arrow), and seminal vesicle cyst (#), assessed by "end fire" probe in sagittal scan. Lower figure, ejaculatory duct cyst (*). SV, seminal vesicle; Pr, prostate; U, urethra; B, bladder

subject), suggesting chronic/past but not acute/subacute inflammation. Subsequently, increasing evidence suggested a TRUS role in identifying current prostate inflammation by evaluating "dynamic" CDUS findings such as hyperemia and high peak systolic velocity (PSV) detected in prostatic arteries. Cho et al. suggested ≥15 parenchymal Doppler spots as indicative of prostate hyperemia (Lotti and Maggi 2015). Berger et al. suggested a PSV >15 cm/s, evaluated in the arteries of the transitional zone, to define prostatic inflammation in men with age >45 years and benign prostatic hyperplasia (Lotti and Maggi 2015). Lotti et al. reported, in an evidence-based way, that a prostatic artery PSV >11 cm/s could identify prostatitis-like symptoms in relatively young subjects (males of infertile couples) (Lotti et al. 2014b) (see Table 6.3). Accordingly, in healthy fertile men, the recent EAA US study found a prostatic artery PSV <11 cm/s (Table 6.2). As a corollary, some authors demonstrated that prostatic arterial PSV was positively associated with a score related to premature ejaculation severity, confirming from an US point of view that clinical prostate inflammation is an organic cause of premature ejaculation (Lotti and Maggi 2015).

6.3.3.1.3 Ejaculatory Duct (ED) Obstruction/Abnormalities

Ejaculatory duct obstruction (EDO) affects 1–5% of infertile men and may be congenital or acquired. Congenital causes include EDs atresia/stenosis, midline prostatic cysts, or ED congenital cysts (Lotti and Maggi 2015; Singh et al. 2012). Acquired causes may be secondary to infection/inflammation, post-infective/inflammatory midline prostatic cysts, calcifications, or iatrogenic. Detection of bilateral EDO by TRUS is useful in defining the diagnosis of obstructive azoospermia and its clinical management, considering surgical treatments if specific abnormalities are found (see below and Table 6.3). Subjects with congenital or noninfectious causes of EDO, or with partial EDO, have better improvements in semen parameters after treatment than those with infectious causes or complete EDO. TRUS findings in suspected EDO include midline prostate cysts and ED dilation, calcifications, or cysts (Lotti and Maggi 2015). Dilated SV (anterior-posterior diameter >15 mm) and enlarged deferential ampulla (diameter >6 mm) have also been previously suggested as EDO-related findings (see below). As a corollary, Lotti et al. (2012) proposed a new parameter related to the SV emptying capacity, "SV ejection fraction," reporting a cutoff (21.6%) suggestive of complete or partial EDO. However, further studies are needed to assess the clinical relevance of this parameter.

Intraprostatic cysts can be classified as congenital or acquired, or, based on their position within the prostate, as midline, paramedian, and lateral cysts (Singh et al. 2012; Lotti and Maggi 2015). *Midline cysts* affect 1–5% of men, with a higher frequency in infertile men. They may cause partial or complete EDO, with reduced sperm count or obstructive azoospermia, respectively, often associated with SV obstruction/dilation, reduced ejaculate volume, and pH. At TRUS, they appear as roundish or pear/oval-shaped anechoic formations in transversal and longitudinal scans, respectively. According to previous studies, two main different cystic entities have been recognized. The first, **Müllerian cyst**, is thought to arise from a regression failure of the Müllerian ducts, causing a focal saccular dilation. This cyst is located at midline or slightly lateral to midline, is large and may extend above the base of the prostate, does not communicate with the urethra or contain spermatozoa, and may be associated with various genitourinary abnormalities. However, it eventually may erode ED and include sperm. The second, **utricular cyst**, is thought to derive from dilation of the prostatic utricle, is strictly midline, smaller than the

former, and confined to the prostate; it communicates with the urethra and usually contains spermatozoa. Both midline cysts may cause EDO by deviating or compressing ED. Midline cyst-related EDO may be diagnosed only after TRUS-guided cyst aspiration (Singh et al. 2012; Lotti and Maggi 2015; Lotti et al. 2018), which will allow cyst reduction and restore semen emission. This is of clinical relevance, since aspiration of large cysts in subjects with obstructive azoospermia may lead to semen parameter improvement (Table 6.3). However, after this procedure, midline cysts may enlarge and lead to EDO and azoospermia again, after variable times. In this case, TRUS should be considered to evaluate cyst recurrence (Table 6.3). Various complications may be associated with prostate cysts, besides infertility, such as urinary tract infection, pain, recurrent epididymitis or prostatitis, and hemospermia (Singh et al. 2012). A recent study (Lotti et al. 2018) performed on a large series of males of infertile and fertile couples reported that infertile men with a midline prostatic cyst (MPC) showed a lower seminal volume and sperm count and a higher prevalence of azoospermia than the rest of the infertile sample or fertile men, and a higher frequency of US signs suggestive of EDO. A midline prostatic cyst with a volume >0.117 ml or a transversal diameter >5 mm identified subjects with severe oligo- or azoospermia with an overall accuracy of ~75%. Accordingly, in fertile men, the highest MPC volume was 0.117 ml, suggesting it as a biological threshold not compromising semen quality. Eleven men with infertility, semen abnormalities, and large MPC (>0.250 ml) underwent TRUS-guided cyst aspiration, which led to sperm count improvement in all patients and natural pregnancy in some cases. Finally, the authors proposed an algorithm, based on semen parameters, useful in identifying a MPC in males of infertile couples. Hence, midline prostatic cyst size cutoffs for complete or partial EDO are now available, and large cysts must be considered as a possible easily treatable causes of obstructive infertility. In fact, TRUS-guided cyst aspiration can be suggested for a cyst volume >0.250 ml (and, eventually, for a volume >0.117 ml) in subjects with suspected obstructive severe oligo- or azoospermia, especially with FSH <8 U/l, to obtain seminal parameters improvement and eventually natural pregnancy.

Ejaculatory duct (paramedian) cysts may be congenital, originating from Wolffian ducts, or acquired, and may be related or not to cystic fibrosis (Singh et al. 2012). Uni- or bilateral, they may lead to obstructive azoospermia. In this case, cyst detection by TRUS is useful in clinical management (Table 6.3), their surgical treatment often restoring semen emission (Lotti and Maggi 2015). **Ejaculatory duct dilation** has been defined as an ED diameter >2 mm (Lotti and Maggi 2015; Singh et al. 2012) and may be related to inflammatory distal stenosis, which is often difficult to detect. **Ejaculatory duct calcifications** may be associated with EDO, but are not a reliable indicator of it (Lotti and Maggi 2015). They have also been associated with hemospermia and prostatitis-like symptoms (Lotti et al. 2014b; Lotti and Maggi 2015). Accordingly, EDO may be associated with hemospermia, prostatitis, and painful ejaculation. In select cases, transurethral resection of EDs resulted in marked improvement in semen parameters, and pregnancies have been achieved (Lotti and Maggi 2015).

6.3.3.1.4 Prostate Cancer

Prostate cancer is usually suspected on the basis of digito-rectal examination and/or increased PSA levels. At TRUS, prostate cancer is often seen as a hypoechoic lesion in the peripheral zone of the gland; however, it can be isoechoic or hyperechoic, and it is not always anatomically well defined (Sarkar and Das 2016). Definitive diagnosis depends on histopathological verification of cancer in prostate biopsy cores or specimens from transurethral resection of the prostate or prostatectomy for benign prostatic enlargement (Mottet et al. 2021). According to EAU guidelines, gray scale TRUS is not reliable in detecting prostate cancer (Mottet et al. 2021). Thus, there is no evidence that US-targeted biopsies can replace systematic ones and there is not enough evidence for TRUS routine use in prostate cancer assessment. Nowadays, multiparametric MRI (mpMRI) is considered the best imaging tool to evaluate prostate cancer with high aggressiveness (Mottet et al. 2021). Adherence to PI-RADS guidelines for mpMRI acquisition and interpretation is strongly recommended.

6.3.3.2 Seminal Vesicles and Deferential Ampullas

6.3.3.2.1 US Anatomy

Seminal vesicles (SV) are paired and saccular structures, which lie superior and posterior to the prostate between the bladder and the rectum. They produce an alkaline fluid contributing ~80% of the ejaculate volume. At TRUS, SV have a typical "bow-tie" appearance in transversal scans, and a tennis-racket shape in longitudinal scans (Lotti et al. 2012; Lotti and Maggi 2015). SV echotexture is characterized by homogenous fine echoes and is slightly less echogenic than the prostate. In relatively young subjects, SV volume is negatively associated with age and tends to shrink after the fifth decade, showing a significant reduction in the eighth compared to the fourth decade. SV volume increases with sexual abstinence, whereas it decreases in current smokers as a function of smoking habit and of lifetime exposure to cigarette smoking. SV volume is also positively regulated by testosterone (Lotti and Maggi 2015), prolactin (Lotti et al. 2013), and free triiodothyronine (fT3) levels (Lotti et al.

2016). While most of the available studies assessed **SV diameters**, Lotti et al. (2012) proposed to calculate **SV volume** by measuring the maximum longitudinal and anterior–posterior diameters, using the "ellipsoid/prolate spheroid" mathematical formula. SV volume varies with ejaculation and is positively related to the **ejaculate volume**, but not with sperm parameters. SV emptying with ejaculation is positively related to fT3 levels, and subjects with subclinical hyperthyroidism show a higher reduction of SV longitudinal diameters after ejaculation as compared with eu- and hypothyroid men (Lotti et al. 2016). The **deferential ampullas** appear at TRUS as oval structures medial to the SV in transversal scans, cephalic to the prostate, or as distal VD enlargements in longitudinal scans (Lotti and Maggi 2015). They have an echotexture similar to that of SV.

6.3.3.2.2 US Normal and Abnormal Patterns

Volume

SV volume abnormalities include dilation and hypoplasia. **SV dilation** has been defined, based on SV diameters, as a SV anterior–posterior diameter >14 mm or 15 mm, suggestive of EDO (Lotti and Maggi 2015). Lotti et al. (2012) proposed an algorithm calculating SV volume. So far, a volumetric cutoff for SV dilation is lacking. However, the EAA US study recently reported the reference ranges of SV diameters and volume in healthy, fertile men, suggesting the higher SV reference values evaluated after ejaculation as possible cutoffs distinguishing normal and dilated SV (Table 6.2). A higher post-ejaculatory SV volume has been associated with a higher prevalence of SV abnormalities (see below), a higher prostate volume, and detection of a prostatic midline cyst (Lotti et al. 2012), causing partial or complete EDO (Lotti et al. 2018), as well as signs suggestive of upstream MGT dilation, such as higher deferential and epididymal tail diameters (Lotti et al. 2012).

SV hypoplasia has been defined as a SV anterior–posterior diameter <5 mm or <7 mm or as SV longitudinal diameter <25 mm (Lotti and Maggi 2015). The term mainly refers to congenitally small SV, although an acquired form may be associated with testosterone deficiency. So far, a SV volume cutoff for SV hypoplasia is lacking. Similarly, to as reported above for SV dilation, the EAA US study suggested the lower reference values of SV diameters and volume evaluated after ejaculation as possible cutoffs distinguishing normal and hypoplastic SV (Table 6.2).

6.3.3.2.3 Echopattern Abnormalities

Several US features are suggestive of SV abnormalities and have been associated with inflammation or stasis. In particular, "SV areas of endocapsulation," that should be assessed after ejaculation (Lotti et al. 2012; Lotti et al. 2018), are considered a feature suggestive of EDO (Lotti and Maggi 2015).

6.3.3.2.4 Obstruction-Related Findings

Enlarged SV anterior-posterior diameter, as well as "SV areas of endocapsulation" (see above), has been related to partial EDO (Lotti and Maggi 2015). Diagrams showing partial EDO percentage probability in function of SV anterior-posterior diameter variation have been reported. Reduced "SV ejection fraction" (<21.6%) is suggestive of impaired SV emptying and partial EDO and is associated with higher prevalence of SV giant cysts and ED abnormalities (dilation, calcifications, or cysts) (Lotti et al. 2012).

Reduced or absent contraction of SV during ejaculation without a clear obstructive cause has been defined as "functional SV atony." Signs suggestive of SV atony have been reported in subjects with type 2 diabetes mellitus with or without diabetic neuropathy (Lotti and Maggi 2015).

6.3.3.2.5 SV Agenesis, Hypoplasia, and Cysts

SV congenital abnormalities include defects in number (agenesis, fusion), maturation (hypoplasia), and canalization (cysts) of the glands (Lotti and Maggi 2015). Their detection is clinically relevant, because they are often associated with abnormal development of other mesonephric/metanephric derivatives, such as the vas deferens, ureter, and kidney, which should be evaluated by US. **Unilateral SV agenesis** arises if an insult occurs before the seventh week of gestation, when the ureteric bud arises from the mesonephric duct (Kim et al. 2009; Lotti and Maggi 2015). It is often associated with ipsilateral renal agenesis (~80%) or other renal abnormalities (Kim et al. 2009). **Bilateral SV agenesis** is associated with CFTR mutations in ~70% of cases, with CBAVD in half of the cases and with normal kidneys (Kim et al. 2009). **Congenital SV hypoplasia** may be isolated or associated with other congenital genitourinary anomalies (Kim et al. 2009). **SV cysts** are rare and may be congenital or acquired (Lotti and Maggi 2015). Congenital SV cyst may be isolated or, more frequently, associated with other genitourinary anomalies. They are mainly secondary to EDO caused by development abnormalities of the distal portion of the mesonephric duct. They are associated with ipsilateral renal agenesis (Zinner syndrome) or dysgenesis in two-thirds of cases (Lotti and Maggi 2015). Ectopic ureteral insertion into the SV, ED, vas deferens, or prostatic urethra or vas deferens agenesis may also be present (Kim et al. 2009). Bilateral SV cysts have been reported to occur in about half of patients with autosomal-dominant polycystic kidney disease (Lotti and Maggi 2015). Hence, detection of SV cysts by TRUS is clinically relevant, leading to careful evaluation of the urinary tract by US. Acquired cysts are usually unilateral and associated with inflammation-related EDO (Lotti and Maggi 2015). Cystic SV dilatation has been associated with perineal pain.

6.4 Specific Applications of Scrotal and Transrectal US

6.4.1 Sensitivity and Specificity in Discriminating Obstructive and Nonobstructive Azoospermia

Du et al. (2010) reported that performing simultaneous scrotal and transrectal US, assessing TV and genital tract obstruction-related findings, respectively, discriminates obstructive and nonobstructive azoospermia (OA and NOA, respectively) with 95% sensitivity and 97% specificity and may be of help in evaluating OA etiology. Abdulwahed et al. (2013) reported that scrotal US is more sensitive for OA and specific for NOA detection, while TRUS showed the opposite trend. Both imaging examinations had greater specificity than sensitivity for OA, indicating that US is better able to exclude, more than to diagnose, OA. However, US is still unlikely to replace testicular biopsy.

6.4.2 Testis US and Surgical Sperm Retrieval in Azoospermic Subjects

Sperm retrieval by testicular surgery has been reported in 50–60% of men with NOA and in almost all with OA (Lotti and Maggi 2015). Among the different CDUS characteristics investigated as predictors of successful sperm retrieval by testicular biopsy in azoospermic men, testicular parameters such as TV and vascularization have shown increasing evidence in the last few years, although still controversial and with limited clinical utility (see below). Azoospermic men with normal FSH levels and positive sperm retrieval have higher TV compared to those with a negative harvesting (Lotti and Maggi 2015). TV in OA has been reported as higher than in NOA patients (Du et al. 2010). Subjects with CBAVD-related OA usually have testes with normal volume and function (Singh et al. 2012) and sperm retrieval by testicular biopsy is virtually certain. In NOA subjects, most, but not all, studies reported higher TV in subjects with a positive sperm retrieval (Lotti and Maggi 2015). TV has been reported as an independent parameter related to testicular biopsy outcome. A total TV of 16 ml or a mean TV of 9.5 ml have been proposed as a cutoff for a positive sperm retrieval (Lotti and Maggi 2015). However, other authors report that TV is not a useful parameter for sperm retrieval prediction (Lotti and Maggi 2015). In fact, sperm can be retrieved by surgery even in men with very small testis, such as those with Klinefelter's syndrome (Lotti and Maggi 2015). On the other hand, subjects with spermatogenic arrest, usually showing normal TV and FSH levels, are characterized by a poor surgical outlook. Hence, even if US has some prognostic value in surgical sperm retrieval outcomes, it is limited, playing a limited role in the workup of the infertile male. Interestingly, some vascular parameters detected by testicular CDUS have been associated with sperm quality (Lotti and Maggi 2015; Lotti et al. 2021a), suggested as useful in discriminating OA and NOA or residual spermatogenic areas in NOA. However, at present, they have been evaluated only for research purposes, with no impact on the clinical management of the azoospermic men.

6.4.3 Scrotal and Transrectal US and Hormonal Treatments

The US assessment of testis, prostate, and SV characteristics before the beginning and/or during hormonal treatment represents an effective tool in evaluating the response of target organs and in monitoring suspicious findings. Baseline US-assessed TV represents one of the main determinants of gonadotropin responsiveness in subjects with hypogonadotropic hypogonadism (HH). In fact, a better response in terms of sperm output and ongoing pregnancy has been observed for basal TV >4 ml, although a subsequent meta-analysis failed to find any significant association between TV and appearance of spermatozoa in the semen upon gonadotropin therapy. In addition, US has been performed by some authors to evaluate TV increment during hormonal treatment. GnRH or gonadotropin treatments in HH subjects were associated with a TV increase. A 12-week treatment with FSH in men with idiopathic infertility demonstrated a TV increase of 5 ml compared to baseline (Lotti and Maggi 2015). Considering that among infertile and azoospermic men, the risk of testicular malignancy is higher, scrotal US could be performed with prevention purpose in azoospermic and/or HH subjects unresponsive to hormonal treatment (Lotti and Maggi 2015). However, at present, no agreement on US testis surveillance in these subjects is available. **TRUS** is a useful tool in evaluating **prostate response to hormonal treatment** in HH subjects under gonadotropin, GnRH, or T treatment (Behre et al. 1995; Lotti and Maggi 2015), monitoring their possible effect on volume increment. In addition, SV volume shows changes at US during hormonal supplementation of hypogonadal men or hypogonadic subjects with Klinefelter syndrome (Lotti and Maggi 2015).

6.5 Penile US

6.5.1 Penile US: Indications

Indications for performing penile US are reported in Table 6.1.

6.5.2 Penile US: Methodological Standards

Although penile US assessment is not fully standardized, efforts toward methodological standardization and interpretation of findings have been made (Aversa and Sarteschi 2007; Patel et al. 2012; Sikka et al. 2013). In particular, **standard operating procedures** (SOPs) for the **vascular assessment of ED** have been reported (Sikka et al. 2013). An isolated, quiet room with dim light is required. The patient must be lying on his back and a 7–15 MHz linear probe must be used and located at the peno-scrotal junction with the penis resting on the soprapubic region. The position of the sample volume should be at the peno-scrotal junction, because measurements at more distal sites are influenced by the intracavernosal pressure degree, leading to lower PSV detection. The sampling of the main cavernous artery must be verified to avoid secondary vessel or a penetrating branch from the dorsal artery. The angle of insonation should be between 40° and 60°, preferably 60°. The sample volume and angle correction should follow the direction of vascular flow. In our opinion, vascular parameters should be evaluated in the first linear part of the cavernosal artery, immediately after the end of the cavernous artery bending in the cavernous body, avoiding ascents and descents of "corkscrew" arteries characterized by flow turbulence. Although some authors (Corona et al. 2008; Corona et al. 2010; Rastrelli et al. 2014) report that flaccid PCDU could be performed alone or before dynamic evaluation, the universal consensus is to perform dynamic PCDU under pharmacologically induced erection (Sikka et al. 2013; Salonia et al. 2021). For this purpose, an **intracavernosal injection (ICI)** of a single or combination of vasoactive agents (e.g., **prostaglandin E1** [PGE1], phentolamine, and/or papaverine) is administered. ICI with 10 mcg PGE1 is frequently used in clinical practice and in most of the available studies. After vasodilating agent injection, **Doppler measurements** of vascular parameters should be monitored for at least 20 (Aversa and Sarteschi 2007) - 30 (Sikka et al. 2013) minutes. In fact, the highest PSV develops after 5–6 minutes from the vasodilating agent injection in four out of five subjects, while about 20% of patients have a greater latency period (range of 1–18 min). Patient's anxiety should be avoided or reported in the medical report, since its related sympathetic stimulation can lead to vasoconstriction and false low PSV measurement in healthy men (Aversa and Sarteschi 2007; Patel et al. 2012; Sikka et al. 2013). The most frequently used vascular parameters considered to evaluate ED in dynamic conditions are **peak systolic velocity (PSV)** and **end-diastolic velocity (EDV)**. In most of the studies, a PSV <35 cm/s is considered suggestive of arterial insufficiency (Aversa and Sarteschi 2007; Sikka et al. 2013; Patel et al. 2012), while a PSV <25 cm/s is indicative of "severe" arterial insufficiency. An EDV >5 cm/s is suggestive of **veno-occlusive dysfunction (VOD)** (Aversa and Sarteschi 2007). Although the aforementioned cutoffs are commonly used, the EAU suggests a PSV <30 cm/s and EDV >3 cm/s as indicative of arterial insufficiency and VOD, respectively (Salonia et al. 2021). Attention should be paid to diagnosis of VOD. Primary VOD is in fact relatively rare, usually due to tunica albuginea degenerative changes (e.g., in Peyronie's disease, diabetes, elderly men) or traumatic injury (penile fracture), structural alterations in the fibroelastic components of the trabeculae, cavernous smooth muscle, and endothelium (e.g., in Peyronie's disease), or acquired venous shunts (e.g., after surgical correction of priapism) in presence of normal arterial supply. More frequently, VOD is secondary to arterial insufficiency (Sikka et al. 2013). In this case, VOD does not represent the cause of ED, but a PCDU finding associated with low arterial flow. In fact, reduced arterial flow cannot guarantee blood supply enough to allow a complete swelling of the corpora cavernosa, leading to low or absent compression of peripheral veins against the tunica albuginea and venous leakage (Patel et al. 2012). Finally, in flaccid conditions, a PSV <13 cm/s is considered indicative of arterial insufficiency (Corona et al. 2008), while a FPA <1.17 m/s (Lotti et al. 2021a) is suggestive of severe arterial insufficiency and is considered a predictor of forthcoming MACE (Rastrelli et al. 2014).

6.5.3 Penile US: Anatomy, Normal and Abnormal Patterns, Clinical Utility, and US Standards

Figure 6.4 shows some normal and abnormal vascular and structural patterns of the penis.

6.5.3.1 US Anatomy and Normal Patterns

A detailed description of penile US anatomy is available in previous studies (Patel et al. 2012; Jung et al. 2018). The penis consists of two paired **corpora cavernosa**, which are the main erectile bodies, and the corpus spongiosum, which contains the urethra. The corpora cavernosa are wrapped in the tunica albuginea, a resistant, inextensible, connective envelope, and are separated by the intracavernosal septum. The intercavernous septum is perforated, allowing for communication of blood (and injected pharmacological agents) across the midline. Proceeding from the inside out of the penis, Buck fascia and dartos fascia are located below the skin. Between the tunica albuginea and the Buck fascia, in the dorsal portion of the penis, two dorsal penile arteries run, the deep dorsal vein and paired neurovascular bundles. The superficial dorsal veins are contained between the Buck fascia and the outer dartos fascia. The inner portion of the corpora consists of interconnected sinusoids separated by smooth muscle trabeculae and is crossed by cavernosal arteries in the central part. The primary source of blood supply to

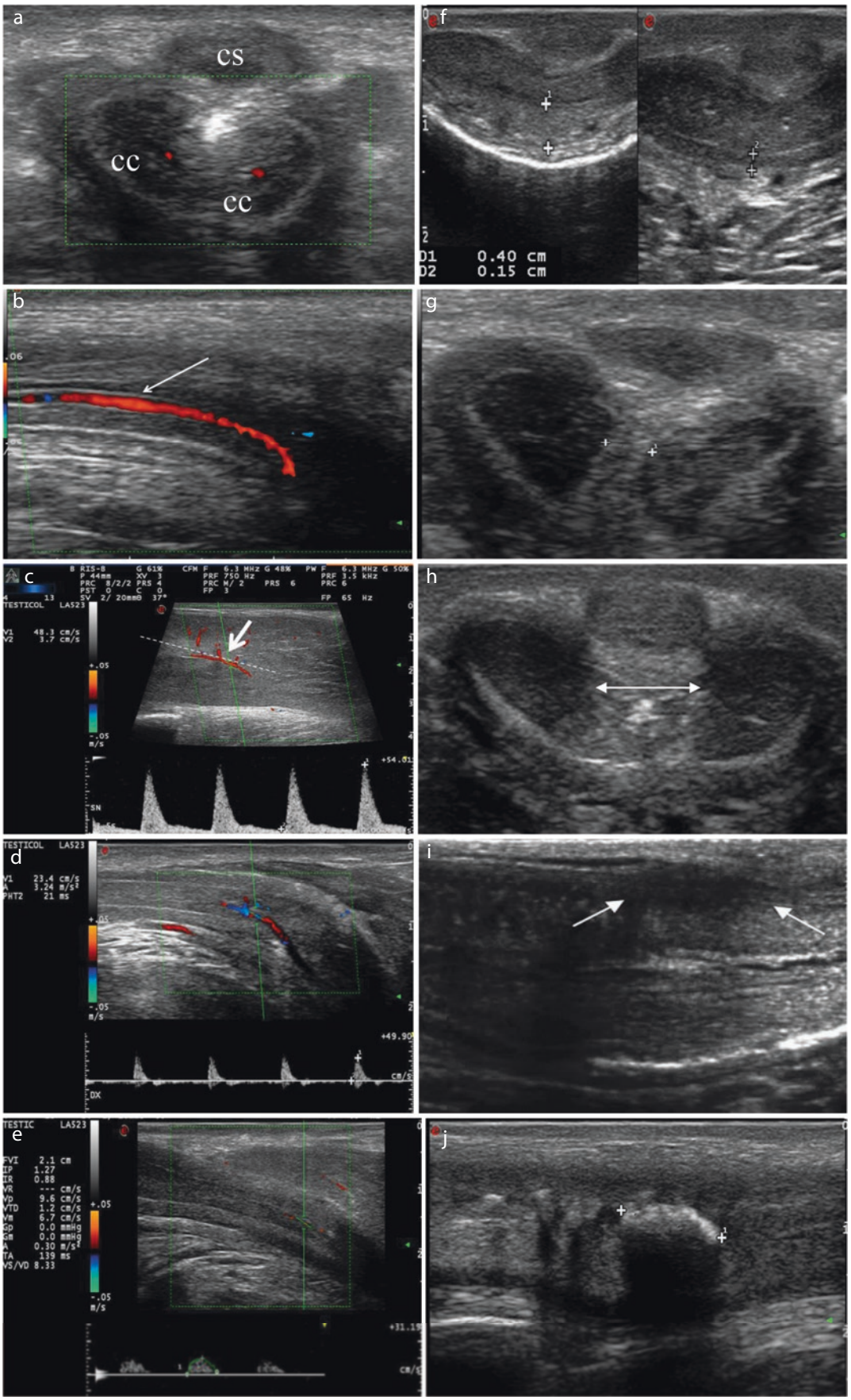

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Fig. 6.4 Normal and abnormal US patterns of the penis. **Panels (a–e): V**ascular anatomy end evaluation of erectile dysfunction. **Panel (a)**. Corpora cavernosa (cc) and corpus spongiosum (cs) of the penis in transversal scan. Cavernosal arteries are revealed by CDUS as red dots in the center of the corpora cavernosa. **Panel (b).** Normal cavernosal artery detected in sagittal scan. Arrows indicated the right position to locate the sample volume to evaluate vascular CDUS parameters. **Panel (c).** The sample volume and angle correction (preferably 60°) should follow the vascular flow direction. A normal waveform at dynamic PCDU is shown, characterized by a rapid ascent (steep rise, culminating in a sharp peak) and a relative rapid descent, with a normal peak systolic velocity (>35 cm/s) and end-diastolic velocity (<5 cm/s). **Panel (d).** A normal waveform at flaccid PCDU is shown, characterized by a rapid ascent (steep rise, culminating in a sharp peak) and a relative rapid descent, resembling a "mountain," with a normal peak systolic velocity (>13 cm/s) and acceleration. **Panel (e).** An abnormal waveform at flaccid PCDU is shown, characterized by a slow ascent and slow descent, resembling a "hill," with an abnormal peak systolic velocity (<13 cm/s) and acceleration (<1.17 m/s^2) in a subject with erectile dysfunction and type 2 diabetes. **Panels (f–j):** US characteristics of Peyronie's disease. **Panel (f).** Abnormal/thickened (left side) and normal (<2 mm) tunica albuginea (transversal scan). **Panel (g).** Thickened, hyperechoic, intercavernosal septum (transversal scan). **Panel (h).** Thickened intercavernosal septum with a calcification and periseptal fibrosis (slightly hyperechoic regions of the corpora cavernosa) (transversal scan). **Panel (i)**. Hypoechoic plaque of the tunica albuginea in a subject with recent onset of Peyronie's disease (longitudinal scan). **Panel (j)**. Hyperechoic, calcified plaque under the tunica albuginea, involving the corpora cavernosa, in a subject with long-lasting Peyronie's disease (longitudinal scan)

the penis is usually through the internal pudendal artery, which arises from the anterior division of the internal iliac artery. The internal pudendal artery becomes the common penile artery after giving off a branch to the perineum. The branches of the penile artery are the dorsal artery, the bulbourethral artery, and the cavernosal artery. During erection, the **cavernosal artery** is responsible for the **tumescence** of the corpus cavernosum during erection, and the dorsal artery and the bulbourethral artery are responsible for engorgement of the glans penis. Distally, three branches of penile arteries join to form a vascular anastomotic ring near the glans. Variants in arterial anatomy exist in up to 20% of men, but their relevance to clinical practice is not fully established. The cavernosal artery gives off many helicine arteries, which in turn flow into the sinusoids via multiple arterioles supplying the sinusoids and the trabecular erectile tissue. The helicine arteries are contracted and tortuous in the flaccid state and become dilated and straight during erection. **Venous drainage** from the three corpora originates in the tiny venules beneath the tunica albuginea, forming the subtunical venular plexus before exiting as the emissary veins. Outside the tunica albuginea, a prominent deep dorsal vein provides the main venous drainage via the spongiosal, circumflex, and cavernosal veins. Then, a single deep dorsal vein runs upward behind the symphysis pubis to join the periprostatic venous plexus.

PCDU dynamic "phases" and related changes in the waveform have been well described elsewhere (Aversa and Sarteschi 2007; Patel et al. 2012). In the **flaccid penis**, the resting smooth muscle tonicity of the cavernosal arterioles and sinusoids is elevated, leading to a high-resistance vascular bed with resultant low volume inflow and outflow. When stimulated to **erection**, relaxation of the smooth muscle occurs through increased parasympathetic drive from the sacral nervous plexus. This relaxation results in increased cavernosal artery flow, leading to engorgement of the cavernosal sinusoids and penile lengthening and tumescence. The engorging sinusoids compress the exiting venules and emissary veins, leading to passive limitation of venous outflow. The high inflow and restricted outflow rapidly increase intracorporal pressure leading to erected penis rigidity. When intracorporal pressure nears systolic pressure, reduction in inflow will also normally occur. When intracorporal pressure exceeds systolic pressure for a long time, as in the case of ischemic priapism, reduction in inflow occurs.

At US, in sagittal scans, the corpora cavernosa are depicted as longitudinally orientated vascular beds of mixed echogenicity, with the tunica albuginea visualized as a thin echogenic envelope, usually <2–3 mm thick (Patel et al. 2012; Jung et al. 2018). In the absence of cavernosal fibrosis, the interface between the tunica and the underlying cavernosal tissue is distinct. The corpus spongiosum is visualized on the ventral surface and is of slightly higher reflectivity than the corpora cavernosa. The cavernosal arteries can be identified within the corpora cavernosa at US as parallel hypoechoic lines inside a hyperechoic binary. In transversal scan, the two corpora cavernosa are displayed as two circles of mixed echogenicity, with the surrounding tunica albuginea and a hypoechoic central spot with a thin hyperechoic labrum (section of the cavernosal artery), while the corpus spongiosum appears as a smaller median circle between and below their ventral and median surfaces. At rest, the normal cavernosal artery has a diameter of 0.3–0.7 mm (Patel et al. 2012), and an intima-media thickness (IMT) <0.3 mm (Caretta et al. 2019). At color Doppler, in longitudinal scan, a colored (red) line can be observed running across each corpora cavernosa, representing the cavernosal artery, which, in transversal scan, appears as a red dot in the center of the corpora cavernosa circular section 2 (Patel et al. 2012; Jung et al. 2018). During erection, the normal cavernosal artery diameter can increase up to 1 mm, and its ramifications can be well identified. At rest, sampling the cavernosal arteries, the normal PSV is >13 cm/s (Corona et al. 2008), while during erection the normal PSV and EDV are >35 cm/s (or 30 cm/s according to EAU guidelines) and <5 cm/s (or 3 cm/s according to EAU guidelines), respectively (Aversa and Sarteschi 2007; Patel et al. 2012).

6.5.3.2 US Abnormal Patterns

6.5.3.2.1 Erectile Dysfunction (ED)

As reported above, PCDU is considered a second-level procedure to assess ED (Sikka et al. 2013; Salonia et al. 2021). PCDU can be performed in flaccid or dynamic conditions. According to international guidelines, **dynamic PCDU**, performed after ICI (usually of PGE1, 10 mcg), is considered the **gold standard** method to assess vasculogenic ED (Sikka et al. 2013; Salonia et al. 2021). At rest (flaccid PCDU), ED due to arterial insufficiency is defined for a PSV <13 cm/s (Corona et al. 2008) and/or a FPA <1.17 m/s (Rastrelli et al. 2014). The aforementioned thresholds are also considered predictive of forthcoming **MACE** (Corona et al. 2008; Rastrelli et al. 2014) (see above). After ICI (dynamic PCDU), arterial insufficiency is defined for a PSV <35 cm/s (or 30 cm/s according to EAU guidelines), while "severe" arterial insufficiency is defined for a PSV <25 cm/s (Aversa and Sarteschi 2007; Corona et al. 2010; Patel et al. 2012). A PSV <25 cm/s is considered predictive of forthcoming MACE (see above). In addition, recently, some authors reported that a cavernosal artery IMT >0.3 mm, evaluated in dynamic state, can be considered as an additional parameter indicating an "unhealthy" artery, predictive of forthcoming MACE (Caretta et al. 2019). In fact, vasculogenic ED can manifest earlier than coronary, carotid, or femoral artery diseases because the smaller penile arteries reach critical narrowing, with insufficient blood flow, earlier than larger vessels ("**artery size hypothesis**") (Corona et al. 2010). Accordingly, a previous study reported, in a limited number of subjects, that men with positive ischemic heart disease (IHD) at stress electrocardiography show lower PSV and a significant higher prevalence of PSV <25 cm/s than those with negative IHD (Shamloul et al. 2004). Accordingly evaluating 60 men (25 with IHD and ED, 25 with IHD alone and 10 with ED alone) with invasive angiography for coronary arteries and aorto-ilio-pudendal arteries and dynamic PCDU, some authors reported that men with ED alone had a higher rate of peripheral lesions than patients with coronary heart disease alone and that 10% of patients with ED alone had coronary heart disease (Sanad et al. 2020). In addition, men with penile arterial insufficiency show carotid and/or lower limb US abnormalities in 75% of cases (Vicari et al. 2005), and subjects with higher cavernous artery IMT show higher IMT in common carotid and femoral arteries (Caretta et al. 2019).

As a corollary, other parameters can be considered suggestive of arterial insufficiency at PCDU. In a qualitative analysis, both at rest and in dynamic conditions, a normal waveform resembles a "mountain," with a rapid ascent (steep rise, culminating in a sharp peak) and a relative rapid descent, while an abnormal one resembles a "hill." In addition, at dynamic PCDU, when diffuse arteriolar damage is present, cavernosal arteries ramifications are reduced (Aversa and Sarteschi 2007; Patel et al. 2012).

Arterial insufficiency can be associated with "secondary" **veno-occlusive dysfunction** (VOD), which can be defined only during dynamic PCDU when EDV is >5 cm/s (or >3 cm/s according to EAU guidelines) beside low PSV (secondary VOD) (Aversa and Sarteschi 2007; Patel et al. 2012). Conversely, "primary" VOD can occur in specific cases (Aversa and Sarteschi 2007; Bertolotto et al. 2009; Patel et al. 2012), including tunica albuginea degenerative changes (e.g., in Peyronie's disease, diabetes, old men) or traumatic injury (penile fracture), structural alterations in the fibroelastic components of the corpora cavernosa (e.g., in Peyronie's disease) or acquired venous shunts (e.g., after surgical correction of priapism), leading to lack of complete compression of exiting venules and emissary veins against the tunica albuginea (see above "methodological standards"). In these cases, there is a normal arterial supply (normal PSV) but an elevated EDV, suggesting venous outflow as the primary cause of vasculogenic ED.

6.5.3.2.2 Peyronie's Disease

Peyronie's disease (PD) is a connective tissue disorder characterized by the occurrence of **fibrous plaques in the tunica albuginea** surrounding the corpora cavernosa, possibly associated with localized partial fibrosis of the corpora cavernosa (Bertolotto et al. 2009; McCauley and Dean 2020). PD is characterized by pain with erection (especially at PD onset), palpable penile nodules/plaques, abnormal curvature of the penis and/or penile loss of girth, shortening or deformity/indentation, evident in the erected penis, and can lead to ED (see below). **Pharmacologically induced erection** is the gold standard to evaluate PD plaques as well as to evaluate the curvature, severity, and morphological abnormalities of the penis when a sufficient erection is achieved after ICI (Bertolotto et al. 2009; McCauley and Dean 2020). However, in our opinion, plaques and other US abnormalities (see below) can be evaluated even in the **flaccid state**, and penile curvature or abnormalities can be assessed by **photographic documentation** performed during erection induced with autoeroticism by the patient at home. PD plaques are identified as localized or diffuse areas in which the tunica albuginea is thickened (>2 mm) (Bertolotto et al. 2009; McCauley and Dean 2020). The plaques are more often located on the dorsal part of the penis, but they can also be found ventrally or laterally (Bertolotto et al. 2009). Some authors report the identification of hypoechoic plaques at PD onset (acute phase); however, a normoechoic or slightly hyperechoic plaque (tunica albuginea thickness) is often observed both in acute and in chronic PD phases (Bertolotto et al. 2009; McCauley and Dean 2020). Along with tunica albuginea thickness, other abnormalities can be observed in PD, including micro- or macrocalcifications within the tunica albu-

ginea, the intercavernosal septum or the corpora cavernosa; septal fibrosis/thickness; intracavernous diffuse or partial fibrosis, characterized by slightly hyperechoic areas within the corpora cavernosa; tunica albuginea partial or extended (up to circular) deformity leading to an objectively localized indentation of the penis or to hourglass/bottleneck morphology, respectively (Bertolotto et al. 2009; McCauley and Dean 2020). The aforementioned abnormalities can sometimes be detected alone, representing US findings of PD with no clear tunica albuginea thickness/plaques. When severe tunica albuginea degenerative changes or intracavernous diffuse fibrosis (extending to the smooth muscle of the corpora) occur, a patient with normal cavernosal arterial supply can experience ED due to primary VOD (Aversa and Sarteschi 2007; Patel et al. 2012) (see above). However, rarely, fibrosis can involve the media of penile arteries leading to PD-related vasculogenic ED (McCauley and Dean 2020). More often, ED in patients with PD can be a consequence of aging and/or other comorbidities (McCauley and Dean 2020), and a mixed ED (including both arterial insufficiency and VOD) can be observed.

6.5.3.2.3 Penile Mondor's Disease (Superficial Dorsal Vein Thrombophlebitis)

Penile Mondor's disease (PMD) is the **thrombophlebitis** of the superficial dorsal vein of the penis (Bertolotto et al. 2009). It usually presents with a sudden hard "rope" on the penile dorsal surface, in the location of the dorsal vein, usually associated with pain, especially during erection (Avery and Scheinfeld 2013). PCDU represents an important tool in diagnosing the condition and monitoring patients (Bertolotto et al. 2009; Avery and Scheinfeld 2013). A lack of blood flow and noncompressibility of the dorsal vein indicate thrombosis. PMD can be self-limited; however, anticoagulative agents or low-molecular-weight heparin can be used to treat the thrombophlebitis. Resolution leads to flow restoration in the dorsal vein. Sometimes outcomes of a thrombus can remain visible as hyperechoic linear findings lying on the dorsal venous wall.

6.5.3.2.4 Priapism

PCDU can be useful in evaluating priapism. Priapism is defined as a persistent (>4 h) **painful erection not related to sexual desire** (Silberman et al. 2021). It can be distinguished into two forms, "**low-flow**" and "**high-flow**" (Silberman et al. 2021). Low-flow or ischemic priapism is the more frequent form, characterized by a disorder of venous blood outflow and a full painful erection. It is characterized by a very low-velocity high-resistance flow, with low PSV (resembling that observed at PCDU in flaccid condition) and negative EDV (Bertolotto et al. 2009; Avery and Scheinfeld 2013), similar to a long-lasting severe phase 5 of the dynamic PCDU phases (Patel et al. 2012) (see above). The specific waveform observed depends on the fact that intracorporal pressure exceeds systolic pressure; hence, no venous outflow is possible (similar to what is observed during a full, normal, rigid erection) and arterial inflow is low (reduced respect to a full, normal, rigid erection). Low-flow priapism is a true **emergency**. In fact, prolonged obstruction of venous outflow causing sustained high cavernous pressures and arterial ischemia can lead to irreversible ischemic changes and permanent ED (Avery and Scheinfeld 2013; Silberman et al. 2021).

High-flow priapism is the less frequent form, characterized by unregulated arterial inflow and a partial, incomplete erection (Silberman et al. 2021). Its etiology includes pelvic or penile trauma or surgery, often leading to a cavernosal artery tear or a cavernosal artery-lacunar fistula. A fistula, characterized by vascular turbulence, can be often observed at PCDU, as well as cavernous hematoma, appearing as a hypoechoic area within the corpora cavernosa. A low-resistance flow is present, with high PSV and positive EDV (Bertolotto et al. 2009; Avery and Scheinfeld 2013), similar to a phase 2 of the dynamic PCDU phases (Patel et al. 2012) (see above).

6.5.3.2.5 Penile Trauma

Penile injury may result from penetrating or blunt trauma (Bertolotto et al. 2009; Avery and Scheinfeld 2013). Patients with penile injury usually present with painful swelling and deformity of the shaft, either diffuse or circumscribed. Imaging may be required to evaluate the extent of the penile injury, locate hematomas, identify involvement of the penile urethra, or rule out albugineal disruption (Bertolotto et al. 2009; Avery and Scheinfeld 2013). US allows differentiation of **cavernosal, septal, and extra-albugineal hematomas**, which appear as hypoechoic areas; recognition of associated vascular injury, which may be seen as a cavernosal artery-lacunar fistula with flow turbulence (see also above, "high flow priapism"); identification of a tunical tear as an interruption in the echogenic interface of the normally appearing albuginea (Bertolotto et al. 2009; Avery and Scheinfeld 2013). **MRI** is more accurate in location of penile hematoma and evaluation of tunical disruption (Bertolotto et al. 2009; Avery and Scheinfeld 2013). **CT** can be requested in polytrauma patients, in whom urethral injuries are associated with pelvic bone fractures (Bertolotto et al. 2009; Avery and Scheinfeld 2013).

6.5.3.2.6 Penile Tumors

Penile tumors may be primary or secondary (Bertolotto et al. 2009; Avery and Scheinfeld 2013). Primary penile tumor usually manifest as an area of induration or a warty exophytic growth (Bertolotto et al. 2009; Avery and Scheinfeld 2013). In patients with primary penile tumors, imaging is indicated for **staging purposes**, and the relationships between the tumor and adjacent structures are

better evaluated in the erected penis (after ICI) (Bertolotto et al. 2009; Avery and Scheinfeld 2013). At US (Bertolotto et al. 2009; Avery and Scheinfeld 2013), primary penile tumors usually have variable echogenicity and poor vascularization. Inflamed tumors may demonstrate increased vascularity and may be painful. When there is initial infiltration of the tunica albuginea, the latter may be in contact with the lesion and focally thickened with decreased echogenicity, showing no interruption. When infiltration of the corpora cavernosa occurs, an interruption of the interface of the tunica albuginea can be detected. Secondary tumors depend on **metastatic invasion** of the corpora cavernosa by a malignant neoplasm and can manifest as painful penile induration, either circumscribed or diffuse (Bertolotto et al. 2009; Avery and Scheinfeld 2013). At US, metastatic lesions may appear as circumscribed tumor nodules within the corpora cavernosa, diffuse infiltration, or both. Venous stasis and thrombosis can be associated due to infiltration of the normal venous drainage (Bertolotto et al. 2009; Avery and Scheinfeld 2013). Direct infiltration of the tunica albuginea can be identified as a tunical interruption. Although US is more accurate than clinical examination for measuring the extent of the tumor, **MRI** is the standard imaging modality for staging penile malignancies (Bertolotto et al. 2009; Avery and Scheinfeld 2013). Usually, US has a role in tumor staging when there are contraindications to MRI.

6.6 Male Breast US

6.6.1 Male Breast US: Indications

When clinical examination is equivocal, male breast US can be useful to distinguish gynecomastia and lipomastia (Niell et al. 2018; Kanakis et al. 2019) (Table 6.1). In addition, if there is clinical suspicion of breast malignancy, US can help to characterize the clinical finding (Niell et al. 2018; Kanakis et al. 2019) (Table 6.1).

6.6.2 Male Breast US: Methodological Standards

Male breast US is not completely standardized (Chau et al. 2016; Kim and Kim 2019; Swerdloff and Ng 2019; Önder et al. 2020). Breast US is performed with the patient supine. Most authors define **gynecomastia** as the presence of increased breast tissue, without specifying any cutoff (Chau et al. 2016; Kim and Kim 2019; Önder et al. 2020). Some authors have proposed to define gynecomastia as the presence of breast tissue with an anteroposterior depth >1 cm measured from the skin surface under the nipple to the anterior surface of the pectoralis muscle (Dialani et al. 2010). Pure **lipomastia** is defined at US as the presence of increased subcutaneous adipose tissue with no evidence of increased male breast tissue (Dialani et al. 2010; Chau et al. 2016; Kim and Kim 2019; Önder et al. 2020). Sometimes gynecomastia and lipomastia can be observed together in the same subject.

6.6.3 Male Breast US Anatomy, Normal and Abnormal Patterns, Clinical Utility, and US Standards

Figure 6.5 shows some normal and abnormal US patterns of the male breast.

6.6.3.1 Male Breast US Anatomy and Normal Pattern

Male breast anatomy before and after puberty has been well described elsewhere (Swerdloff and Ng 2019). US anatomy of the normal male breast consists mainly of subcutaneous fat and a modest subareolar button of breast tissue, slightly hypoechoic ducts (Chau et al. 2016; Kim and Kim 2019; Önder et al. 2020). Although normal breast tissue is virtually absent (small breast button) and asymptomatic in males, various abnormalities can affect the male breast, including benign findings, such as gynecomastia or lipomastia, or, rarely, malignancy.

6.6.3.2 Male Breast US Abnormal Patterns

The most recent guidelines on male breast abnormalities support breast imaging only when clinical examination is equivocal (Niell et al. 2018; Kanakis et al. 2019). Breast clinical examination represents the gold standard to evaluate possible male breast abnormalities, confirming the presence of palpable glandular tissue to discriminate **gynecomastia** from **lipomastia** (pseudogynecomastia) and ruling out the suspicion of malignant breast tumor (Niell et al. 2018; Kanakis et al. 2019).

6.6.3.3 Gynecomastia

Gynecomastia is defined clinically as generalized enlargement of **male breast tissue** possibly accompanied by discomfort or pain (especially at onset) (Swerdloff and Ng 2019). It can occur normally during three phases of life: after birth, at puberty, and in elderly men (Swerdloff and Ng 2019). The mechanisms underlying the "physiologic" development of gynecomastia, reported above, as well as diseases and drugs associated with gynecomastia have been described elsewhere (Swerdloff and Ng 2019). When gynecomastia is found, a **testicular exam** is essential, and if a testicular mass is suspected, **testicular US** is mandatory to investigate possible estrogen-secreting neoplasms, especially Leydig-,

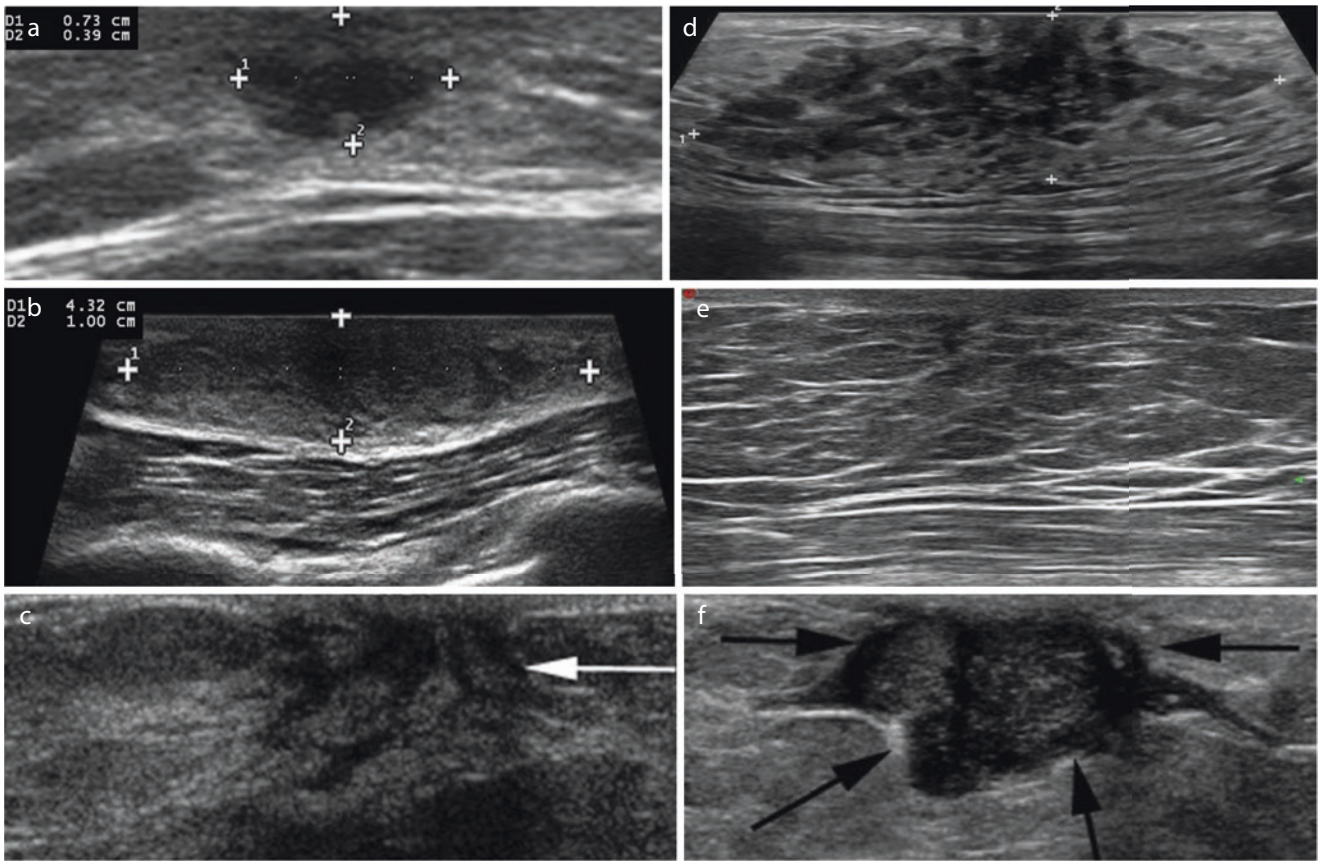


Fig. 6.5 Normal and abnormal US patterns of the male breast. **Panel** (**a**)**.** Normal male breast button. **Panel** (**b**)**.** Gynecomastia (nodular pattern). **Panel** (**c**)**.** Gynecomastia (dendritic pattern). **Panel** (**d**). Gynecomastia (diffuse pattern). **Panel** (**e**)**.** Lipomastia. **Panel** (**f**). Male breast cancer

Sertoli-, and granulosa-cell tumors (Kanakis et al. 2019; Swerdloff and Ng 2019).

Clinically, gynecomastia is defined as subareolar breast tissue of ≥2 cm in diameter (Swerdloff and Ng 2019). Histologically, there are three phases of proliferative changes described in gynecomastia, including "florid," "intermediate/transition," and "fibrous/inactive" gynecomastia, well described elsewhere (Chau et al. 2016; Kim and Kim 2019; Swerdloff and Ng 2019). At US, gynecomastia is defined by some authors as the presence of increased breast tissue described as anteroposterior depth >1 cm from the skin surface under the nipple to the anterior surface of the pectoralis muscle (Dialani et al. 2010). Three patterns of gynecomastia can be observed: nodular, dendritic, and diffuse, reflecting underlying histologic changes within the male breast (Dialani et al. 2010; Chau et al. 2016; Kim and Kim 2019; Önder et al. 2020). **"Nodular" gynecomastia** occurs in the early (florid) phase of gynecomastia and is indicative of the early phase of ductal and stromal proliferation. It typically correlates with symptomatic duration of <1 year. US demonstrates a subareolar fan- or disk-shaped area of hypoechoic tissue that may be slightly hypervascular due to the proliferation of stroma. **"Dendritic" gynecomastia** occurs with long-standing gynecomastia that has been present for 1 year or longer, is quiescent and fibrotic, and has been associated with stromal fibrosis and dilated ducts. It typically presents as a flame-shaped, serpiginous hypoechoic area with finger-like projections in the subareolar region. Due to stromal fibrosis, this subtype is typically irreversible. "Diffuse" gynecomastia is usually seen in the setting of high-dose estrogen therapy or exogenous hormone use. It presents with increased volume and echogenicity of breast tissue, identical to female breasts.

In nodular gynecomastia of recent onset (less than 6 months), under medical treatment responders usually improve with reduced pain within 1 month (Swerdloff and Ng 2019), and a reduction in breast tissue can be observed at US after 3–6 months. However, if gynecomastia is established since a long time (dendritic form), medical treatment often fails to produce breast regression, and surgery can be considered (Kanakis et al. 2019; Swerdloff and Ng 2019).

6.6.3.4 Pseudogynecomastia/Lipomastia

Pseudogynecomastia is characterized by increased subareolar fat without enlargement of the breast glandular component. It is a frequent finding in obese men. US is typically not

necessary, but if performed due to clinical concern, it will demonstrate subcutaneous adipose tissue.

6.6.3.5 Male Breast Cancer

Male breast cancer is very rare and is suspected if an immobile firm mass, usually painless, is found on physical examination. At US, most invasive carcinomas appear as solid, hypoechoic, irregularly shaped masses with spiculated or microlobulated margins in the subareolar region, often eccentric to the nipple (Chau et al. 2016; Kim and Kim 2019; Önder et al. 2020).

6.6.3.6 Specific Use of Male Breast US

Male breast US can be used to evaluate suspected gynecomastia in men with **advanced prostate cancer** under androgen deprivation treatment (Swerdloff and Ng 2019). In addition, male breast US can be used to evaluate breasts in **transgender subjects** (Phillips et al. 2014). In transgender men, US can be useful to evaluate postmastectomy complications, including hematoma, seroma, and abscess formation. In addition, breast cancer may occur in residual breast tissue of those patients who underwent bilateral mastectomies (Phillips et al. 2014). In transgender women, breast tissue will increase over time under hormonal therapy, reaching maturity by 2–3 years with a more pronounced nipple-areola complex (Phillips et al. 2014). The breast tissue that develops should not be considered as gynecomastia, since it contains lobules and resembles breast tissue seen in natal women. Breast cysts and fibroadenoma can develop, and breast cancer, although rare, can occur in transgender women (Phillips et al. 2014). In addition, breast augmentation with implants is commonly used as part of sex reassignment surgery. Hence, training as female breast sonographers is suggested for sonographists evaluating transgender subjects.

Key Points

- Scrotal US is useful to assess reproductive health, scrotal pain, masses, and trauma.
- Regarding reproductive health, scrotal US can detect testicular location, hypotrophy, inhomogeneity, microlithiasis, lesions, and varicocele as well as epididymal abnormalities and congenital bilateral absence of vas deferens.
- Regarding scrotal pain, scrotal US can detect testicular or epididymal inflammation (hyperemia), torsion (absent vascularization), lesions, and other abnormalities.
- Contrast-enhanced US can improve the differential diagnosis of scrotal diseases, especially focal testicular lesions.
- Sonoelastography can improve the differential diagnosis of focal testicular lesions in conjunction with other US techniques.
- Transrectal US is useful to assess obstructive azoo-/oligospermia, especially through detection of congenital bilateral absence of vas deferens, midline prostatic cysts, or ejaculatory duct obstruction.
- Transrectal US has assumed a growing relevance in chronic pelvic pain assessment, detecting signs of chronic or current prostate inflammation.
- Penile US, performed in flaccid or dynamic state, can be used to investigate erectile dysfunction, structural penile abnormalities (fibrosis, trauma, tumors), dorsal vein thrombosis, and priapism.
- Breast US can assess male breast abnormalities, including gynecomastia, lipomastia, and lesions.
- Thanks to the efforts of different international societies, including the European Academy of Andrology, several standards and reference ranges in US have been achieved.

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Endocrine Laboratory Diagnosis

7

Manuela Simoni and Eberhard Nieschlag

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Abstract

The measurements of serum gonadotropins, testosterone, SHBG, and calculated free testosterone have a central role in the endocrine diagnostics of male hypogonadism and infertility. Additional hormone determinations include the newest specific markers of Sertoli (inhibin B, anti-mullerian hormone) and Leydig (insulin-like factor 3) cell function. Dynamic tests (GnRH and hCG stimulation tests) are useful in the assessment of pituitary and testis reserve capacity. In selected cases, molecular biology techniques may be helpful in the endocrine workup.

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7.1 Gonadotropins

The evaluation of serum levels of LH and FSH in combination with testosterone provides important information to pinpoint the localization of hypogonadism, which is then decisive for adequate therapy.

High gonadotropin levels in serum in combination with low testosterone levels indicate testicular origin of hypogonadism (**primary hypogonadism**); low gonadotropin levels point to a central cause (**secondary hypogonadism**).

For differentiation between low normal and pathologically low LH and FSH levels, highly sensitive third-generation immunometric assays are recommended.

When interpreting basal **LH** values, the physiological **pulsatility of pituitary secretion** with ensuing oscillations of serum levels has to be considered. The combination of pulsatile release and short circulatory half-life (60 min) is the basis for **LH pulsatility**. A normal man shows approximately 8–20 LH pulses per day with measured LH variations

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of 100% and more between peak and trough levels. For this reason, repeated sampling may be necessary. Patients with primary hypogonadism have increased average serum concentrations, as well as elevated LH pulse frequency. When hypothalamic GnRH fails to be secreted, only sporadic LH pulses or none at all can be measured. High LH levels occurring in combination with high testosterone serum concentrations indicate an androgen receptor defect (**androgen resistance**) (see Chap. 31).

Due to its longer half-life of 4–6 h, **FSH** displays only minor serum level oscillations, and therefore a single-point measurement is representative. **To a certain extent, FSH serum concentrations mirror spermatogenesis.** High FSH levels in the presence of small, firm testes (< 6 ml) and azoospermia are diagnostic indicators for Klinefelter syndrome; low FSH levels indicate a hypothalamic or pituitary deficiency (Fig. 7.1). If testicular volume exceeds 6 ml and azoospermia or severe oligozoospermia is simultaneously present, then elevated FSH indicates primary impairment of spermatogenesis. Within wide margins, the extent of FSH elevation is correlated with the number of seminiferous tubules lacking germ cells (**Sertoli cell-only tubules**) (Fig. 7.2) (von Eckardstein et al. 1999). Normal FSH levels, however, may be found in patients with azoospermia or severe oligozoospermia due to defects at late, postmeiotic spermatogenetic stages, which are FSH-independent (Bergmann et al. 1994).

Normal FSH values in combination with azoospermia, normal testicular volume, and low levels of glucosidase in the ejaculate, raise suspicion of an obstruction or aplasia of the deferent duct (Fig. 7.1). This constellation, possibly accompanied by ambiguous findings, justifies bilateral testicular biopsy; in case of normal testicular histology, reconstructive surgery of the epididymis or deferent duct or techniques of assisted reproduction are indicated (see Chaps. 27 and 42).

For the determination of gonadotropin levels in serum, sensitive noncompetitive **immunoassays** such as immunoradiometric assays (IRMA), immunofluorometric assays (IFMA), or enzyme-linked immunosorbant assays (ELISA) are available (Sussman et al. 2008). The **ultrasensitive, third-generation LH assays** are useful in cases of low LH levels such as prepubertal state or hypogonadotropic hypogonadism (Sequera et al. 2002).

Mutations of gonadotropin genes are rare. Inactivating mutations of the LH-ß subunit lead to infertility and failure of spontaneous puberty to occur. Inactivating mutations of the FSH-ß subunit cause azoospermia and, hence, infertility (Simoni et al. 2020). Similarly, rare mutations of the **gonadotropin receptor genes** are divided into activating and inactivating mutations. Activating LH receptor mutations cause precocious puberty, whereas inactivating mutations cause Leydig cell hypoplasia and hypogonadism. Inactivating FSH

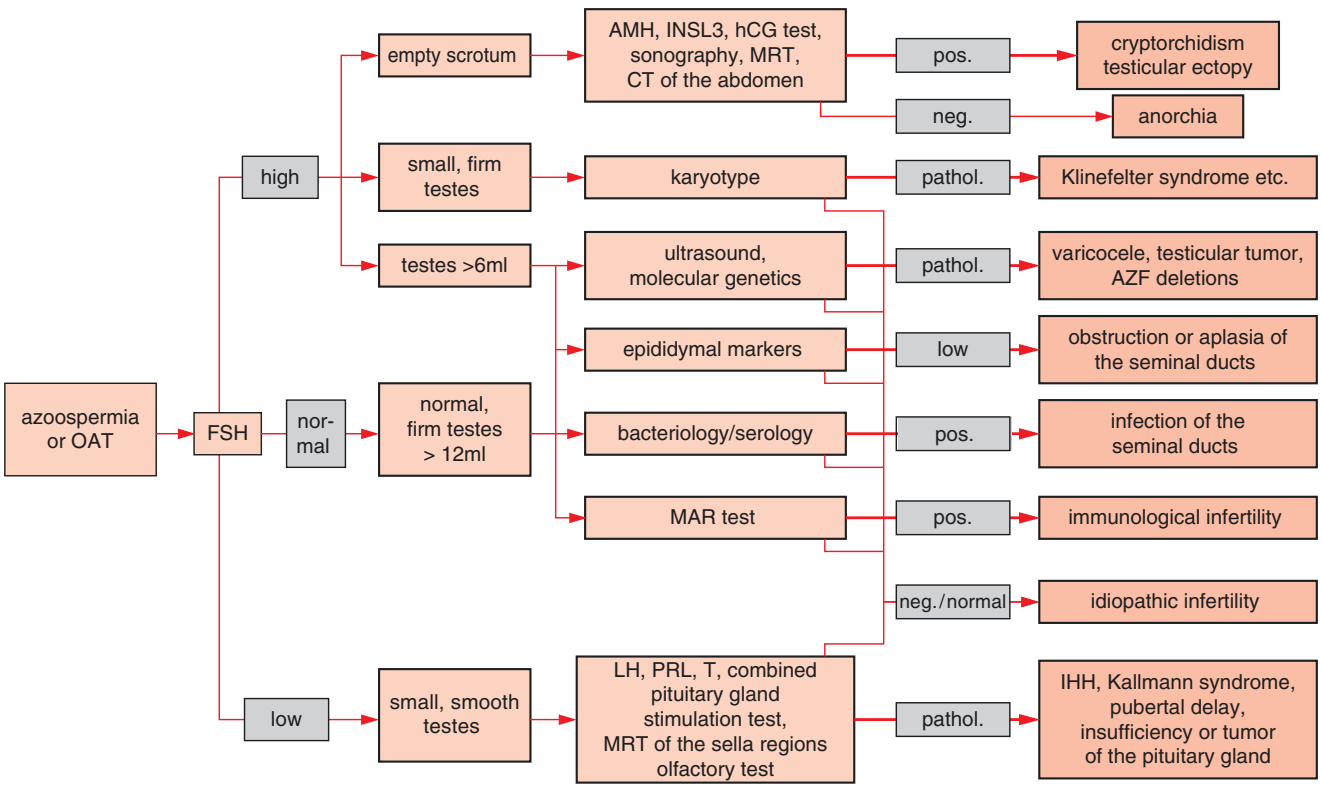


Fig. 7.1 Algorithm for differential diagnosis of male fertility disorders indicating the importance of FSH, along with semen analysis

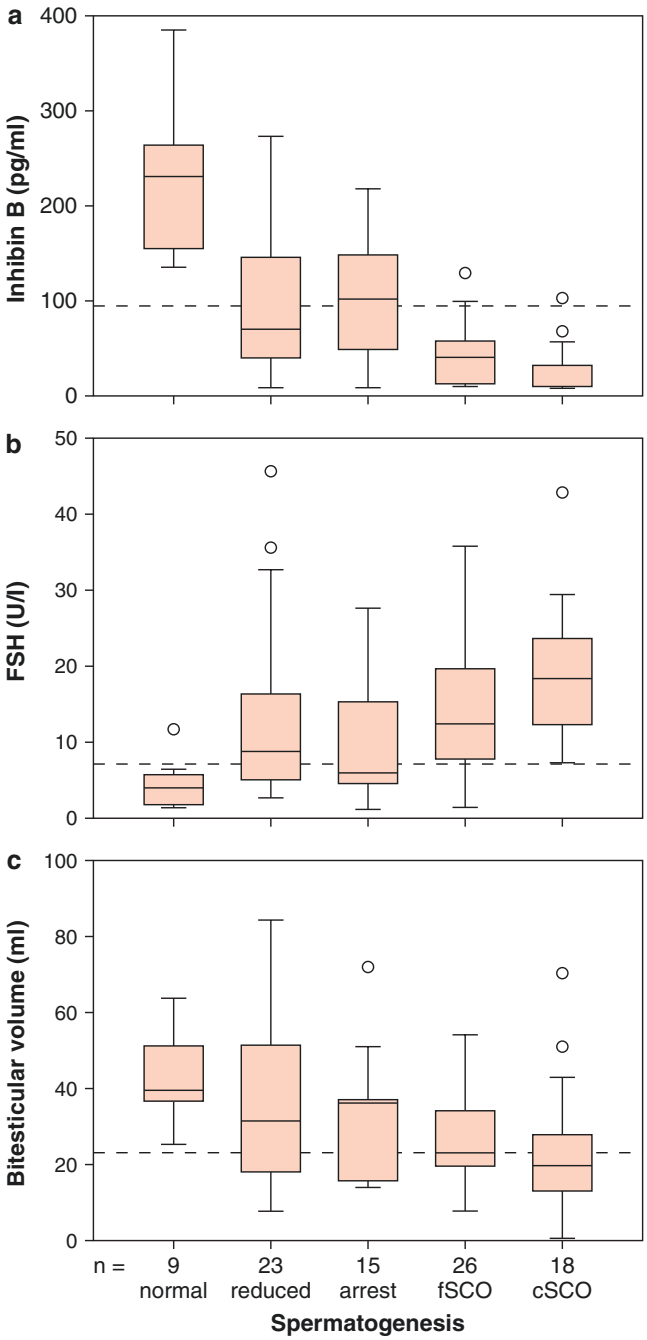


Fig. 7.2 Box plot showing serum concentrations of inhibin B (**a**) and FSH (**b**) as well as total testicular volume (**c**) in 91 infertile men divided into 5 groups according to testicular histology (*normal*: normal spermatogenesis; *reduced*: qualitatively reduced spermatogenesis; *arrest:* spermatogenic arrest; *fSCO*: focal Sertoli-cell-only syndrome; *cSCO*: complete Sertoli-cell-only syndrome). Broken line shows lower normal levels for inhibin B and total testicular volume as well as upper normal levels of FSH (von Eckardstein et al. 1999)

receptor mutations lead to variable disturbances of spermatogenesis (Huhtaniemi and Alevizaki 2006). The activating FSH receptor mutations described so far, in only few cases, maintained spermatogenesis in hypophysectomized males. (overview in Casarini et al. 2015).

7.2 GnRH, GnRH Test, Kisspeptin

Because of extremely low serum concentrations and short half-life, GnRH cannot be measured in peripheral blood by immunoassay.

The **GnRH test** is performed to determine the gonadotropin reserve capacity of the pituitary and is particularly indicated for low-normal LH and FSH values, which cannot always be differentiated from pathologically low basal values. However, the combination of elevated LH and FSH with low testosterone makes the GnRH-test redundant, as this indicates a primary hypogonadism.

The rise of LH should be at least threefold 30–60 min after injection of 100 µg GnRH; the increase in FSH should be 1.5 times over basal. However, the results should be judged by an experienced clinician. When basal gonadotropin levels are high, which points to a primary testicular disorder, no additional information can be gained from a GnRH test.

The **GnRH-agonist test** is indicated for the differentiation of constitutional delay of puberty and congenital hypogonadotropic hypogonadism (CHH) and replaces the former GnRH pump test. Between 7.00 and 9.00 h, a basal blood sample is collected for determination of LH and testosterone and a GnRH agonist is injected (e.g., buserelin 1 µg/kg, maximal 100 µg). Following 4 and 24 h, blood samples are collected again for LH and testosterone determination. A LH rise of >4 IU/L is an indication for constitutional delay of puberty and <4 IU/L for CHH (Diederich et al. 2020).

Mutations in the **GnRH receptor** gene as well as in other genes involved in the control of GnRH secretion (*GPR54*, *FGFR1*, etc.; see Chap. 2) were also identified as the cause of hypogonadotropic hypogonadism (Cangiano et al. 2020). The molecular genetic diagnostic procedures of these mutations are reported in Chap. 8.

Kisspeptins are a group of neuropeptides mediating GnRH release and thereby regulating reproductive functions such as puberty and fertility in males and females.

In males, kisspeptin stimulates the hypothalamo-pituitory-testicular axis, resulting in increased testosterone. Fertile men have higher serum kisspeptin levels than infertile men (Trevisan et al. 2018). So far measurement and application of kisspeptin is reserved for research and clinical trials.

7.3 Prolactin

The determination of **prolactin** in male patients of an infertility clinic does not play such a pivotal role as it does in the female. Prolactin should be measured in case of acquired hypogonadotropic hypogonadism to rule out a pituitary adenoma (Colpi et al. 2018). Unclear fertility disorders, erectile dysfunction and loss of libido, gynecomastia, galactorrhea, or other symptoms that indicate a pituitary disorder and suspicion of pituitary tumor should prompt prolactin serum measurements. Prolactin is measured by competitive or noncompetitive immunoassays. When interpreting results, it should be remembered that numerous drugs, particularly psychiatric medication (dopamin antagonists and catecholamine depletors), and stress increase prolactin secretion (see Chap. 15). In case of suspicion of prolactinoma, the diagnostic workup should continue with imaging of the hypothalamo-pituitary region by magnetic resonance and, if an adenoma is confirmed, with endocrine testing of the remaining pituitary function.

It is important to point out that the current prolactin assays, although generally robust and reliable, are susceptible to interference, especially due to the presence in the serum of some individuals of a high molecular weight form of prolactin conglomerated to IgG, but biologically inactive (*macroprolactin*). Some assays do not allow correct differentiation of patients with true hyperprolactinemia from those with macroprolactinemia, estimated to represent about 10% of all cases of biochemical hyperprolactinemia. The macroprolactin autoantibodies can be removed by polyethylene precipitation (PEG) and Good Clinical Practice recommends that all samples with elevated prolactin levels should undergo PEG-precipitation to differentiate between biologically active prolactin and macroprolactin to avoid this diagnostic pitfall (Fahie-Wilson and Smith 2013).

7.4 Testosterone, Free Testosterone, Salivary Testosterone, SHBG

Testosterone in serum is the laboratory value most important for confirming a clinical suspicion of hypogonadism and for monitoring testosterone substitution therapy. When interpreting testosterone values, **diurnal variations** should be considered as they cause morning serum concentrations to be approximately 20–40% higher than evening values (Fig. 7.3). All reference values are therefore derived from samples obtained in the morning hours (7–11 a.m.) after overnight fasting.

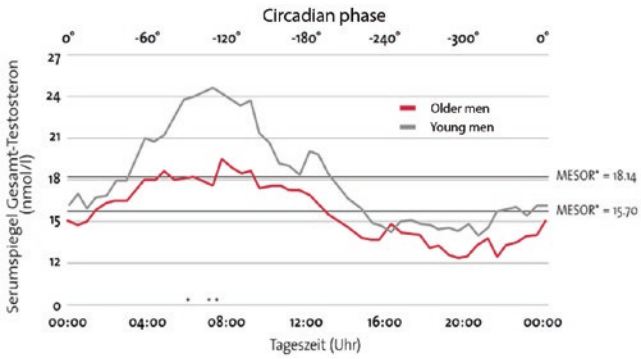


Fig. 7.3 Circadian rhythm of serum total testosterone in 10 young (23–33 years) and 8 elderly men (55–64 years). Highest values are seen between 6.00 and 10.00 in the morning and lowest levels between 17.00 and 21.00 h in the evening. Elderly men maintain the diurnal rhythm seen in the younger men, although on a lower level and with a lower amplitude (data adapted from Diver et al. 2003)

Serum testosterone levels are influenced by many factors under physiological and pathological conditions (Zitzmann and Nieschlag 2001). Short, intensive **physical exercise** can increase serum testosterone concentrations, whereas extended exhausting physical exercise, high-performance sports, and overtraining can bring about a decrease and result in hypogonadism (Schürmeyer et al. 1984; Hackney 2020). **Food intake** can lower testosterone levels (Lehtihet et al. 2012; Caronia et al. 2013). Therefore, serum samples for testosterone determination should be collected after overnight fasting to observe standard conditions. Nearly all **chronic diseases**, particularly those of the liver, kidneys, and the cardiovascular system, as well as **stress**, **anesthesia**, **drugs,** and certain **medications** (e.g., ketoconazole), can prompt a decrease in testosterone levels (see Chap. 34).

Serum testosterone levels, especially free testosterone, decrease with age (Zitzmann and Nieschlag 2003; Araujo et al. 2007; Bae et al. 2019) (Fig. 7.4). Although it is not clear whether such age-related decrease is physiological or caused by multimorbidity (functional hypogonadism) or a genuine late-onset hypogonadism (LOH) (Nieschlag 2019), when serum testosterone levels are below the lower cutoff of the young adult range they may be associated with increased frailty and symptoms of hypogonadism requiring clinical attention. No separate reference range for aging men exists and the lower cut-off levels are equal for all adults. However, there is not one threshold between normal and pathological, but that there are symptom-specific thresholds for the various signs of hypogonadism in aging men (Zitzmann et al.

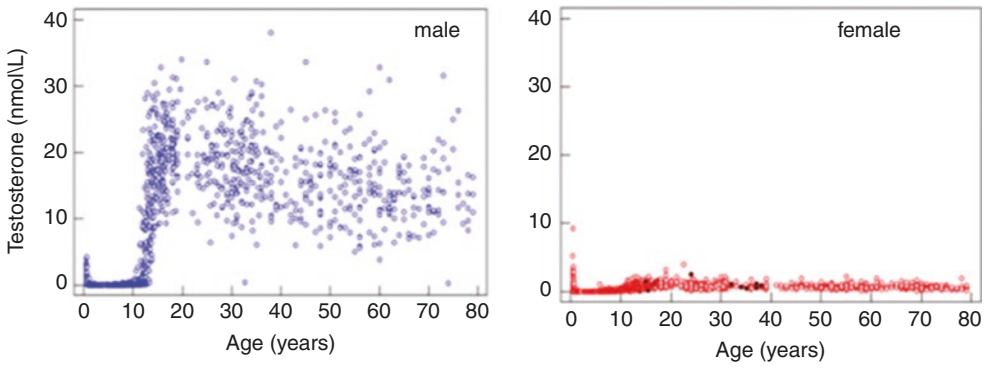


Fig. 7.4 Serum testosterone in 2512 healthy males and 2166 healthy females in relation to age in years. (Fig. provided by Prof. J. Kratzsch, coauthor of Bae et al. 2020)

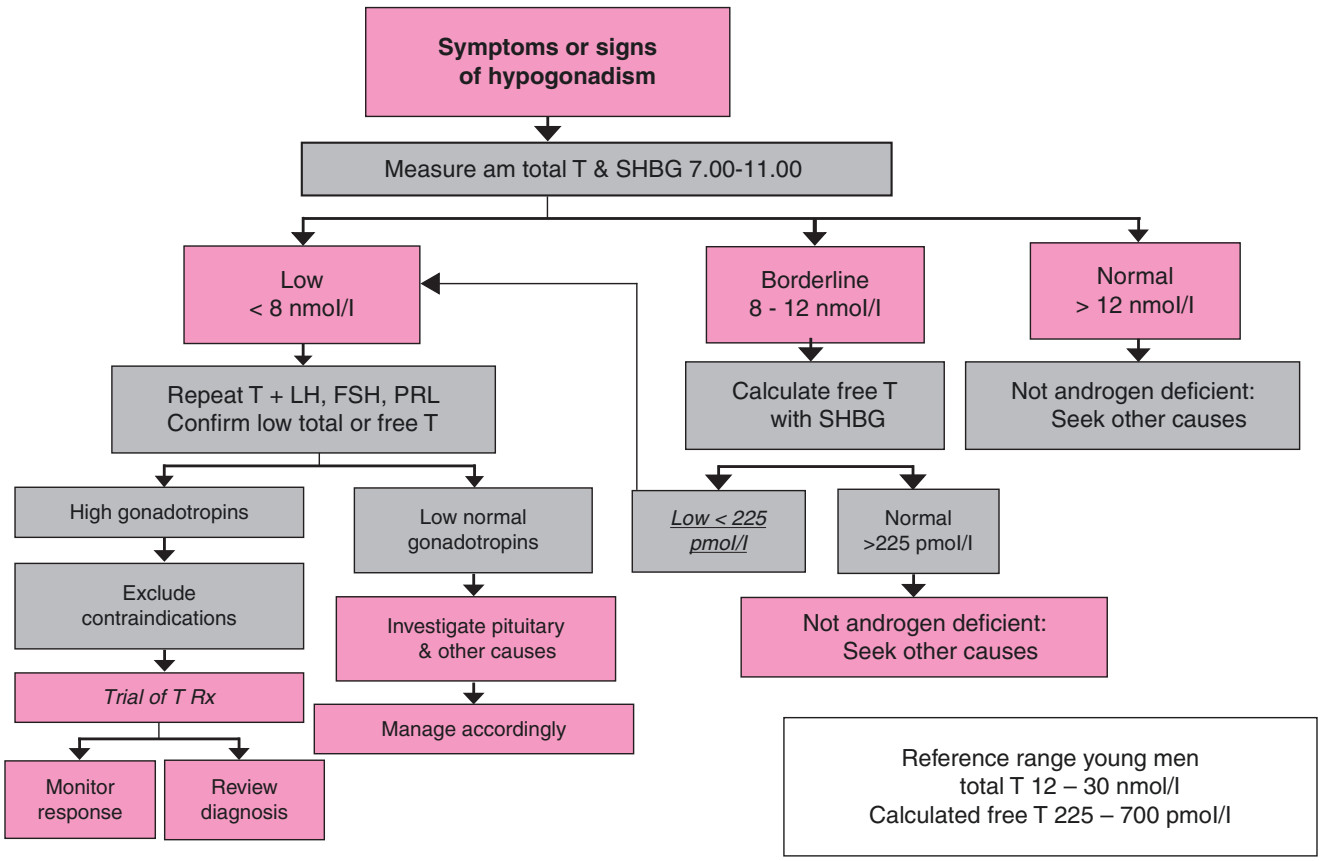


Fig. 7.5 Algorithm for diagnosis in treatment of hypogonadism emphasizing the importance of total testosterone and SHBG measurements

2006a) as well as in younger men (Kelleher et al. 2004) (see Chaps. 25 and 36).

Considering these factors, normal serum testosterone concentration in the adult male of all ages lies between 12 and 30 nmol/l during the first half of the day. Prepubertal boys and castrates have serum levels below 4 nmol/l. An algorithm for the diagnoses of hypogonadism, emphasizing the central role of testosterone and SHBG, is shown in Fig. 7.5.

The threshold levels for the diagnosis of hypogonadism have varied between countries (Nieschlag et al. 2004), but have been harmonized in more recent guidelines based on new evidence (Table 36.2) (e.g., Bhasin et al. 2011; Frost et al. 2013; Bae et al. 2019) so that the lower limit of normal is generally accepted as 12 nmol/L. Failure of the serum testosterone levels of a patient to match the clinical picture is an indication for the determination of the **androgen receptor CAG repeats**. For example, symptoms of hypogonadism in the presence of normal serum testosterone levels may be caused by long CAG repeats.

In most laboratories serum total testosterone concentrations are determined by radioimmunoassay, enzyme immunoassay, fluoroimmunoassay or chemiluminescence assay by direct methods without extraction (Simoni et al. 2012). However, the current direct immunoassays for testosterone are inaccurate especially at low testosterone concentrations (prepubertal boys, women). Therefore, high throughput liquid chromatography followed by tandem mass spectrometry (LC-MS/MS) is becoming increasingly popular. In addition, LC-MS/MS allows the simultaneous accurate identification and quantification of several steroids in the same sample (e.g., DHT). LC-MS/MS is the method of choice for detecting testosterone and anabolic steroids in blood and urine samples for doping control purposes (Schänzer and Thevis 2012). One journal has even adopted the policy of accepting publications only when testosterone has been measured by LC-MS/MS (but is not very strict about it).

Testosterone in serum remains stable even after repeated freezing and thawing. However, repeated thawing may reduce the volume of the sample and thereby increase the testosterone concentration. Normally, a single morning blood sample is usually sufficient for the assessment of testosterone serum levels.

In blood, testosterone is bound to protein, specifically to **sex hormone binding globulin (SHBG).** Only approximately 2% of testosterone is unbound and available as free testosterone for biological activity. The concentration of **free testosterone**, as a measure of biologically available testosterone, is determined by equilibrium dialysis or as the serum fraction of total testosterone which is not precipitated by ammonium sulfate. These methods, however, are too complicated for clinical routine. Calculating free testosterone from the serum concentration of total testosterone and SHBG using a standardized formula has proved to be a simple and reliable method for estimating testosterone bioactivity (Vermeulen et al. 1999). The **free androgen index (FAI)** should not be used in males because of several inaccuracies (Ly et al. 2010).

Since generally total testosterone is well correlated with free testosterone, separate determination of free testosterone is only necessary in certain cases. As an example, hyperthyroidism and antiepileptic and other drugs and conditions cause an increase in SHBG levels and thereby increased testosterone concentration in serum (Table 7.1), without a parallel increase of the biologically active free testosterone fraction. In **extreme obesity** low testosterone levels are measured; however, in combination with low SHBG values; accordingly, the free testosterone fraction remains normal, although in the low-normal range (Wu et al. 2008). However, free testosterone should not be measured with the current immunoassays because they are inaccurate and other ways to measure free testosterone (e.g., dialysis and MS) are not available in most andrology centers.

Table 7.1 Influences on SHBG concentrations

Stimulation of SHBG production	Inhibition of SHBG production
Estrogen application	Androgen therapy
Hyperthyroidism	Hypothyroidism
Lack of androgens	Gestagens
Growth hormone deficiency	Acromegaly
Hepatitis	Nephrotic syndrome
Liver cirrhosis	Glucocorticoids
Phenytoin application	Hyperinsulinaemia
Thinness	Obesity
Genetic polymorphism	Genetic polymorphism

Testosterone can also be measured in **saliva** by direct immunoassays (normal range 200–500 pmol/l). Salivary concentrations are correlated with free testosterone in serum. However, the values are widely scattered interindividually and are therefore not suited for the diagnosis of hypogonadism (Hayes et al. 2015). This method would be especially suited for monitoring testosterone substitution therapy since the patient can produce samples without the help of medical personnel, but has many drawbacks (e.g., contamination with blood) which limits its application to clinical routine (Lood et al. 2018).

7.5 hCG Test

The **endocrine reserve capacity of the testis** can be tested by stimulation with human chorionic gonadotropin (hCG). hCG has predominantly LH activity and stimulates testosterone production of the Leydig cells. Today, the test is predominantly used for **differentiation between cryptorchidism or ectopy of the testis** (rise in testosterone levels present, but diminished) and anorchia (absent testosterone rise), also in cases of 46,XY DSD (Bertelloni et al. 2018; Diederich et al. 2020) (Fig. 7.1).

On the first day of the examination, basal blood samples are obtained between 8.00 and 10.00 h; immediately thereafter, a single injection of 5000 I.U. hCG/m^2 body surface is given IM. Further blood samples are obtained after 48 and/or 72 h. The rise of testosterone should be 1.5–2.5-fold. Lesser values indicate primary hypogonadism; higher values signal secondary hypogonadism. Failure of values in the castrate range to increase indicates anorchia or complete testicular atrophy. Decreasing reserve capacity of the Leydig cells is a characteristic of the elderly man (see Chap. 25).

7.6 Anti-Mullerian Hormone, Insulin-like factor 3

After testis formation, further development of a male phenotype (masculinization) is driven by three hormones from the fetal testis: **anti-mullerian hormone (AMH), insulin-like**

factor 3 (INSL3), and testosterone. AMH**,** also known as **mullerian inhibiting substance (MIS)**, is secreted by the immature Sertoli cell and is responsible for the regression of the mullerian ducts in the male fetus (Fig. 7.6).

In women, AMH is an important parameter for the presence of follicle (ovarian reserve). In the prepubertal male, determining serum levels of AMH is a sensitive and specific test providing evidence for the existence of a testis. Normal values for boys show the presence of testicular tissue; undetectable serum concentrations indicate **anorchia.** Compared to the hCG test, the AMH test is equally specific, but more sensitive, and hence its predictive value is higher in prepubertal boys (Josso et al. 2013) (Fig. 7.5).

AMH serum concentrations are significantly higher in prepubertal boys with normal testicular function than in boys with disturbed testicular function. Extremely high values are seen in men with congenital hypogonadotropic hypogonadism, where they are caused by deficient pubertal maturation of the Sertoli cell and where they are comparable to values seen in prepubertal boys. hCG or testosterone therapy reduces these levels significantly, while FSH treatment increases AMH levels in seminal plasma (Sinisi et al. 2008). AMH serum levels are not significantly altered in men with impaired spermatogenesis, but appear to be correlated with spermatogenic parameters in men with maldescended testes, in whom AMH could serve as a Sertoli cell marker.

AMH levels in seminal plasma are weakly positive correlated with sperm count and motility (Andersen et al. 2016). However, in men undergoing testicular sperm extraction (TESE), AMH measurement does not improve the predictive value of serum FSH, since AMH levels are widely scattered in the low range (Toulis et al. 2010).

Insulin-like factor 3 (INSL3) is a peptide of the relaxin-like hormone family. In the male, it is expressed in testicular fetal and adult Leydig cells and is directly responsible, together with testosterone, for the process of abdominal descent of the testes towards the scrotum during male development. Mutations of the *INSL3* gene or its receptor *RXFP2* cause high intra-abdominal cryptorchidism due to a differentiation failure of testicular ligaments, the gubernacula.

Several mutations of these two genes resulting in non-functional proteins have been described in patients with testicular maldescent as a **marker of Leydig cell function** independent of testosterone. In boys, early postnatal INSL3 is high, which decreases later in childhood and increases again at puberty under the differentiating action of LH on Leydig cells. Cord blood and serum INSL3 are reduced in cryptorchid boys (Sansone et al. 2019).

Since INSL3 is a specific marker of Leydig cells (it is not measurable in women), it can be used to demonstrate the presence of testicular tissue in boys with bilateral cryptorchidism and its absence in serum suggests anorchidism. Serum INSL3 declines significantly with age, independently of tes-

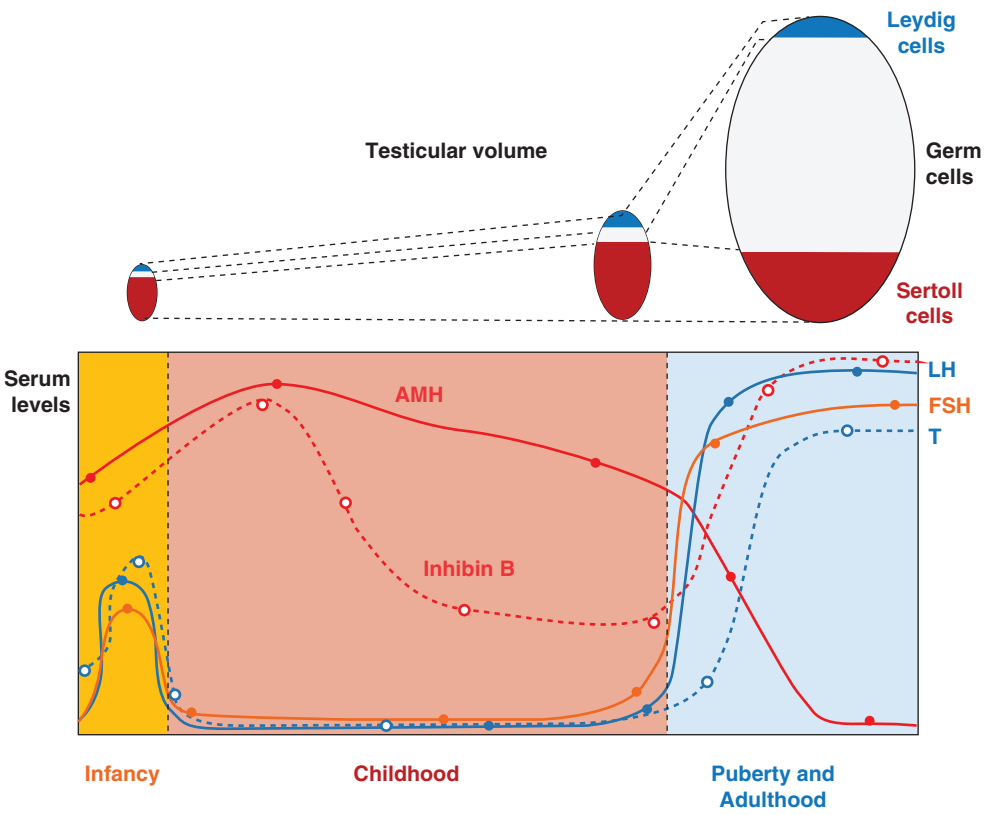


Fig. 7.6 Testicular development paralleled by the hormones LH, FSH, testosterone, AMH, and Inhibin B (Rey 2020 ©2020 Rodolfo A. Rey, published by De Gruyter. This work is licensed under the Creative Commons Attribution 4.0 Public License)

tosterone (Anand-Ivell et al. 2006). At present, INSL3 determinations have no practical application in the diagnosis of male infertility or hypogonadism.

7.7 Inhibin B

Inhibin B is produced by the Sertoli cells in the testes and plays a role in the regulation of FSH secretion in the pituitary (see Chap. 2). Inhibin A is not produced in the male. Inhibin B is a **marker of spermatogonial proliferation** and interaction with Sertoli cells and its concentration in serum drops quickly when spermatogonial proliferation stops (Meachem et al. 2001). Overall, inhibin B levels decrease and, consequently FSH concentrations increase in the presence of germ cell depletion (O'Connor and De Kretser 2004) (Fig. 7.5).

Inhibin B shows a marked **circadian rhythm**, with high values in the morning, descending to lowest levels in the late afternoon. In healthy and infertile men, morning serum concentrations of inhibin B are correlated with FSH serum levels, sperm concentration, and testicular volume (Fig. 7.2) (von Eckardstein et al. 1999). As a direct secretory product of the Sertoli cell, inhibin B is slightly more sensitive than FSH as a parameter for evaluating spermatogenesis (von Eckardstein et al. 1999). However, neither inhibin B nor FSH nor a combination of the two can accurately predict the presence of sperm in testicular biopsies, information which is of great relevance for azoospermic patients who are to undergo testicular sperm extraction (TESE) prior to ICSI therapy. Rather, FSH levels alone seem to be a better predictor of ICSI success (von Eckardstein et al. 1999; Zitzmann et al. 2006b).

7.8 Further Endocrine Diagnosis

Determining serum concentrations of **17ß estradiol, hCG, androstenedione** or **5α-dihydrotestosterone (DHT),** and **5α-reductase** activity in skin fibroblasts may be necessitated by particular findings, e.g., gynecomastia, suspected testicular tumor, enzyme defects in testosterone biosynthesis, or resistance of androgen target organs (see detailed descriptions in Chaps. 8 and 31).

Key Points

- The main constituent of endocrine laboratory diagnosis of testicular disorders is the determination of the gonadotropins LH and FSH as secretions of the anterior pituitary, of testosterone as the most important secretion of the Leydig cells, and of inhibin B as the product of the Sertoli cells.
- When hypothalamic or pituitary diseases are suspected, a GnRH stimulation test should be performed and prolactin should be measured.
- The hCG test evaluates the endocrine reserve capacity of the testes and helps to differentiate between cryptorchidism and anorchia.
- For special diagnostic questions, additional hormone determinations are performed, e.g., determinations of prolactin in all cases of hypo- or normogonadotropic hypogonadism, prolactin and estradiol in the case of gynecomastia, or hCG and estradiol upon suspicion of testicular tumor.
- Circulating anti-mullerian hormone (AMH) and insulin-like factor 3 (INSL3) represent additional markers of Sertoli and Leydig cell function, respectively.
- For disturbances of sexual differentiation, different steroids are measured in order to localize enzyme defects. Sequencing of the androgen receptor gene or androgen metabolizing enzyme (e.g., 5α-reductase) genes may be necessary (see detailed descriptions in Chaps. 2, 8 and 31).

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Cytogenetic and Molecular Genetic Diagnostics

8

Frank Tüttelmann and Albrecht Röpke

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Abstract

Genetic investigations are now indicated in almost all andrological topics ranging from isolated infertility to specific clinical entities such as hypogonadotropic hypogonadism. The spectrum of methods includes the classical cytogenetic examinations, such as the determination of the karyotype, as well as molecular cytogenetic procedures (e.g., FISH), and in particular, molecular genetic examinations (e.g., analysis of microdeletions, sequencing of genes). So-called next-generation sequencing opens up new possibilities and is rapidly changing diagnostics (and research). The results of genetic testing and their implications for affected individuals, their offspring, and families should be discussed during genetic counselling.

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8.1 Introduction

Infertility, hypogonadism, and other andrological syndromes may be (co-)caused genetically. While "clinical diagnoses" often tend to be descriptive, such as azoospermia as the cause of infertility, the causes of such phenotypes can partly be clarified by cytogenetic and molecular genetic methods. New molecular genetic methods, based on so-called next-generation sequencing (NGS), open up entirely new diagnostic possibilities and are becoming increasingly important. Accordingly, genetic testing is now indicated for almost all andrological issues, from isolated infertility to specific clinical entities such as hypogonadotropic hypogonadism.

When initiating genetic analyses and reporting findings, the legal framework must be taken into account, in Germany in particular the **Gene Diagnostics Act** (GenDG). In accordance with the GenDG, any physician can initiate genetic analysis to clarify a suspected diagnosis. However, in order to report suspicious findings, an additional qualification, "specialized genetic counselling" is required, which can be acquired by specialists in urology with an additional qualification in andrology, among others. Alternatively, specialists in human genetics are available to discuss the results of genetic testing and their implications for affected individuals, their offspring, and families.

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Genetic diagnosis is of central importance by definitively confirming suspected diagnoses such as Klinefelter syndrome, identifying causes for certain conditions such as sperm immotility, and/or assessing risks to offspring and relatives. In addition, genetic diagnoses increasingly influence the course of treatment, as they have prognostic value for, e.g., the chances of testicular biopsy or the success of IVF/ICSI.

8.2 Human Genome and Variability

The haploid human genome, which is present after the first meiotic division, has a chromosome set of $n = 23$. The somatic cells as well as the spermatogonia and oogonia have a diploid chromosome set ($2n = 46$). Most human genes are located on the chromosomes of the nucleus (nuclear genome), whereas some genes are located in the mitochondria (mitochondrial genome). The human haploid genome consists of about 3 billion base pairs (bp), with only about 1% consisting of coding sequences that make up the approximately 20,000 protein-coding genes. In contrast, the number of proteins they form is much higher due to alternative splicing. The nuclear genome is composed of singular and repetitive sequences. The singular segments contain coding sequences, regulatory sequences, and spacer DNA as elements interspersed between regulatory sequences and promoters. Repetitive sequences include satellite DNA, retroposons, endogenous retrovirus DNA, and transposons.

The human genome exhibits extraordinary variability at all scales of magnitude, and the following forms of variation can be distinguished:

- **Numerical Chromosomal Variations**; e.g., Klinefelter Syndrome (47,XXY).
- **Copy Number Variations** (CNVs), i.e., deletions or duplications of larger DNA segments ranging from a few kilobases (kb) to a few megabases (Mb); e.g., the deletion of the *CATSPER2*/*STRC* locus leading to the deafness infertility syndrome.
- **Variable Numbers of Tandem Repeats** (VNTRs), which are changes in the number of repetitive sequences; e.g., the CAG repeat unit (repeat) in exon 1 of the androgen receptor (AR) gene, which modulates testosterone action.
- **Single Nucleotide Variations** (SNVs), which are exchanges of single or a few nucleotides; e.g., point mutations in the *CFTR* gene that cause **obstructive azoospermia**.

Genetic methods are now available that can detect almost all of the genomic variations mentioned. It is important to know the limitations of the respective analyses (Fig. 8.1).

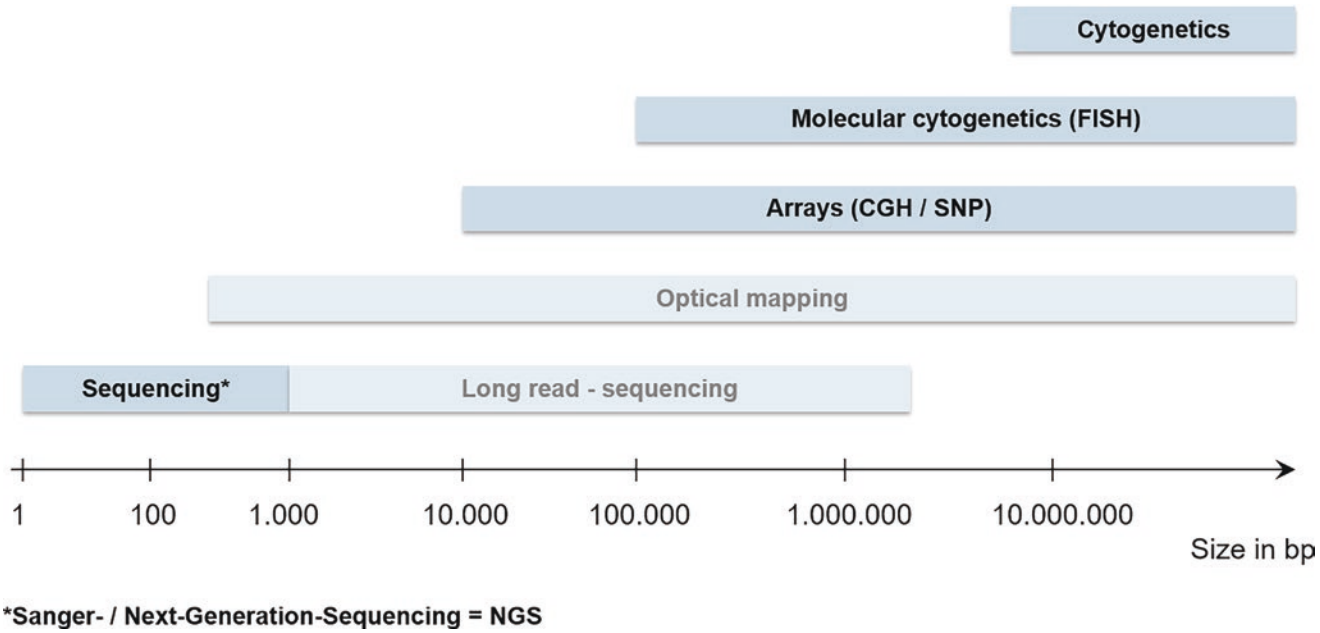


Fig. 8.1 Detection limits of genetic analyses. Gray: new methods, which are currently still mainly used in research

8.3 Methods of Investigation

8.3.1 Conventional Cytogenetics

Chromosomes are best visualized in metaphase, since they reach their maximum condensation during this phase of cell division. In this routine test, the chromosome set is determined from the lymphocytes of a Li heparin blood sample. The addition of the mitogen phytohemagglutinin stimulates mitotic division of the lymphocytes. Typically, a maximum of cell divisions is reached after 72 h. To arrest the cells in metaphase, the spindle toxin colchicine is added. Subsequent treatment with hypotonic saline and a mixture of acetic acid and methanol allows the chromosomes to be spread by dripping the cell suspension onto a slide. After appropriate staining, the chromosomes can be assessed by light microscopy at 1000× magnification.

The most commonly used banding technique is GTG banding (G banding after treatment with trypsin and staining with giemsa), which allows identification of chromosomes. The dark bands are AT-rich, have an increased degree of folding, and contain fewer genes than the light bands, which are GC-rich. To create the karyotype ("chromosome formula"), appropriate metaphases are imaged and chromosomes are sorted into groups (A to G as well as sex chromosomes) according to size, centromere location, and banding pattern, resulting in a karyomgram (Fig. 8.2).

The description of a karyotype follows an internationally standardized scheme (International System for Cytogenetic Nomenclature, ISCN, current version 2020). First, the chromosome number is given, then—separated by a comma—the constellation of sex chromosomes (gonosomes), and then any aberrations (numerical aberrations, deletions, duplications, translocations inversions, insertions, or marker chromosomes) are listed.

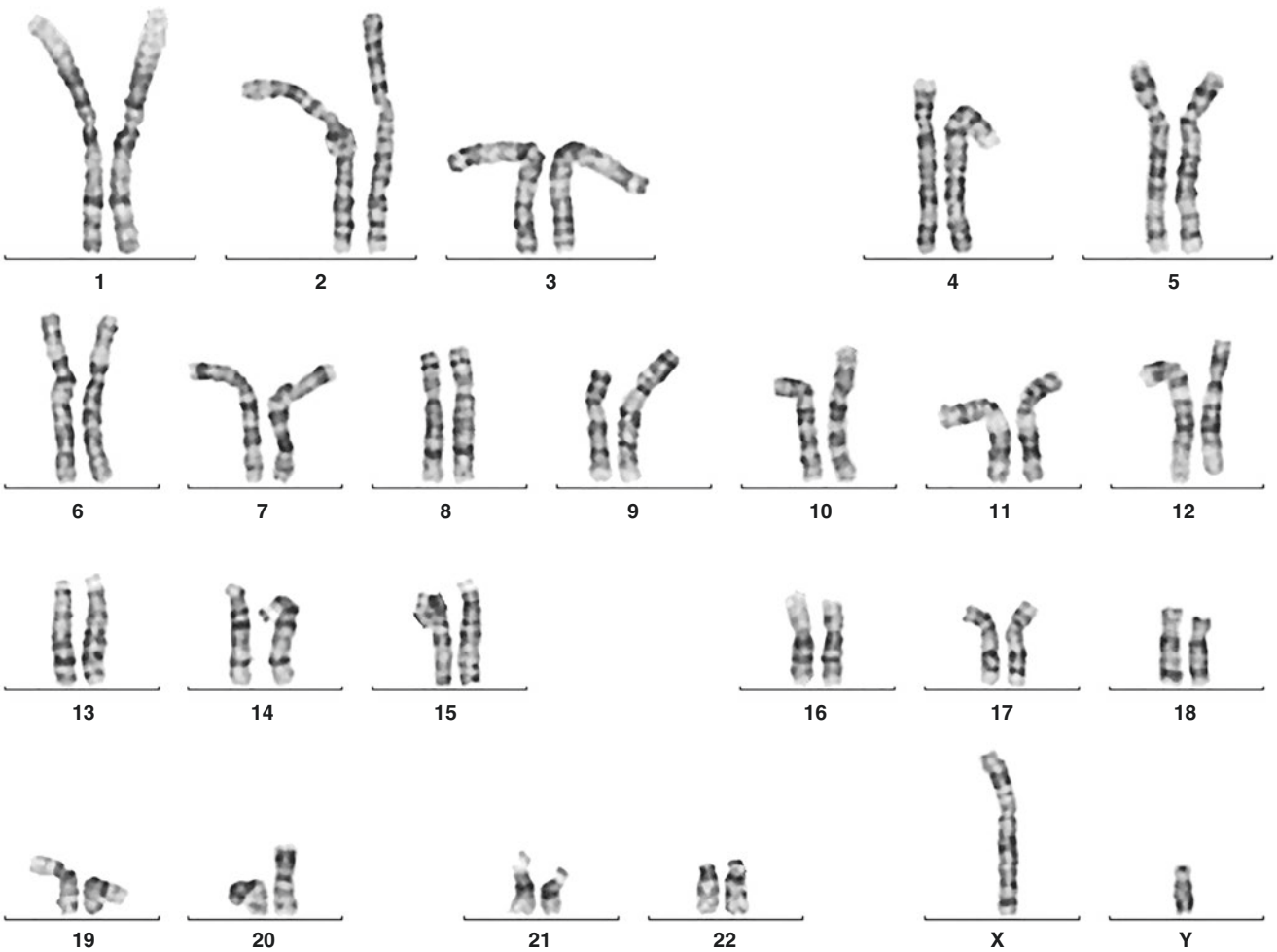


Fig. 8.2 Karyogram with normal male chromosome set 46,XY (GTG banding)

In a cytogenetic report, the number of metaphases examined, the staining procedures used, and the band level obtained are given. The chromosome number should be determined from at least 10 metaphases. If a mosaic is suspected, i.e., a mixture of cells with different sets of chromosomes, more metaphases should be analyzed numerically. Of these metaphases, at least 5 should also be analyzed structurally. The band level is of importance to estimate to what extent structural aberrations can be detected by this analysis. As a minimum standard, a banding level of 500 per haploid chromosome set is required for postnatal examinations. By conventional cytogenetic examination, deletions or duplications can be detected from a size of about 8 Mb (depending on the localization).

8.3.2 Fluorescence In Situ Hybridization

Fluorescence in situ hybridization (FISH) diagnostics combine classical cytogenetics with molecular genetic techniques and represents the basic method of molecular cytogenetics. **FISH** is based on the ability of single-stranded, fluorescently labelled DNA segments (probes) to selectively bind to single-stranded chromosomal regions according to base complementarity. For this purpose, chromosomal DNA is denatured and hybridized with the fluorescently labelled DNA probe. The fluorescent regions are then detected using a microscope equipped with appropriate filters (Fig. 8.3).

FISH is used as a complement to conventional cytogenetics because it allows higher resolution (up to 100 kb) and can

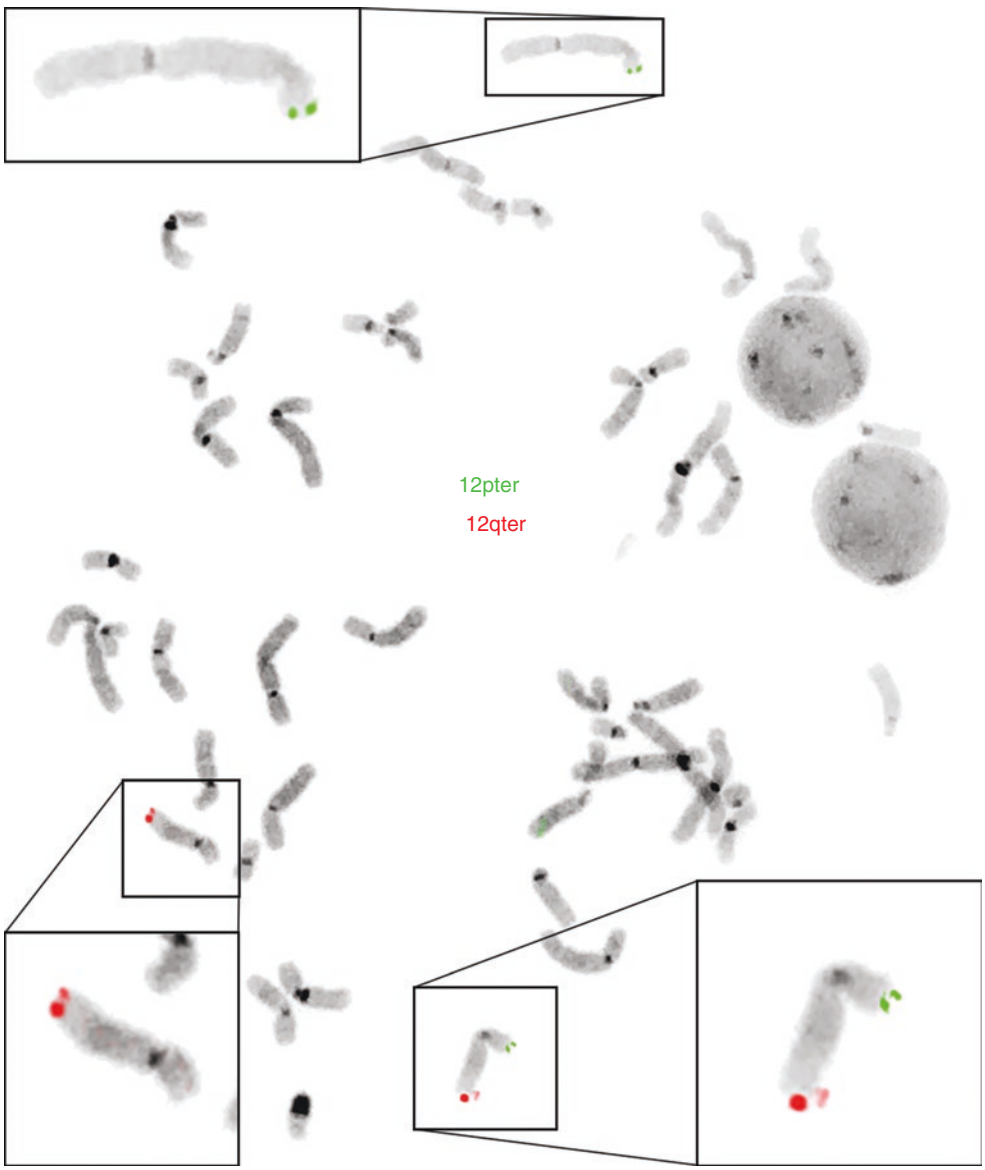


Fig. 8.3 Fluorescence in situ hybridization (FISH) in a male with oligozoospermia and detection of a translocation between a chromosome 2 and a chromosome 12 (green: telomere probe short arm = 12pter, red: telomere probe long arm = 12qter). The karyotype is 46,XY,t(2;12)(q37.3;p13.33)

be used on interphase nuclei (i.e., without prior cell culture). A variety of different probes are available for FISH analyses, staining specific regions of a chromosome (locus-specific probes for the detection of microdeletions/duplications), the area close to the telomere (subtelomere probes, e.g., for the detection of translocations) or whole chromosomes (whole chromosome painting, WCP, for the detection of chromosome rearrangements).

8.3.3 Array Analysis

Array analysis is used to identify smaller chromosomal imbalances that cannot be detected by conventional chromosome analysis. Thus, genome-wide gains (duplications) and losses (deletions) can be detected from a size of about 20 kb, thus closing the gap between classical cytogenetics and molecular genetics. It is therefore virtually a high-resolution chromosome analysis (**molecular karyotyping**), although balanced, structural changes, such as translocations, cannot be detected. Two methods are used, CGH arrays (Comparative Genomic Hybridization) and SNP arrays (Single Nucleotide Polymorphisms). The starting material for both analyses is a DNA sample from a patient, usually extracted from an EDTA blood sample. Microdeletions and duplications can be the cause of many different syndromes, ranging from **syndromic diseases**, such as heterozygous duplications in region 17q24.3 adjacent to the SOX9 gene in XX males, to milder diseases that may be relevant in infertile males, such as **deafness-infertility syndrome** (DIS) with deletions of the *CATSPER2* and *STRC* genes in region 15q15.3 (Fig. 8.4).

8.3.4 Microdeletions of the Y Chromosome

Microdeletions of the so-called **azoospermia factors (AZF)** on the long arm of the Y chromosome play a special role in andrology/male infertility. These deletions, along with Klinefelter syndrome, are among the few certain and long-known causes of severe spermatogenesis disorder. Analysis with respect to AZF deletions is performed according to the laboratory guidelines of the European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN) by means of two multiplex PCR reactions using a limited number of robust and validated primers covering the three relevant AZF regions with the inclusion of control samples (Krausz et al. 2014). The absence of PCR amplification in the basic analysis indicates a microdeletion and is confirmed by an extended analysis that simultaneously confirms the extent of the deletion (complete/incomplete). This protocol is sufficient to detect at least 95% of clinically relevant microdeletions. In addition to the classical PCR approach, commercial kits, arrays, MLPAs (multiplex ligation-dependent probe amplification), and sequencing methods are also used. The use of an unnecessarily large number of markers is problematic, as this introduces addi-

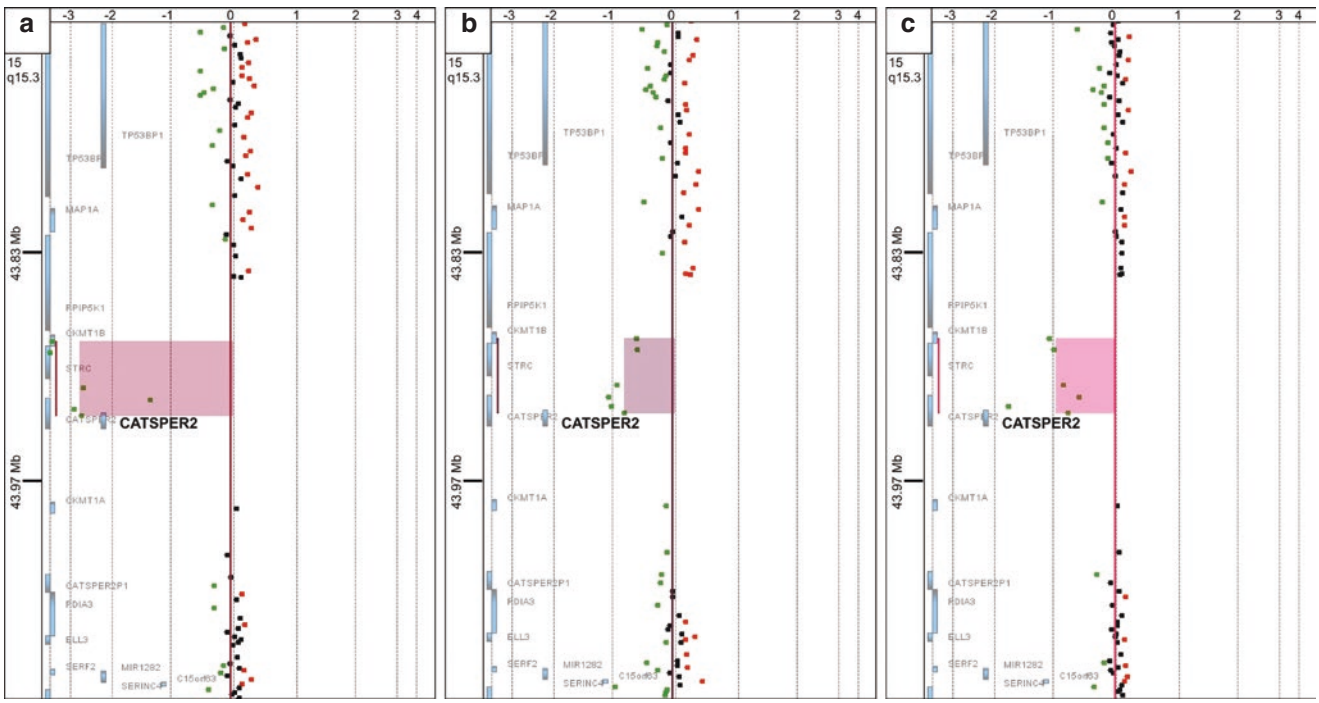


Fig. 8.4 Array CGH showing homozygous deletion from *CATSPER2*/*STRC* in a patient with deafness infertility syndrome (**a**), and his heterozygous parents (mother **b**, father **c**). The red shaded areas correspond to the size of the deletion

tional possibilities for error without increasing the diagnostic quality. An external quality control program (proficiency testing) is offered by EAA/EMQN to check diagnostic accuracy and correct interpretation of findings (https://www.emqn.org/).

8.3.5 Molecular Genetics: Sequencing

In recent decades, molecular genetics has developed by leaps and bounds. The complex, classical Sanger sequencing using gel electrophoresis was initially replaced by automated "dye-terminator sequencing". In recent years, **"next-generation sequencing**" (NGS) has become a much more efficient technique. Ultimately, all these techniques aim to "read" DNA sequences, i.e., to identify the base sequence and thus deviations (variants/disease-relevant mutations). The starting material is usually DNA (more rarely RNA), which in most cases is extracted from EDTA blood, but can also be obtained from almost any other tissue. The details of each method are hardly relevant for the evaluation of collected findings, but a basic understanding of the differences and limitations of the analyses is useful.

In (automated) **Sanger sequencing**, previously enriched, specific DNA fragments of approximately 300–1000 bases are sequenced. In contrast, in NGS much shorter fragments of 100–150 bases, but in many times greater numbers ("massive parallel sequencing"), are sequenced. These small fragments must then be assembled ("aligned") using a reference sequence in order to detect deviations from the normal sequence. The (bio)informatic processing of the data sets collected by NGS has therefore become increasingly important in recent years. Continuous further development and the associated steady decline in the price of NGS mean that fewer and fewer individual genes/gene segments (exons) are being sequenced, but increasingly "gene panels", i.e., all genes simultaneously that are associated with a particular disease, e.g., 30–50 genes in Kallmann syndrome/congenital hypogonadotropic hypogonadism. The transformation of modern molecular genetics is in full swing towards standard sequencing of the exome (nearly all coding genes; whole-exome sequencing, WES) or even the whole genome (**whole-genome sequencing**, WGS). Apart from nucleotide variations, genome sequencing can also be used to detect structural variants, so that some of the methods described above are already losing importance.

Thanks to the new sequencing techniques, almost all genes can now be sequenced in any number. The data can be generated within a very short time (a few days). The laboratory effort thus recedes into the background, while the interpretation of the data is by far the greater challenge. Since the human genome, as described above, is characterized by diverse variation, deviations from the "normal" base sequence are also regularly detected during sequencing. Most of these deviations represent so-called polymorphisms, i.e., norm (benign) variants, which are not relevant to disease. Only a small proportion is "pathogenic", i.e., causative for a disease. The classification of variants requires a high level of expertise with the aid of bioinformatic tools, large databases and, if necessary, further (functional) analyses. For the evaluation of variants, the following classification according to the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) has become the gold standard (ACMG-AMP Guidelines, Richards et al. 2015): benign - likely benign - uncertain significance - likely pathogenic - pathogenic. For many genes/diseases, there is not yet enough data, so it is not uncommon for "variants of uncertain (clinical) significance" (VUS) to appear in the findings. These are not suitable for clinical/diagnostic decisions. Due to the continuous increase in knowledge, it is necessary to reassess these variants on a regular basis according to the ACMG Guidelines mentioned above.

8.3.6 New Methods

Technological developments in genetics continue to advance rapidly. Examples of new analytical methods include "optical mapping" and "long-read sequencing." Using these methods, among others, structural variants can be detected at nearly all orders of magnitude including balanced translocations and other rearrangements. In parallel, NGS technologies are combined with microfluidics to enable, e.g., single-cell RNA analysis (scRNAseq). Initially, all these techniques have expanded the possibilities of genetic research, but are also now rapidly finding their way into clinical diagnostics, allowing a move towards "personalised medicine" with examples such as individualized cancer therapy. It is only a question of time until the first comparable approaches will also find their way into andrology.

8.4 Indications for Genetic Testing

After a long time during which only expert recommendations were available for genetic diagnostics in the field of andrology and reproductive medicine, a current AWMF guideline on "Diagnostics and therapy prior to assisted reproductive medical treatment" was published in 2019 (DGGG 2019). The recommendations for genetic diagnostics in cases of infertility and recurrent miscarriages (habitual abortions) are summarized in Wyrwoll et al. (2021). An overview of the diagnostic algorithm is given in Fig. 8.5.

In men, the frequency of chromosomal abnormalities increases with decreasing sperm count and reaches about

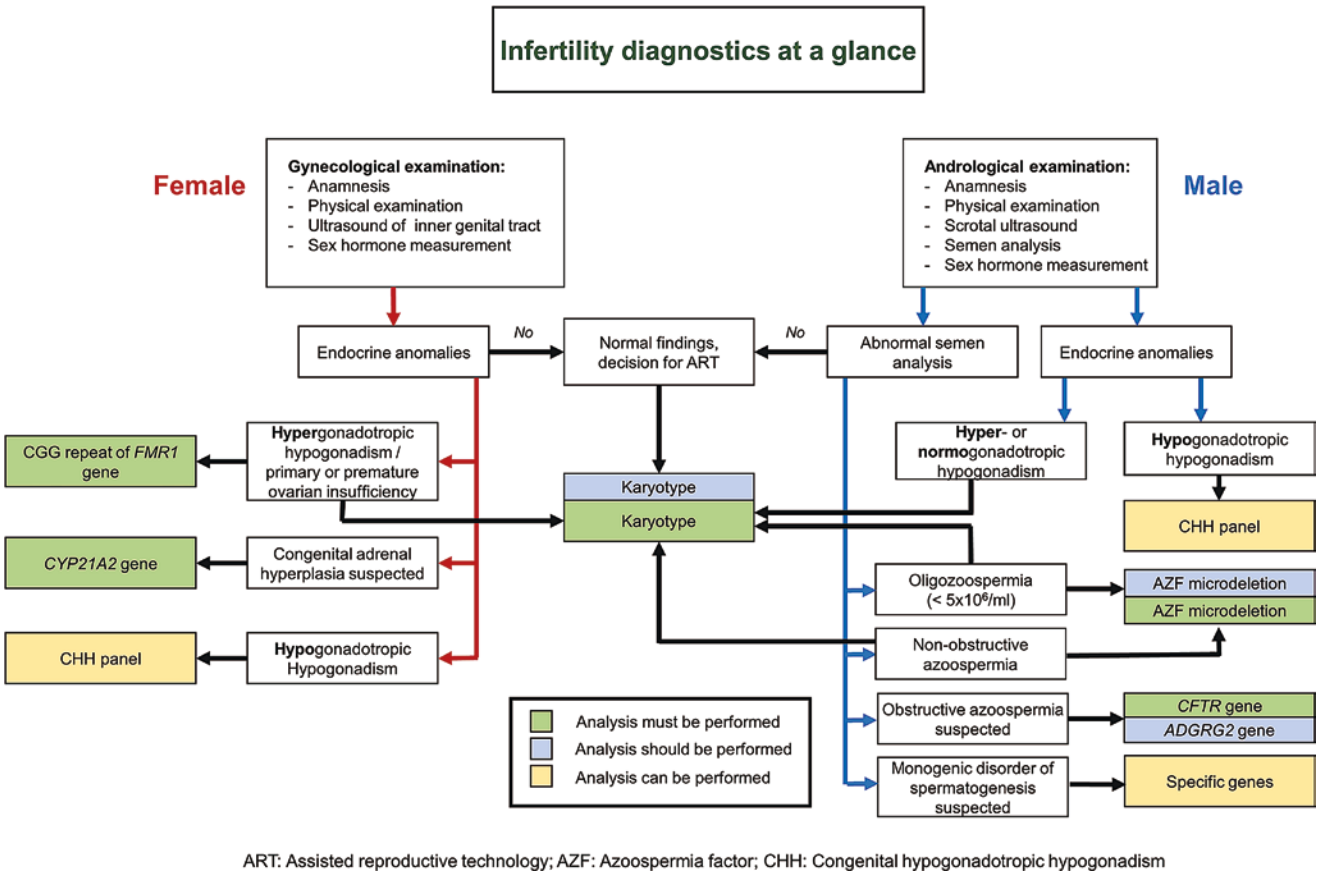


Fig. 8.5 Genetic diagnostic procedures for couple infertility according to the current AWMF guideline (Wyrwoll et al. 2021, adapted from DGGG 2019)

15% in azoospermia (Tüttelmann et al. 2011). Even if all clinical findings are inconspicuous, i.e., normozoospermia was detected in the male, there is still an increased risk of chromosomal alterations, e.g., translocations, so that chromosomal analysis should be performed even then. In 4–5% of couples with two or more miscarriages, a chromosomal abnormality is present in one of the two partners. In some rare diseases, chromosomal alterations may be the cause, e.g., in variants of sex differentiation (also in German-speaking countries "DSD" after English: Differences [formerly Disorders] of Sex Development, Chap. 31).

In cases of infertility or recurrent miscarriages, chromosomal analysis should be performed in both partners regardless of other clinical findings (DGGG 2019).

Klinefelter syndrome, a numerical chromosomal change (karyotype 47,XXY), is a common cause for azoospermia (Chap. 21); much less frequently, a chromosome set 46,XX is detected (XX male, Chap. 22). In cases of oligozoospermia, but also in cases of inconspicuous findings in semen diagnostics and couple infertility as well as in cases of recurrent miscarriages, mainly structural chromosomal alterations such as translocations, inversions, and insertions are detected (Chap. 23). Apart from the causal diagnosis, it is of particular importance to identify patients with chromosomal alterations such as translocations or inversions, because in case of pregnancy (whether spontaneous or, e.g., after ICSI) the probability of unbalanced chromosomal defects in children is significantly increased. This is associated with a significantly increased risk of miscarriage/stillbirth or the birth of a child with a usually severe mental and physical developmental disorder. In this context, the possibilities of prenatal and preimplantation genetic diagnosis (PND/PID) must be explained to the couple.

Males with azoospermia or severe oligozoospermia should additionally be screened for Y-chromosomal AZF microdeletions. Complete deletions of the AZFa or AZFb regions regularly lead to azoospermia and there is virtually no chance to obtain sperm at testicular biopsy/TESE. Men with a complete AZFc deletion may have severe oligozoospermia or azoospermia, and in the latter case have about a 50% chance of successful TESE. AZFc deletions can be

inherited (by ICSI, sometimes spontaneously) by sons. Thus, the diagnosis of AZF deletions has both prognostic value and influence on therapy (see also Chap. 23).

In men with sperm concentrations <1 × 10^6/ml, analysis should be performed with regard to AZF deletions; above this limit, deletions are extremely rare (DGGG 2019).

If obstructive azoospermia is suspected (normal FSH, normal testicular volume), a congenital absence of the vas deferens (**Congenital Bilateral Aplasia of the Vas Deferens, CBAVD**) may be the cause. CBAVD is most often caused by alterations in the *CFTR* gene (Chap. 27), which should be analyzed accordingly. Mutations in the *ADGRG2* gene also lead to CBAVD, so this gene can also be investigated.

If obstructive azoospermia is suspected, analysis of the *CFTR* gene and, if necessary, the *ADGRG2* gene should be performed (DGGG 2019).

Indications for (larger) sequencing analyses of gene panels up to the exome and, in some cases, special investigations (e.g., methylation analyses) are

- congenital hypogonadotropic hypogonadism (CHH) incl. Kallmann syndrome (Chap. 12),
- rare forms of secondary hypogonadism (Chap. 13),
- various forms of spermatogenesis disorders such as globozoospermia and marked astheno/teratozoospermia (Primary Ciliary Dyskinesia, PCD and Multiple Morphological Abnormalities of the Sperm Flagella, MMAF) (Chap. 20),
- Nonobstructive azoospermia (NOA) after exclusion of other causes (Chap. 20).

In men with rare disorders such as Kallmann syndrome, specific spermatogenesis disorders such as globozoospermia, or nonobstructive azoospermia, and gene panel/exome sequencing may help to clarify causal factors.

Key Points

- Genetic testing is indicated for almost all andrological issues ranging from isolated infertility to specific medical conditions.
- The spectrum of methods includes classical cytogenetic investigations, molecular cytogenetic procedures, and especially molecular genetic investigations.
- Next-generation sequencing opens up new possibilities and is rapidly changing diagnostics (and research).
- The results of genetic testing and their consequences should be discussed in genetic counselling.
- In cases of infertility or recurrent miscarriage, chromosomal analysis should be performed in both partners regardless of other clinical findings.
- In men with sperm concentrations <1x10^6/ml, analysis should be performed with regard to AZF deletions.
- If obstructive azoospermia is suspected, an analysis of the *CFTR* gene and, if applicable, the *ADGRG2* gene should be performed.
- In men with rare diseases such as Kallmann syndrome, specific spermatogenesis disorders such as globozoospermia, or nonobstructive azoospermia, gene panel/exome sequencing may help to clarify the underlying cause.

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Semen Analysis

9

Verena Nordhoff, Elisabetta Baldi, Barbara Hellenkemper, and Eberhard Nieschlag

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Abstract

Semen is analyzed for both diagnostic and prognostic purposes. Whether spermatozoa are present, in what numbers and in what condition (viable, motile, morphologically normal), are useful indicators that reflect testicular, epididymal, and accessory gland function and provide information on the quality of semen transferred at coitus. As variation in semen quality produced by the same man on different days is high, the use of standard methods for collecting and quantifying semen constituents and sperm characteristics is obligatory for an objective interpretation of the semen analysis result. The major semen analysis tests are described here along with the terminology and reference values provided by the sixth edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen (2021).

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9.1 Introduction

Semen analysis is performed for evaluation of fertility disorders with or without symptoms of androgen deficiency. The quality of semen analysis varies depending on how and where they are collected, and also on the analytical methods employed. As there are both biological and analytical causes for variations in semen quality, standardizing procedures for the collection and analysis of semen should render results from different laboratories comparable. A minimum number of spermatozoa need to be assessed to achieve acceptable values for total sperm numbers per ejaculate and percentages of motile, viable and morphologically normal cells. The assessment of motility and the rationale for seeking, and how to recognize, morphologically normal spermatozoa are provided.

9.2 Semen Collection

For comparability of results and adherence to reference values reported in the sixth edition of the WHO manual (WHO 2021), patients should observe a period of abstinence of between 48 h and 7 days. As semen quality reflects the state of sexual arousal and the time it takes to produce the sample (Pound et al. 2002; Elzanaty 2008), the conditions of collection are important. The ejaculate should be obtained at the clinic in a suitable room with adjacent toilet and hand basin. This eliminates any effect of temperature and time on a sample that is produced at home and brought to the laboratory; it also ensures a standard time of analysis after collection. The room should contain appropriate illustrated literature, background music, and/or video recordings to provide a suitable atmosphere. Semen collection can also be performed in a different place or at home. In such a case, semen should be kept at body temperature or at least at 30 °C and has to be brought to the lab within 30 min after the collection. Because of the normal variability of the various parameters, an estimation of fertility potential should be based on at least two, and preferably three, semen analyses performed after abstention times of 2–7 days.

Men are also requested to fill out a questionnaire including questions regarding abstinence time (days), recent fever, eventual inflammatory infections, smoking habits, recent use of drugs (especially antibiotics), and exact time of collection.

The semen sample should be obtained by masturbation. Formerly it was recommended to use a wide-mouthed clean, graduated glass cylinder. However, today the use of a disposable wide-mouthed plastic non-sperm-toxic container has become established in laboratory routine. For semen volume determination, the container is weighed prior to obtaining the ejaculate and the net weight is recorded on the container. Sample containers of a batch may differ in weight, so weighing is mandatory for each container. After sampling, the container is reweighed. As the density of the ejaculate is about 1 g/ml, weight corresponds to volume. The usage of pipettes or syringes for transferring the specimen into other containers for volume determination is strongly discouraged as a reasonable amount of the sample will be lost (Cooper et al. 2007). It is important to collect the total ejaculate, especially the first sperm-rich fraction; any deviation should be recorded. Collected semen should be stored during analysis at 37 °C (incubator) until complete liquefaction and evaluation of sperm motility (between 30 and 60 min).

9.3 Semen Analysis

The examination of the ejaculate should always be performed according to the Guidelines of the World Health Organization, which are explained in detail in the WHO Laboratory Manual for the Examination and Processing of Human Semen, sixth edition (WHO 2021). This laboratory handbook should be available in every andrology laboratory and is to be considered a supplement to this chapter. Only the most important of the standard and optional tests and selected sperm function tests are described in this chapter. The handbook considers semen analysis at three levels:

- standard tests: macroscopic appearance (appearance, liquefaction, semen volume, and pH), microscopic appearance (aggregation, agglutination, occurrence of non-sperm cells), sperm motility, sperm vitality (if motility is below 40%), sperm numbers, sperm morphology, count of leukocyte (by peroxidase activity) and immature germ cells (if 3–4 non-sperm cells (400x magnification) are present), mixed antiglobulin reaction test (especially if sperm agglutination is present),
- optional tests: teratozoospermia indices; pan-leukocyte CD4 staining, biochemical assessments of seminal plasma, sperm DNA fragmentation, aneuploidies,
- research tests: for instance, reactive oxygen species, spontaneous and induced acrosome reaction tests, assesment of sperm chromatin, Computer-aided-sperm analysis (CASA), transmembrane ion flux and transport in sperm.

Semen analysis according to WHO guidelines comprises standard methods, which are robust procedures for determining routine semen variables; optional tests may be used in certain situations or by choice of the laboratory; research tests exist that are not currently regarded as routine.

9.3.1 Macroscopic Appearance of the Ejaculate

A normal ejaculate has a homogenous grey-opalescent appearance and usually liquefies at room temperature within 15–30 min, when microscopic examination can begin. A whitish color may indicate high sperm numbers or presence of leukocytes; a yellowish appearance and purulent smell indicate infections; a reddish-brown color indicates the presence of red blood cells (haemospermia). For all the subsequent examinations, the liquefied ejaculate has to be mixed well so that representative samples are obtained. If the pH, measured soon after liquefaction, exceeds 8, infection may be suspected, although the pH rises with time after production as CO_2, supporting the buffering capacity, is lost. pH-values (measured with pH paper) lower than 7.0 together with azoospermia indicate malformation or obstruction of the epididymis, the deferent ducts, the seminal vesicles, or the ejaculatory ducts (Rudnik-Schöneborn et al. 2020).

9.3.2 Initial Microscopical Examination

Microscopical examination can be accomplished with a phase contrast microscope at 20 or 40x magnification on a warm plate at 37 °C on wet preparations of undiluted semen. For these, a volume of semen (e.g., 10 µl) is placed on a pre-warmed slide under a cover-slip of appropriate size (e.g., 22 mm × 22 mm) to produce a 20 µm deep chamber (other slides have to be used with other volumes, see WHO 2021). This provides sufficient depth for unrestricted 3-dimensional sperm motion meanwhile allowing the spermatozoa to remain in focus.

9.3.2.1 Sperm Aggregation and Agglutination

Aggregation, the adherence of immotile spermatozoa to debris or other elements of the ejaculate, or motile spermatozoa with non-sperm cells, has to be distinguished from agglutination of motile spermatozoa with each other. Aggregation is not considered further, since these spermatozoa cannot contribute to fertility. However, if in combination with agglutination, it should be noted. Agglutination in the fresh semen sample is suggestive of immunological infertility and is graded according to the type and extent of interaction. Extensive agglutination should be followed by the Mixed Agglutination Reaction (MAR) test to detect anti-sperm antibodies.

9.3.2.2 Non-sperm Cells

Besides spermatozoa, the ejaculate contains epithelial cells of the urogenital tract and so-called round cells (spermatogenic cells and leukocytes).

> Preliminary microscopic observations reveal whether spermatozoa are agglutinated or aggregated, whether non-sperm cell (epithelial cells or round cells [leukocytes, germ cells]) are present, and what dilution is needed to assess sperm concentration accurately.

9.3.3 Further Microscopical Analysis

9.3.3.1 Sperm Motility

Sperm motility is examined in the freshly liquefied semen sample at a magnification of ×200–400 . This can be performed at room temperature (between 20 °C and 24 °C), but preferably at 37 °C, normally 30 min to latest 60 min after ejaculation on the wet preparations described above. The quality of motility is expressed as the percentage of cells displaying the distinct classes of motility according to the WHO 2021 handbook:

- **A**: Rapidly progressive (≥25 µm/s): spermatozoa moving actively, either linearly or in a large circle, covering a distance, from the starting point to the end point, of at least 25 µm (or ½ tail-length) in one second.
- **B**: Slowly progressive (5 to <25 µm/s): spermatozoa moving actively, either linearly or in a large circle, covering a distance, from the starting point to the end point, of 5 to <25 µm (or at least one head length and less than ½ tail-length) in one second.
- **C**: non-progressive motility: motion on the spot flagellation or motion in small circles
- **D**: immotile: no motion visible.

In the 5th edition of the laboratory manual (2010) the motility was subdivided into only three categories, progressive (PR, or A and B), non-progressive (NP or C) and immotile (IM or D) spermatozoa. In the latest edition, this was changed and reverted to the familiar classification from the previous WHO manuals (WHO 1993, 1999).

The heads of the normally motile spermatozoa appear globular under light microscopic examination of the ejaculate. So-called pinhead sperm are actually sperm tails without heads and can be very motile. As they carry no genetic information, they are not included in sperm count, motility, or morphology assessments. Similarly, heads without a tail are not included in the evaluation of motility or morphology.

9.3.3.2 Total Sperm Numbers

The number of spermatozoa in the ejaculate can be used both prognostically, as it reflects what a man can provide to his partner at coitus, and also diagnostically, since it reflects his

testicular sperm output, the patency of the ductal system, and epididymal sperm reserves. As the sperm concentration reflects the extent of dilution by accessory gland fluids of the sperm pellet emitted from the epididymides through the urethra at the time of ejaculation, it provides no biologically relevant information about the testis or the accessory glands. It is measured in order to calculate the total sperm number in the ejaculate, which is obtained by multiplying the sperm concentration by the semen volume.

Sperm concentration is determined in a 100 µm deep counting chamber, preferably a Neubauer improved chamber (Zuvela and Matson 2020), after suitable dilution in a bicarbonate-formalin solution (to fix the cells; this may contain gentian violet or trypan blue solution, to help visualize the spermatozoa). Only intact spermatozoa, with a head and tail (comprising the mid-, principal-, and end-piece), are counted. Dilutions, which are designed to permit at least 200 spermatozoa to be observed in the chamber, are chosen according to the number of sperm seen in a x400 high power field of the fresh sample. The total number of at least 400 spermatozoa counted ensures a counting error of 5% for the estimated value. Sperm concentration is derived from the number of cells counted in a known chamber volume and multiplied by the dilution factor.

The number of spermatozoa obtained in an ejaculate is partly related to the abstinence time (Ayad et al. 2018, Hanson et al. 2017 Fig. 9.1) and to the testicular volume (Fig. 9.2). Multiple ejaculates per day further increase the cumulative number of spermatozoa recovered (Fig. 9.1), since the epididymis is not emptied at one ejaculation (Cooper et al. 1993). Men with small testicles do not display an abstinence-related increase in sperm numbers (Fig. 9.2). Devices or apps determining the presence of spermatozoa per semen are available (Agarwal et al. 2018). However, these tests do not always quantify the amount of spermatozoa and the visit in a specialized center is of utmost importance (Drobnis and Rothmann 2018).

When few spermatozoa are found in semen, it may not be necessary to determine their numbers accurately, if sufficient are available for intracytoplasmic sperm injection (ICSI). In this case, the report should indicate that the number of spermatozoa is below 2 Mill/ml. For contraceptive studies or assessing postvasectomy semen, counting accurately may be required. In these cases, semen should be diluted (1:2 to 1:25 maximum) before counting in a Neubauer chamber. Azoospermia, defined as the absence of spermatozoa from the ejaculate is classically assessed by evaluating the presence of spermatozoa in a centrifuged semen pellet (WHO 2021) to distinguish with cryptozoospermia. However, whether azoospermia is recorded or not depends on the centrifugation force and time; and not all spermatozoa are brought down at g forces obtainable in routine bench centrifuges (Lindsay et al. 1995; Jaffe et al. 1998; Corea et al. 2005). As examination of the pelleted spermatozoa can therefore never be quantitative; alternative methods are used in which the sensitivity is higher. For this, semen is diluted (1 + 1) and the entire Neubauer chamber (all 9 grids) is examined for spermatozoa; if fewer than 25 are observed in this volume, the sensitivity of the method (56,000 cells/ml semen) is reported; larger chambers can be used to increase

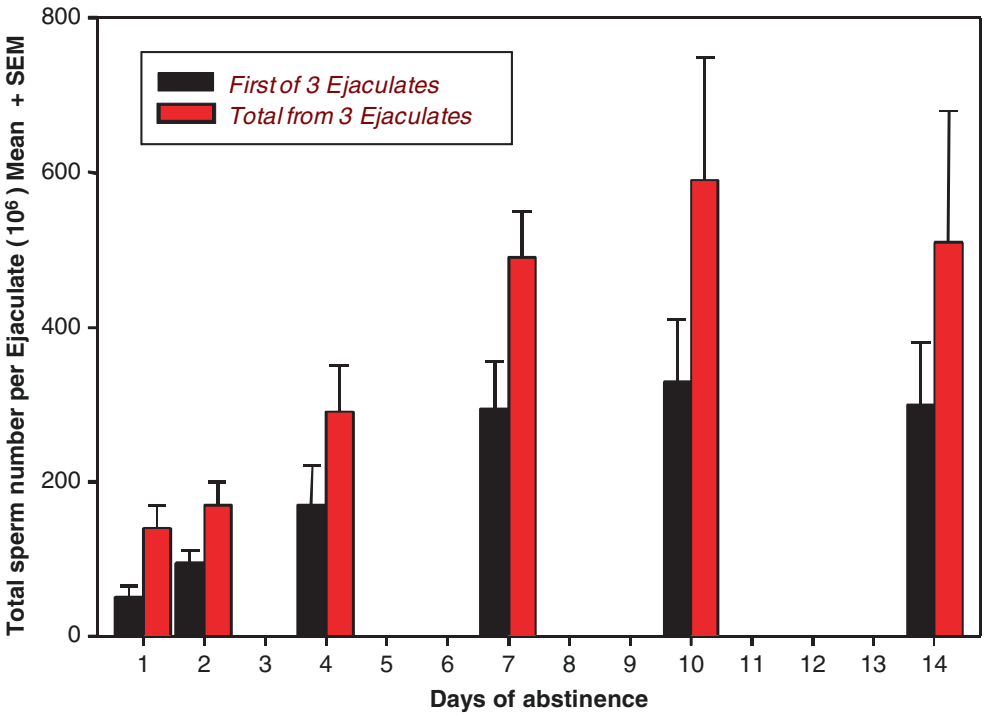


Fig. 9.1 Numbers of spermatozoa in the ejaculates from healthy donors after epididymal emptying (3 ejaculates within 4 h on 1 day) followed by a further three ejaculates per day on each day after abstinence times of 1, 2, 4, 7, 10, and 14 days. Total sperm numbers in the first ejaculate (white columns) and in all three ejaculates (red columns). Adapted from Cooper et al. (1993)

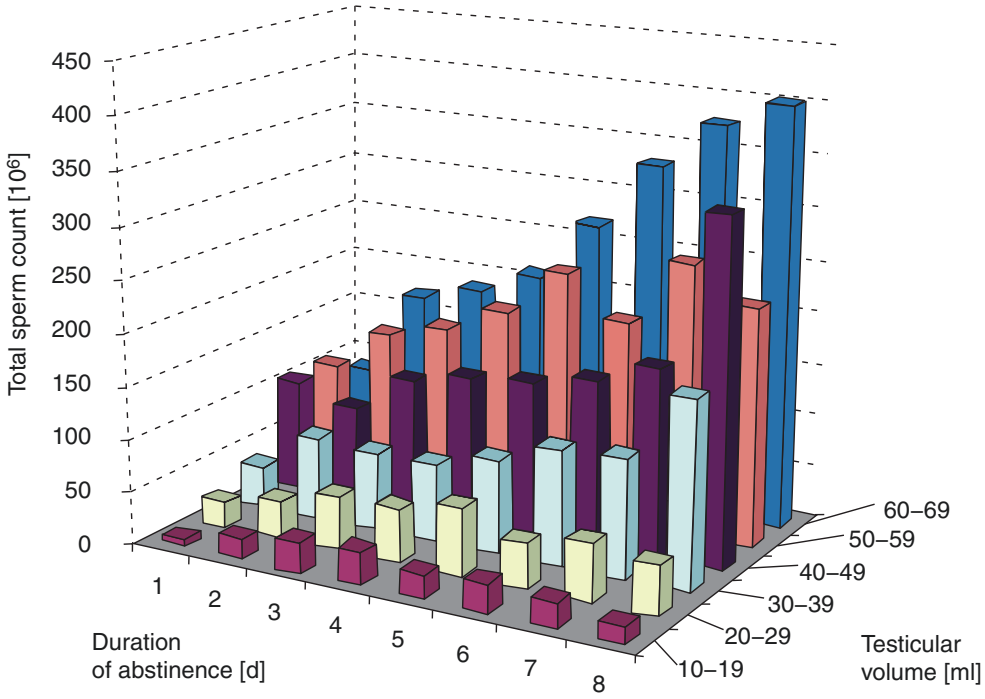


Fig. 9.2 Relationship between testicular volume, abstinence time, and total sperm number in 30,965 semen samples from 11,062 infertile men

the sensitivity of the assay to 2000 cells/ml semen (Cooper et al. 2006). It should be noted that centrifugation may also alter the ability of spermatozoa to move, therefore, if evaluation of motility is needed, centrifugation should not exceed 500 × *g*.

9.3.3.3 Sperm Morphology

As semen is viscous, it cannot be fixed before making a usable microscopical examination smear. Thus, sperm morphology is routinely examined in an air-dried, fixed, and stained semen smear. Unfortunately, spermatozoa in air-dried smears are smaller than those in living wet preparations, immature sperm heads swell under these conditions, and osmotically sensitive cytoplasmic droplets (present on the majority of motile spermatozoa) are rarely preserved, whereas excess residual cytoplasm (on abnormally formed spermatozoa) is retained. Nevertheless, such smears can provide preparations of clearly separated spermatozoa preferentially exposing the flat plane of their heads. The WHO-recommended Papanicolaou, Shorr and Diffquik staining procedures provide adequate coloration for spermatozoa and allows distinguishing between immature germ cells and granulocytes (the distinction of rarely occurring monocytes could be difficult).

A normal spermatozoon consists of a head, a midpiece, and a tail. Normal spermatozoa sought in the ejaculate are those that are biologically selected by reaching endocervical mucus. These spermatozoa, recovered and stained in air-dried fixed smears, exhibit no obvious defects of the head or tail. A similar population of morphologically normal spermatozoa are those that are tightly bound to the zona pellucida ("zona-preferred" spermatozoa, Garrett et al. 2007). Determining the extent of the mucus-defined subpopulation is the aim of sperm morphology assessment. They have a regular oval-shaped head with an intact and slender midpiece and principal piece displaying no abrupt breaks. The acrosome is clearly visible and covers 40–70% of the sperm head area (see Fig. 9.3). Vacuoles (a large or several small ones) should not exceed 20% of the acrosomal area and are not present in the post-acrosomal region. Excess cytoplasm occupying less than 30% of the head volume are considered cytoplasmic droplets and the spermatozoa are normal. If the size is more than 30% or it has an irregular shape, it is considered excess residual cytoplasm and the spermatozoon is considered abnormal. Men produce few potentially fertilizing spermatozoa since only low proportions of seminal spermatozoa can bind to the zona pellucida (range 8–25%, Liu et al. 2003). The ability to detect the presence of the acrosome by lectins (WHO 2021) and specific antibodies (Ito and Toshimori 2016) can be used when specific information about this organelle is required.

Many abnormal forms of spermatozoa are found in semen: large or small oval heads and tapering, pyriform, and vacuolated heads (>20% of the head area occupied by unstained vacuolar areas). A specific head variant are spermatozoa without acrosomes; if they show globular heads in a high percentage, the patient is affected by globozoospermia. Some spermatozoa have double heads and should not be included in the morphological evaluation. The midpiece and principal piece can show defects; tails can be coiled, broken,

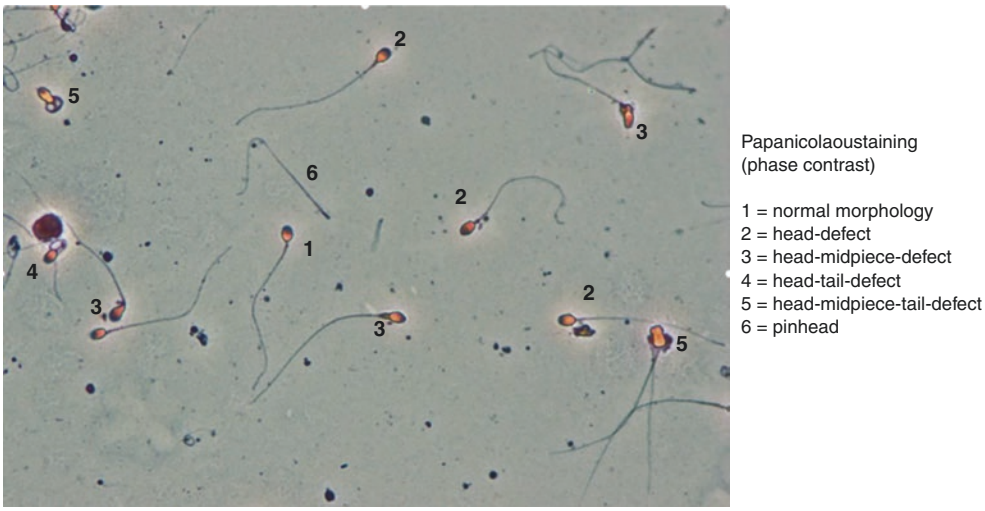


Fig. 9.3 Semen smear after Papanicolaou staining observed with phase contrast optics. Spermatozoa with normal heads (1), abnormal head size or shape (2), cytoplasmic droplet (3a), midpiece defect (3b), excess residual cytoplasm (3c), both head and midpiece defects (4), head and tail defects (5) and head, midpiece, and tail defects (6)

or doubled. Heads can be separated from tails (decapitation forms) or tails may occur without heads (pinheads), but these are not considered spermatozoa (which are defined as having a head and a tail) for the purposes of counting or morphological assessment. Additionally, noting the nature of these abnormal forms and calculating morphological indices may be useful in diagnosis.

Standard semen analysis provides information on the total number of spermatozoa per ejaculate as well as the percentage of motile and morphologically normal forms.

9.3.4 Additional Analyses

9.3.4.1 Sperm Vitality

When the percentage of progressively moving spermatozoa is below 40%, sperm vitality tests are used to determine whether spermatozoa have lost their flagellation because of metabolic dysfunction (with a functional axoneme) or axonemal defects (with normal or abnormal metabolism), or are simply dead.

The eosin test is based on the fact that membrane-impermeant eosin is excluded by membranes of living cells. The damaged cell membrane of dead spermatozoa can be penetrated by the dye and therefore the heads of such cells are specifically stained red. These can be observed in wet preparations or in smears in which a suspension of nigrosin is included to make sperm heads more readily identifiable. The latter can be scored at 1000× magnification in bright field and provide samples that can be stored for internal quality control purposes.

The hypo-osmotic swelling (HOS) test is a simple test of the integrity of the semipermeable plasma membrane of the sperm tail (Jeyendran et al. 1984). Semen is diluted with a hypotonic solution so that water enters spermatozoa osmotically. Intact spermatozoa are revealed by swelling-related changes in cell shape that occur to minimize cell area and which enforce a coiling of the flagellum. Swelling is completed by 5 min, and by 30 min, the various swelling patterns have stabilized and can be quantified by scoring at 400x phase contrast microscopy. In contrast, dead cells with damaged membranes provide no osmotic gradient for swelling to occur.

With the availability of ICSI, especially in conjunction with testicular sperm extraction (TESE), the HOS test is of interest, since dead cells can be distinguished from vital but poorly motile or immotile cells without the need for a stain, and such a cell can be used for ICSI (Check et al. 2013). In this case, culture medium is diluted 1 + 1 to create hypotonicity and only 5 min incubation is employed. Still, this method may result in heavily swollen sperm which might not fit into the ICSI needle anymore. Therefore, other optional vitality tests which leave the spermatozoon intact and usable for ICSI could be used: among them the modified HOS-test, the use of chemical compounds for motility indication, the sperm-tail flexibility test (STFT), or the application of a laser shot to the tip of the tail (Nordhoff 2015).

9.3.4.2 Round Cells

The detection of peroxidase in wet preparations, which specifically stains granulocytic leukocytes, is a simple test for the presence of leukocytes. Their concentration can be measured, as for spermatozoa, from the number of cells in a known chamber volume and taking into account the dilution.

A preliminary distinction between leukocytes and spermatogenic cells may be achieved in stained semen smears by the more bluish staining of the former and more pinkish staining of the latter with Papanicolaou staining. In addition, morphological appearance after staining might be of help in

distinguishing granulocytes from germ cells. Further investigations of the semen for the presence of leukocytes can be made by using, e.g., the pan-leukocyte marker CD45.

Leukocyte concentration can be assessed relative to the number of spermatozoa per unit area (field of view, e.g., one leukocyte per 100 sperm) and from the sperm concentration. The total number of leukocytes per ejaculate is obtained by multiplying their concentration by the semen volume.

9.3.4.3 Immunological Tests

Sperm agglutination in the fresh semen sample may be indicative of the presence of specific sperm antibodies; however, agglutination may not always be caused by antibodies and not all antibodies directed against spermatozoa cause agglutination; others are cytotoxic and interfere with motility disorders.

For the determination of antibodies of the IgA or IgG class directed against sperm antigens, the mixed antiglobulin reaction (MAR) test has proved to be useful as a screening test. For this, a fresh semen sample and IgG- or IgA-coated latex particles or erythrocytes are mixed together with antiserum directed against IgA or IgG antibodies. If the respective antibodies are present on the sperm surface, the particles or cells will be bound to the spermatozoa by the antiserum. The proportion of these particle-bound motility sperm can then be quantified. If more than 50% of the spermatozoa are IgG or IgA antibody-coated, immunological infertility is likely (Vicram et al. 2019). If positive, the results may be complemented by sperm mucus interaction tests (postcoital test, Kremer test) (Talwar and Hayatnagarkar 2015); however, this test is not very common anymore.

More sensitive immunobead tests on washed spermatozoa can be made and the indirect immunobead test permits antisperm antibodies in cell-free fluids to be assessed.

9.4 Biochemical Analyses of Seminal Fluid

Different chemical substances in the ejaculate, secreted by specific reproductive organs, can be measured in the ejaculate to serve as markers for these organ's function. As a general principle, a decrease in the concentration of the seminal markers indicates a dysfunction of the secreting organs or an obstruction of the duct system. The determination of specific substances for each level of the post-testicular duct system allows a rough localization of the disorder. However, it should be considered that in the case of bilateral organs, only bilateral dysfunction may cause significant changes of the biochemical markers.

Prostatic function can be gauged by the measurement of zinc, citric acid, and acid phosphatase. Prostaglandins and fructose are secreted mainly by the seminal vesicles. Low fructose concentrations in the seminal plasma may indicate bilateral agenesis or severe dysfunction of the seminal vesicles or obstruction of the ejaculatory ducts. In cases of low fructose levels, transrectal ultrasonography of the seminal vesicles before and after ejaculation should be performed for further diagnosis.

L-carnitine, glycerophosphocholine (GPC) and neutral α-glucosidase are markers of epididymal function, but carnitine and GPC are also secreted by the seminal vesicles and acid glucosidase by the prostate. Neutral α-glucosidase is specific for the epididymis and thus has a higher specificity and sensitivity for judgement of epididymal function (Cooper et al. 1990). A severely diminished amount of neutral α-glucosidase in the presence of normal FSH and normal testicular volume is an indication that azoospermia might be caused by bilateral epididymal obstruction or obstruction or agenesis of the vasa deferentia (e.g., CBAVD).

> Seminal biochemistry can provide extra insight into the state of the accessory glands by providing specific information on the seminal vesicles (fructose), prostate (zinc), and epididymis (neutral α-glucosodase), when interpreted together with other clinical parameters.

9.5 Microbiological Tests

Nowadays, the classic venereal diseases such as gonorrhea or syphilis are of minor importance in infertile men in Europe. The predominant microorganisms found are *Chlamydia trachomatis* or *Ureaplasma urealyticum*, as well as gram-negative bacteria typical of urogenital infections (Solomon and Henkel 2017). These microorganisms can be identified directly in the urine, ejaculate, prostate expiate, or urethral swab. Leukocyte concentrations higher than one million/ml ejaculate alone or with a significant growth of microorganisms in the ejaculate culture may indicate an infection of the efferent seminal system (WHO 2021). Whereas the determination of different microorganisms in an aerobic ejaculate culture is unproblematical, the proof of *Chlamydia* requires special examination techniques. The direct determination of *Chlamydia* in the ejaculate by means of PCR (polymerase chain reaction) is considered the "gold standard".

9.6 Objective Semen Analysis

In clinical practice, the classic investigation of the ejaculate retains its central role in the assessment of couple infertility. However, systematic investigations have shown that the estimation of concentration, motility, and morphology of spermatozoa is influenced by significant subjective factors. In addition to standardization of ejaculate analysis by uniform laboratory methods, achieved by use of the WHO Laboratory Manual (WHO 2021), efforts are being made to further establish objective laboratory methods.

9.6.1 Sperm Concentration

The use of DNA flow cytometry to permit objective and precise determination of spermatozoa is based on their extent of DNA staining. When incubated with fluorescent propidium iodide (PI) or 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) stains, spermatozoa are characterized by highly condensed (HC) (or condensed chromatin [CC]) peaks, which are less stained and thus lie to the left of the expected haploid (1C or IN) peak. Since this method allows several thousand cells to be counted in very short time, high precision is possible because statistical counting errors are minimized (Hacker-Klom et al. 1999; Eustache et al. 2001). However, this technique has been brought into question since (1) it is not possible to ascertain if the heads assessed have a tail, i.e., are by definition spermatozoa; (2) the volume of liquid assessed, and hence the calculated sperm concentration may not be accurate. Calibration is often performed by co-measurement of fluorescent beads whose concentration is determined by electronic sizer; (3) the HC(CC) peak is not composed solely of spermatozoa: some cells differ in size or granularity and lack mitochondria (Yeung et al. 2007).

Sperm concentration can also be automatically analyzed by Computer-Aided Sperm Analysis (CASA) systems (for selection see Table 9.1). Counting the number of sperm cells identified by the size and contrast of the digitized video image of the sperm heads has been hampered by the presence of non-sperm particles (including round cells in the semen recognized by the programme as spermatozoa). Attempts to overcome this problem posed by this contamination include requiring additional criteria such as (1) accepting only oval head shapes, based on measurements of the major and minor axis and their ratio (e.g., Hamilton-Thorne Systems), (2) providing a tail-detection algorithm that scans for projections along the axis of the sperm head (QualiSperm), and (3) recognizing condensed chromatin from fluorescent staining of DNA (Hamilton-Thorne Systems). However, the reliability of these methods to determine sperm concentrations accurately, especially for samples with few spermatozoa, has yet to be established and is hampered by the low depth chambers in which streaming of semen upon filling the chamber causes unequal distribution of spermatozoa (Douglas-Hamilton et al. 2005; Mortimer et al. 2015). Because of these difficulties, the use of CASA systems for routine semen analysis is not recommended by the WHO.

Table 9.1 Selection List of some EQC programmes for semen analysis (homepage last visited 04.01.2021)

Country	Programme	Address	URL
Australia	External Quality Assurance Schemes for Reproductive Medicine	Fertility Society of Australia, PO Box 162 Northlands WA Australia	http://www.eaasrm.com.au
Denmark	Dansk Institut for Ekstern Kvalitetssikring for Laboratorieri	Rigshospitalet-Glostrup, Valdemar Hansens Vej 1-23, indgang 8,1., 2600 Glostrup	http://www.deks.dk/
Germany	QuaDeGA	Quality Control Program of the German Society of Andrology c/o: Universitätsklinikum Münster Center for Reproductive Medicine and Andrology, Albert-Schweitzer-Campus 1, Building Domagkstrasse 11, D-48129 Münster, Germany	http://www.quadega.de
Great Britain	UKNEQAS Schemes for Andrology	Department of Reproductive Medicine, St. Mary's Hospital, Whitworth Park, Manchester M13 0JH, United Kingdom	http://www.uknequas.org.uk
Italy	Valutazione Esterna di Qualità	Gruppo Controllo Qualità Analitico Azienda Ospedaliero-Universitaria di Bologna, Policlinico Sant'Orsola-Malpighi	http://legacy.aosp.bo.it/veq/qm/veq-proflili/veq_profili-andrologico.htm
Scandinavia	NAFA (Nordic Association for Andrology)	Andrology Unit, Reproductive Medicine Centre, Karolinska Hospital, PO Box 140, SE-171 76 Stockholm, Sweden	https://www.eshre.eu
USA	American Association Bioanalysts Proficiency Testing Service	1931 Wickchester Lane, Suite 200 - Houston, Texas 77043, USA	https://www.aab-pts.org

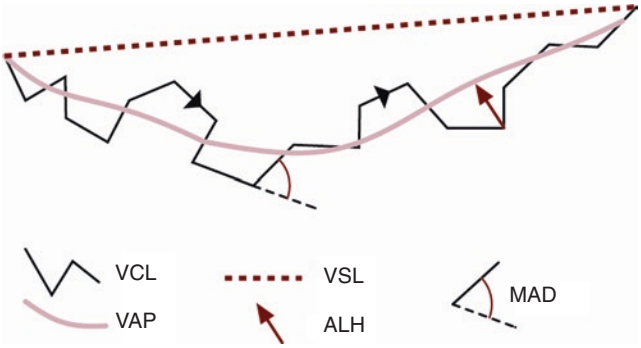


Fig. 9.4 Sperm movement parameters recorded by computer-assisted sperm analysis (CASA). *VCL*: curvilinear velocity (μm/s); *VAP*: averaged path velocity (μm/s); *VSL*: straight-line velocity (μm/s); *ALH*: amplitude of lateral head displacement (μm); *MAD*: mean angular displacement (°). Derived parameters: *LIN*: linearity (VSL/VCL); *STR*: straightness (VSL/VAP); *WOB*: wobble (VAP/VCL)

9.6.2 Sperm Motility

Among the various methods for objective measurements of sperm motility, the tracking of video images of individual sperm cells by CASA provides objective data on velocity. In addition to determining the proportion of motile sperm, these systems allow measurements of kinematic parameters, such as sperm velocity, linearity, amplitude of lateral head displacement, and beat cross frequency of the head, although the values are dependent on the settings of the machine (WHO 2021, Gallagher et al. 2018; see Fig. 9.4). Although some studies showed a correlation with pregnancy rates in vivo or after medically assisted reproduction (MAR) (Garrett et al. 2003; Youn et al. 2011), the predictive value of CASA for fertility prediction, e.g., in IUI, is still questionable (Fréour et al. 2010).

9.6.3 Sperm Morphology

Standardization of sperm morphology is the least developed of all parameters. Computer-aided video techniques are unlikely to be applied soon as improvements. By focusing through the sample, technicians may well be better able to determine if a sperm head is normal, whereas CASA equipment so far depends on images in one plane of focus. Confusion with background seminal protein is also a problem experienced in some semen samples, especially those that do not liquefy easily; problems not present in washed or swim-up samples. Examination of unfixed samples may be useful (Berkovitz et al. 2005), but remain experimental. The achievement of objectivity in the assessment of ejaculate parameters has been only partially successful. Therefore, in clinical practice, well-controlled conventional semen analysis remains fundamental in the evaluation of fertility.

However, CASA investigations are to be considered optional and supplementary for the routine examination of an infertile couple.

9.7 Quality Control in the Andrology Laboratory

The need for effective quality control of ejaculate analysis was demonstrated by a significant discrepancy between different evaluations of sperm concentration and morphology of identical samples analyzed by different laboratories (Neuwinger et al. 1990). Since then a lot of steps have been taken to establish a strict quality control programme in the andrology laboratory (Pacey 2009).

9.7.1 Internal Quality Control

Internal quality control of semen analysis is important for the continuous maintenance of standards throughout the year and for coping with errors arising from changes in personnel. It includes regular determinations of the inter- and intra-technician coefficients of variations for the evaluation of sperm concentration, motility, viability, and morphology of the same samples by different technicians (Pacey 2009). Where samples can be stored (morphology and viability slides, fixed semen samples, video recordings of motility), variation from target (known) values can be performed. In this case, the use of video recording or cryopreserved samples is advantageous. In large andrology laboratories, the calculation of monthly means of all the various determinations can be used for early recognition of a systematic bias in the methods used, provided there is no gross change in clientele (WHO 2021). Determinations of biochemical marker substances in seminal plasma are subjected to the usual internal quality controls of the clinical laboratory.

Special requirements for internal quality control exist, e.g., in Germany. The directive of the Federal Medical Association B4 (Rili-BÄK 2019) regulates quality assurance

for ejaculate analysis and obliges every laboratory producing spermiograms to carry out internal and external quality control. For internal quality control, no special test materials or additional samples are analyzed, but patient samples are subject to an evaluation emphasizing two issues: first, the basic parameters of the spermiogram, i.e., sperm motility, sperm concentration, and morphology, are assessed in duplicate. The difference between the two values must lie within the 95% confidence range. Instructions are given in the tables of the Appendix of the WHO Laboratory Manual (WHO 2021) or can be calculated by a formula (Rili-BÄK 2019). If the formula or the table indicates that the difference between the two assessments is too high, the sample must be reanalyzed. Second, internal quality control calculates and assesses the mean of the differences of the duplicate determinations of a control period. A control period consists of 50 pairs of measured values and is determined separately for each parameter using a formula (Rili-BÄK 2019). Discrepancies indicate a systematic error. The German Medical Board (Bundesärztekammer) offers an Excel table for internal quality control; the formulas can also be implemented within existing patient documentation systems.

9.7.2 External Quality Control

All andrology laboratories should enrol in an external quality control scheme (Table 9.1). In Germany, participation in external quality control is mandatory for each laboratory performing human semen analysis (RiliBÄK 2019). These external control programmes include determination of sperm concentration, sperm motility, and morphology; some additionally include sperm vitality. Regular information from laboratory technicians regarding results of quality control promotes a continuous subjective assessment of the different variables of the ejaculate (Cooper et al. 1999, 2002, 2007) and increases comparability of values measured by different laboratories (Mallidis et al. 2012; Nieschlag et al. 2017). These are essential for conducting multicenter studies.

The German Quality Assurance Scheme (QuaDeGA) was founded in 2001 by the German Society for Andrology and was appointed as a Reference Insitute by the German Medical Board in 2011. Two ring trials consisting of two different samples (A and B) are offered each year. Sperm concentrations are to be evaluated using the preserved samples; from the fixated smears provided, following staining by the participating lab, the percentage of morphologically normal spermatozoa is to be determined. For assessment of motility, video sequences of native ejaculate samples are provided on the internet and are available to participants of the ring trial. For the three parameters, sperm concentration, normal morphology, and progressive motility, medians of the values obtained by those laboratories indicating that they adhere strictly to WHO guidelines the 2.5 and 97.5 percentiles are used to construct Youden plots. Those participants whose results for all parameters lie within the Youdon plot windows receive certificates of passing the external quality control. Only laboratories with a valid certificate can claim reimbursement from insurances.

About 700 laboratories from Germany and abroad regularly participate in the QuaDeGA programme (Nieschlag et al. 2017).

9.8 Documentation, References Values, Nomenclature, and Classification of Semen Parameters

The documentation of semen quality and serum hormones is best managed on an evaluation sheet containing several columns in which the values of ejaculate examinations at different time-points can be entered (Table 9.2). This enables straight-forward evaluation and comparison of the results of several examinations. The values in Table 9.3 are the lower reference limits in semen from recent fathers whose partners had a time-to-pregnancy of 12 months or less (recently confirmed by Campbell et al. 2021). Samples with values above or below the lower reference limit can be described by the terminology given in Table 9.4.

The investigation of ejaculate parameters plays a central role in the evaluation of male fertility. Since the occurrence of a pregnancy depends on many factors, particularly on the reproductive functions of the female (see Chaps. 1 and 41), examination of the ejaculate parameters is only one of many prognostic tests of the couple's fertility. A sharp discrimination between a fertile and infertile male is not possible on the basis of the parameters and diagnoses mentioned above. This is theoretically only possible in the case of azoospermia (but see the problems in assessing this above) and in rare cases of 100% necrozoospermia, globozoospermia, or in the total absence or severence of tails. Otherwise, semen parameters have value only in relation to female reproductive functions since subnormal semen parameters can be compensated by optimal reproductive functions of the female partner and may be compatible with fertility.

Table 9.2 Specimen documentation form (example)

Name		
Code	Date	Date
Collection at Laboratory (1) or at home (2)		
Collection time (hour:min)		
Sample delivered (hour:min)		
Start analysis (hour:min)		
Patient		
Abstinence time		
Medication		
Difficulties in collection		
Semen		
Volume (ml)[>1.5)		
Treatment (e.g., Bromelain)		
Complete (1: complete; 2: incomplete)		
Appearance (1: normal; 2: abnormal)		
Viscosity (1: normal; 2: abnormal)		
Liquefaction (1: normal; 2: abnormal)		
Agglutination (%)		
Aggregation (%)		
pH		
Spermatozoa		
Total number (10^6/ejaculate)[>39]		
Concentration (10^6/ml)[>15]		
Vitality (%live)[>58]		
Motile category A (%)		
Motile category B (%)		
A + B=PR (%) [>32]		
Motile category C=NP (%)		
Total motility A + B + C=PR + NP (%) [>40]		
Motile category D=IM (%)		
Normal forms (%) [>3]		
Abnormal heads (%)		
Abnormal midpieces (%)		
Abnormal principal pieces (%)		
Excess residual cytoplasm (%)		
Direct MAR-Test IgG (%) (3 or 10 min) [<50)		
Direct MAR-Test IgA (%) (3 or 10 min) [<50)		
Non-sperm cells		
Peroxidase-positive cell total number (10^6/ejaculate)		
Peroxidase-positive cell concentration (10^6/ml)		
Accessory gland function		
Zinc (µmol/ejaculate) [≥2.4]		
Fructose (µmol/ejaculate) [≥13]		
α-Glucosidase Zinc (neutral)(mU/ejaculate) [≥20]		
Technician		

Table 9.3 Lower reference limits for semen parameter for couples with a time to pregnancy of 12 months or less (according to WHO 2021 manual) and in brackets recently confirmed by Campbell et al. (2021)

Parameter	Lower reference limit
Semen volume (ml):	1.5 (1.4)
Total sperm number (10^6/ejaculate)	39 (39)
Sperm concentration (10^6/ml)	15 (16)
Total motility (PR + NP, %)	40 (42)
Progressive motility (PR, %)	32 (30)
Vitality (live, %)	58 (53)
Sperm morphology (normal, %)	4 (4)

Table 9.3 (continued)

Parameter	Lower reference limit
Other consensus threshold values	
pH	≥7.2
Peroxidase-positive leucocytes	$<1 \times 10^6$/ml
MAR test (spermatozoa with bound beads)	<50%
Immunobead test (spermatozoa with bound beads)	<50%
Zinc (µmol/ejaculate)	≥2.4
Fructose (µmol/ejaculate)	≥13
Neutral α-glucosidase (mU/ejaculate)	≥20

Table 9.4 Descriptive terminology of semen

Terminology	Description
Normozoospermia	Total number of, and percentages of progressively motile and morphologically normal, spermatozoa above the lower reference limits.
Oligozoospermia	Total number of spermatozoa below the lower reference limit.
Asthenozoospermia	Percentage of progressively motile spermatozoa below the lower reference limit.
Teratozoospermia	Percentage of morphologically normal spermatozoa below the lower reference limit.
Oligoasthenozoospermia	Total number of, and percentage of progressively motile, spermatozoa below the lower reference limits.
Oligoteratozoospermia	Total number of, and percentage of morphologically normal, spermatozoa below the lower reference limits.
Asthenoteratozoospermia	Percentages of both progressively motile and morphologically normal spermatozoa below the lower reference limits.
Oligoasthenoteratozoospermia	Total number of, and percentages of both progressively motile and morphologically normal, spermatozoa below the lower reference limits.
Cryptozoospermia	Spermatozoa absent from fresh preparations but observed in a centrifuged pellet ($3000 \times g$ for 15 min).
Necrozoospermia	Low percentage of live, and high percentage of immotile, spermatozoa in the ejaculate.
Azoospermia	No spermatozoa in the ejaculate. Given as the lower limit of quantification for the counting method used.
Leukospermia	Presence of leukocytes in the ejaculate. (leukocytospermia, pyospermia).
Haemospermia	Presence of erythrocytes in the ejaculate. (Haematospermia).
Aspermia	No semen (no or retrograde ejaculation).

Key Points

- Semen analysis is used for diagnosis and prognosis of male fertility.
- The presence, number, and quality of spermatozoa (vital, motile, morphologically normal) are useful indicators that reflect the function of the testis, epididymis, and accessory sex glands and provide information about the quality of semen transmitted during coitus.
- High intra-individual variability demands standardized methods for obtaining and quantifying the components of the ejaculate. The characteristics of spermatozoa are necessary to allow objective interpretation of the data obtained.
- The main tests for semen analysis, as well as terminology and reference values, are described here according to the fifth edition of the WHO Laboratory Manual for the Examination and Preparation of Human Ejaculate (2021).

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Sperm Quality and Sperm Function Tests

10

Verena Nordhoff

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Abstract

Despite a plethora of methods that provide further information on spermatozoa quality and functions, only a few sperm function tests are routinely used in the andrology laboratory. Numerous of these tests for diagnosing fertility disorders examine only single isolated aspects of spermatozoa and do not necessarily reflect the situation in vivo. Moreover, some sperm function tests are not feasible due to technical or ethical limitations and more experimental tests cannot be performed in a routine andrology laboratory due to lack of technical expertise. However, tests for analysis of sperm DNA quality are problematic because they are invasive and destroy the analyzed sperm, making them unavailable for use, for example in ICSI.

10.1 Introduction

It is generally agreed that the routinely measured **classical sperm parameters (number, motility, viability, morphology)** do not provide sufficient information about actual sperm function. Similarly, they are not useful for accurately predicting fertility (Snow-Lisy and Sabanegh 2013). Moreover, classical ejaculate analysis is dependent on inter- and intra-individual assessment; and it cannot provide information on sperm dysfunction at the cellular or molecular level (Esteves 2014). And even the reference values established by WHO do not apply dichotomously to all men, as semen parameters of fertile and infertile men cannot be clearly distinguished. Moreover, especially idiopathic cases of infertility cannot be explained by the classical routine analyses (Barbăroșie et al. 2020; Hamada et al. 2011). Therefore, it is useful to perform tests that can assess specific **spermatozoa functions** and thus contribute to more accurate diagnostic and prognostic clarification of the man or the couple before natural or assisted reproduction (Oehninger et al. 2014, Fig. 10.1). These additional tests should ideally be able to distinguish between fertile and infertile men and explain the cause of infertility in order to propose a rational therapy for the man or the couple (Kızılay and Altay 2017). Here, the various cellular aspects required for successful fertilization will be described that can be investigated with appropriate tests. However, not all of the sperm function tests presented here are suitable for routine use; some tests have their justification only in an experimental setting.

10.1.1 Sperm Function in General

Natural sperm function consists of a chain of complex processes that have to occur in temporal and spatial coordination (see Chap. 3). Spermatozoa possess unique and highly specific functions to ultimately deliver the paternal genome to the egg.

A spermatozoon consists of distinct compartments whose different functions are similarly compartmentalized (see Chap. 3). The individual sperm function must be transmitted at the right place at exactly the right time; the function can be transmitted, for example, by secondary messengers, modification of lipids in cell membranes, ion flow through membranes, or posttranslational modification of proteins. Thus, for example, certain molecules are localized on cellular structures such as the ring fibers and the acrosome to serve as scaffolds and sites for interaction between signal transducers and neighboring structures (see Chap. 3). Some sperm function tests target specific aspects of the fertilization cascade, e.g., sperm interaction with cervical mucus or sperm-ovum interaction, while other tests examine particular organelles or specific components of a spermatozoon. Some cellular components even play a role only after entry into the egg, such as the centrosomes in syngamy or at initiation of embryonic development.

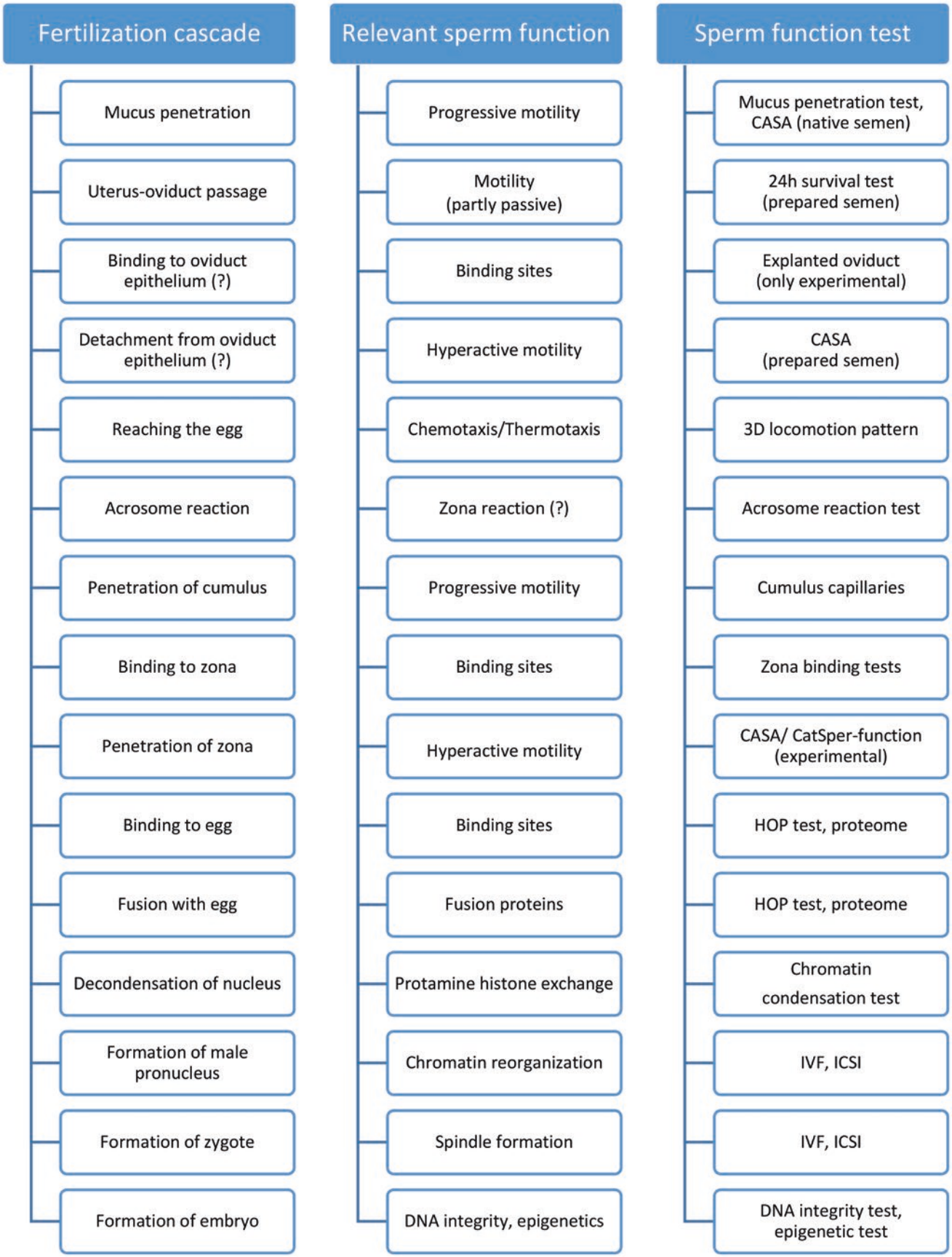


Fig. 10.1 Classification of sperm function tests in the fertilization cascade. Not all sperm functions have been clearly proven or run exactly in this order

10.2 Sperm Survival and Viability

10.2.1 Sperm Survival

The assessment of **sperm survival** (duration of motility or viability) in seminal fluid is of low predictive value for the corresponding survival in the female genital tract; changes in pH due to bacterial colonization vary among samples and thus results cannot be accurately interpreted. In contrast, testing the survival of washed specimens may be useful, for example, after 24 h of overnight incubation similar to the survival detection after in vitro fertilization on the next morning. However, to date there is no clear evidence that the result of a 24 h survival test is actually predictive of the success of in **vitro fertilization (IVF**). However, this test could indicate that **intracytoplasmic sperm injection (ICSI**) is the better choice if survival values are repeatedly low. The survival test may also serve as a toxicity test for antibiotic-contaminated sperm preparations (Hossain et al. 2010).

10.2.2 Sperm Vitality

Only a vital sperm can fertilize an egg; however, an immotile spermatozoon can also be vital (Nordhoff 2015). **Sperm vitality** can be investigated with different tests. Classically, this is done by **eosin or eosin-nigrosin staining** according to the WHO manual (2021). Dead sperm with disintegrated membranes take up the dye eosin and appear red to pink.

However, this kind of vitality test is only suitable for diagnostic purposes. If the tested sperm are to be used for artificial insemination, the test should not stain or destroy the sperm. Alternative methods include the modified **hypoosmotic swelling test (HOS test)**, the sperm tail flexibility test (STFT), the use of chemical substances that can activate motility, or the use of a laser (laser-assisted immotile sperm selection, **LAISS**) as an alternative to the HOS test. In the HOS test, sperm are exposed to a hypoosmotic medium that causes the tail to swell or curl (Barbăroșie et al. 2020). Only vital spermatozoa exhibit this specific response and, to still be usable for ICSI, must be quickly returned to a normo-osmotic environment. **STFT** uses tail flexibility in vital sperm as a selection marker. Activating agents, often methylxanthine derivatives, increase intracellular cAMP concentration and thereby stimulate sperm motility. The **LAISS** applies a laser pulse to the tip of a spermatozoon's flagellum and results in a curling of the flagellum only of vital spermatozoa, similar to the HOS test (Nordhoff 2015).

10.3 Function of the Flagellum

Sperm require their motility to move through the female cervical mucus in order to enter the uterus and subsequently the fallopian tubes; once there, they must penetrate the *cumulus oophorus* and ultimately the *zona pellucida* to successfully fertilize the egg. In order to penetrate the egg, sperm must be "activated" in the female reproductive tract. This activation process is a complex metabolic and physiological remodelling, called **capacitation** (see Chap. 3), and results in a **hyperactivation** of spermatozoa, the ultimate requirement for fertilization. Hyperactivated spermatozoa have specific flagellar patterns that can be evaluated by different techniques.

10.3.1 Assessment of Sperm Motility by CASA

In addition to the routine, rather subjective, microscopic examination of sperm motility (see Chap. 9), different sperm motility patterns can be analyzed using a ***c*omputer-*a*ssisted *s*perm *a*nalysis (CASA) system**. In this way, a wide variety of kinematic parameters can be quantified. Modern CASA programs can distinguish between progressive motility, which is necessary for penetration of the cervical mucus, and hyperactivation, which is necessary for penetration of the cumulus and *zona pellucida*. Parameters in this respect are a high **curvilinear velocity ("VCL")**, a low linearity (LIN) as a measure of the elongation of the curvilinear pathway (expressed as ratio of VSL (progressive velocity, "straight-line velocity") and VCL), and from the amplitude of lateral head motion (ALH, see Chap. 9 for definitions of kinematographic parameters). Data obtained by CASA correlate with natural pregnancy rates or success rates after IVF (Garrett et al. 2003, 2007).

10.3.2 Sperm Motility After Washing

After processing (e.g., washing, swim-up, or density gradient, WHO 2021) of sperm, the extent of hyperactivity is correlated with the capacity for zona-induced acrosome reaction (Liu 2011). Accordingly, the detection of a low percentage of hyperactivated spermatozoa could be a prognostic indicator of the inability to penetrate the zona, indicating fertilization failure both in vitro and in vivo. Determination of hyperactivation in washed and processed spermatozoa may be useful to aid in decision-making: if VCL levels are low, ICSI might be indicated instead of in vitro fertilization; in ICSI the lack of hyperactivity is not an important factor as sperm are injected directly into the egg.

10.3.3 Assessment of Sperm Motility by Physiological Assays

So far three different physiological mechanisms have been postulated that sperm probably use on their way to the egg: rheotaxis, thermotaxis, and chemotaxis. In **rheotaxis**, sperm

can use the ability to swim against a flow over longer distances, **thermotaxis** can be used by sperm for goal-directed locomotion based on a temperature gradient, and by using **chemotaxis**, sperm may find their way to the egg in a biochemical gradient (Yan et al. 2020). These three mechanisms could be useful in identifying 3D locomotion patterns and for selecting motile sperm; selection can also occur in submillimeter chambers using **microfluidics** (Yan et al. 2020).

The ability for using rheotaxis is present in all spermatozoa; however, only capacitated spermatozoa appear to possess thermotaxis or chemotaxis. Here the calcium ion plays a central role, but not only in capacitation, but also in navigation through the female reproductive tract, in the acrosome reaction, and for hyperactivation. The cation-specific sperm channel "CatSper", as mediator of calcium signalling, plays an important role in sperm function and male fertility. The lack of a functional CatSper channel can lead to infertility (Singh and Rajender 2015; Rahban and Nef 2020).

To test for a functional CatSper channel, the so-called "patch-clamp" technique can be used (Lishko et al. 2010; Strünker et al. 2011). Here, a fluid-filled pipette is directly connected to the sperm cytoplasm, either at the neck region or at the cytoplasmic droplet, after rupturing the membrane. Electrical currents, as a measure of ion transport through ion channels in the plasma membrane between the pipette and the bathing solution, can thus be measured via an amplifier. This is indicative for the **functionality of the CatSper channel**. However, this test is not a routine test, but is rather used in a research setting, mainly due to the high effort involved.

10.3.4 Mobility in the Female Mucus

The first hurdle that sperm must overcome in the female genital tract is the cervical mucus. To analyze whether sperm can penetrate the mucus, the so-called **Kremer test** can be performed (Talwar and Hayatnagarkar 2015). Cervical mucus is collected approximately 2 h after coitus and the presence of motile sperm is analyzed. Alternatively, this test can be experimentally performed in a microfluidic chamber in which methyl cellulose mimics the female mucus (Eamer et al. 2015; Yan et al. 2020).

The purpose of these tests is to obtain information about flagellar movement and sperm hydrodynamic capabilities. However, these mucus penetration tests do not provide information on whether antisperm antibodies may also be present. In addition, these tests are rarely used clinically now because after three unsuccessful attempts of hormonal stimulation together with timed intercourse at ovulation, the woman proceeds to the next stage, **intrauterine insemination (IUI)**. In IUI, processed ejaculate is introduced directly into the uterus, bypassing the need for sperm to penetrate mucus.

In highly viscous fluids, differences in flagellar movement can be detected that may not be visible in classical semen analysis.

10.3.5 Antisperm Antibodies

Antisperm antibodies account for approximately 9.5% of infertility in men with impaired semen quality (Munuce et al. 2020). They can affect motility and the ability to penetrate cervical mucus (Barbăroșie et al. 2020), and also sperm-ovum binding (Agarwal and Said 2009). Various tests (immunobead assay, ELISA, "tray agglutination test") have been developed; however, the **"mixed antiglobulin reaction test" (MAR test)**, which is also recommended by WHO (2021) (see Chap. 3 and 28), is the one most frequently used. Antisperm antibodies can negatively correlate with sperm concentration or motility (Cui et al. 2015). If more than 50% of sperm exhibit IgG or IgA antibodies, immunological infertility is likely (Vicram et al. 2019).

Determination of antisperm antibodies is part of the classic ejaculate analysis and is helpful in diagnosing immunological infertility.

10.4 Mitochondrial Function

Mitochondria are structurally and functionally distinct organelles that produce adenosine triphosphate by oxidative phosphorylation to provide energy for sperm (Park and Pang 2021). Mitochondrial function can be determined by measuring the **mitochondrial membrane potential (MMP)** using fluorescence microscopy or flow cytometry (Barbăroșie et al. 2020). The MMP is visualized using specific fluorescent cation stains, e.g., JC-1 or the mostly preferred TMRM (Uribe et al. 2017). Correlations between membrane potential and various ejaculate parameters have been described (Espinoza et al. 2009; Zou et al. 2010; Uribe et al. 2017). While some researchers have found no differences in mitochondrial activity of fertile or infertile males (Meseguer et al. 2004), mutations of mitochondrial genes have been detected in a Tunisian population, which are most likely causative for motility impairment in males with **asthenozoospermia** (Baklouti-Gargouri et al. 2013).

10.5 The Cytoplasm

Human spermatozoa in wet preparations may have a so-called **cytoplasmic droplet** extending from the neck to the midpiece. In air-dried sperm preparations, one can distin-

guish between the cytoplasmic droplet in normal spermatozoa and the excess cytoplasm (excess residual cytoplasm) of abnormal spermatozoa (see Chap. 9). Because cytoplasmic droplets respond to osmotic influences, they burst during sperm preparation and are therefore normally not well preserved in stained preparations.

10.5.1 The Cytoplasmic Droplet as a Normal Structure

Cytoplasmic droplets are observed in the majority of motile spermatozoa in ejaculate preparations from proven fathers or normozoospermic subjects (Fetic et al. 2006). This contradicts the often-held view that the cytoplasmic droplet indicates dysfunction. Since the cytoplasmic droplet plays a role in sperm volume regulation (Yeung and Cooper 2008), and this is likely where osmotic exchange occurs, it has an important functional role in volume regulation of fertilizable sperm (Cooper 2011).

10.5.2 Excess Residual Cytoplasm

An increased excess residual cytoplasm (>30% of head size) in ejaculated sperm may indicate testicular dysfunction; normally testicular Sertoli cells phagocytise this residual structure (Cooper 2011). The excess cytoplasm is often associated with excessive production of free oxygen radicals ("**reactive oxygen species", ROS**), which can affect sperm lipid membranes, proteins, and DNA. Thus, in the ejaculate, normal spermatozoa may be impaired in their function by sperm with excess residual cytoplasm producing ROS (Cooper 2005, 2016).

Since spermatozoa from proven fathers and healthy subjects can better regulate their volume than those sperm from infertile patients, a closer observation and the sizing of excess cytoplasm may provide further diagnostic information.

10.5.3 "Reactive Oxygen Species" (ROS) and Lipid Peroxidation

Sperm function may be disrupted by deficient or suboptimal protective mechanisms in the ejaculate, or in the sperm themselves. Although spermatozoa produce "reactive oxygen species" (ROS) themselves to support the fertilization process (Robert et al. 2020), excessive production of ROS by cytoplasmic remnants or leukocytes in the seminal plasma can cause oxidative damage (Cannarella et al. 2020) and thus be a possible cause of male infertility (Agarwal and Sengupta 2020). ROS can be measured by direct (e.g., chemiluminescence, cytochrome C reduction assay, or oxidation-reduction potential), or indirect methods that determine antioxidant concentrations or ROS-induced damage (e.g., end-tidal assay, ROS-TAC score, lipid oxidation products, or protein modifications using proteomics) (Robert et al. 2020).

The **lipid membrane of sperm** is particularly sensitive to oxidative stress (Ritchie and Ko 2021). The damage caused by oxidation can be measured, for example, by **chromatography** or by **spectrofluorometry**. Oxidative end products in higher concentrations have been detected in oligo- and asthenozoospermic samples (Robert et al. 2020). Oxidative damage leads to disrupted sperm membranes and the loss of motility and viability; this of course disrupts the fertilization cascade. Oxidative damage to sperm DNA can be measured (see Sect. 10.14), although it is unclear what effect the disruption has on fertilization or embryo development.

Measurement of ROS production may identify a possible source of sperm dysfunction.

10.6 Capacitation

Sperm must undergo a capacitation phase in order to fertilize the egg. Only successfully capacitated sperm acquire the ability to respond to physiological signals leading to hyperactivity and acrosome response. To find out whether sperm have the ability to capacitate, they can, for example, be placed in a capacitating medium (Agarwal et al. 2016).

Another test detects and analyzes the localization of hydrophobic regions of the cell membrane (called "lipid rafts") as a marker of the ability to fertilize an egg (Moody et al. 2017). A **capacitation test** may be a useful diagnostic test, as a correlation with an increased probability to reach a pregnancy has been postulated (Martinez and Majzoub 2020).

10.7 Interaction with the Fallopian Tube Epithelium

Prior to ovulation, sperm presumably bind to the fallopian tube epithelium and might detach once ovulation products (e.g., progesterone, produced by the cumulus cells surrounding the egg) stimulate hyperactive motility (see Chap. 3). Experimentally, the binding of spermatozoa to tubal epithelium can be observed in vitro with homologous explants of oviducts (Reeve et al. 2003).

This interaction test is not a routine test, as it requires human tissue, which is very difficult to obtain, and therefore currently remains of academic interest.

10.8 Interaction with the *Zona Pellucida*

In order for sperm to fertilize an egg, they must first "recognize" or localize it. After localizing the egg, a sperm can bind to the egg shell, the *zona pellucida*, via specific receptors in order to penetrate it. Spermatozoa that are bound to the *zona pellucida* tend to have normal morphology (Liu and Baker 1994), are capacitated (Liu et al. 2006), and contain normal chromatin (Liu and Baker 2007). They are referred to as "zona-preferred" (Garrett et al. 2007). Abnormal sperm-zona interaction may prevent regular fertilization and can lead to failure of intrauterine insemination (IUI) or in vitro fertilization (IVF) (Zini and Sigman 2009; Barbăroșie et al. 2020).

10.8.1 Zona-Binding Assays

Two zona-binding assays have been described: the **hemizona assay**, in which a human *zona pellucida* is divided and each half is incubated with control or treated sperm, respectively; and the **competitive zona-binding assay**, in which a non-vital egg is incubated with equal numbers of motile sperm from the control and test populations, with each population pre-labelled with different fluorescent colors (Barbăroșie et al. 2020). In both cases, the number of spermatozoa bound to either the half or the whole zona is counted and presented as a quotient to the proportion of control sperm.

Because human sperm cannot bind to the zonae of other mammals, human zonae have to be used for this test. However, since zonae are available only in limited quantities or even not at all, these tests have not found their way into clinical routine.

10.8.2 Hyaluronic Acid as Zona Surrogate

Hyaluronic acid (HA) is produced by the cumulus-ophorus complex (COC). Spermatozoa can bind to HA and the subsequent hyperactivation leads to penetration of the *zona pellucida* of the egg (Kirkman-Brown et al. 2019). In vitro, this process can be mimicked in a culture dish (Huszar et al. 2003): spermatozoa whose heads bind to hyaluronic acid are morphologically normal (Prinosilova et al. 2009); they are more vital and less acrosome-reacted (Huszar et al. 2003), have more compacted chromatin (Sati et al. 2008) than an unselected sperm population, and show lower rates of aneuploidy (Parmegiani et al. 2010). However, a large randomized trial failed to show that HA-based sperm selection could increase live birth rates, but showed reduced abortion rates (Kirkman-Brown et al. 2019).

The injection of hyaluronic acid-selected spermatozoa versus those prepared with density gradient centrifugation or swim-up does not appear to increase pregnancy rates.

10.9 Acrosome Reaction

The acrosome reaction is a prerequisite for a sperm to penetrate the *zona pellucida* of the egg. Methods such as flow cytometry, fluorescence microscopy, or electron microscopy can examine and describe acrosomal function more accurately (Barbăroșie et al. 2020). These acrosome response tests determine the percentage of spontaneously or in vitro-induced acrosome responsive sperm (Snow-Lisy and Sabanegh 2013) and thus describe the **acrosome reaction inducability**. However, these methods are very expensive and tend not to be routinely applicable (Barbăroșie et al. 2020).

Easier to apply is an assay that uses the Ca^{2+} ionophore A23187 **(ARIC assay)**. The ionophore induces changes in intracellular calcium and subsequently mimics a pH change, which then induces the acrosome reaction and can be detected by using fluorescence (Zeginiadou et al. 2000). The acrosome reaction can also be evaluated by **polarization light microscopy** (Pinto et al. 2020). The specific birefringence pattern not only shows the status of the acrosome reaction, it can also be taken as an indicator of normal sperm structure (Gianaroli et al. 2008; Magli et al. 2012). However, it is unclear whether birefringence is associated with a higher or lower incidence of DNA fragmentation (Petersen et al. 2011; Crippa et al. 2009).

Acrosome reaction tests may be a predictive parameter for IVF success (Oehninger et al. 2000) and couples with a low acrosome reactivity should therefore be referred to ICSI (Kızılay and Altay 2017).

Acrosome reaction tests may indicate the sperm's functional capacity for acrosome reaction.

10.10 Sperm-ovum Fusion

10.10.1 Hamster Ovum Penetration (HOP) Test or Sperm Penetration Assay (SPA)

After penetration of the *zona pellucida*, the acrosome-reacted sperm fuses with the oolemma of the egg in the equatorial region. Since zona-free human eggs are not available, hamster eggs can be used as a surrogate (mentioned in WHO 2010). If swollen and decondensed sperm are found within the egg, this provides evidence of penetration ability. Non-penetrated, still condensed sperm heads bound to the oolemma show to which extent the acrosome reaction has occurred. However, this assay is no longer commonly used, because assay performance and evaluation are extremely variable.

Provided that sufficient motile sperm are available, in vitro fertilization itself is the best proof for sperm fusion and the HOP test is no longer used as a surrogate test.

10.11 Sperm Centrosome

The **sperm centrioles**, which together form the centrosome, are essential for human fertilization. This centrosome is responsible for the formation of spindle fibers in the fertilized human egg and controls pronuclear migration. Interestingly, mouse sperm centrioles are degraded after fertilization, suggesting that mouse oocytes synthesize centrioles de novo to form spindle fibers for cell divisions (Avidor-Reiss et al. 2019). Changes of the centrioles appear to play a role in male infertility and are associated with miscarriages. Although ICSI can "fix" male infertility in most cases, this is not possible if centriole dysfunction is the cause for infertility.

To test for proper sperm centriole function, human sperm can be injected, for example, into bovine eggs, where the formation of the spindle fibers can be detected by immunofluorescence (Yoshimoto-Kakoi et al. 2008). However, how exactly sperm centrioles control human embryonic development is still unclear; research in this field is difficult, especially since the mouse cannot be used as a model for the reason mentioned above (Avidor-Reiss et al. 2019).

The high technical complexity of these functional tests, such as injecting sperm into bovine eggs to observe spindle formation, prevents its clinical application.

10.12 Sperm Chromosomes

The correct amount (euploidy) and integrity of the chromosomal content of a sperm is of great importance for subsequent embryonic development. Therefore, it may be quite useful to analyze the genetic information of spermatozoa. **Fluorescence in situ hybridization** (FISH) can be used to screen individual spermatozoa for aneuploidy; however, this technique only provides information on **numerical chromosomal abnormalities** and cannot analyze all chromosomes simultaneously. More recent techniques, such as "**next generation sequencing**" (NGS), can simultaneously examine all chromosomes, this not only on a population of sperm, but through refined methods also at the single cell level. Thus, not only numerical but also other **changes at the DNA level**, such as deletions, insertions, duplications, even down to single mutated bases, can be detected. However, it must be mentioned that all these methods are sperm-consuming. In vivo selection of chromosomally normal sperm has not yet been achieved. One experimental approach is the use of **Raman spectromicroscopy**, where sperm are still intact after analysis (Mallidis et al. 2014). However, further research is needed to make this method applicable for routine use in the future (see also Chap. 8).

Although sperm chromosomes provide important diagnostic information, it is not possible to nondestructively select chromosomally normal spermatozoa for therapeutic purposes.

10.13 Sperm DNA

Spermatozoa contain both mitochondrial and nuclear DNA, and disorders in either type can lead to infertility.

10.13.1 Mitochondrial DNA (mtDNA)

Mitochondrial DNA (mtDNA) is a circular, double-stranded structure with protective histones that is replicated independently of the nuclear DNA (nDNA). The mDNA, together with the nDNA, encodes for proteins involved in oxidative phosphorylation of the electron transport chain to ensure ATP-dependent sperm motility (Piomboni et al. 2012). Spermatozoa have approximately 70 mitochondria with approximately 1000 to 1500 copies of mDNA (St John et al. 2000). **Deletions in mtDNA** are possibly responsible for some cases of male infertility, as these alterations can lead to disruptions in sperm flagellar movements (Karimian and Babaei 2020).

Mutations in mitochondrial genes may possibly alter flagellar movement in some cases of infertility.

10.13.2 Nuclear DNA (nDNA)

The integrity of the paternal genome is the basis of an intact pregnancy. **Sperm nuclear DNA (nDNA)** is well protected from interference due to the almost complete replacement of histones by protamines (approximately 85%, Hammoud et al. 2009) and the associated high chromatin compaction (Ward 2010). Nevertheless, nDNA is exposed to intrinsic (e.g., oxidative stress, apoptosis, aberrant germ cell maturation, chromatin reorganization) and extrinsic (e.g., environmental, lifestyle, chemotherapeutic) factors. Such disorders can occur in the testis, but also post-testicularly, and can affect regular fertilization or normal embryonic development (Agarwal et al. 2020; Panner Selvam et al. 2020). If the DNA has low integrity, fertility may be affected (Simon et al. 2017). Studies have found an association of increased **sperm DNA fragmentation** with the risk of miscarriage, both after IVF and ICSI therapies. Nevertheless, the predictive power of a DNA integrity test and the occurrence of a miscarriage is rather low (Practice Committee of ASRM 2013).

A number of tests for assessment of sperm DNA integrity are available (see following chapters); there are assays that detect preexisting DNA damage, while others indicate single or double DNA strand breaks or specific chromosomal problems (Dutta et al. 2020). Additionally, there are assays that provide only indirect evidence of potential damage by examining the compaction of chromatin.

10.14 DNA Fragmentation

10.14.1 Chromatin Condensation

The chromatin of spermatozoa before fertilization is highly condensed; this is due to sulfur cross-links between the remaining histones. After sperm penetration into an egg, the chromatin is decondensed, allowing the male pronucleus to form. To evaluate the decondensation potential of the sperm genome, the sulfur cross-links can be experimentally solubilized and subsequently lead to cell swelling with normozoospermic samples showing more than 70% of swollen cells (Talwar and Hayatnagarkar 2015).

The chromatin decondensation assay gives indications of the genome integrity; however, it is rarely used in routine practice.

10.14.2 Aniline Blue and Toluidine Blue Assay for Determination of Compaction

To examine compaction, the dye aniline blue can be used. Aniline blue binds to the lysine residues of remaining histones and an increased staining is indicative of lower compactness of the chromatin and suggests higher immaturity of spermatozoa (Dutta et al. 2020). There are no uniform standard values for the **aniline blue test**; for IVF an upper limit of 20–28% is given and for ICSI a value of 29% is stated (Hammadeh et al. 1996, 1998; Haidl and Schill 1994).

To determine the acidic cellular components, such as the phosphate residues of immature DNA, toluidine blue staining is used. Sperm heads with normal DNA integrity are stained light blue in the **toluidine blue assay**, while heads with damaged DNA or lower chromatin compaction appear purple (Ajina et al. 2017). Thus, the intensity and color of sperm-bound toluidine blue is an indicator of chromatin status (Erenpreisa et al. 2003) and correlates with classical ejaculate parameters such as morphology, motility, and viability (Ajina et al. 2017). A cut-off value for predicting male infertility is reported to be 45% (Tsarev et al. 2009).

However, it is not entirely clear for either test to what extent a specific value can be considered a predictive parameter for ART success (Dutta et al. 2020).

Staining of DNA in routine sperm smears using metachromatic dyes can provide information on the compaction of chromatin and thus potential damage to nDNA.

10.14.3 Staining of Nucleic Acid: CMA3, Acridine Orange, and SCSA® Assays

10.14.3.1 CMA3 Test

The dye **chromomycin A3 (CMA3)** binds specifically to the guanine-cytosine (GC)-rich sites in DNA. The dye competes with the protamine-binding sites of DNA (indirect test) and indicates protamine deficiency of chromatin (Barbăroșie et al. 2020). CMA3-positive spermatozoa are stained bright yellow or green and indicate inadequate protamine levels (Nijs et al. 2009; Pourmasumi et al. 2017). Sperm with low color intensity have normal protamine levels and show a tendency to higher fertilization rates (Barbăroșie et al. 2020).

10.14.3.2 Acridine Orange and SCSA® Assays

The acridine orange (AO) assay is an indirect test and measures **sperm DNA integrity**. AO is a metachromatic fluorescent dye that intercalates into both double-stranded (dsDNA,

appears green in the assay) and single-stranded (ssDNA, appears red in the assay) DNA (Barbăroșie et al. 2020; Sharma et al. 2020). The intensity of the AO staining is analyzed, either subjectively by fluorescence microscopy as a simple acridine orange assay, or objectively by flow cytometry as a "sperm chromatin sensitivity assay" (SCSA®). The ratio of red-stained spermatozoa (R) to the total number of cells stained red and green (G) [R/R + G] is recorded and thus provides the DNA fragmentation index (DFI). The DFI documents the extent of strand breaks in DNA (Evenson et al. 2002). Likewise, the proportion of highly stained DNA, which contains few protamines and many histones, is reported as the "high DNA stainable" (HDS) value. The SCSA® is a well-established test with recognized clinical cut-off values (Evenson et al. 2007) and is considered the gold standard of DNA fragmentation tests (Ribeiro et al. 2017). Studies have shown that infertile males have higher amounts of AO-positive, or red-appearing, sperm compared to fertile controls; this correlates inversely with classical ejaculate parameters (Saleh et al. 2003). Detection of ssDNA also shows an inverse correlation with success after IVF (Dutta et al. 2020). However, it is unclear whether and which DFI threshold predicts a pregnancy in vivo or a successful IVF or ICSI; studies show different results (Bungum et al. 2008; Giwercman et al. 2010; Zini 2011); however, ICSI is recommended if the DFI exceeds 30% (Bungum et al. 2007).

Staining with specific fluorescent dyes provides information about sperm chromatin and may indicate DNA damage as a possible cause of infertility.

10.14.4 Dispersion of DNA: Sperm Chromatin Dispersion (SCD) and Comet Assay

10.14.4.1 Sperm Chromatin Dispersion (SCD) Assay

In the **sperm chromatin dispersion assay**, nuclear proteins are removed due to an acid denaturation of sperm DNA; this results in the release of DNA loops and reflects the status of the chromatin. Only cells with normal DNA exhibit these DNA loops and can be microscopically visualized as a central nucleus with a surrounding halo (Barbăroșie et al. 2020; Sharma et al. 2020). However, if the DNA is fragmented, the nucleus appears either without, or only with a small halo. The SCD is an indirect test, is easy to perform, and inexpensive. The DFI value measured by SCD has the potential to distinguish infertile from fertile males (cut-off value 26.1%; Wiweko and Utami 2017). However, a meta-analysis showed that the SCD has only a low power to predict a pregnancy after IVF (Cissen et al. 2016).

This relatively simple assay provides information on the percentage of sperm with poorly packed chromatin, but may not predict DNA damage.

10.14.4.2 Comet Assay

The **comet assay**, also called single cell gel electrophoresis, is based on the permeability and electrophoretic migration of fluorescently labelled fragmented DNA.

DNA forms a comet-like tail in an electric field; this tail consists of fragmented and fluorescently labelled ssDNA and dsDNA that migrates in an electrophoretic field away from the center with intact DNA (Enciso et al. 2009). The longer the tail, the more DNA is possibly damaged; the length of the comet tail can serve as an index of DNA damage (Simon et al. 2017; Dutta et al. 2020). The comet assay is performed under neutral or alkaline pH conditions. In the neutral version, only dsDNA loops migrate away from the nucleus as an unwound tail, whereas in the alkaline version, also as 2 T comet assay variant, dsDNA and also ssDNA strand breaks form the tail (Cortés-Gutiérrez et al. 2017).

Accurate cut-off values that serve as diagnostic predictive parameters of infertility have not been established (Simon et al. 2017), yet the assay is simple, sensitive, but requires a high level of expertise in interpreting the results (Dutta et al. 2020).

Dispersion assays reflect the percentage of sperm with actual or potential DNA damage.

10.14.5 "In Situ Nick Translation Assays" or TUNEL Assay

Free ends of denatured single-stranded and double-stranded DNA indicate DNA fragmentation and can be stained with fluorescein thiocyanate (FITC)-dUTP. This **"terminal desoxynucleotidyl transferase-mediated dUTP nick-end labeling" (TUNEL) assay** is a direct test and quantifies the number of fluorescent cells microscopically (simple, inexpensive, less sensitive) or flow cytometrically (complex, costly, higher sensitivity) (Sharma et al. 2016, 2020). The percentage of damaged spermatozoa correlates with sperm morphology, motility, and concentration (Carrell et al. 2003; Avendaño et al. 2009). The TUNEL assay has varying cut-off values from 20% to up to 36% depending on how it is performed. It has only a varying efficiency as miscarriage prediction parameter (Benchaib et al. 2003; Henkel et al. 2004; Sergerie et al. 2005), but possibly helps to find an explanation for, e.g., repeated miscarriages (Opuwari and Henkel 2016).

10.14.6 Prognostic Value of DNA Tests

The use of the various DNA fragmentation tests as a routine test in sperm analysis is discussed controversially worldwide, also by professional societies (Tharakan et al. 2021). Many studies show that sperm DNA fragmentation correlates with male infertility, and the impact of DNA damage on natural conception or assisted reproduction has also been extensively studied. Although the value of DNA testing is certainly high, the role of fragmentation testing in routine clinical practice is still not well-understood (Panner Selvam et al. 2020).

A direct comparison between these tests is also problematic, as the method protocols are not standardized and adequate intra- and inter-individual standardization is lacking; this leads to a considerable limitation of results (Esteves et al. 2017). Moreover, internal and external quality controls are not established (Esteves et al. 2017); therefore, there is no international guidance on which test should be used predominantly (Dutta et al. 2020). There is also frequent confusion as to which test is the correct one: a test that measures chromatin condensation or a test that examines DNA damage. And those that examine DNA damage can be further subdivided into those that measure damage directly, or those that measure a potential risk of DNA damage. Similarly, one must distinguish whether the assay analyzes direct damage to DNA, or whether it rather examines DNA-DNA or DNA-protein cross-links (Aitken et al. 2009). Thus, comparability has not been achieved to date.

Therefore, it is necessary to develop guidelines for the clinical application of DNA fragmentation tests, to identify precisely those patients who will benefit from the analysis. This is the only way to decipher the underlying mechanisms so that rational treatment strategies can be developed (Dutta et al. 2020).

Correlations among the results of different DNA fragmentation tests are difficult because large intra- and inter-individual variations do not allow comparison. Therefore, routine analysis has been discouraged to date. However, sperm DNA fragmentation tests are quite useful to complete the clinical picture of male infertility (Toth et al. 2019).

10.15 Epigenetics

Epigenetic modifications, which include histone modification, noncoding RNAs, and DNA methylation, do not alter the sequence of the DNA in the genome (Berger et al. 2009), but have the ability to regulate the transcription of genes. Epigenetic modifications, particularly **aberrant DNA methylation** in sperm, appear to play a role in male idiopathic infertility (Carrell 2012) and in reproduction (Laurentino et al. 2016). DNA methylation of cytosines at so-called cytosine-phosphate-guanine (CpG) dinucleotides is found primarily in CpG-rich regions (often promoter regions) and is associated with gene inactivation (Jones 2012). In addition, epigenetic modifications are necessary for germline reprogramming, thus removing parental methylation patterns and establishing new ones. Excluded from a postfertilization demethylation wave are "imprinted" genes, which are monoallelically methylated depending on the parent from whom the chromosome originates (Masahiro 2011; Laurentino et al. 2016). Epigenetic changes can be intrinsic, or extrinsic, e.g., influenced by environmental or lifestyle factors (Cescon et al. 2020). There is a strong association between aberrant DNA methylation of imprinted genes and classical ejaculate parameters such as number, motility, and morphology. However, not all spermatozoa in an ejaculate show these aberrations and rather display an epigenetic heterogeneity (Laurentino et al. 2016).

Epigenetic modifications are more common in infertile males than in normozoospermic controls.

10.16 Sperm RNA Assays

It has been assumed that spermatozoa are transcriptionally and translationally inactive, at least at the nucleus level, as during spermiogenesis chromatin is actively reorganized and condensed by the exchange of histones by protamines (Oliva 2006), and most cytoplasm is actively extruded. However, spermatozoa can also acquire new RNAs post-testicularly via exosomes from the epididymis or the seminal plasma (Jodar 2019).

In fact, during sperm maturation, many of the existing sperm RNAs are lost, but a small but complex population of RNAs remains in mature sperm (Jodar et al. 2013). These include long and short **noncoding RNAs, "micro" RNAs, "small interfering" (si) RNAs, and "transfer" RNAs**. Many of these RNAs are believed to have no post-testicular function, but there are RNAs that play an important role in early embryogenesis and can modulate gene expression. These coding and noncoding RNAs can, directly or indirectly (after translation in the egg), regulate embryonic gene expression; for example, paternal RNAs can transmit epigenetic modifications. However, it is unclear whether sperm RNAs interact directly with chromatin or other structures, or even how they effect a modification (Gòdia et al. 2018).

10.17 Translation Products

Spermatozoa do not have active protein biosynthesis, but show dynamic changes in their protein profile. These changes are likely a consequence of protein transfer from vesicles of different origins (epididymis, prostate, or seminal plasma; Castillo et al. 2018) or may originate from posttranslational changes (Samanta et al. 2016). The protein signature (proteome) of spermatozoa and seminal plasma is very similar (Jodar et al. 2017), but appears to be modifiable by oxidative stress such that protein metabolism, folding, or degradation can be affected in infertile patients, as opposed to fertile controls (Agarwal et al. 2015).

The **sperm proteome** now contains over 6800 proteins (Castillo et al. 2018), which are involved in a wide variety of functions. For example, there are proteins involved in membrane trafficking, apoptosis, cell cycle, meiosis, capacitation and acrosome response, sperm-ovum fusion, early embryogenesis, and many other functions (Amaral et al. 2014). There are, e.g., proteins which are important for motility acquisition, such as the sperm-specific cation channel CATSPER (Williams et al. 2015), or the protein IZUMO1 (sperm-egg fusion protein 1), which is essential for sperm binding to the egg (Bianchi et al. 2014), or phospholipase C zeta (PLCZ1), which triggers calcium oscillations in the egg for its activation and initiation of further development (Yoon and Fissore 2007).

The prospective further characterization of the sperm proteome may decipher the molecular aspects of sperm function (Amaral et al. 2014).

Prospective research will provide insights into whether analysis of the transcriptome, the proteome, or the (epi-)genome can distinguish fertile from infertile males. In addition, these future results could be the basis for the development of new methods for noninvasive diagnostics to assess male fertility.

10.18 Conclusion and Future Developments

Since the classical ejaculate parameters have relatively low value in predicting a pregnancy, a sperm function test prior to medically assisted reproduction (MAR) would provide helpful information on which treatment is optimal for the infertile couple.

The **disadvantages of the sperm function tests** listed here are mainly that they do not capture the totality of the *in vivo* situation in its sequential order of events, but only reflect isolated steps. Even if some tests can detect sperm defects (e.g., zona-binding defects), the option to perform these tests is often lacking (e.g., the lack of human zonae or even nonexisting technical requirements). Thus, for example, in the case of limited DNA quality as a possible cause of natural fertilization failure, ICSI is often recommended directly (Bungum et al. 2007) (see Sect. 1.7). Moreover, although this procedure has relatively high success rates, selection of sperm without DNA damage still remains impossible until now. Only a few tests guarantee the intactness of the spermatozoon after successful selection, so that it can still be used for ICSI afterwards.

It is still unclear whether transcriptome, proteome, or (epi-)genome analysis will provide a **new dimension to sperm diagnostics**. The widespread use of medically assisted reproduction (MAR), especially the **ever-growing use of ICSI** even in non-male infertility, has greatly minimized the interest in sperm function testing. Nevertheless, the sperm that penetrates the egg must always have a functioning centrosome and an intact genome with normal epigenetic properties.

Despite a variety of methods that provide additional information on sperm function, there are only a few tests that have found their way into the andrology laboratory, in addition to classical ejaculate analysis. Moreover, some of the test techniques are very complicated and require such a high level of technical equipment that a routine andrology laboratory would not be able to perform them. Therefore, many of the tests presented here find their application in an experimental setting.

Key Points

- There are many different sperm function tests; however, every one examines only one aspect of the fertilization cascade; none can represent the totality of the *in vivo* situation in its sequential order of events.
- Some tests are designed to predict a specific function, while others only indirectly detect sperm damage.
- There are sperm function tests that have found their application in routine practice; others are very complex and tend to be applied in an experimental setting.
- Many of the tests are sperm-consuming; only a few tests leave the spermatozoon intact for subsequent use, e.g., for ICSI.

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Biopsy and Histology of the Testis

11

Daniela Fietz and Sabine Kliesch

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Abstract

The basis of correct and meaningful testicular histology is the qualitative and quantitative analysis of the testicular cell populations as well as a semiquantitative score evaluation. Based on the resulting biopsy report, critical counseling of patients regarding successful treatment of infertility with TESE and ICSI becomes possible. In addition, detailed and subsequent analysis of testicular sperm or testicular tissue may improve assisted reproduction. Testicular biopsy is an invasive surgical procedure and has a direct impact on the patient and further course of treatment. It may be performed only in strict compliance with the indications and with validated surgical and examination techniques. It is recommended to be performed in qualified andrological center, certified by the European Academy of Andrology (EAA).

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11.1 Indication for Testicular Biopsy

Testicular biopsy may have purely diagnostic goals or may be performed therapeutically to perform testicular sperm extraction (TESE) and is—in these cases—combined with diagnostic testing. The current AWMF Guideline on Diagnostics and Therapy Before Assisted Reproduction Treatments (Toth et al. 2019; Köhn et al. 2020) as well as the guidelines of the "European Association of Urology" (EAU) (Jungwirth et al. 2018) recommend the following **indications** for testicular biopsy:

- **Testicular biopsy** must be performed in combination with TESE and cryopreservation of sperm as a therapeutic testicular biopsy in patients with an unfulfilled desire to have children. Cryopreservation of sperm obtained during TESE enables infertility treatment by intracytoplasmic sperm injection (ICSI). It can be performed in both obstructive (OA) and **nonobstructive azoospermia (NOA)**. In the presence of hypergonadotropic NOA, at least focally impaired spermatogenesis is likely; focal or even total germ cell aplasia (Sertoli cell-only phenotype, SCO) is often present. In the presence of areas with at least qualitatively preserved but quantitatively impaired spermatogenesis (hypospermatogenesis), sperm for subsequent IVF/ICSI treatment can be obtained by **TESE** in approximately 50% of NOA cases (Corona et al. 2019).

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Testicular tissue is always cryopreserved for later use in assisted reproduction, although the techniques used here may vary (Jungwirth et al. 2018; Kliesch 2020).

- Therapeutic testicular biopsy is also indicated in the presence of **Klinefelter syndrome** (karyotype 47,XXY) (Schlatt et al. 2010). Although most adults with Klinefelter syndrome reveal total atrophy with partly focal SCO and Leydig cell hyperplasia, focal qualitatively preserved, quantitatively usually severely restricted hypospermatogenesis can be detected in about 50% of adult and especially in adolescent patients and young adults under 25 years of age with Klinefelter syndrome. This then allows successful sperm retrieval for subsequent therapy with TESE and ICSI (Rohayem et al. 2015; Corona et al. 2017; Ragab et al. 2018).
- The indication for purely **diagnostic testicular biopsy** is suspected **preinvasive germ cell neoplasia in situ (GCNIS)** (Berney et al. 2016; Batool et al. 2019; Kliesch et al. 2021), e.g., when an inhomogeneous ultrasound finding (microlithiasis) occurs (Köhn et al. 2020). In the presence of a clinically manifest germ cell tumor, at least a double biopsy of the contralateral testis is performed, as the prevalence of contralateral GCNIS is 5–6% (Dieckmann et al. 2007). Nevertheless, bilateral testicular biopsy is debated due to its invasiveness (Heidenreich 2009; Paffenholz et al. 2020). Furthermore, GCNIS occurs in approximately 2–4% of all men with adult cryptorchidism (Giwercman et al. 1989; Jungwirth et al. 2018).

The indications for testicular biopsy are summarized in Table 11.1.

Table 11.1 Indications and consequences for testicular biopsy

Indication	Clinical finding	Clinical relevance
Infertility	Hypergonadotropic azoospermia	Decision for TESE and ICSI procedures
	Non-reconstructible obstructive azoospermia	Decision for TESE and ICSI procedures
	Obstructive azoospermia	Exclusion of spermatogenic defects
	Klinefelter syndrome (47,XXY)	Decision for TESE and ICSI procedures
Sonographic microlithiasis	Histological analysis to exclude GCNIS	Early prevention of testicular tumor development
Adult cryptorchidism	Histological analysis of spermatogenesis and to exclude GCNIS	Decision for TESE and ICSI procedures Early prevention of testicular tumor development
Manifest testicular tumor	Histological analysis to detect or exclude GCNIS in the contralateral testis	Early prevention of secondary tumor development in the contralateral testis

11.2 Surgical Procedure and Tissue Preparation

Preoperative screening includes palpation of the genital organs, ultrasonography of the testes, and determination of ejaculate parameters and hormone levels as indicated. The patient's written consent to the surgical procedure (open biopsy with or without microsurgical dissection) is a prerequisite for any surgical procedure. The details of the surgical procedure are described in Chap. 40.

Sterile conditions are a matter of course during removal of testicular tissue as well as during its transfer. In addition, tissue transfer should be performed as gently as possible without traumatizing the tissue ("no-touch technique") in order to minimize mechanical damage to the sensitive testicular tissue and thus allow maximum quality of the histological examination or TESE. If the TESE is performed as **microsurgical TESE**, the microsurgical instruments ensure the most atraumatic tissue removal possible. If the testicular biopsy is performed without a microscope, gentle removal of the tissue with scissors and gentle stripping on, e.g., a Roux is a prerequisite for preserving the tissue structure. Immediately after removal from the testis, the tissue samples are picked up, e.g., with a sterile needle tip and transferred into the analysis media, i.e. both into sterile tubes with medium for further processing for TESE (cryopreservation and sperm extraction) and into tubes with fixative for further **histological analysis**. Specimen identification includes name, date of birth, date of surgery, and surgical site. Transfer of tissue for TESE and transport to the processing laboratory can be optimized by using portable mini-incubators to control temperature (Fig. 11.1a, b).

11.2.1 Surgical Techniques

Various techniques are used to obtain the testicular tissue. At the inception of TESE for later use in ICSI, percutaneous testicular fine needle aspiration (TEFNA) was recommended for detection of spermatogenesis as well as for sperm retrieval in NOA (Craft et al. 1997; Lewin et al. 1999). In a controlled animal model, it was shown that TEFNA can also lead to widespread destruction of testicular structure with irreversible damage to the germinal tubules. This damage was not limited to the needle channel, but led to chronic inflammation, necrosis, and **total germ cell aplasia**, especially after repeated punctures—in addition, the number of sperm retrieved by TEFNA was smaller than by TESE (Shufaro et al. 2002; Leung et al. 2014). In humans, especially in the presence of NOA, blind puncturing with TEFNA results in only a low sperm recovery rate. Spermatozoa were found in only 63 of 452 (14%) patients after TEFNA, whereas

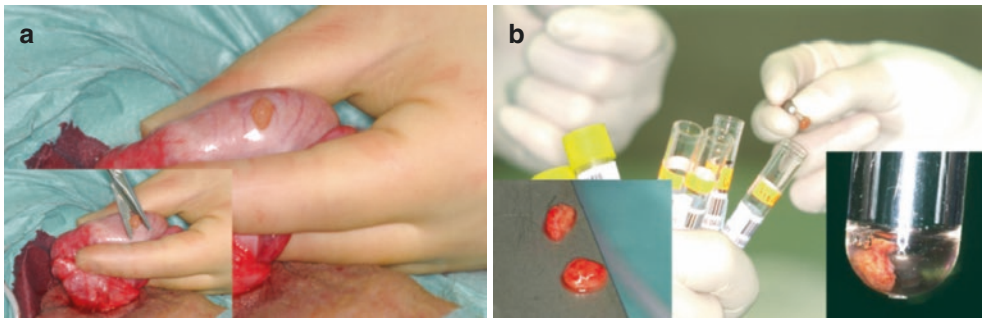


Fig. 11.1 Surgical testicular biopsy for TESE using multiple biopsy collection as an example. (**a**) Scrotal incision of the tunica albuginea after scrotal exploration and excision of a small biopsy with scissors (insert). (**b**) Transfer of testicular biopsy (insert on the left) using "no-touch technique" in medium. The biopsy must be completely covered by medium/fixative for transfer and storage (insert right)

spermatozoa were successfully recovered in 228 men (50%) with open surgical TESE (Mercan et al. 2000). Another disadvantage of TEFNA is that the tissue obtained is not suitable for histological examination. In summary, this technique falls behind **conventional (c-TESE)** or **microscopic-assisted TESE (m-TESE)** (Bernie et al. 2015) and is no longer considered in the current guidelines of the European Society of Urology and Andrology (Jungwirth et al. 2018).

Patients with hypergonadotropic NOA as well as patients with Klinefelter syndrome usually show a **partial** or even **complete SCO**. However, areas with at least qualitatively intact spermatogenesis are common. Furthermore, preinvasive GCNIS are also heterogeneously distributed in the testis (Kliesch et al. 2003). Therefore, in case of NOA, **multiple testicular** biopsies are recommended to increase the chance of successful sperm retrieval and detection of GCNIS (Kliesch et al. 2003; Dieckmann et al. 2007; van Casteren et al. 2008). This is especially true in the presence of multiple risk factors for GCNIS (Kliesch et al. 2021). The association of male infertility with a manifest testicular germ cell tumor has an increased incidence of 0.3% (10 of 3847 men) compared with the normal incidence of approximately 10:100,000 (0.0001%) in men (Raman et al. 2005; Kliesch et al. 2021). Infertility is therefore accepted as a risk factor for germ cell tumors. Using multiple testicular biopsies, as first proposed by Jezek et al. (1998), cryopreservation, TESE, and histology can be combined by multifocal biopsies (Fig. 11.1).

Using the **microdissection technique**, in which individual dilated tubules are selectively dissected under a surgical microscope (microscopic-TESE, m-TESE), the success rate of TESE can be improved in severe spermatogenesis disorders because microscopic magnification allows better differentiation between stromal and germinal parts. Peter Schlegel's research group was the first to describe and develop this technique (Schlegel and Li 1998; Amer et al. 2000; Amer et al. 2008). Measurement of tubule diameter can be helpful as a supporting parameter (Amer et al. 2008; Yu et al. 2019). The advantage of micro-TESE over standard multifocal TESE has been comparatively studied in only a few papers and depends on the underlying patient selection in recent meta-analyses (Colpi et al. 2018; Corona et al. 2019).

11.2.2 Fixation

Fixation in 10% formaldehyde or 4% paraformaldehyde solution, which is commonly used in pathology, cannot be recommended for testicular biopsies because severe shrinkage artifacts occur after formalin fixation and poor nuclear morphology makes detailed histological examination impossible. Therefore, the recommended fixative for testicular biopsies is **Bouin** or **Stieve solution** followed by embedding in kerosene wax (Bergmann 2006; McLachlan et al. 2007; Dhakal et al. 2019). This processing not only allows precise identification of all cellular components through good structural preservation, but also immunohistochemical detection of markers (Dhakal et al. 2019). The semi-thin section technique with the option of ultrastructural analysis after fixation in glutaraldehyde and embedding in Epon resin leads to optimal structural preservation, which particularly simplifies the diagnosis of GCNIS, as the atypical germ cells can be quickly identified by their nuclear morphology and the accumulation of glycogen granules in the cytoplasm (Holstein et al. 1988). Nevertheless, the laborious fixation and processing of this material makes it less suitable for rapid histology. In addition, the tissue is no longer suitable for further studies, such as immunohistochemistry or in situ hybridization, and also for gene expression analyses, whereas fixation in Bouin solution allows immunohistochemical detection of specific GCNIS markers, such as placental alkaline phosphatase (PlAP) (Fig. 11.2a, b and c) (Bergmann 2006; McLachlan et al. 2007).

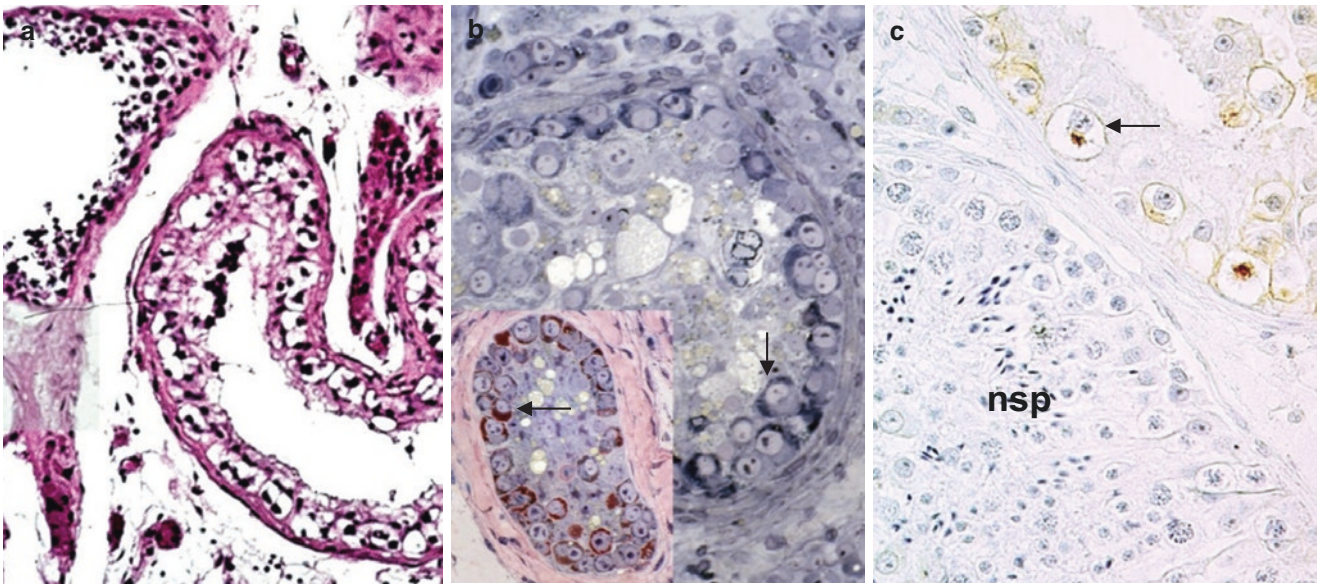


Fig. 11.2 (**a**–**c**) Seminiferous tubules with GCNIS. (**a**) Fixation in formalin (paraffin section, hematoxylin-eosin stain; primary magnification x20). Note severe shrinkage artifacts of the tissue. (**b**) Fixation in glutaraldehyde (semi-thin section, methylene blue stain; primary magnification ×40). Note the characteristic large nuclei with multiple nucleoli as well as dark cytoplasmic glycogen deposits (arrow). Insert: These could be selectively stained with Periodic Acid Schiff (PAS) (arrow). (**c**) Fixation in Bouin solution (paraffin section, PlAP immunohistochemistry; primary magnification ×40). Note the membrane-bound labeling of the GCNIS cells (arrow). nsp, germinal epithelium showing normal spermatogenesis

11.3 Histology

11.3.1 Definitions

A prerequisite for a sound histological evaluation is knowledge of the process of normal spermatogenesis and the relevant terminology in order to write a biopsy report that interprets the findings correctly. The term **"spermatogenesis"**encompasses the entire development of male germ cells within the germinal epithelium from the diploid spermatogonia contacting the basal lamina to the release of the differentiated haploid spermatozoa into the lumen of the seminiferous tubules (germinal tubules). This process includes proliferation and differentiation (type A to type B) of spermatogonia, meiotic divisions of primary and secondary spermatocytes, and transformation of haploid early round spermatids into spermatozoa. The differentiation of spermatids is called **"spermiogenesis"**(synonymously: spermatohistogenesis). At the end of this differentiation, the elongated spermatid is released into the lumen of the germinal tubules ("spermiation") and is called a "spermatozoon" (sperm). The process of normal spermatogenesis including the "stages of spermatogenesis" of the different species is described in detail in Chap. 2 with the exception of the human **spermatogenesis stages**.

11.3.2 Evaluation

The testicular biopsy is examined on a histological section and includes evaluation of (1) the **histology** of each germinal tubule for the presence of spermatogonia, spermatocytes, round and elongated spermatids, and Sertoli cells, (2) the **morphology** of the tubule wall (lamina propria), and (3) the **composition** of the interstitial tissue with respect to the number and morphology of Leydig cells and the presence and composition of immune cell infiltrates.

Within a germinal tubule, the **stages of germ cell development** are assessed and whether the arrangement of germ cells represents one of the six stages of spermatogenesis defined by Clermont (Clermont 1963) for the normal germinal epithelium. These stages are defined by a characteristic arrangement of germ cells from basal to adluminal, resulting from the observation that several waves of spermatogenesis occur simultaneously within the germinal epithelium (Fig. 11.3).

Stage I is the first stage after the second division of maturity, so early round spermatids with an acrosomal vesicle appear here. It consists of type A and type B spermatogonia, pachytene primary spermatocytes, round (step 1) and elongated (step 7) spermatids (Fig. 11.3a).

Stage II is the stage at the end of which **spermiation** occurs. It consists of type A and type B spermatogonia,

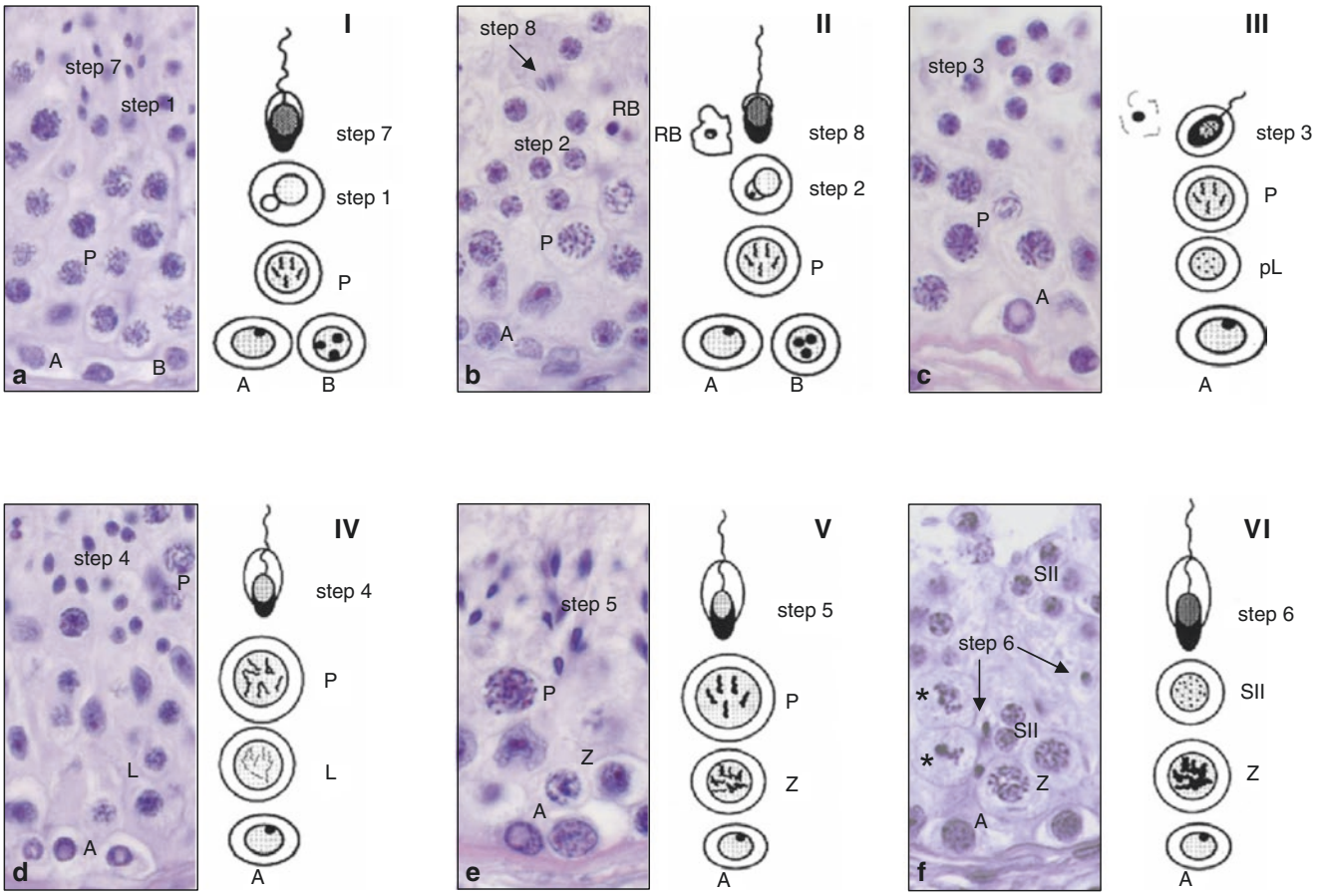


Fig. 11.3 (**a**–**f**) Normal spermatogenesis. (**a**) stage I, (**b**) stage II, (**c**) stage III, (**d**) stage IV, (**e**) stage V, and (**f**) stage VI. A, B, spermatogonia type A/type B; P, pachytene primary spermatocytes; L, leptotene primary spermatocytes; Z, zygotene primary spermatocytes; SII, secondary spermatocytes; step1–8, round to elongated spermatids; RB, residual bodies; * cells in first maturation division. Bouin fixation, paraffin section, hematoxylin-eosin stain, primary magnification ×40

pachytene primary spermatocytes, round (step 2) and elongated (step 8) spermatids. The Sertoli cells show the "residual bodies," strangulated cytoplasmic remnants of spermatids phagocytosed by Sertoli cells. Step 8 elongated spermatids are inside the germinal epithelium with only the sperm head while the flagella are already reaching into the tubular lumen. At the end of the stage, elongated sperm is released into the tubular lumen (spermiation) (Fig. 11.3b).

Stage III is characterized by the onset of **condensation** of spermatid nuclei (step 3) and the entry of spermatogonia into meiosis (preleptotene) (Fig. 11.3c).

Stages IV and V show further condensation of spermatid nuclei (step 4 and step 5), pachytene primary spermatocytes, and can be distinguished by the presence of leptotene (stage IV) and zygotene (stage V) primary spermatocytes. After stage V, pachytene primary spermatocytes undergo diakinesis and the first meiotic division (Fig. 11.3d and e).

Stage VI is the last spermatogenesis stage and accordingly characterized by the appearance of **secondary spermatocytes** that undergo the second meiotic division after only a short interphase of about 6 h. Due to this rapid development, stage VI is very rarely seen in histological sections. Furthermore, type A spermatogonia, zygotene primary spermatocytes, and elongated spermatids are found in this stage (step 6). Mitotic figures are very common.

If most of the germ tubules of a histological section clearly show one of each of these six spermatogenesis stages, one can speak of **qualitatively and quantitatively intact spermatogenesis**.

The Sertoli cells in qualitatively and quantitatively preserved spermatogenesis show a rather triangular nucleus and usually a prominent nucleolus (Sharpe et al. 2003). The cytoplasm of a Sertoli cell cannot be assessed by the intercalation of the various germ cell stages in the presence of intact spermatogenesis. The presence of a tubule lumen is due to intact Sertoli cell function. Due to the formation or closure of the

blood-testis barrier around the time of pubertal onset, not only does the tubular lumen form as a result of directional fluid transport, but also a structural and functional division of the germinal epithelium occurs (Pelletier 2011). While the spermatogonia and preleptotene primary spermatocytes present in the basal compartment are freely accessible to the immune system, the meiotic and postmeiotic germ cells are located in the adluminal compartment. There, they are protected from the immune system by the intact blood-testis barrier; the testis is therefore also described as an **immunoprivileged organ** (Fijak et al. 2011; Loveland et al. 2017). This is necessary due to the appearance of new antigens during spermatogenesis, which are presented to the immune system long after the formation of auto-tolerance and thus could lead to an autoimmune reaction that would destroy the germ cells.

Part of the immune privilege is the presence of specific **immune cells** that establish an "anti-inflammatory immune milieu." Part of this milieu are, first, the immune cells themselves. In the case of intact spermatogenesis, these are primarily macrophages, mast cells, and individual T lymphocytes. In addition to the cellular component, immunomodulators, specific cyto- and chemokines play a crucial role in maintaining immune tolerance (Loveland et al. 2017).

Leydig cells in qualitatively and quantitatively preserved spermatogenesis occur in the human testis as single cells or as Leydig cell groups. They are characterized by a large, euchromatin-rich nucleus and a distinct cytoplasm. Lipid droplets, signs of **steroid hormone biosynthesis** occurring in these cells, are frequently found within this cytoplasm (Prince 2007; Fietz and Bergmann 2017).

In the context of histological evaluation of testicular biopsies, the reliable diagnosis of pathohistological findings and their classification is crucial. The following **histopathologies** must be considered:

Hypospermatogenesis: In **qualitatively preserved, quantitatively restricted spermatogenesis**, the number of elongated spermatids in the germinal epithelium is reduced and the cellular composition of the germinal epithelium is incomplete or disorganized (Johnson et al. 1992) (Fig. 11.4a).

Maturation arrests: In spermatogenic arrests, germ cell development may stop at the level of different germ cells, e.g., at the level of round spermatids (Fig. 11.4b), primary spermatocytes (Fig. 11.4c), or spermatogonia (Fig. 11.4d).

Sertoli cell-only phenotype (SCO): In so-called **germ cell aplasia**, there are no more germ cells in the germinal epithelium, but only Sertoli cells are found. This phenomenon may be generalized (Fig. 11.4e) or focal. Focal SCO may also manifest in the setting of **mixed atrophy** (Fig. 11.7).

Tubular shadows (tubule atrophy): In case of a complete loss of germ cells and Sertoli cells, a thickening of the lamina propria due to the incorporation of light microscopic amorphous material (**hyalinization**) is common. This phenomenon can also be complete (total tubular atrophy) or focally expressed (Fig. 11.4f).

Descriptive evaluation of germinal epithelium histology should assess not only the presence or absence of germ cells, but also their **nuclear abnormalities**. For example, the presence of multinucleated spermatids may indicate defects in spermiogenesis (Holstein et al. 1988; Wang et al. 2016). Multinucleated spermatogonia can also be frequently observed (Fig. 11.5a). Abnormal nuclear sizes should be noted. So-called **megalospermatocytes** may indicate a meiosis defect (Holstein and Eckmann 1986; Johannisson et al. 2003) (Fig. 11.5b). Infertile males may also reveal Sertoli cell maturation defects. Unlike the physiological nuclear morphology in intact spermatogenesis, **immature Sertoli cells** show a more round or oval, relatively dark nucleus. Such fetal or prepubertal Sertoli cells express fetal cell markers, such as anti-Müllerian hormone (AMH), and show persistent proliferation (Steger et al. 1999; Sharpe et al. 2003; Kruse et al. 2009; Brehm et al. 2006). Furthermore, **prepubertal germinal cords** characteristically do not show a tubular lumen because the blood-testis barrier is not yet closed in immature Sertoli cells (Fig. 11.5c). Another anomaly is the appearance of **spherical concrements**, which may also be referred to as intratubular concentric microliths. These are formed by invagination of the basement membrane and can reach different sizes (Nistal et al. 1995) (Fig. 11.5d). The presence of spherical concrements, tubular shadows, thickening of the lamina propria, and also interstitial fibrosis may be associated with ultrasonographically diagnosed microlithiasis and, consequently, may be indicative of spermatogenesis disorder or else germ cell neoplasia in situ (Barbonetti et al. 2019; Rassam et al. 2020).

Assessment of testicular biopsies also consistently reveals accumulations of immune cells. In the vast majority of cases, this is not an acute inflammation but a **chronic event** (Schuppe et al. 2008). The immune cell infiltrates present cannot be further differentiated in the standard hematoxylin-eosin stain, but should be assessed according to their **density** (single immune cells, sparse, or dense immune cell infiltrates) and **localization** (peritubular, intratubular, perivascular, or interstitial). These infiltrates are particularly common in preinvasive and manifest tumor disease (see below), but also occur regularly in infertile patients (Schuppe and Meinhardt 2005; Pilatz et al. 2019). Mast cells in particular are significantly increased in patients with a SCO (also associated with Klinefelter syndrome) (Roaiah et al. 2007; Willems et al. 2020).

Special attention should be paid to the presence of **preinvasive tumor cells** as an obligate precancerous condition when evaluating testicular biopsies; this is not only true for patients biopsied because of abnormal ultrasound findings and suspected presence of a testicular tumor. Rather, it is well known that tumor diseases and their precursors occur in

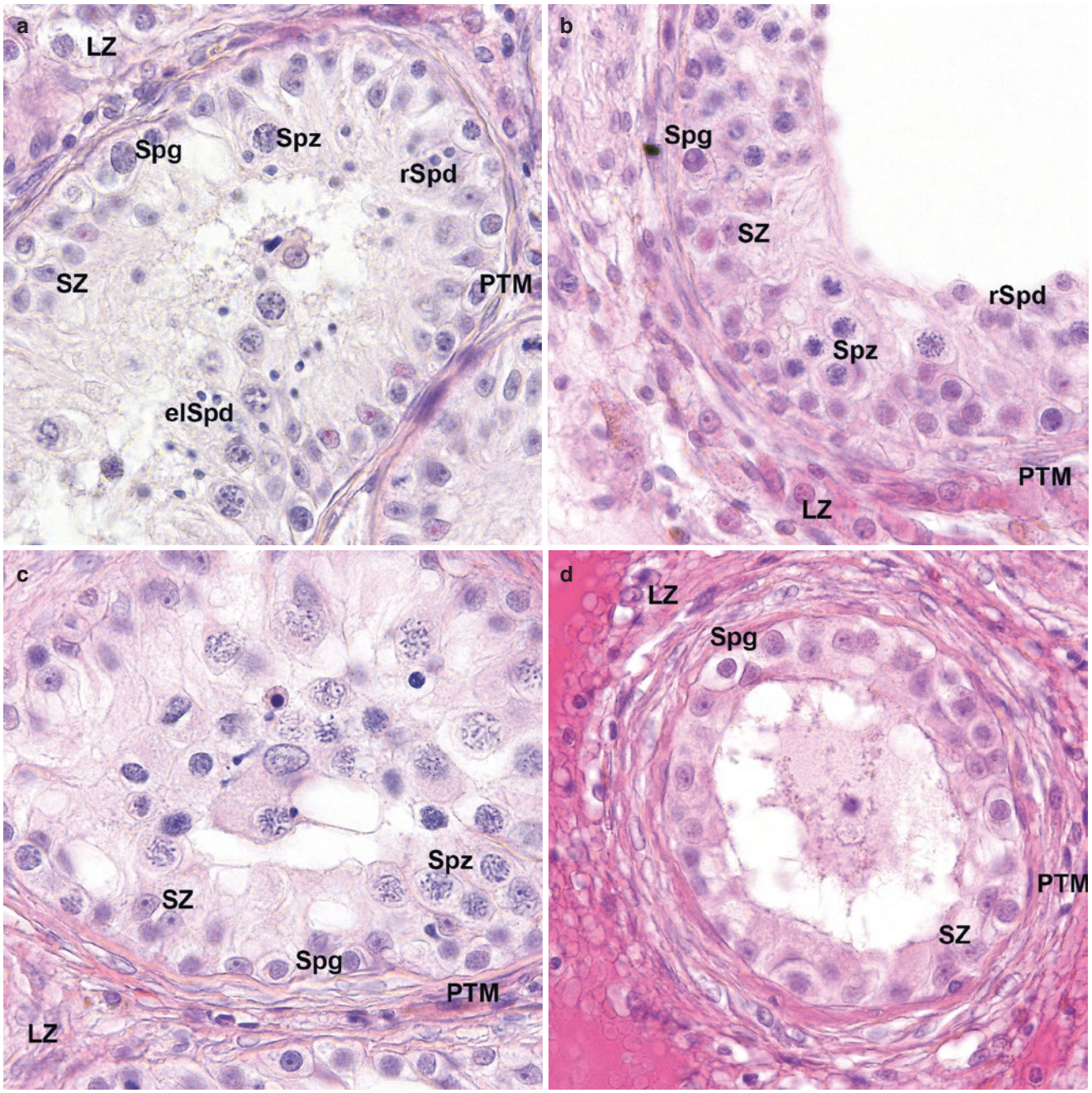


Fig. 11.4 Examples of different histopathologies. (**a**) Hypospermatogenesis: The germinal epithelium shows qualitatively intact spermatogenesis with a reduced number of elongated spermatids (elSpd). (**b–d**) Arrest of spermatogenesis at the stage of round spermatids (**b**), primary spermatocytes (**c**) or spermatogonia (**d**). Germ cell maturation stops with the development of round spermatids (rSpd), primary spermatocytes (Spz), or. spermatogonia (Spg), resp. (**e**) Sertoli cell-only phenotype: In SCO, there are no germ cells, but only Sertoli cells (SZ) in the germinal epithelium. Peritubular myoid cells (PTM) can be found within the lamina propria (Lp). (**f**) Tubular shadows: When germ and Sertoli cells are lost, there is massive thickening of the lamina propria (Lp). The remnants of the germinal tubules are then referred to as tubular shadows (ts). Bouin fixation, paraffin section, hematoxylin-eosin stain, primary magnification ×40

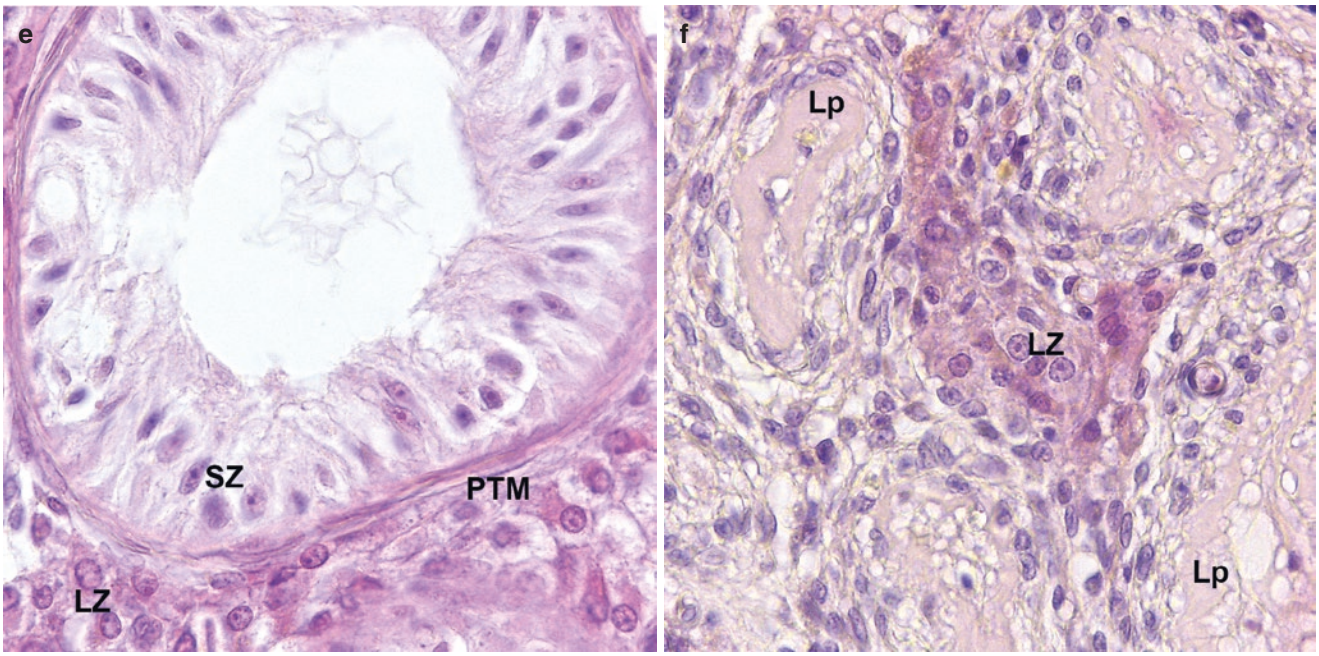


Fig. 11.4 (continued)

an increased incidence in patients with a desire to have children (Kliesch et al. 2021).

Preinvasive germ cell neoplasia in situ (GCNIS) (Berney et al. 2016) is the cellular precursor of all **seminomas** and also **nonseminomas** (Dieckmann and Loy 1996; Dieckmann et al. 2007; Kliesch et al. 2021) with the exception of spermatocytic testicular tumor (formerly spermatocytic seminoma) (Moch et al. 2016). In paraffin sections, atypical spermatogonia/GCNIS cells can be readily distinguished with some experience from normal spermatogonia by their size, "empty" cytoplasm, and large irregular nuclei with many nucleoli (Fig. 11.6a). A definitive diagnosis should nevertheless be confirmed by immunohistochemical detection of specific markers, such as membrane placental alkaline phosphatase (PlAP, Fig. 11.6b) (McLachlan et al. 2007), OCT4 (POU5F1), NANOG, AP-2γ (TFAP2C), LIN28, or even c-kit (Rajpert-De Meyts et al. 2015; Kliesch et al. 2021). GCNIS is characterized by an **intact basement membrane** as a preinvasive tumor precursor, and the development of seminoma or nonseminoma often occurs with a time delay of sometimes several years. In the development of manifest seminoma (Fig. 11.6c) or nonseminoma, GCNIS cells leave the germinal tubule by overgrowth and degradation of the basement membrane and are found in the interstitial tissue. This finding is often described pathohistologically as early invasive seminoma. Dense interstitial immune cell infiltrates are often seen in GCNIS or manifest seminoma or nonseminoma. Immunohistochemical studies show a massive accumulation of CD68+ macrophages and CD3+ T lymphocytes (Fig. 11.6d and e).

In addition, the morphology of Leydig cells should be assessed in the interstitium, which may appear as nodular hyperplasia. Differentiation between **Leydig cell hyperplasia** and a (also mostly benign) **Leydig cell tumor** is difficult and can be done by supplemental immunohistochemical analysis (Kliesch et al. 2021) (see also Chap. 24) (Fig. 11.6f).

11.3.3 Score-Count Evaluation

The most common histological diagnosis in the presence of azoospermia is **mixed atrophy** (Sigg 1979; McLachlan et al. 2007). In this case, different histopathologies, such as hypospermatogenesis, SCO, tubular shadows, or different maturation arrests occur side by side (Fig. 11.7). In the presence of mixed atrophy in particular or for objective evaluation of obstructive and nonobstructive azoospermia in general, a **score-count evaluation** is performed.

Score-count analysis according to Bergmann and Kliesch (1998) is used to objectively determine the percentage of tubules with elongated spermatids, i.e. the **quantitative analysis score** can provide a better estimate of how likely a positive testicular sperm extraction and thus a subsequent potentially successful ICSE can be performed, depending on the surgical procedure (Fig. 11.8).

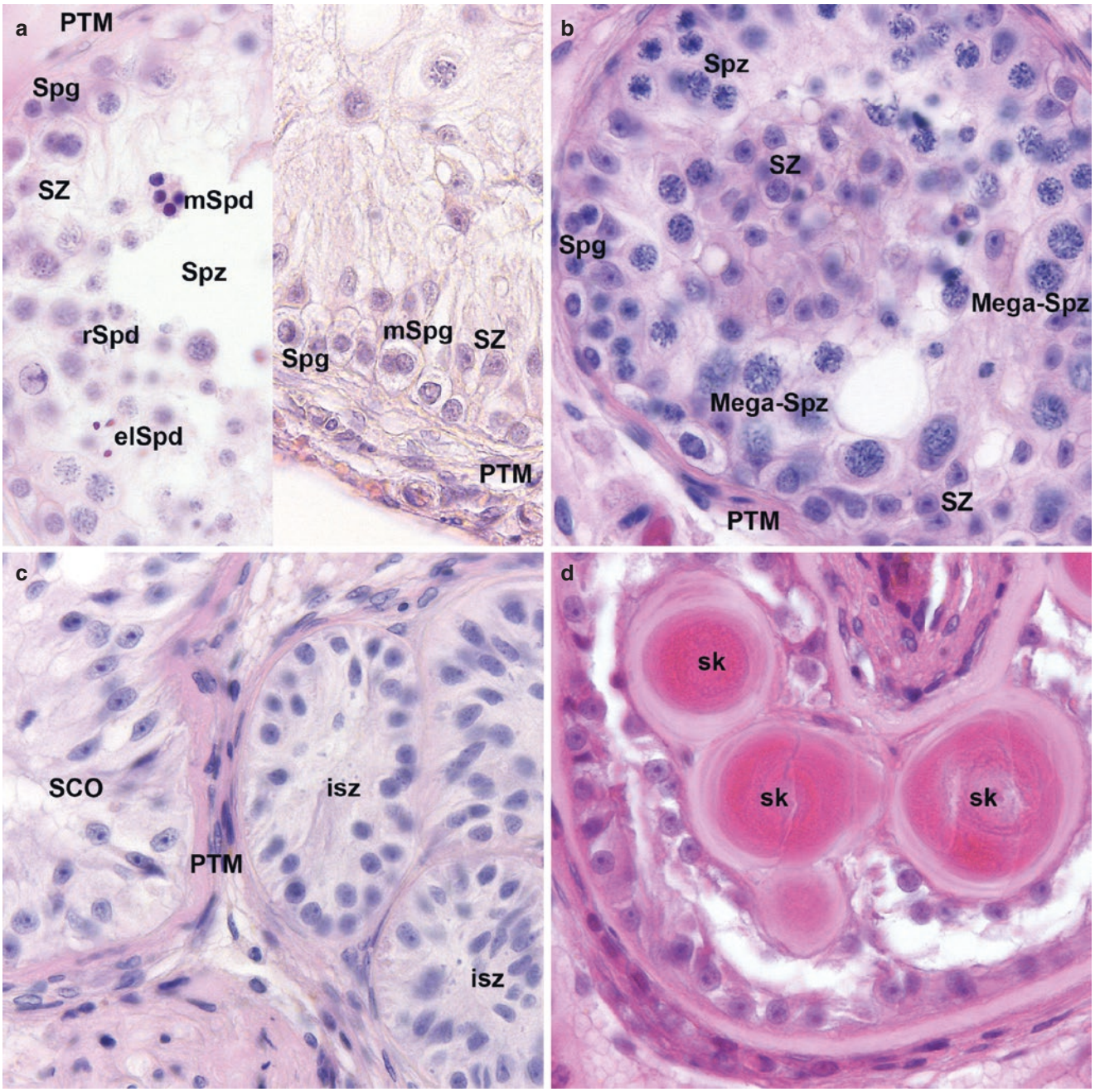


Fig. 11.5 Examples of specific histopathologies. (**a**) Multinucleated spermatids/spermatogonia: Multinucleated spermatids (mSpd) may occur as a result of spermiogenesis disorders; multinucleated spermatogonia (mSpg) indicate the existence of intercellular bridges. (**b**) Megalospermatocytes: compared to normal pachytene spermatocytes (Spz), megalospermatocytes are significantly larger (Mega-Spz). (**c**) Immature Sertoli cells: Immature Sertoli cells (isz) exhibit a round, dark nucleus; no tubular lumen is usually found in prepubertal germ tubules. (**d**) Spherical concrements: Striking concentric layering of intratubular microliths (sk) resulting from invagination of the basement membrane. PTM peritubular myoid cells. Bouin fixation, paraffin section, hematoxylin-eosin stain, primary magnification ×40

One of the first scores for histology assessment was developed by Johnsen (1970), which assessed spermatogenesis primarily qualitatively and was modified by De Kretser and Holstein (Jezek et al. 1998). This score assigns a value to each germinal tubule in a given histological section, so that there is a good correlation between score and sperm count, especially in **oligozoospermic patients**. However, nowadays **oligozoospermia** is no longer an indication for testicular biopsy (Table 11.1). Especially in the presence of nonobstructive azoospermia, which is by far the most common clinical diagnosis in infertile patients compared to obstructive azoospermia, the modified

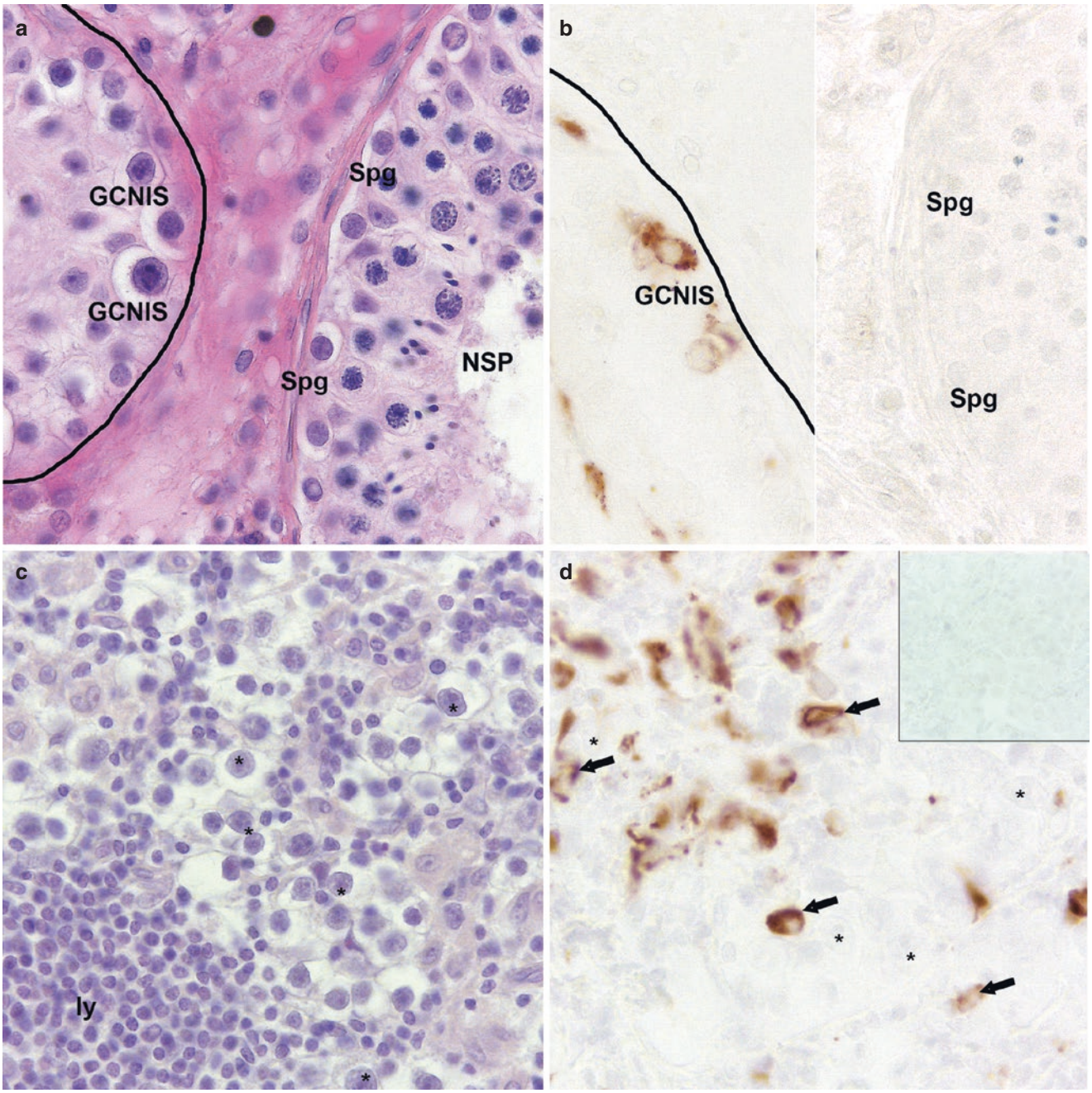


Fig. 11.6 Examples of malignant and benign tumors. (**a**) GCNIS: The tumor cells (GCNIS, left) are very large, show an "empty" cytoplasmic ring and abnormal nuclear morphology compared to normal spermatogonia (Spg, right) in a tubule with normal spermatogenesis (NSP). However, the GCNIS cells are always located within the germinal tubules (line). (**b**) PlAP: immunohistochemical detection of PlAP in GCNIS (left) is positive but negative in normal spermatogonia (right). (**c**) Seminoma: in a manifest seminoma, the invasive tumor cells (*) are no longer located within the germinal tubules but in the interstitium. Frequently, lymphocytic infiltrates also occur here (ly), which are already clearly visible in the hematoxylin-eosin stain. (**d, e**) Immunohistochemistry: By examining specific immune cell markers, interstitial CD68+ macrophages (arrow) (**d**) and CD3+ T lymphocytes (arrow) (**e**) can be easily diagnosed between the tumor cells (*). (**f**) Leydig cell hyperplasia: In Leydig cell hyperplasia, there is a usually benign proliferation of interstitial, hormone-producing Leydig cells. This can be diffuse (Lhyp) or nodular. Here, the Leydig cell hyperplasia surrounds multiple SCO tubules (SCO). Bouin fixation, paraffin section, hematoxylin-eosin stain, primary magnification ×40 (**a**–**c**) or ×20 (**f**) or immunohistochemistry (ABC, AEC; **d** and **e**), primary magnification ×40, insert: negative control without first antibody

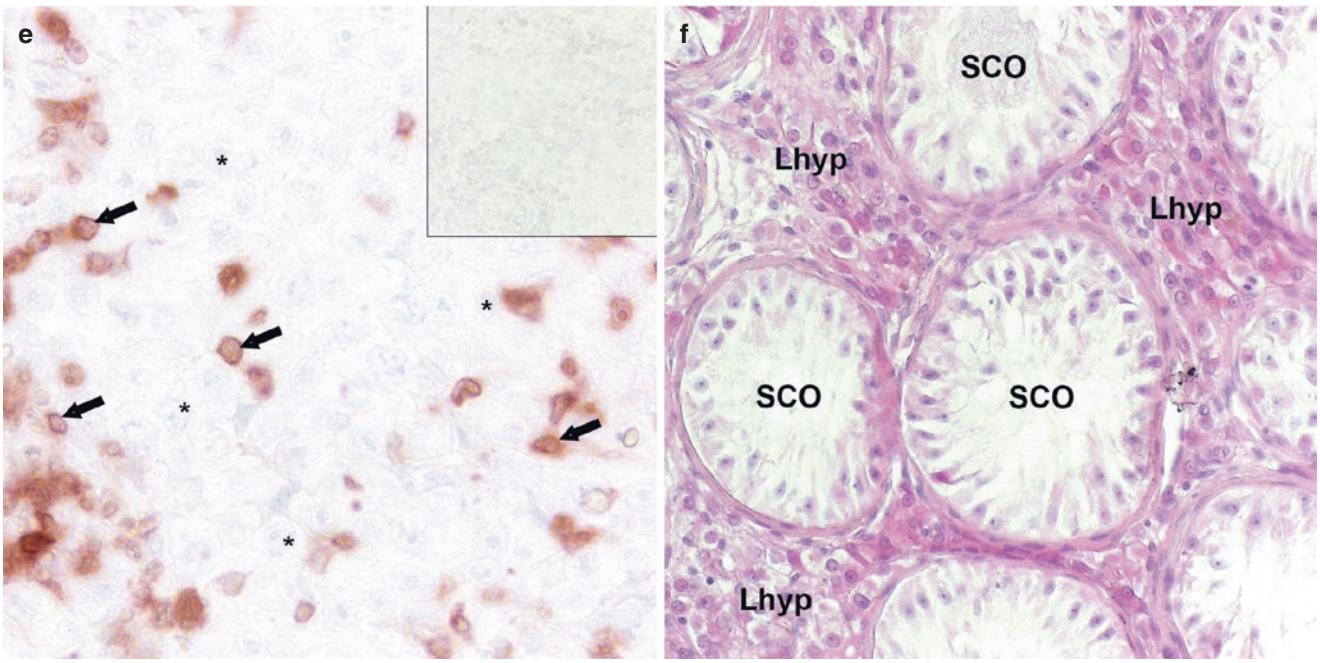


Fig. 11.6 (continued)

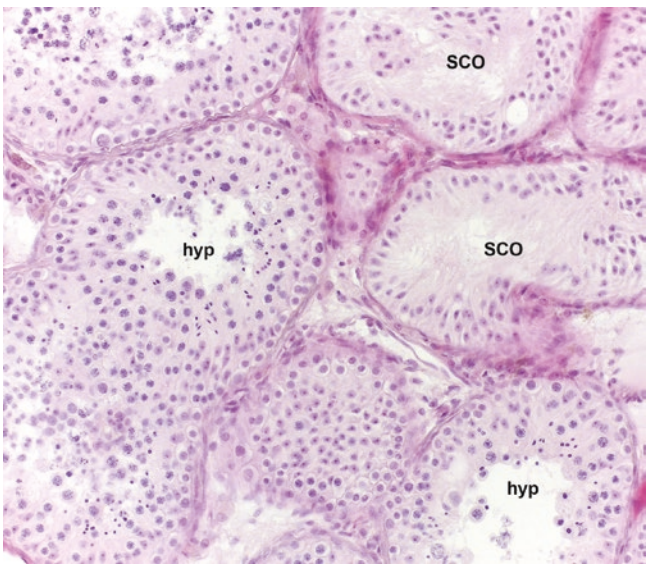


Fig. 11.7 Mixed atrophy of spermatogenesis. In mixed atrophy of spermatogenesis, different histopathologies are present in one histological section. In the present figure, tubules with hypospermatogenesis (hyp) and tubules with a Sertoli cell-only syndrome (SCO) are adjacent. In addition, tubular shadows (ts) and other pathologies may also be present. Bouin fixation, paraffin section, hematoxylin-eosin stain, primary magnification ×20

Johnsen score does not correctly represent the actual presence of elongated spermatids. For example, a mean score of 3 can occur both on biopsy with or without elongated spermatids. In contrast, the score according to Bergmann and Kliesch (1998) determines the percentage of germinal tubules with elongated spermatids and accordingly provides an estimate of how high the probability of successful TESE is considered to be (Fig. 11.8). Each tubule of a section is evaluated individually and noted on a data sheet. Finally, the histological evaluation also includes cytological changes such as meiosis (megalospermatocytes) or spermiogenesis defects (multinucleated spermatids) (Fig. 11.9). By determining the absolute number of tubules analyzed, the percentage of tubules with elongated spermatids is calculated: A score of 10 means 100% of tubules contain elongated spermatids, while a score of 1 means only 10% of tubules contain elongated spermatids. A score of 0 means no elongated spermatids are present in the histology section. Subclassification may be useful, especially when TESE/ICSI treatment is planned, and can be provided to the clinician (Table 11.2).

While the patient has a total SCO in the right testis, mixed atrophy is present in the left biopsy: Here, there is predominantly a SCO with areas of qualitatively preserved, quantitatively reduced spermatogenesis (hypospermatogenesis).

The use of Bouin's solution as fixation and the embedding in paraffin also allows further investigations on the basis of the testicular biopsies, such as structural and functional **gene and protein expression analyses**. By using **specific anti-**

Germ cells inside tubules	Patient X				Patient Y			
	No. of tubules		Johnsen Score	Total scores	No. of tubules		Johnsen Score	Total scores
many elongated spermatids	5	x	10	50	-	x	10	-
few elongated spermatids	5	x	8	40	-	x	8	-
round spermatids	-	x	6	-	-	x	6	-
primary spermatocytes	-	x	5	-	20	x	5	100
spermatogonia	-	x	3	-	30	x	3	90
only Sertoli cells	20	x	2	40	20	x	2	40
tubular shadows	10	x	1	10	0	x	1	0
Total	40			140	70			230

Johnsen score

$$\frac{\text{Total scores}}{\text{Total tubules}} = \frac{140}{40} = 3{,}5$$

Johnsen score

$$\frac{\text{Total scores}}{\text{Total tubules}} = \frac{230}{70} = 3{,}0$$

Bergmann/Kliesch score

$$\frac{\text{Tubules with elongated spermatids}}{\text{Total tubules}} = \frac{10}{40} = 2{,}5$$

Bergmann/Kliesch score

$$\frac{\text{Tubules with elongated spermatids}}{\text{Total tubules}} = \frac{0}{70} = 0$$

Fig. 11.8 Score-count analysis according to Johnsen (1970) and Bergmann and Kliesch (1998)

bodies, not only can the diagnosis of GCNIS be confirmed, but also the analysis of cell cycle-specific proteins allows the investigation of spermatogenesis defects and thus male infertility. For example, detection of the proliferation marker Ki-67 demonstrated that the proliferation rate of spermatogonia is reduced in defective spermatogenesis (Steger et al. 1998). In humans, studies of the expression and especially the **ratio of the nuclear proteins,** protamine-1 and protamine-2 have contributed to an expanded understanding of nuclear condensation and its involvement in the development of male infertility (Steger et al. 2000; Steger et al. 2001). The use of many markers for germ cells and Sertoli cells may help to elucidate the underlying mechanisms in patients with NOA and unusual histology findings (Fietz et al. 2020). Differentiation of maturation arrests has also become possible using IHC and in combination with genetic analyses (Yatsenko et al. 2015; Tüttelmann et al. 2018; Wyrwoll et al. 2020; Krenz et al. 2020; Oud et al. 2021; Rudnik-Schöneborn et al. 2021). When testicular biopsies are fixed with Bouin's solution, further investigation of gene expression is possible in addition to the application of immunohistochemical methods. For example, detection of RNA in the histology section can be performed by **in situ hybridization (ISH)** (Fietz et al. 2016; Pleuger et al. 2017). The use of histology sections for reverse transcription PCR (RT-PCR) is also possible and regularly used (Hartmann et al. 2016).

Key Points

- The basis of correct and meaningful testicular histology is the qualitative and quantitative analysis of the testicular cell populations as well as a semi-quantitative score evaluation.
- Through the resulting biopsy report, critical counseling of patients regarding successful treatment of infertility with TESE and ICSI becomes possible.
- In addition, the detailed and also further analysis of testicular sperm or testicular tissue enables the improvement of assisted reproduction and also the research of spermatogenesis damage.
- Testicular biopsy is an invasive surgical procedure and has a direct impact on the patient and the further course of treatment. Therefore, it may be performed only in strict compliance with the indications and with validated surgical and examination techniques.
- Therefore, it is recommended to be performed in qualified andrological centers, for example, certified by the European Academy of Andrology (EAA).

Date of evaluation: xx.xx.xxxx **Histo No:** xxx/xx

Evaluation of testicular biopsy

Name, prename: xx **Date of birth:** xx.xx.xxxx

Date of biopsy: xx.xx.xxxx

Clinical diagnosis: hypergonadotropic azoospermia

criteria	Right biopsy			Left biopsy		
semi quantitative analysis	Number of tubules (n)			Number of tubules (n)		
Spermatogenesis up to	1	2	3	1	2	3
elongated spermatids				11		11
round spermatids						6
primary spermatocytes						13
Spermatogonia						
Sertoli cell only	42	23	10	18	37	35
tubular shadows		20				
total	42	43	10	29	37	65
score	0	0	0	4	0	2
Morphological analysis						
tubules containing						
multinuclear spermatids						
multinuclear spermatocytes						
multinuclear spermatogonia						
megalospermatocytes						
megalospermatogonia						
degenerative germ cells						
tubular diverticle						
thickening of lamina propria	partly					
morphology of Sertoli cells	normal			normal		
morphology of Leydig cells	normal			Normal		
interstitium						
peculiar features						
diagnosis	SCO Focal total atrophy			SCO Focal qualitatively preserved, quantitatively reduced spermatogenesis		

Fig. 11.9 Evaluation of the biopsy of a patient with hypergonadotropic azoospermia

Table 11.2 Classification of the score-count analysis

Percentage of tubules with elongated spermatids	Score	Classification
100–95	10	Normal spermatogenesis
94–85	9	
84–75	8	
74–65	7	Mixed atrophy
64–55	6	
54–45	5	
44–35	4	
34–25	3	
24–15	2	
14–10	1	
9–1	0.9–0.1	Predominant atrophy of germinal epithelium with isolated formation of elongated spermatids
0	0	Sertoli cell-only, maturation arrest, testicular atrophy: tubular shadows.

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Part III

Clinics in Andrology: Secondary Hypogonadism

Congenital Hypogonadotropic Hypogonadism of Hypothalamic Origin

12

Julia Rohayem, Frank Tüttelmann, Eberhard Nieschlag, and Hermann M. Behre

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Abstract

Testicular dysfunction of hypothalamic and/ or pituitary origin is referred to as "hypogonadotropic hypogonadism, HH."

Hypothalamic GnRH deficiency results in inadequate pituitary gonadotropin stimulation of the testes and entails both impaired testosterone secretion and inadequate spermatogenesis.

Congenital forms of hypothalamic hypogonadotropic hypogonadism (CHH) occur with or without associated congenital impairments of olfaction. If anosmia is present, the condition is termed "Kallmann syndrome."

Rarely, CHH is associated with primary adrenocortical insufficiency.

CHH may also be present in syndromic disorders.

Acquired hypothalamic hypogonadotropic hypogonadism may result from tumors or injuries within the

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suprasellar region, e.g., as a consequence of surgical interventions, irradiation, inflammation or ischemia in this area.

Acquired hypothalamic HH may also result from suppression or downregulation of the central hypothalamic-pituitary-gonadal (HPG) axis. This condition is potentially reversible if its cause is removed, and is therefore referred to as "functional hypogonadotropic hypogonadism."

Effective hormonal treatment strategies are available for males with testosterone deficiency and infertility due to hypogonadotropic hypogonadism.

To date, the genetic origin of CHH and Kallmann syndrome can successfully be uncovered in up to 50% by molecular genetic analyses, most importantly next-generation sequencing (NGS). Men attempting biologic paternity should receive genetic counseling on the risks for transmission of their genetic variant(s) in a timely manner.

12.1 Introduction

12.1.1 Definition of Terms

"Hypogonadotropic hypogonadism (HH)" is the term used to describe **hypothalamic** or **pituitary-derived** testicular dysfunction. This chapter focuses on hypothalamic disorders of the hypothalamic-pituitary-gonadal (HPG) axis.

Testicular dysfunction of pituitary origin is treated in Chap. 15.

12.1.2 Causes of Hypothalamic Hypogonadotropic Hypogonadism

HH of hypothalamic origin is either **congenital or acquired**.

If **congenital** hypogonadotropic hypogonadism occurs without additional disturbances, the condition is termed "**congenital hypogonadotropic hypogonadism (CHH)"**; if it is associated with a disturbed sense of smell, i.e., **anosmia** or **severe hyposmia**, it is termed "**Kallmann syndrome."**

Acquired forms of **hypothalamic HH** are caused by **tumors, injuries**, including surgery or irradiation **of the suprasellar region**, as well as **inflammation** or **ischemia** in this area.

HH may also result from **reversible suppression or downregulation of the central HPG axis** (see also 12.6).

12.1.3 Epidemiology

In **childhood**, congenital forms of hypothalamic HH result, for the most part, from **genetic causes**.

The most common cause of acquired HH, combined with other pituitary axis failures with onset at 5–14 years is **craniopharyngiomas**, suprasellar neoplasms that develop from nests of epithelium derived from Rathke's pouch.

The prevalence of CHH in males is 1:10,000 (Fromantin et al. 1973); males are affected four times more frequently than females (Seminara et al. 1998). In half of all subjects with CHH, anosmia is additionally present, giving rise to **"Kallmann syndrome."**

In **adulthood**, **acquired origins** of hypothalamic disorders **prevail** over congenital causes. Tumors or injuries within the suprasellar region, caused by surgery, irradiation, inflammation or ischemia are at the origin of acquired hypothalamic dysfunction.

Late manifestations of congenital HH during adulthood after spontaneous normal puberty are rare.

12.2 Pathophysiology

Hypothalamic HH is characterized by reduced pituitary **secretion of LH and FSH** in response to **quantitatively inadequate or inadequately effective pituitary GnRH stimulation** (Spratt et al. 1987; Waldstreicher et al. 1996). As a result of inadequate gonadotropin amplitudes and/or frequencies, the testes are not sufficiently stimulated: reduced LH stimulation of Leydig cells entails **impaired testosterone secretion**, and insufficient FSH stimulation results in **spermatogenic failure.**

In both CHH and Kallmann syndrome the underlying pathology is either **disordered migration of GnRH neurons** to the hypothalamus during the embryonic period (Casoni et al. 2016) and/or **dysfunctional GnRH secretion or action** (Belchetz et al. 1978). In Kallmann syndrome, there is an **additional disorder of migration of olfactory neurons** through the lamina cribrosa, which results in **aplasia or hypoplasia of the olfactory bulbs** (Takeda et al. 1992).

12.2.1 Genetic Causes of CHH and Kallmann Syndrome

Many forms of CHH and Kallmann syndrome have a **common genetic basis**, i.e., can be caused by variants in the same genes. However, there are variants in genes that lead exclusively to CHH, whereas variants in other genes exclusively cause Kallmann syndrome.

CHH and Kallmann syndrome occur sporadically or clustered in families; familial occurrence is observed in 30–40%.

More than 50 disease-associated genes have been identified to date (Seminara et al. 1998; Dwyer and Raivio 2015; Maione et al. 2018; Cangiano et al. 2021). The mode of inheritance of CHH may be autosomal-dominant, autosomal-recessive, or X-linked (Mitchell et al. 2011; Costa-Barbosa et al. 2013). The underlying genetic alteration may be **monogenic** or **oligogenic**.

Mutations in the following genes are found **exclusively in Kallmann syndrome**: ***ANOS1 (KAL1), SEMA3A, SOX10, FEZF1, HESX1*** (Dattani et al. 1998), ***IL17RD,*** and ***NELF*** (see Table 12.1).

Mutations in the ***anosmin-1*** (***ANOS1)*** gene (formerly termed ***KAL1*** gene) are detectable in 10–50% of familial cases with Kallmann syndrome and X-linked recessive inheritance. ***ANOS1*** **gene** variants are also detected in 15% of sporadic cases. The encoded protein **anosmin-1** regulates axon growth. Its alteration affects the migration of GnRH precursor neurons and olfactory neurons (Franco et al. 1991).

Mutations in the following genes may result in **both CHH or Kallmann** syndrome: ***FGFR1 (=KAL2); FGF8; PROK2 (=KAL3); PROKR2 (=KAL4); HS6ST; AXL; WDR11;*** and ***CHD7*** (Table 12.1).

Table 12.1 Genetic causes of hypothalamic hypogonadotropic hypogonadism

Kallmann syndrome	***ANOS1 (=KAL1)*** ***SOX10, FEZF1, IL17RD,*** ***NSMF(=NELF), SPRY4, FLRT3,*** ***FGF17*** *CCKBR, FGF13, GAP43, PALM2,* *PLEKHA*
Kallmann syndrome and CHH	***FGFR1 (=KAL2), FGF8, FGF17*** ***PROK2 (=KAL3), PROKR2 (=KAL4),*** ***HS6ST1, AXL, WDR1, SEMA3A,*** ***DUSP6, CHD7, HESX1***
CHH	***KISS1, KISS1R = GRP54,*** ***TAC3, TACR3,*** ***GNRH1, GNRHR,*** ***FSHB, LHB,*** ***LEP, LEPR*** ***PSCK1*** *AMN1, CRY1, CXCR4, GLI3, JAG1,* *MASTL, NOS1, NOTCH1, NRP2* *PDE3A, RD3, TRAPPC9, SEMA7A,* *SEMA3E, PLXNA1*
CHH with congenital adrenal hypoplasia	***NROB1 (=DAX1)***
Syndromic CHH in **CHARGE syndrome** **Bardet-Biedl syndrome** **Prader-Willi syndrome**	***CHD7*** ***BBS 1-21*** Lack of expression of genes on the paternal chromosome (15q-11-q13-region)

Heterozygous ***fibroblast growth factor receptor 1 (FGFR1 = KAL2)*** mutations underlie CHH or Kallmann syndrome in up to 15%. Due to the gene's **incomplete penetrance** in **autosomal-dominant inheritance,** familial or sporadic cases are possible (Dode et al. 2003).

Genes encoding **FGFR1** or its ligand, **fibroblast growth factor 8 FGF8**, are involved in organogenesis (Falardeau et al. 2008). **Anomalies of the phalanges**, such as polydactyly and camptodactyly appear to be exclusive to ***FGFR1*** and ***FGF8*** **mutations** and may therefore be useful for prioritizing genetic studies (Costa-Barbosa et al. 2013).

PROK2 (=KAL3) encodes **prokineticin 2,** a chemical messenger that acts on neural progenitor cells. Mutations in this gene, as well as mutations in the gene coding for its receptor ***prokineticin receptor2 PROKR2 (=KAL4),*** may be causative of CHH or Kallmann syndrome (Dode et al. 2006).

Pathogenic variants in the gene ***chromodomain helicase DNA-binding protein 7, CHD7*** also cause both CHH and Kallmann syndrome (6% of sporadic cases) (Kim et al. 2008). In addition, it is at the origin of **CHARGE syndrome**, an autosomal-dominantly inherited multisystem disorder with CHH.

Mutations in the following genes have been described **exclusively in normosmic patients with CHH**: ***KISS1, KISS1R (=GRP54), TAC3, TACR3, GNRH1, GNRHR, FSHB, LEP*** (Strobel et al. 1998), ***LEPR*** (Clement et al. 1998), ***LHB*** (Table 12.1).

Mutations in ***GNRHR*** are among the most common genetic causes of CHH. They lead to **GnRH insensitivity of gonadotropic cells** within the anterior pituitary, thereby entailing **relative LH and FSH deficiency of varying severity** (de Roux et al. 1997; Layman et al. 1998; Bedecarrats and Kaiser 2007; Beneduzzi et al. 2014). The mode of inheritance is **autosomal-recessive**. Accordingly, symptomatic CHH occurs sporadically, i.e., as isolated cases in families.

The discovery that mutations in the gene encoding the ***human G-protein-coupled receptor 54 GRP54 (=KISS1R***) entail CHH, has contributed to the knowledge that **GPR54 and its ligands, the kisspeptins**, are essential for the release and action of GnRH (de Roux et al. 2003; Seminara et al. 2003). The neuropeptide **neurokinin B** represents another important **central regulator of GnRH secretion**; accordingly, mutations of the encoding gene ***TAC3*** or the gene encoding its receptor ***TACR3*** cause CHH (Topaloglu et al. 2009).

If CHH is associated with **primary adrenocortical insufficiency**, mutations of the ***NROB1 (group B, nuclear receptor subfamily 0, member 1)***, previously termed ***DAX1*** gene ***(dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1)*** may be causative (Habiby et al. 1996) (see Chap. 13).

12.2.2 Genetic Basis of Syndromic Hypothalamic Hypogonadism

The term **CHARGE syndrome** is an acronym and refers to hypothalamic congenital hypogonadism associated with the following congenital developmental disorders: **c**oloboma, **h**eart (cardiac) defects, choanal **a**tresia, **r**etardation of growth and developmental, **g**enital and **e**ar abnormalities (see Chap. 13). The syndrome is caused by mutations in the ***CHD7*** gene (Janssen et al. 2012).

Congenital central hypogonadism of both hypothalamic and pituitary origin, and in addition combined with primary gonadal dysfunction occurs in **Prader-Willi syndrome** (see Chap. 13). PWS is caused by decreased or absent expression of genes on the paternal **chromosome on 15q-11-q13** (Angulo et al. 2015).

Hypogonadism can also manifest as a symptom of **Bardet-Biedl syndrome (BBS).** However, this is only partially based on hypothalamic-pituitary dysfunction, but is primarily due to gonadal dysfunction. The cause of the autosomal-recessively inherited multisystem disorder is genetically heterogeneous; alterations in the ***BBS 1-21*** genes are possible causes, in addition, a "triallelic" mode of inheritance may be underlying (Khan et al. 2016) (see Chap. 13; Table 12.1).

12.3 Clinics

12.3.1 Symptoms of CHH/Kallmann Syndrome in the Newborn, Infant, and Prepubertal Boy

Dysfunction of the central HPG axis during fetal life may **impair testicular descent**, as this process is regulated by androgens (Chap. 17). In addition, intrauterine testosterone deficiency (resulting in deficiency of its bioactive metabolite dihydrotestosterone (DHT)) results in **inadequate penile growth** in the male fetus. A "**micropenis**" is present in a male newborn, if his stretched penile length is below 2.5 cm.

Inguinal testes and/or a micropenis in a male neonate thus represent valuable early indicators of potential CHH. However, this combination of symptoms may also be present in other **variants of sex development** (**DSD**) that also have to be considered as a differential diagnosis (Chap. 31).

During **"minipuberty"**, i.e., during the first 3–6 months of life, the physiologic activation of the HPG axis is lacking in **male infants with CHH**. This can be evidenced by **subnormal increases in serum LH, FSH, and testosterone** concentrations. Minipuberty is thought to be important for **Sertoli cell maturation and proliferation** in the testes (Grinspon et al. 2010) and thus for future fertility, as the number of Sertoli cells determine the **spermatogenetic potential of the testes**.

In both CHH and Kallmann syndrome, **nonreproductive features** may in addition be diagnostic of the condition. These include **dental and finger anomalies, clefting** of lips or palate, **iris colobomas**, and **agenesis of one kidney.** Other symptoms associated with CHH/Kallmann syndrome may become prominent later, such as involuntary movements **(synkinesia)** of the other hand or, rarely, **cerebral ataxia**.

12.3.2 Consequences of CHH/Kallmann Syndrome for Pubertal Development

If central hypogonadism is not recognized in the newborn or infant, diagnosis of CHH will be retarded, as the HPG axis enters a physiologic phase of quiescence during prepuberty. Therefore, functional impairment of the central HPG axis may remain unnoticed until pubertal ages. Olfactory dysfunction that could indicate Kallmann syndrome at prepubertal ages is not always noticed.

> Boys with **Kallmann syndrome** are often unaware of their olfactory impairment. Although they are unable to smell **aromatic substances** (coffee, perfumed soaps) mediated by the olfactory nerve, they appear to be capable of smelling, because they perceive "**trigeminal irritants,**" such as vinegar or ammonia.

Semi-quantitative olfactometry [e.g., by "Sniffin' Sticks" or the "University of Pennsylvania Smell Identification Test"(UPSIT)] not only reliably allows diagnosis of smell impairment, but also enables the differentiation between **hyposmia** and **anosmia** (Chap. 7).

> The **leading symptom** of CHH and Kallmann syndrome is absent or arrested **pubertal development**.

Delayed puberty refers to an onset of puberty that is 2.5 standard deviations beyond the population mean (Chap. 14). The mean age norm for pubertal entry in German boys is 12.5 years. According to this definition, delayed puberty is present if testicular volumes are below 4 ml each side after the 14th birthday, or if the passage through puberty lasts more than 5.5 years.

Thus, a lacking onset of male puberty is still "normal" until age 14 years is reached. Rarely, spontaneous onset of puberty may still occur later in "very late bloomers" with extreme "**constitutional delay of growth and puberty,**

CDGP." The clinician's challenge is to distinguish CDGP that is temporary in nature from permanent CHH, in order to allow for timely and adequate treatment of boys with persistent absence of puberty (Chap. 14).

12.3.2.1 Absence of Puberty

If spontaneous puberty fails in boys with CHH or Kallmann syndrome, the lack of pubertal increases in LH and FSH in the blood will lead to a **persistence of prepubertal testosterone levels** in the adolescent male. The **testes will not show pubertal growth** to above 4 ml beyond the 14th birthday. Pubertal penile growth will not occur, **voice mutations will not appear,** and the **sparse body hair pattern typical for childhood** will persist. Likewise, there will be no **beard growth** and **musculature** will remain prepubertally "underdeveloped". With progressing age, the young male will experience a **change in body shape** toward **longer extremities, broader hips, and accumulation of subcutaneous fat deposits around trunk and hips**.

Of note, **pubic and axillary hairs** do develop spontaneously in adolescents with CHH, albeit at a sparse level, as their growth results from **gonadotropin-independent activation of androgen synthesis during adrenarche**. However, pubic hair in males with untreated CHH never extends to the lower abdomen.

The lack of physical virilization during puberty is accompanied by a lack in **psychosexual maturation**. Frequent spontaneous (nocturnal) erections do not occur, libido does not awaken (thus the adolescent's desire for regular masturbation remains absent). Even if provoked, there are no **ejaculations**, i.e., only "dry orgasms" without emissions of semen.

12.3.2.2 Arrested Puberty

In contrast to adolescents with complete absence of puberty, boys with **arrested puberty** experience a **slight pubertal growth of both testes.** However, there is no further increase in testicular volumes, and adult testicular sizes are not reached. The penis enlarges slightly but also arrests in growth. **Serum testosterone** concentrations may reach **midpubertal levels**, but drop again to prepubertal concentrations afterwards. Spermatogenesis remains insufficiently stimulated. Ejaculations rarely occur, and if present, very small volumes are characteristic.

The diagnosis of pubertal arrest is challenging, not only as pubic hair can be misinterpreted as a sign of puberty but also, as the decline of a freshly awakened libido and the cessation of ejaculations tend to be underreported.

12.3.2.3 Consequences of a Late Diagnosis of CHH/Kallmann Syndrome

Late diagnosis of CHH or Kallmann syndrome, at a time when other adolescents have already undergone puberty, has a **negative impact on body and soul** of affected males: Insufficient sex steroid concentrations in the blood result in **delayed fusion of the epiphyseal joints**. As a consequence, tubular bones continue to grow longer than usual, giving rise to **eunuchoid tall stature** with long arms and legs. In addition, there is a broadening of the hips. Male sex steroid deficiency during adolescence induces an **increase in subcutaneous fat** around the **hips and abdomen,** and favors the development of **lipomastia**. Bone mineralization is reduced, reducing peak bone mass ("**peak bone mass"**) that is achieved in the second decade of life. **Osteopenia or osteoporosis** are the consequences later in life.

Sex steroid hormone deficiency also may entail **depressive mood** with **social withdrawal and permanent impairment of self-esteem**, with **fears regarding intimate contacts**. This can lead to lifelong partnerlessness.

12.4 Diagnostics

12.4.1 Medical History

A detailed medical history should inquire about the presence of **undescended testes at birth**, or **a small phallus size**. The age at which testicular position was corrected and whether this was done by hormone therapy or orchidopexy is also of interest (Fig. 12.1).

Preexisting **conditions that could affect hypothalamic-pituitary function** should be explored, such as tumors, surgery, inflammation, irradiation or **chronic diseases**. Eating disorders or **recent weight loss**, possibly in combination with **extreme exercise**, should be sought. Inquiries on **olfactory disorder, congenital abnormalities** such as finger and tooth or kidney anomalies, cleft lip, jaw, and/or cleft palate complete the history. Problems due to involuntary comovements of a hand (**synkinesia**) or due to gait unsteadiness (**cerebellar atax**ia) can also be addressed by physical examination.

Current medication should be documented, specifically, **previous testosterone therapy**. Use of **opioids for pain medication** should also be inquired for.

If there is a suspicion of secondary GnRH failure after spontaneous virilization at puberty, it is essential to direct the question toward **drug abuse** or the use of **anabolic steroids** (injections, protein shakes).

Family history elicits whether parents, siblings or other relatives are also affected by absent or delayed sexual development. The **age of the mother's menarche** and the **age of the father's voice change** can be indicative. **Unintentional childlessness** and **limited smelling ability** within the family can also be addressed. Information on **parental height** allows for the calculation of **parental target height.** In boys, the following formula may be used: father's height + mother's height/2 ± 6.5 cm (standard deviation ±7 cm).

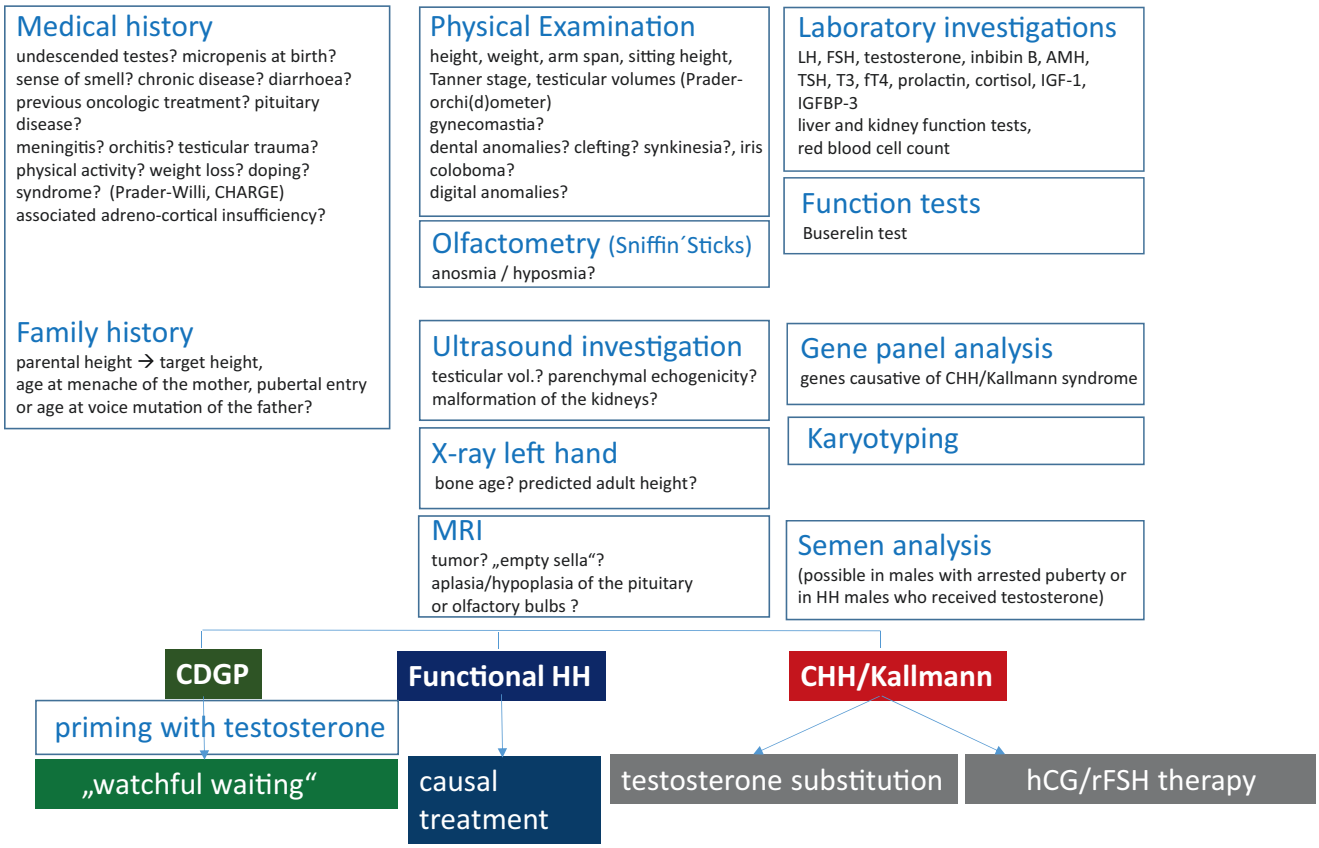


Fig. 12.1 Diagnostics, differentials, and treatment in males with hypogonadotropic hypogonadism

12.4.2 Physical Examination and Ultrasound Imaging

The physical examination documents **anthropometric data** , such as height, sitting height, arm span and body weight, hip and abdominal circumferences. It also documents **beard growth, body hair, presence of voice mutation**, the **pubertal Tanner stage,** and **testicular volumes**, estimated using a Prader orchi(d)ometer.

Ultrasound investigation of the testes provides information on testicular parenchymal echogenicity and texture, as well as on volumes of both testes. Testicular size may be measured with the help of the volume formula for ellipsoids. However, this is accurate only if the testes have reached a pubertal size. The volume of small/ prepubertal testes may therefore rather be calculated using the following formula: (volume (ml) = length (cm) × width (cm) × depth (cm) × 0.52). Ultrasound investigation also visualizes the epididymis (see Chap. 6).

12.4.3 Laboratory Diagnostics, Functional Testing, and Genetic Diagnostics

Hormone concentrations are measured in serum. LH, FSH, total testosterone, and the Sertoli cell markers inhibin B and AMH allow assessment of HPG axis function (see Chap. 7). Additional analysis of the hormone concentrations of other hypothalamic-pituitary axes (TSH, T3, fT4, IGF1, IGFBP3, prolactin, cortisol) may be useful.

Functional testing with the GnRH agonist buserelin, with determination of basal and stimulated LH concentrations in serum is helpful for differentiating between the CDGP and CHH (see Chap. 14).

The **GnRH (=LHRH) test** is not suitable for distinguishing CHH from CDGP, as the results display a large overlap: in both conditions, LH and FSH can either not respond to stimulation at all or show a weak response.

The **GnRH pump test** is indicated only if the localization of a central HPG axis disorder has to be determined in order to explore whether GnRH pump therapy can be used effectively for treatment. The test consists of s.c. applications of 5 µg GnRH pulses every 90–120 min via a minipump for 7 days, and of assessment of pituitary LH and FSH responses. Hypothalamic dysfunction is likely to be the cause of hypogonadism, if gonadotropin secretion is successfully stimulated by the test; otherwise, pituitary

dysfunction or pituitary GnRH receptor resistance is the likely cause of CHH.

Karyotyping is of diagnostic value only if additional chromosomal abnormalities are suspected in CHH or Kallmann syndrome, or if the intention is to detect ***ANOS1 (KAL1)*** deletions on the X-chromosome.

Molecular genetic analyses (usually by means of "**next-generation sequencing,**" **NGS**), examine genes listed in Table 12.1 and potentially additional novel candidates. It may be helpful for confirming CHH and provides a tool for its differentiation from CDGP. Detection of pathogenic variants enables genetic counseling regarding the risks of transmission to offspring.

12.4.4 Magnetic Resonance Imaging (MRI) and Complementary Diagnostics

Absent or arrested puberty or secondary failure of gonadotropin secretion justifies the need for **imaging of the hypothalamic-pituitary region by MRI**. Thereby, an intracranial tumor is visualized or excluded. MRI may also detect **malformations**, including aplasia or hypoplasia of the pituitary gland or the olfactory bulbs.

Bone age is determined by X-ray of the left hand and additional comparison of the radiograph with images of the standard bone age atlas of Greulich and Pyle. It allows for estimation of the **longitudinal growth potential** of adolescents. Predicted adult height is calculated according to the method of Bayley and Pinneau.

Determination of **bone density by DEXA scan** is indicated if HH has remained untreated for a long time.

Assessment of smell by the "University of Pennsylvania Smell Identification Test" (UPSIT) or by "Sniffin' sticks" allows identification of hyposmia or anosmia.

Semen analysis is rarely possible in males with untreated CHH/Kallmann syndrome, as the male excretory glands are immature or inactive. In males previously treated with testosterone or in those with arrest after spontaneous partial puberty, it may be possible to obtain a semen sample for analysis.

12.5 Treatment of CHH

Gonadotropin replacement in boys with HH and absent or arrested puberty aims to **initiate or complete normal pubertal maturation**. Maturation of male secondary characteristics (i.e., virilization), activation of spermatogenesis, and psychosexual maturation take about 2.5 to 5 years. Therefore, central hormone replacement has to be carried out for at least 2, rather 3 years, rarely even longer. Maturity for semen analysis is achieved after a period of at least 2 years of combined gonadotropin substitution (see Chap. 38).

In CHH males without desire for progeny, maintenance of virilization may also primarily be achieved by **testosterone replacement**. However, testes will then remain immature and thus of prepubertal sizes. If biologic paternity is desired later, there is still a high chance for successful initiation of spermatogenesis by replacement of central hormones.

Gonadotropin replacement is also applied in men in whom **secondary central HPG axis dysfunction and thus infertility** occurred **after spontaneous puberty,** if there is a wish for **reinduction of fertility**.

If hypothalamic dysfunction is the **exclusive origin of HH**, **GnRH pump therapy** can alternatively be used for spermatogenic induction. Fertilization successes of gonadotropin and GnRH replacement are comparably effective (see Chap. 38).

Cryobanking of semen is an option for CHH males who have completed pubertal and testicular maturation. Spermatogenesis will arrest as soon as treatment is switched to testosterone replacement. If spontaneous conception is desired in the future, to achieve biologic fatherhood, treatment has to be switched back to gonadotropin replacement (or GnRH-substitution). Alternatively, cryopreserved spermatozoa may be used for impregnation of the partner by intracytoplasmic sperm injection (ICSI).

12.6 Special Aspects: Functional Hypogonadotropic Hypogonadism

Functional hypogonadotropic hypogonadism . is a potentially reversible condition resulting from **downregulation or suppression of GnRH pulsatility** and consequently reduced secretion of pituitary LH and (to a lesser extent) of FSH.

12.6.1 Hypogonadotropic Hypogonadism Due to Inadequate or Excessive Nutrient Intake or/and Sport Excess

Functional hypogonadism can be observed in cases of **severe malnutrition**, **due of restrictive eating disorders** (anorexia nervosa or starvation) (Wabitsch et al. 2001), **malabsorption** (inflammatory bowel diseases, celiac disease or cystic fibrosis) or in patients with **cachexia due to chronic or malignant diseases**.

On the other hand, functional hypogonadism can also be induced by **excessive obesity** with or without associated type 2 diabetes (Dhindsa et al. 2010). However, herein the suppression of the central HPG axis is less severe, as it results from inhibitory effects of enhanced endogenous production of estrogens in adipose tissues.

GnRH pulse generator function may also be downregulated in athletes with extreme training/**overtraining** with and without **dietary restrictions** (Nieschlag and Vorona 2015; Tenforde et al. 2016). It can also be induced by **severe stress**, including social deprivation or depression.

12.6.2 Drug-Induced Hypogonadotropic Hypogonadism

Abuse of anabolic androgenic steroids (AAS) or testosterone exerts suppressive effects on the central HPG axis (Nieschlag and Vorona 2015) (see Chap. 37). Downregulation of pituitary LH and FSH secretion is reversible if androgen applications are ceased. However, reactivation of the GnRH pulse generator takes 3–24 months (Liu et al. 2006). During this time, serum testosterone concentrations remain at hypogonadal levels, with all consequences regarding symptoms of androgen deficiency.

Likewise, the use of **opioids (morphine, heroin, methadone**) activates inhibitory pathways in the hypothalamus, thereby suppressing GnRH secretion (Cicero et al. 1974).

Of note, androgen deficiency symptoms in the context of medication with opioids for chronic pain can easily be confused with those resulting directly from chronic pain, such as depression and loss of libido.

Key Points

- Congenital hypogonadotropic hypogonadism (CHH) and Kallmann syndrome are closely related. The common pathophysiological feature is a congenital impairment of the secretion or action of hypothalamic GnRH, which results in hypogonadism. In addition, patients with Kallmann syndrome are affected by anosmia or hyposmia.
- More than 50 genes have been identified to be causative of CHH; in up to 50%, a pathogenic mutation can be assigned by molecular genetic analyses.
- Undescended testes and the presence of a micropenis at birth are indicators of CHH or Kallmann syndrome. However, the leading clinical symptom is absent or arrested pubertal development.
- Constitutional delay of growth and puberty (CDGP) is an important differential diagnosis of CHH in adolescence.
- Functional and thus reversible forms of HH due to malnutrition or weight excess are further differential diagnoses to CHH.
- Rarely, congenital central disturbances of the HPG axis are associated with congenital primary adrenocortical insufficiency. Mutations of the *NROB1 (DAX1)* gene may be causative.
- Acquired forms of hypothalamic hypogonadism dominate the spectrum of causes in adulthood. The differentials include brain tumors, abuse of androgens or opioids.
- Infertility and undervirilization due to hypothalamic HH are successfully treatable by GnRH or gonadotropin replacement.

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13 Congenital Hypogonadotropic Hypogonadism of Pituitary Origin and Rare Syndromes with Central Hypogonadism

Julia Rohayem, Carl-Joachim Partsch, and Eberhard Nieschlag

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Abstract

Hypogonadotropic hypogonadism (HH) can be of hypothalamic or pituitary origin. In hypogonadism caused by the pituitary gland, pulsatile LH and FSH release from gonadotropic cells of the anterior pituitary is quantitively or qualitatively altered, despite uncompromised hypothalamic GnRH stimulation.

Congenital forms of pituitary-derived hypogonadism result either from malformations of the anterior pituitary or from aplasia of the pituitary stalk. These entities are generally associated with impairments of other pituitary-endocrine axes, such as the somatotropic, thyrotropic, and corticotropic axis. By contrast, the lactotropic axis is rarely involved in congenital multiple pituitary hormone deficiencies.

Peripartum pituitary trauma is a rare cause of early acquired hypopituitarism with congenital multiple pituitary insufficiencies.

X-linked adrenal hypoplasia congenita (AHC) is caused by a deletion or mutation in the *NROB1(=DAX1)* gene. It entrains adrenocortical insufficiency, combined with dysfunction at all levels of the hypothalamic-pituitary gonadal (HPG) axis.

Syndromal disorders arising from chromosomal anomalies or disease-causing gene mutations may also involve hypogonadism. There may either be an impairment of the central HPG axis or primary gonadal dysfunction, or a combination of both.

In Prader-Willi(-Labhart) syndrome, both central and gonadal HPG axis functions are altered.

In CHARGE syndrome, hypogonadotropic hypogonadism is of hypothalamic origin.

In Bardet-Biedl syndrome, hypogonadism is most likely of combined central and gonadal cause.

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In Gordon-Holmes syndrome, Boucher-Neuhauser syndrome, and Oliver-Mc-Farlane syndrome, hypogonadotropic hypogonadism of pituitary origin is typical, in addition to cerebellar ataxia.

13.1 Introduction

Hypogonadotropic hypogonadism (HH) of pituitary origin arises if the pulsatile release of LH (and/or FSH) from gonadotropic cells of the anterior pituitary is **quantitatively inadequate,** despite uncompromised hypothalamic GnRH stimulation. Further, pituitary-derived hypogonadism occurs if the secretion of gonadotropins is **qualitatively altered**, resulting in LH (and/or FSH) with **decreased bioactivity**.

Gonadotropin deficiency develops if more than 75% of the gonadotropic pituitary tissue is absent or functionally impaired.

The cause of pituitary-derived hypogonadotropic hypogonadism may be either **congenital or acquired**.

The **acquired forms** are described in Chap. 15; they are the most common cause of central hypogonadism **in adults**.

In children, the origin of hypopituitarism involving the gonadotropic axis is **acquired in only 30%**; in these subjects, oncologic disease and/or its treatment are mostly causative.

The dominating **congenital** forms of HH **in children** are caused by pituitary malformations and therefore entrain **multiple pituitary hormone deficiencies (MPHD)**.

Rarely, congenital disorders of the central gonadotropic axis are additionally associated with **impaired gonadal and/or adrenocortical function**.

In **rare syndromes with** concomitant **hypogonadism**, central or combined central and primary gonadal dysfunction may be present.

All these entities are presented in the following chapter.

13.2 Congenital Hypogonadism of Pituitary Origin

13.2.1 Pathophysiology

Congenital forms of HH of pituitary origin are generally associated with impairments of other pituitary-endocrine axes, giving rise to **multiple pituitary hormone deficiencies (MPHD)**. While pituitary developmental disorders, such as hypoplasia or aplasia, are the origin of MPHD in around 70%, dysgenesis of the pituitary stalk is causative in 20%, and perinatal pituitary injury in 10% (Wang et al. 2019).

Clarification of the **genetic cause** of congenital MPHD is successful in only 10–20%, even if next-generation sequencing (NGS) techniques are used. To date, disease-causing mutations have been identified in genes encoding transcription factors involved in pituitary development: these include *PROP1, PIT1 (POU1F1), LHX3, LHX4, GLI2, FGF8, KAL4 = PROKR2*, and *HESX1* (Pfaffle and Klammt 2011; Giordano 2016).

The *PROP-1 gene* encodes the transcription factor *PROP-1* (acronym for "prophet of Pit"), that is required for the expression of *PIT1(=POU1F1)*. *Pit1* is important for the development of gonadotropic, as well as somatotropic, thyrotropic, and variably also lactotropic and corticotropic cells of the anterior pituitary gland (Wu et al. 1998).

Table 13.1 shows transcription factors important for human pituitary development and the respective potentially associated phenotype in case of a pathogenic mutation in the corresponding gene.

Haploinsufficiency: dominant gene action, in which a single copy of the wild-type allele at a locus in heterozygous combination with a variant allele is insufficient to produce the wild-type phenotype.

13.2.2 Clinic and Treatment

The hypothalamic-pituitary-endocrine axes are differentially susceptible to impairment: The **somatotropic axis** is the most sensitive; however, during the first year of life, linear growth is growth hormone-independent. Therefore, growth hormone deficiency due to dysregulated pituitary development causes growth failure only after 1 year of age.

TSH deficiency entrains reduced serum thyroid hormone concentrations due to decreased TSH stimulation of the pituitary gland. **Thyrotropic axis** dysfunction often becomes evident after the initiation of growth hormone therapy. L-thyroxine treatment is then indicated.

During adolescence, failure of the **gonadotropic axis (HPG axis)** becomes obvious by absent spontaneous puberty, accompanied by prepubescent LH/FSH and testosterone serum concentrations. Gonadotropin replacement can be initiated if both puberty and fertility induction are desired. Alternatively, virilization may be induced by testosterone substitution. However, with the latter option, the testes will remain immature (see Chap. 38).

The **corticotropic axis** is particularly robust, so that ACTH deficiency either does not develop at all or that it appears relatively late, in association with congenital combined pituitary deficiencies (Rohayem et al. 2016). If adrenal cortisol production drops below the normal range (which can be evidenced by measurement of 24-h urinary cortisol secretion), lifelong replacement with hydrocortisone, divided into 2–3 single doses daily, is indicated. There is a need for stress dosing (i.e., to increase hydrocortisone single doses to 2–5 times the amount of the usual dose) in the case of fever, surgery or accidents.

Table 13.1 Transcription factors of pituitary development and phenotypic characteristics as a consequence of pathogenic mutations in the respective genes (modified after Xatzipsalti et al. Hormone Metab Res Xatzipsalti et al. 2019)

Transcription factor of pituitary development	Inheritance	Hypothalamic-pituitary axis impairment	Potential phenotypic characteristics
PROP1	AR	GH, TSH, PRL, LH, FSH, variable ACTH	Hypoplasia of the pituitary gland (potentially after transient pituitary hyperplasia)
Pit1/POU1F1	AD/AR	GH, TSH, PRL	Hypoplasia of the pituitary gland
LHX3	AR	GH, TSH, FSH/LH, ACTH (53%)	Pituitary hypoplasia or hyperplasia; abnormal head and neck rotation; spinal anomalies; hearing loss
LHX4	AD	GH or MPHD	PSIS: triad of aplasia or hypoplasia of the pituitary stalk, hypoplasia of the anterior pituitary gland, ectopic posterior pituitary lobe; corpus callosum hypoplasia or Chiari syndrome; respiratory distress
Gli2	haploinsufficiency	MPHD	Holoprosencephaly, midline defect, ectopic pituitary lobe, polydactyly
HESX1	AD/AR	GH o. MPHD	Septo-optic dysplasia (SOD) PSIS: triad of aplasia or hypoplasia of the pituitary stalk, hypoplasia of the anterior pituitary gland, ectopic posterior pituitary lobe; hypoplasia of the corpus callosum
SOX2	AR	LH, FSH, variable GH	Microphthalmia, mental retardation, sensorineural hearing loss, esophageal atresia
SOX3	X-chromosomal	GH or MPHD	PSIS: triad of aplasia or hypoplasia of the pituitary stalk, hypoplasia of the anterior pituitary gland, ectopic posterior pituitary lobe; mental retardation
OTX2	AD	GH or MPHD	Ocular malformations; PSIS: triad of aplasia or hypoplasia of the pituitary stalk, hypoplasia of the anterior pituitary gland, ectopic posterior pituitary lobe; Chiari syndrome
PAX6	AR	GH, FSH,LH, variable ACTH	Pituitary hypoplasia
BMP4	AR	MPHD	Ocular malformations, pituitary anomalies, partial corpus callosum agenesis, cerebellar anomalies, sensorineural hearing loss, developmental delay, spondyloepiphyseal dysplasia tarda
ARNT2	AR	MPHD central diabetes insipidus	Hypoplastic anterior pituitary and thin pituitary stalk, ocular anomalies, microcephaly, renal anomalies

Table 13.1 (continued)

Transcription factor of pituitary development	Inheritance	Hypothalamic-pituitary axis impairment	Potential phenotypic characteristics
FGF8	AD/AR	LH, FSH, TSH, LH, FSH, ACTH, central diabetes insipidus	Semilobar holoprosencephaly, corpus callosum agenesis, hypoplastic optic nerves, Moebius Syndrome
FGFR1	AD	MPHD	Septo-optic dysplasia (SOD) corpus callosum agenesis, other midline defects
SHH	AR	MPHD, central diabetes insipidus	Midline (brain) defects, holoprosencephaly
PROKR2	AR/AD	FSH, LH	PSIS: triad of aplasia or hypoplasia of the pituitary stalk, hypoplasia of the anterior pituitary gland, ectopic posterior pituitary lobe; corpus callosum hypoplasia, anomalies of the optic nerve
CHD7	AD	GH, TSH, FSH, LH	CHARGE syndrome ectopic posterior pituitary lobe
GPR161	AR	GH	PSIS: triad of aplasia or hypoplasia of the pituitary stalk, hypoplasia of the anterior pituitary gland, ectopic posterior pituitary lobe; congenital alopecia
IGSF1	X-chromosomal	GH, TSH, PRL	Macroorchia, delayed adrenarche
Pix2	AD/AR		Axenfeld-Rieger syndrome
NFKB2	AD	GH, TSH, ACTH	Immunodeficiency

AR, autosomal recessive; *AD,* autosomal dominant; *MPHD,* multiple pituitary hormone deficiencies; *PSIS,* pituitary stalk interruption syndrome; *GH,* growth hormone

The **lactotropic axis** may also fail, causing undetectable concentrations of prolactin in serum. However, this does not cause obvious symptoms in boys or men. Therefore, prolactin substitution is not considered necessary.

13.3 X-Linked Adrenal Hypoplasia Congenita (AHC)

13.3.1 Pathophysiology of AHC

X-linked adrenal hypoplasia congenita (AHC) is a rare congenital disorder of adrenocortical function, associated with central and gonadal impairment of HPG axis function.

Clinically, AHC manifests as adrenal insufficiency with hypogonadotropic hypogonadism.

AHC has an estimated incidence of 1:12,500 live births (Laverty et al. 1973).

The cause of AHC are **hemizygous pathogenic *NROB1 (=DAX1)* variants** on the short arm of the X chromosome on Xp21.3-21.2 (Muscatelli et al. 1994; Habiby et al. 1996; Achermann et al. 2001; Jadhav et al. 2011).

The disease may also develop as a consequence of a **microdeletion syndrome (contiguous gene deletion syndrome)**, in which several adjacent genes near Xp21 (including *NROB1*) are missing (Budarf and Emanuel 1997). In these cases, AHC is present in combination with **Duchenne's muscular dystrophy** (due to a deletion of *DMD*) and **glycerol kinase deficiency** (due to a deletion of *GK*). **Developmental delay** is part of the deletion syndrome, if the deletion includes *DMD* or both *IL1RAPL1* and *DMD*.

***DAX1 (NROB1)* mutations** give rise to **adrenal hypoplasia with inadequate steroid biosynthesis**, in addition to **impaired hypothalamic GnRH secretion** (Partsch and Sippell 1989). The GnRH secretion deficit is combined with **reduced pituitary GnRH sensitivity** (Habiby et al. 1996) and **gonadal dysgenesis** (Caron et al. 1999). The importance of the *DAX1* gene for testicular development and function was proven by animal studies (Yu et al. 1998).

13.3.2 Clinic and Treatment of AHC

Sixty percent of newborn boys affected by a *NROB1 (DAX1)* mutation experience a life-threatening **adrenal crisis,** with

salt wasting within the first 2 months of life. As a result of glucocorticoid and mineralocorticoid deficiency, nausea and vomiting occur, leading to dehydration; this causes arterial hypotension that may progress to circulatory shock. Metabolic acidosis, hyponatremia, hyperkalemia, and hypoglycemia characterize the biochemical constellation of this crisis.

There is marked **intrafamily variability** in the clinical symptoms of AHC (Merke et al. 1999). Around 40% of males with residual endogenous cortisol or mineralocorticoid secretion experience **subclinical adrenal insufficiency** that may become symptomatic later in life, either at 1–9 years of age and rarely in young adulthood (Mantovani et al. 2002; Ozisik et al. 2003; Guclu et al. 2010; Kyriakakis et al. 2017). Before the crisis becomes manifest, **hyperpigmentation of the skin** and **mucous membranes** can be observed in these boys or men, as a warning symptom that the central corticotropic axis is maximally activated to compensate for the deficit in cortisol via increased ACTH stimulation of the adrenal gland. Pigmentation occurs, as cleavage of the precursor molecule of ACTH, **proopiomelanocortin** also creates MSH, which in turn induces melanin incorporation into the skin.

Rarely, if milder missense mutations within the *NROB1* gene are present, **spontaneous pubertal development** may occur. **Spermatogenesis** is possible, although at a reduced level, resulting in oligozoospermia in semen. This may enable spontaneous paternity (Raffin-Sanson et al. 2013; Vargas et al. 2020). However, in these men there appears to be a steady decline in spermatogenesis over time (Tabarin et al. 2000; Raffin-Sanson et al. 2013).

In the majority of males affected by pathogenic *DAX1* mutations, **delayed puberty** manifests during adolescence. Puberty either remains completely absent or arrests at an early pubertal stage (Tanner stage 3).

Interestingly, there have also been descriptions of **gonadotropin-independent precocious puberty** in boys with both an isolated X-linked AHC or in those with a *NROB1(DAX1)* deletion as part of a microdeletion syndrome (Domenice et al. 2001; Loke et al. 2009; Nagel et al. 2019). It is speculated that the chronic ACTH stimulus on Leydig cells causes premature testosterone secretion by the testes. Alternatively, an intrinsic gonadotropin- and ACTH-independent activation of steroidogenesis in the testes could cause **pseudo-precocious puberty**.

Treatment of boys with AHC consists of **lifelong gluco- and mineralocorticoid substitution**, as well as **replacement of testosterone from puberty onwards**. Central hormone replacement is indicated, if **induction of testicular maturation and an attempt to achieve fertility** are desired. However, pulsatile administration of GnRH does not result in adequate LH and FSH secretion, due to the **pituitary defect** in AHC (Habiby et al. 1996; Caron et al. 1999). Therefore, treatment with **hCG** (as an LH substitute) and **rFSH** should be preferred in the attempt to stimulate testosterone production in the **dysgenic testes**. Adult testosterone serum concentrations are achieved in the majority of affected individuals by hCG stimulation. However, **no sperm** will be detectable **in semen** in most of the men, even after several years of combined gonadotropin stimulation (Seminara et al. 1999; Mantovani et al. 2006). Testicular histology regularly shows **Sertoli cell-only syndrome (SCO)**. Nevertheless, isolated **foci with spermatogenesis** may be present in testicular tissue, allowing for surgical testicular sperm extraction (TESE) in selected cases. Combined with intraplasmic sperm injection (ICSI), this has already been successful in inducing pregnancy in the partner of men affected by AHC (Frapsauce et al. 2011).

If a pathogenic *NR0B1(DAX1)* variant is recognized in one member of a family, it is useful to **clarify the genetic status of high-risk asymptomatic male relatives** by molecular genetic testing for the presence of a hemizygous mutation, in order to anticipate the manifestation of adrenal insufficiency.

13.4 Rare Syndromes with Central Hypogonadism

13.4.1 Prader-Willi (-Labhart) Syndrome (PWS)

13.4.1.1 Pathophysiology and Epidemiology of PWS

PWS is caused by reduced expression of genes inherited on the paternal chromosome 15q-11-q13 (Butler et al. 2015). Three subtypes of genetic origin are distinguished:

- A paternal 15q11-q13 deletion (in 70%)
- A maternal uniparental isodisomy 15, i.e., expression of both maternal chromosomes 15 (in 20–30%)
- Defects in the "imprinting center" (in 1–3%) (Ohta et al. 1999; Angulo et al. 2015)

The following genes expressed exclusively on the paternal chromosome and absent in PWS, may be involved in the manifestation of PWS: *SNURF-SNRPN, NDN, MAGEL2, MKRN3, C15orf2*, and *HBII* snoRNA cluster. Animal models have clarified that several of these genes are involved in the regulation of neuronal development, hypothalamic GnRH secretion, spermatogenesis, and circadian rhythmicity (Napolitano et al. 2021).

The diagnosis of PWS is made by detection of an abnormal methylation pattern in the critical gene region 15q-11-q13 (Gillessen-Kaesbach et al. 1995). However, this method does not allow differentiation between a deletion, a uniparental disomy or an imprinting defect. To clarify the origin further, molecular genetic studies are necessary, for which additional blood samples of the parents are used.

The estimated prevalence of PWS is 1:10,000 to 1:25,000.

13.4.1.2 Clinic and Treatment of PWS

Newborns and infants with PWS display generalized **muscular weakness** with predominance of the trunk and neck muscles. Weakness in drinking is typical for the "**floppy infant**" with PWS. One-third of PWS children are hypopigmented, with **light hair and blue eyes**. **Almond-shaped palpebral fissures** and a **narrow forehead** are characteristic craniofacial features.

In boys, the **penis and scrotum are hypoplastic** at birth; **undescended testes** are present in 75–95% (Gillessen-Kaesbach et al. 1995; Crino et al. 2003; Eiholzer et al. 2006; Siemensma et al. 2012; Hirsch et al. 2015; Pacilli et al. 2018).

Delayed motor development, **moderate mental retardation** with language developmental delay, dyscalculia, attention problems, and slowness in thought are characteristic features of PWS.

Obesity develops from early childhood onwards due to an insatiable appetite and reluctance to exercise (Cassidy 1997; Eiholzer and Whitman 2004). In up to 25% of PWS patients, type 2 diabetes mellitus arises during adulthood (Partsch et al. 2000; Butler et al. 2002).

The **increased mortality** of PWS is attributed to diabetes mellitus, cardiovascular disease, and obesity-related sleep apnea.

Behavioral disturbances of various types are observed in PWS, including defiance, with temper tantrums in childhood and with feelings of insecurity and anxiety later in life.

If left untreated, **short stature** is also typical for PWS, with male patients barely reaching an adult height of 160 cm (Wollmann et al. 1998).

Boys and men with PWS are affected by a combination of **central and gonadal hypogonadism**: Symptoms of **undervirilization at birth** are followed by an increase of serum LH, FSH, but only subnormal testosterone concentrations during **mini-puberty** (Fillion et al. 2006). During **puberty**, hypogonadism with **incomplete virilization** develops. **Pubertal arrest** occurs at a mean age of 13.5 years, with **low testicular volumes** (mean: 4 ml) and **slowed growth velocity** (Prader et al. 1956; Eiholzer et al. 2006). By contrast, pubic hair develops rather early. A Tanner stage PH 3–5 is generally reached at the time of pubertal arrest (Eiholzer et al. 2006).

At puberty, there is a rise of serum LH to the lower normal range; while FSH increases to normal or elevated levels. Serum testosterone concentrations also increase subnormally.

In **adult** males with PWS, decreased serum testosterone concentrations are typical, accompanied by inadequately normal or inadequately (only slightly) elevated LH serum concentrations and inadequately normal or slightly elevated serum FSH. **Inhibin B** levels are decreased to undetectable (Siemensma et al. 2012; Hirsch et al. 2015).

This constellation reflects the combination of gonadal dysfunction in combination with partial hypothalamic-pituitary regulatory dysfunction in males with PWS. The consequence is **infertility**: to date, there are no reports of PWS men who have fathered a child (Schulze et al. 2001).

Microscopic examination of testicular biopsies obtained during orchidopexies in prepubertal PWS boys has revealed a severely reduced number or complete absence of **spermatogonia** (Vogels et al. 2008; Matsuyama et al. 2019).

Spermatogonia were also undetectable in adult males with PWS who had ectopic testes at birth (Katcher et al. 1977).

To date, there is no causal therapy for PWS (Goldstone et al. 2008).

Pubertal arrest can be treated with **testosterone substitution** (see Chap. 14). A dose of 50–100 mg testosterone enanthate intramuscularly injected every 4 weeks appears reasonable from the age of 13–14 years onwards, if there is pubertal arrest (Heksch et al. 2017). The initial dose is gradually increased, until doses of 250 mg/4 weeks are reached. From age 18 years onwards, testosterone undecanoate 1000 mg every 12 weeks can alternatively be used (see Chap. 36).

Growth hormone therapy not only normalizes final height (Lindgren and Lindberg 2008), but also leads to an increase in muscle mass with a simultaneous reduction in fat mass. It also tends to increase physical activity (Burman et al. 2001; Bridges 2014).

13.4.2 CHARGE Syndrome

CHARGE syndrome (C: coloboma of the eye, H: heart defect, A: atresia of the choans, R: retarded length growth and developmental delay, G: genital malformation, E: ear malformations) is based on an association of the name-giving disorders. Malformations of the outer and inner ear result in **deafness** and balance disorders. The associated **hypogonadotropic hypogonadism** is of hypothalamic origin.

The **prevalence** of CHARGE syndrome is estimated to be 1 in 16,000 (Issekutz et al. 2005; Janssen et al. 2012).

Heterozygous pathogenic variants in the **chromodomain helicase DNA-binding protein 7 (*CHD7*)** gene have been identified in 70–90% of individuals meeting the clinical diagnostic criteria for CHARGE syndrome, making autosomal dominant inheritance likely. However, most cases occur sporadically due to de novo mutations.

CHD7 variants identified in individuals with milder CHARGE phenotypes are missense variants rather than frameshift, nonsense variants, or deletions.

It is possible that a *CHD7* mutation results in HH with anosmia (**Kallmann syndrome**) with **only one single or no additional features** of CHARGE syndrome. Among these single features, the most common are congenital deafness, dysplastic auricles, and/or hypoplasia or aplasia of the cochlea and arcuate ducts, or clefting of lip or palate (Jongmans et al. 2009; Bergman et al. 2012; Marcos et al. 2014).

Hypogonadism is a treatable condition in all male CHARGE patients. **Gonadotropin substitution** enables normal puberty with pubertal testicular growth and activation of spermatogenesis (Rohayem et al. 2017) (see Chap. 38).

13.4.3 Bardet-Biedl Syndrome and Laurence-Moon Syndrome

Multisystemic **Bardet-Biedl syndrome (BBS)** is a rare hereditary **ciliopathy**, characterized clinically by six major defects: **truncal obesity**, **retinal degeneration** (usually in the form of rod-cone dystrophy/retinitis pigmentosa), **learning disabilities**, **renal malformations**, **poly(hexa)dactyly**, and **hypogonadism in males** (Beales et al. 1999).

Other secondary features, not always present in all BBS patients include diabetes mellitus, nephrogenic diabetes insipidus, ocular abnormalities (strabismus, cataract, astigmatism), dental abnormalities (hypodontia, enamel defects, narrow teeth, and dental roots), malformations of the fingers (brachydactyly/syndactyly), cardiovascular anomalies (left ventricular hypertrophy, congenital heart disease), developmental delay, delayed language acquisition, ataxia/coordination deficit/balance disorder, mild lower extremity spasticity, and hepatic fibrosis.

There are interfamily and intrafamily clinical variabilities among BBS patients.

While BBS is used to be equated with **Laurence-Moon syndrome (LMS)**, there is now controversy as to whether BBS and LMS are not two distinct disorders with overlapping features. Features such as polydactyly and obesity are more likely to be present in BBS; spasticity predominates in LMS.

The **prevalence of BBS** in Europe is estimated to be 1 in 125,000 to 160,000 (Beales et al. 1997), but it is significantly higher in Arabic populations and in Newfoundland (at 1 in 13,500 and 1 in 18,000, respectively), which has been attributed to the higher degree of consanguinity in these areas.

BBS is genetically heterogeneous in cause. To date, more than 21 ***BBS1-21* gene mutations** have been described on chromosomes 2, 3, 4, 7, 8, 9, 11, 12, 14, 15, 16, and 17 (Khan et al. 2016). Although the syndrome is considered to be inherited in an autosomal recessive manner, the manifestation of some forms of BBS requires an additional mutation in a second locus, which is referred to as "triallelic inheritance" or "recessive inheritance with a modifier of penetrance" (Burghes et al. 2001; Katsanis 2004).

Hypogonadism and **hypogenitalism** are almost universally present in male BBS patients. Undescended testes are present at birth in approximately 15%. The penis is often small and buried in adipose tissue (Beales et al. 1999).

In contrast to earlier views, hypogonadism in BBS is only **partially** due to **hypothalamic-pituitary dysfunction**, and **primarily due to gonadal dysfunction:** testosterone serum concentrations are within the normal range in most men, with few exceptions. LH levels are also within the normal range, and serum FSH concentrations are normal or elevated. In the GnRH test, gonadotropins can be stimulated adequately (Soliman et al. 1996). Systematic studies on semen parameters or on testicular histology are not available to date. Individual case reports contain evidence of a variably pronounced disorder of spermatogenesis. Spontaneous **paternity** of men with BBS has been reported (Beales et al. 1999).

13.4.4 Cerebellar Ataxias with Pituitary-Induced Hypogonadism

Cerebellar ataxias make up a clinically and etiologically heterogeneous group of diseases (Koeppen 1998). The heredo-ataxias, i.e., the genetically determined forms, dominate by numbers of affected subjects. Pure cerebellar ataxias manifest by cerebellar symptoms only. In addition, numerous forms of ataxia are present in conditions with additional disturbances in other organ systems. The association of cerebellar ataxia and hypogonadism represents one of these associations.

Hypogonadism may either be hypo- or hypergonadotropic. Patients with the autosomal recessive **Marinesco-Sjögren syndrome** or with **ataxia telangiectasia**, have been categorized under the term cerebellar ataxia with **hypergonadotropic hypogonadism**. This heterogeneous and poorly characterized group of patients will not be discussed in detail here.

Gordon-Holmes syndrome, **Boucher-Neuhäuser syndrome**, and **Oliver-McFarlane** syndrome represent a phenotypic cluster within a spectrum of **neurodegenerative disorders**. These syndromes may be caused by mutations in the *PNPLA6* gene; all are associated with **HH**. The *PNPLA6* gene encodes neuropathy-target-esterase (NTE), an enzyme that catalyzes the deesterification of phosphatidylcholine and that is important for membrane integrity. Damage to the *PNPLA6* protein affects a variety of neuronal systems, particularly the retina, cerebellum, motor neurons, and the neuroendocrine system.

13.4.4.1 Gordon-Holmes Syndrome

The association between cerebellar ataxia and hypogonadotropic hypogonadism was first described in a family by Gordon Holmes in 1907 (Holmes 1907). In adults with the syndrome, **cognitive decline** leads to dementia and **neurodegenerative spinocerebellar degeneration** involves ataxia, nystagmus, and **HH** (De Michele et al. 1993; Margolin et al. 2013). Pulsatile administration of GnRH is not successful in inducing adequate LH and FSH responses. Therefore, central hypogonadism seems most likely to be of pituitary origin (Quinton et al. 1999; Seminara et al. 2002).

In addition to mutations in the *PNPLA6* gene, disease-causing mutations have been identified in the following other genes in Gordon-Holmes syndrome: *RNF216, OTUD4,* and

STUB1 (Alqwaifly and Bohlega 2016). Some of these genes are involved in the regulation of autophagy of cellular cytosolic components, a process important for cellular homeostasis.

13.4.4.2 Boucher-Neuhauser Syndrome

Boucher-Neuhauser syndrome was first described in 1969 as a combination of slowly **progressive cerebellar degeneration** with spinocerebellar ataxia, **chorioretinal degeneration/dystrophy**, and **hypogonadotropic hypogonadism** in individual families (Boucher and Gibberd 1969). It was later shown to be inherited in an autosomal recessive way (Rump et al. 1997). Hypogonadism in Boucher-Neuhauser syndrome is characterized by absent or incomplete pubertal development with low levels of LH, FSH, and testosterone. Even if pretreated with GnRH, the increase in gonadotropins remains subnormal after injection of a GnRH bolus. Therefore, pituitary dysfunction seems probable.

The syndrome has been associated with autosomal recessive mutations in the *PNPLA6* gene (Synofzik et al. 2014; Tarnutzer et al. 2015). The majority of mutations most likely inhibit the catalytic activity of *PNPLA6*, which provides the precursor for the biosynthesis of the neurotransmitter acetylcholine.

13.4.4.3 Oliver-McFarlane Syndrome

This syndrome includes growth failure, retinal degeneration, trichomegaly of the eyelashes and thin hair, in association with **HH** (Sampson et al. 1989).

Key Points

- Congenital multiple pituitary deficiencies due to pituitary developmental defects represent the most common cause of pituitary-derived hypogonadism. All endocrine deficiencies are treatable by hormone replacement, except prolactin deficiency.
- X-linked adrenal hypoplasia congenita (AHC) is associated with hypogonadism of hypothalamic, pituitary, and gonadal origin. Therefore, hormonal replacement strategies are rarely successful regarding induction of fertility.
- While CHARGE syndrome is associated with hypogonadism of isolated hypothalamic origin, which is easily treatable, Prader-Willi (-Lenhard) syndrome and Bardet-Biedl syndrome present a more complex disturbance at different levels of the HPG, which significantly limits fertility. While in PWS, paternity has not been reported, in BBS occasional paternity seems possible.
- Neurodegenerative syndromes associated with cerebellar ataxia and hypogonadism of pituitary origin may share a common genetic basis.

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Delayed Puberty in Boys

14

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Abstract

"Puberty" refers to the stage in human life during which maturation from boy to man (or girl to woman) occurs. The primary sexual characteristics of the hitherto infantile organism undergo maturation into the secondary sexual characteristics.

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The initiation of puberty is the result of complex regulatory processes in the neuronal network of the hypothalamus. Pulses of GnRH, increasingly secreted by hypothalamic neurons, induce secretion of gonadotropins in the anterior pituitary. The latter stimulate sex steroid hormone secretion by the gonads. Testicular maturation results in both virilization and production of sperm that are capable of fertilizing eggs. Parallel to this, sex drive awakens. The final stage of pubertal maturation is characterized by sexual maturity and fertility.

The onset and progression of puberty in boys are divided into five stages according to Tanner; testicular volumes are estimated by Prader orchidometry.

"Delayed puberty" is present if pubertal enlargement of the testes to a volume ≥4 ml each side has not yet occurred by the age of 14 years, if the passage through puberty takes more than 5.5 years, or if pubertal development has remained arrested for more than 18 months.

Among all boys with delayed puberty, 70% are "late developers" with constitutional developmental delay (CDGD = constitutional delay of growth and puberty).

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In 18%, the maturational delay is "functional," that is, temporary in nature, provided that the underlying problem is removed or successfully treated.

In about 12%, a permanent disorder is present: 7% of these boys with delayed puberty have a permanent hypogonadotropic, 5% a permanent hypergonadotropic disorder.

The therapeutic approach is based on whether maturational delay is transient or permanent. This differential diagnosis is difficult and requires extensive clinical exploration.

14.1 Introduction

14.1.1 Definition of Terms

"Puberty" refers to the developmental stage of life in humans in which boys mature to men, and girls mature to women. By this process, reproductive capacity is achieved.

The onset of puberty in both sexes has been defined by Marshall and Tanner. They also divided the progression of puberty into five stages (Marshall and Tanner 1970). Criteria for assignment to these "**Tanner stages" of puberty** in boys are penile size (G1–5) and presence and amount of pubic (PH1–5) and axillary hair (A1–2), with stage 1 denoting the infantile stage and stage 5 the mature adult stage (see Fig. 14.1).

Testicular volumes are estimated by **Prader orchidometry** (see Fig. 14.2). By this means, it is possible to clarify whether central puberty has begun and whether pubertal testicular maturation is progressing normally (Prader 1966; Largo and Prader 1983; Willers et al. 1996).

"**Delayed puberty**"in both sexes is defined by the absence of pubertal development at an age that is 2–2.5 standard deviations later than the population mean (Howard and Dunkel 2018).

The **mean age of pubertal onset in boys in central Europe is 11.5–12 years** (Largo and Prader 1983; Juul et al. 2006; Lawaetz et al. 2015). The **onset of male puberty** is defined by **testicular enlargement to a volume of ≥4 ml each (and a Tanner stage G2).** Therefore, the following definition of "delayed puberty" in boys emerges for clinical practice:

"Delayed puberty" is present if pubertal enlargement of the testes to a volume ≥4 ml has not yet occurred by the age of 14 years, if the passage through puberty takes more than 5.5 years, or if pubertal development has remained arrested for more than 18 months.

14.1.2 Epidemiology

By definition, delayed puberty is present in **3 (97th percentile)–10% (90th percentile) of all boys**.

Among these boys, **70% are "late bloomers,**" who experience a delayed pubertal onset, but then spontaneously undergo puberty. This variant is termed **"constitutional delay of growth and puberty" (CDGP**). In **50–75%** of boys with CDGP, a **positive family history** for late pubertal development (late maternal menarche or late paternal voice change) can be elicited (Sedlmeyer et al. 2002).

In **18%** of all boys with uninitiated puberty, maturational delay is "**functional,"** meaning that it is **transient in nature, if the underlying problem is resolved**.

In **12%** of boys with delayed puberty, a **permanent disorder** is present; among these,

7% have **hypogonadotropic hypogonadism (HH),** that is central dysfunction at the hypothalamic or pituitary level and **5%** have **hypergonadotropic** testicular failure (Sedlmeyer et al. 2002).

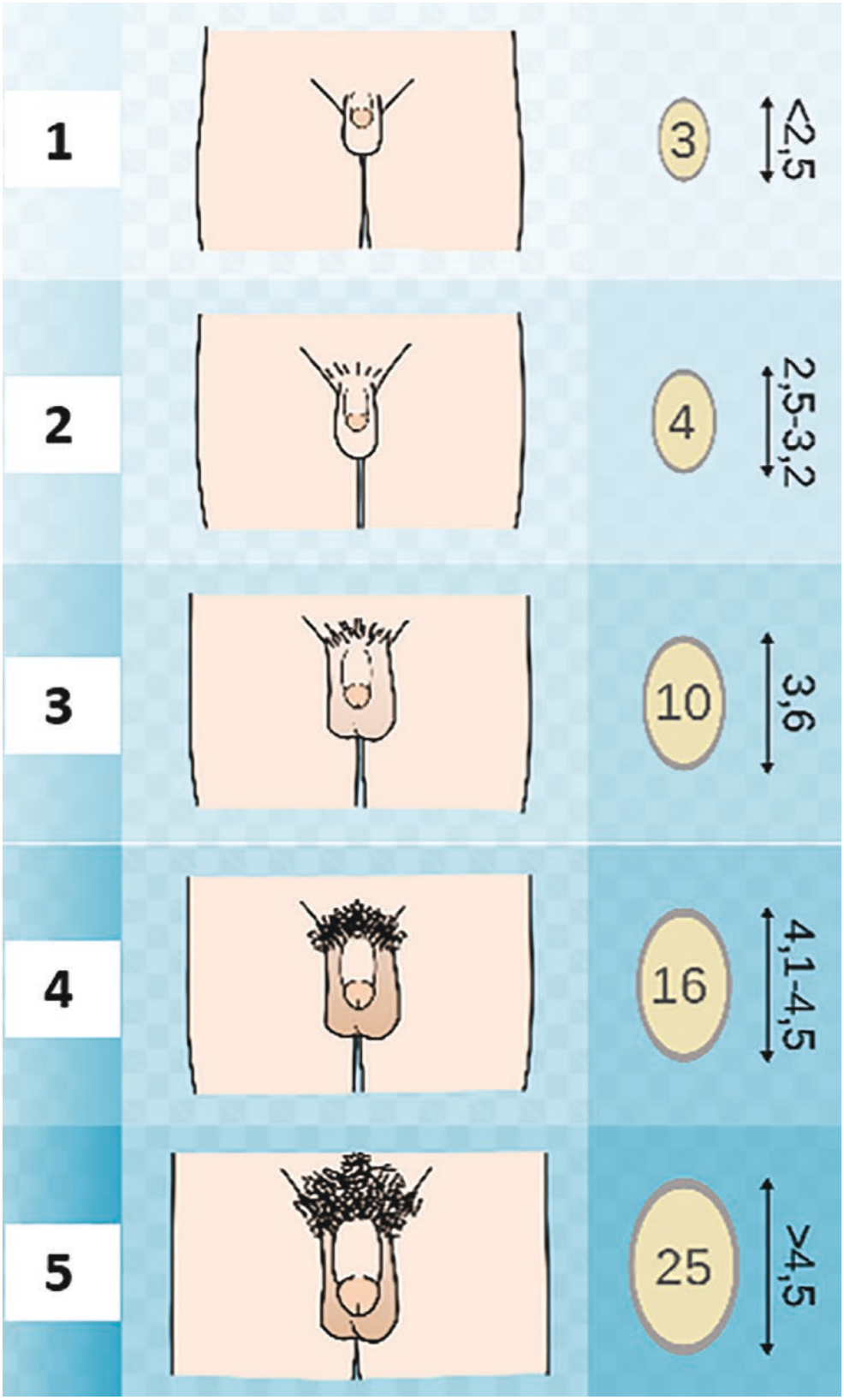


Fig. 14.1 Tanner stages of puberty in boys (http://www.wikiwand.com/de/Tanner-Stadien)

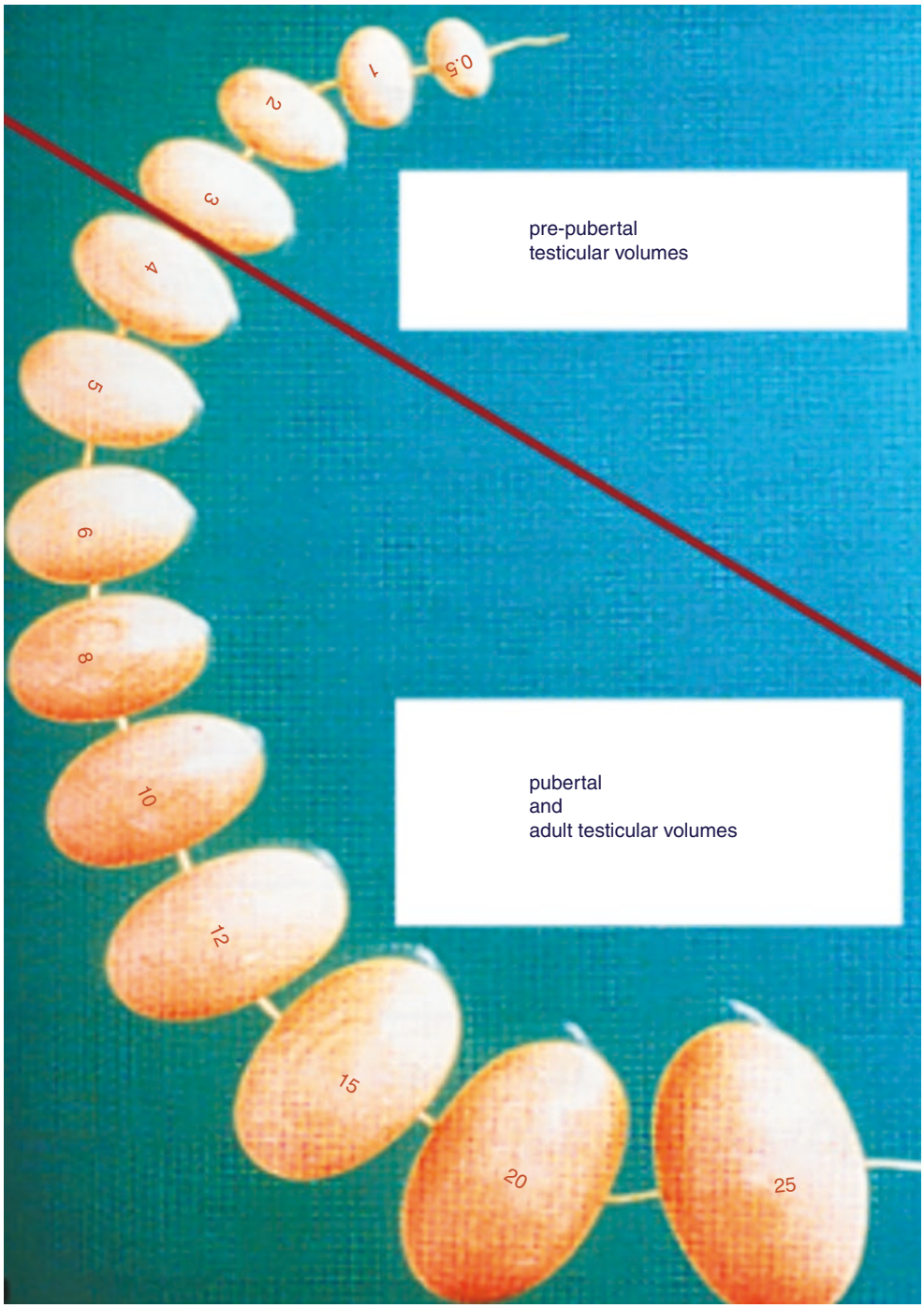


Fig. 14.2 Prader orchidometer

14.2 Physiology and Pathophysiology of Puberty

14.2.1 Physiology of Puberty

The initiation of puberty is the result of complex processes in the hypothalamic neuronal network. At pubertal onset, the inhibitory brake on **hypothalamic GnRH neurons** is removed, giving rise to an increase in **GnRH pulses.** These are secreted into the portal venous bed. Consequently, increasing **gonadotropin pulses** are released from the pituitary, thus increasing **gonadal sex hormone** production and its secretion into the peripheral blood. The primary sexual characteristics of the organism, which until then had been infantile, mature into the secondary sexual characteristics: In boys, there is growth of the penis and the testicles and growth of pubic hair. The longitudinal growth spurt, voice change, and beard growth are also induced by sex steroid hormones. At the same time, the sex drive awakens. The maturation of

the testicles, also known as **gonadarche**, leads to the formation of **sperm capable of fertilization**. The final state of pubertal maturation is popularly known as **"sexual maturity"** or **"capacity to reproduce."**

The maximum of the longitudinal growth spurt occurs around mid-puberty. It ends with the estrogen-induced closure of the epiphyseal joints of the long tubular bones, thereby defining final adult height.

Of note, **emotional, cognitive, and social maturational processes** in the brain, that also occur during adolescence, depend only in part on gonadal sex steroid hormone production (Sisk and Zehr 2005).

Growth of **pubic and axillary hair**, stimulation of the **sebaceous glands** in the hair follicles, **sweat** and **body odor** development for the most part result from **adrenal** androgen production, a process termed "**adrenarche"**. Adrenarche is regulated **independently of gonadotropins**. It precedes central puberty, with a median age at onset of 8 years (Sklar et al. 1980; Wierman et al. 1986).

14.2.1.1 Hormonal Regulation of the Hypothalamic-Pituitary-Gonadal (HPG) Axis During Puberty

Puberty is initiated by a pulsatile release of **GnRH** from neurons of the **hypothalamus** into the portal venous blood. GnRH stimulates LH and FSH secretion from the **gonadotropic cells of the anterior pituitary**. As early as 2–3 years before the first clinical signs of puberty, there is an increase in the **frequency** of **nocturnal LH and FSH pulses**. As puberty progresses, the **amplitude and frequency of nocturnal** gonadotropin **pulses increase**; eventually, LH and FSH pulses also occur regularly **during daytime,** without circadian rhythmicity. The late pubertal and postpubertal stages are characterized by stable adult LH and FSH concentrations in the peripheral blood (Wennink et al. 1989; Wu et al. 1991).

LH stimulates testicular Leydig cells to produce testosterone; the **rise in serum testosterone levels from infantile to adult levels takes an average of 6 years**. It begins at the mean age of about 12 years, increases exponentially at first, and reaches an individual **maximum at about 15 nmo/l (SD: ± 5 nmo/l) at a mean age of 18–19 years** (Knorr et al. 1974). In healthy males, testosterone serum concentrations then **remain at** a slightly lower level **around 12 nmol/**l (SD: ± 4 nmo/l) **for the rest of life** (Kelsey et al. 2014) (see Chaps. 7 and 36).

Circadian rhythmicity of serum testosterone concentrations is present from late puberty onwards, with a **morning peak,** followed by a **slight decrease during the day** (De Lacerda et al. 1973; Simoni et al. 1992) (see Chap. 7).

FSH—together with testosterone—stimulates **Sertoli and germ cells**. Consequently, as puberty progresses, mitotic divisions and meiotic maturational divisions of testicular stem cells (spermatogonia) are gradually initiated in the seminiferous tubules of the testes, resulting in **mature spermatozoa (=sperm capable of fertilization)**. A few years after reaching pubertal Tanner stage 5, sperm concentrations in the ejaculate reach an individual maximum.

The regulatory circuit of the hypothalamic-pituitary-gonadal (HPG) axis is subject to negative feedback via the hormones **testosterone, estradiol, DHT, activin,** and **inhibin B** (see Chap. 2).

14.2.2 Pathophysiology of Puberty

The origin of pubertal delay is reflected by abnormal hormone concentrations in the peripheral blood: if the cause of pubertal impairment is at the central level of the HPG axis, inadequately low LH and FSH concentrations for age are typical. If the origin is at the gonadal level, gonadotropins will rise above the normal range. In both situations, serum testosterone concentrations are inadequately low.

Hypogonadotropic disorders have to be distinguished from constitutionally delayed puberty (CDGP), that is genetically determined maturational delay, with a self-limiting course (Sedlmeyer and Palmert 2002; Wehkalampi et al. 2008).

The differential diagnosis of delayed puberty in boys is shown in Fig. 14.3.

Functional hypogonadism (FH) is an important differential diagnosis of CDGP. In FH, puberty is delayed due to **persistent inhibition** of hypothalamic GnRH secretion. **Malnutrition** (due to chronic disease, eating disorders, or due to malabsorption) and **athletic overtraining with or without doping** are the most common causes of FH. Functional hypogonadism may also be induced by **chronic renal insufficiency** (Haffner and Zivicnjak 2017).

Hypothalamic HH is characterized by permanently impaired GnRH secretion or deficient GnRH action. Regarding GnRH secretion, **congenital or acquired causes** have to be distinguished; by contrast, the origin of GnRH resistance is genetically determined, thus always congenital (see Chap. 12).

Pituitary-derived HH is marked by quantitatively or qualitatively impaired gonadotropin secretion in the anterior pituitary. The origin of this disturbance can be either **congenital or acquired** (see Chaps. 13 and 15).

Primary hypergonadotropic hypogonadism is caused by dysfunction at the gonadal level. LH secretion is upregulated during the course of puberty to compensate for testicular endocrine insufficiency. Endocrine impairment of testicular function is accompanied by spermatogenetic testicular dysfunction. The latter may be reflected by a rise of serum FSH concentrations above the upper limit of the normal adult range.

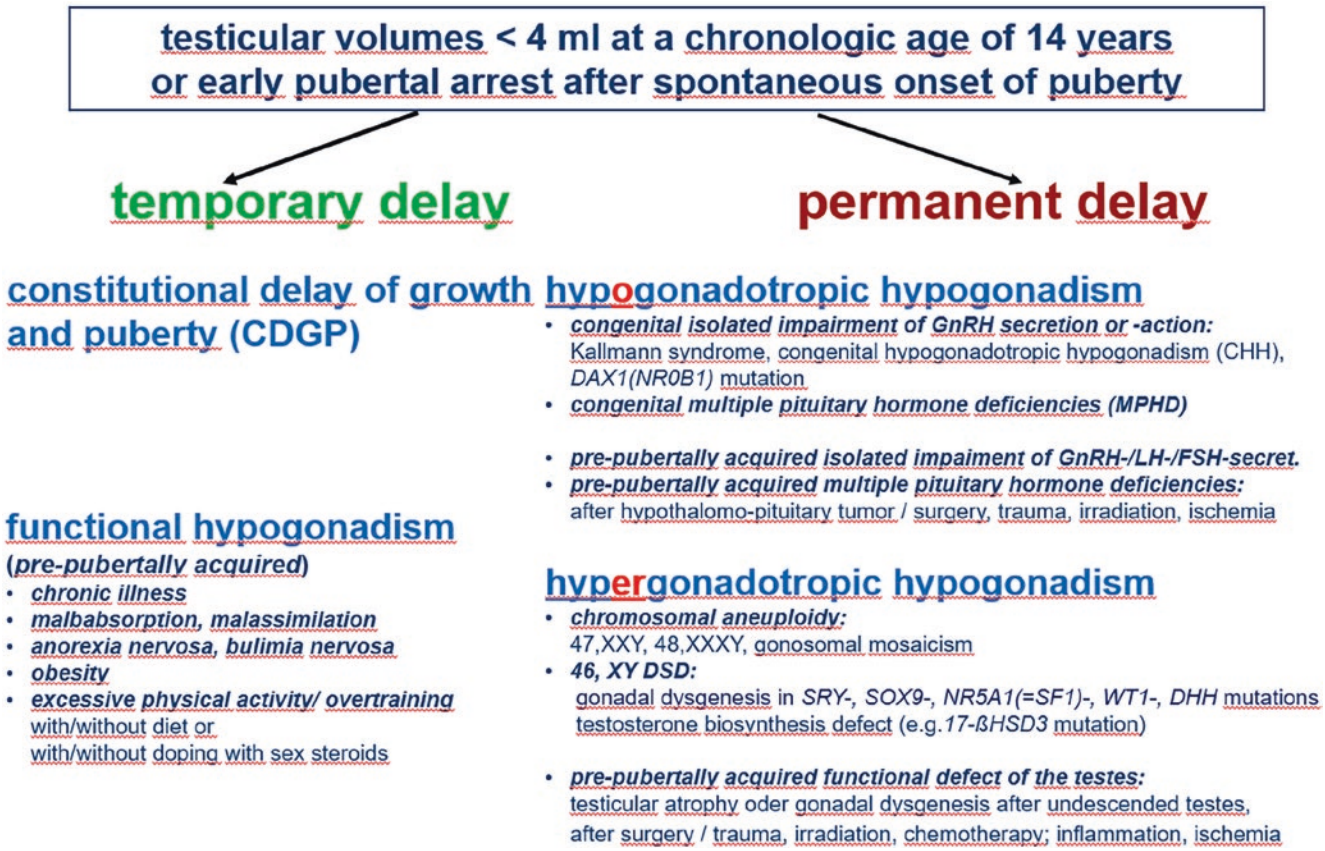


Fig. 14.3 Differential diagnosis of delayed puberty in boys

While **absence** of pubertal development will ensue for the most part if **hypothalamic** dysfunction is present, **testicular causes of hypogonadism** are in general characterized by **pubertal arrest**, occurring after spontaneous initiation of puberty. In some cases, where testicular function is less severely impaired, **normal pubertal maturation** may take place. However, **decompensation of endocrine function** will then occur at a variable age **during adulthood**.

The origins of **pubertal arrest with hypergonadotropic hypogonadism** include:

- **Gonosomal aneuploidy** (see Chap. 21)
- **Testicular atrophy** (with or without residual endocrine function), mostly after surgery on **undescended testes** (see Chap. 38)
- Less commonly, other causes such as
- Testicular **trauma or ischemia after testicular torsion** or
- **Orchitis** can be elicited
- Even more rarely,
- **Congenital gonadal dysgenesis** on the basis of **variant sexual differentiation (DSD)** is causative (see Chap. 31).
- Rarely, primary hypogonadism leads to **complete absence of puberty**. This is the case in
- **Congenital anorchia (so-called "vanishing testes")** or
- **Acquired anorchia after bilateral orchiectomy**. In these males, there is an excessive pubertal increase in serum gonadotropin levels (with much higher FSH than LH serum concentrations), without an accompanying rise in serum testosterone.

14.3 Clinic

The adolescent with **permanently absent and untreated puberty** shows the following clinical abnormalities: While peers are already virilized,

- The penis remains of childlike size.
- There is no or insufficient growth of both testicles.
- The voice continues to be infantile/not mutated.
- Musculature remains poorly developed, while subcutaneous fat tends to accumulate around the belly and hips.

- No male body hair develops.
- Beard growth remains absent.
- The pubertal growth spurt is not initiated, however longitudinal growth continues, eventually giving rise to eunuchoid tall stature, with long arms and legs in relation to the trunk. This disproportion is the consequence of delayed closure of the epiphyseal joints of all long tubular bones.
- Libido does not awaken; spontaneous morning erections remain rare.
- Spermatogenesis is not initiated.
- Ejaculations of semen do not occur.
- Anemia develops in the medium term.
- Bone mineralization is impaired (Gilsanz et al. 2011).

The occurrence of **pubic and axillary hair, sebum production, and sweat and body odor** development result from **adrenarche**. These changes take place, **even if central puberty is not initiated**. Signs of adrenarche may therefore be **misinterpreted** as central puberty.

Adolescents with **constitutional developmental delay (CDGP)** present for two possible reasons:

First, **short stature** in comparison to peers, may cause distress. However, some of these boys may have not yet reached the age of 14 years, defining "delayed puberty."

Second, and for the most part, medical consultation is initiated, because of **absent pubertal development beyond the age of 14**, with all abovementioned symptoms.

In boys with CDGP, **bone age** is always **retarded**. The lag in maturation ranges from over 1 to 5 years. If put into relation to bone age, body height and the pubertal stage of development are within the normal range in these "late bloomers" (Sedlmeyer and Palmert 2002).

In boys with CDGP, the pubertal **growth spurt** is not as marked as in subjects with an earlier onset of pubertal development (Poyrazoglu et al. 2005; Wehkalampi et al. 2007). Therefore, boys with CDGP potentially reach a **final height** slightly below their **parental target height (TH)** expectations (Butenandt et al. 2005).

Boys with CDGP may also exhibit **slight eunuchoid body disproportions,** with longer arms and legs due to the prolonged phase of prepubertal growth reduction and due to delayed closure of the epiphyseal joints of the long tubular bones (Albanese and Stanhope 1994). This disproportion remains recognizable in adulthood as evidence of formerly late pubertal development.

14.4 Diagnostics

The reliable differentiation of permanent pubertal failure (or early pubertal arrest) from temporary CDGP is a challenge for the clinician.

Answers to the following questions are helpful regarding the differential diagnosis of delayed puberty:

- Undescended testes? (clustered in congenital hypogonadotropic hypogonadism (CHH)/Kallmann syndrome)
- Micropenis at birth? (CHH/Kallmann syndrome)
- Olfactory disorder? (anosmia or hyposmia in Kallmann syndrome)
- Chronic disease, diarrhea with weight loss? (functional hypogonadism)
- Extreme physical activity? doping? opiates? (functional hypogonadism)
- Oncological pretreatment? with irradiation of testes or the hypothalamic-pituitary region, or with gonadotoxic chemotherapy, or with a conditioning therapy in the context of stem cell transplantation? (primary or combined hypogonadism)
- Pituitary disease (following traumatic brain injury / pituitary surgery / meningitis)?
- Orchitis? testicular trauma? surgery for undescended testes? (testicular disorder).
- Early childhood developmental delay? attention deficit disorder? (chromosomal anomaly)
- Prader-Willi-Labhard syndrome? CHARGE syndrome? (syndromes with central or combined hypogonadism)
- Impaired vision? (due to optic atrophy in septo-optic dysplasia (SOD) or following craniopharyngioma)
- Combined adrenal insufficiency? *(NROB1 = DAX1* mutation) (see Chap. 13)
- Positive family history for late puberty? (GDGP)

14.4.1 Clinical Examination

Anthropometry includes determination of height, weight, sitting height, and arm span. Current height and bone age are plotted on a **growth chart**.

The pubertal **Tanner stage** is documented, testicular volumes are determined by comparative palpation using a Prader orchidometer.

Physical examination explores whether signs of chronic disease, an eating disorder or physical overtraining are pres-

ent, such as a distended abdomen (possible sign of malabsorption), cachexia with lanugo hairiness, parotid hypertrophy, bradycardia (present in restrictive or bulimic eating disorders).

Phenotypic features that may be associated with CHH or Kallmann syndrome are:

- Dental anomalies (especially missing teeth)
- Finger abnormalities
- Cleft lip and/or palate
- Iris coloboma
- Synkinesia (=mirror movements)
- Ataxia

14.4.2 Laboratory Investigations

Investigations for delayed puberty include determination of the following parameters in serum:

- LH, FSH, testosterone, inhibin B, AMH, TSH, T3, fT4, prolactin, cortisol, IGF-1, IGFBP-3.
- Blood count, liver and kidney function tests, celiac antibodies.
- In case of suspected inflammatory bowel disease, investigations should be expanded accordingly.

14.4.3 Interpretation of Laboratory Findings

In delayed puberty, the growth factors **IGF-1** and **IGFBP-3** may be below the age norm, without raising suspicion of growth hormone deficiency, as the levels are adequate with respect to the prepubertal stage of maturation.

Low levels of the Sertoli cell markers **inhibin B** and **AMH** may be a consequence of failure to prenatal stimulation of testes by the fetal gonadotropins and/or failure of this stimulation during mini-puberty; these findings thus are indicators of CHH (Rohayem et al. 2015; Binder et al. 2015) (see Chap. 7).

A **low T3 syndrome** accompanied by TSH serum concentrations within the normal range and by a relatively **high-serum cortisol concentrations** may indicate malnutrition in eating disorders (or cachexia in chronic disease).

14.4.4 Diagnostic Imaging

Ultrasound investigation of the testes records the following measures:

Testicular volumes (volume (ml) = testicular length (cm) × width (cm) × depth (cm) × 0.52).

A testicular length ≥2–2.5 cm is indicative of a pubertal testicular volume (Koskenniemi et al. 2017).

Ultrasound investigation of the kidneys searches for unilateral renal agenesis (potentially associated malformation in Kallmann syndrome).

X-ray of the left hand is performed to determine **bone age** according to Greulich and Pyle (1959) and to calculate **predicted adult height (PAH)** according to Bayley and Pinneau (1952).

MRI of the hypothalamic-pituitary region explores whether there is a tumor or an "empty sella." Targeted search for aplasia or hypoplasia of the olfactory bulbs should also be made in males with anosmia or hyposmia (Kallmann syndrome).

14.4.5 Functional Tests

Various stimulation tests with GnRH, GnRH-agonists or hCG are tools available for the clinician in order to facilitate the difficult differentiation of late but spontaneous puberty (CDGP) from a permanent hypogonadotropic disorder in boys with absent pubertal development. However, results from these hormone function tests overlap in prepubertal patients with CDGP and in those with CHH, limiting their usefulness. Neither the **LHRH (=GnRH**) test, nor the **hCG test** have proven to be valid discriminators.

The performance of **serum LH overnight profiles** is cumbersome and the determination of **urinary gonadotropins in the first morning urine** is difficult to implement in outpatient clinical care.

However, beyond assessment of

- **Serum inhibin B concentrations**,
  - A **GnRH agonist test with buserelin** with determination of **stimulated LH concentrations** has proven helpful.
  - A baseline serum sample is taken between 8 and 10 am, followed by a single dose of 10 µg/kg bw buserelin s.c.. In the second stimulated blood sample drawn after 4 h, serum LH and testosterone are determined. If the LH increase does not exceed 4 U/l, this is indicative of CHH (Kaspers et al. 2004; Wilson et al. 2006; Harrington and Palmert 2012).

In case of uncertainty, the boy with uninitiated puberty may additionally be subjected to a

- **3–6-month "priming" with low-dose testosterone enanthate i.m.** at 4-weekly intervals.

- This will allow clarification whether the GnRH pulse generator can be activated by sex steroids: If **reevaluation** of testicular volumes and testosterone levels **6 months after the last testosterone injection** shows testicular growth to a pubertal level, and if serum testosterone concentrations have increased markedly, spontaneous further progression of puberty can be expected.
- **Off-label "oral priming" with testosterone undecanoate (Andriol®),** starting at 40 mg once daily may be used alternatively. Depending on chronologic and bone age, the dose may be increased to 40 mg twice daily and then to 80 mg twice daily (Lawaetz et al. 2015). Priming is to be discontinued if there is an increase in serum LH serum concentrations and/or if a pubertal increase in testicular volumes is documented. Both observations indicate an activation of the central HPG axis.

Chromosomal analysis is recommended in the presence of hypergonadotropic delayed puberty, to exclude **gonosomal aneuploidy** (47,XXY; Klinefelter syndrome, 48,XXXY or 48,XXYY; mosaic 46,XY/45,X0 with male phenotype), especially in the presence of concomitant learning difficulties due to dyslexia, attention deficits, and behavioral problems.

A molecular genetic analysis by means of **"targeted multiplex next-generation sequencing" (NGS)** can clarify the genetic origin of CHH or Kallmann syndrome in cases with hypogonadotropic delayed puberty (see Chap. 12) or in those with congenital multiple pituitary hormone deficiencies (MPHD) (see Chap. 13).

Semen analysis is only successful in adolescents who either have experienced spontaneous pubertal development or in those previously treated with testosterone.

In boys with pubertal arrest due to testicular failure, semen analysis is useful prior to hormone replacement, in order to determine the presence of viable sperm that could be cryostored for future use in assisted reproduction.

14.5 Treatment of Delayed Puberty

If puberty is not spontaneously initiated in due time, the therapeutic goal is the age-adapted normalization of serum testosterone levels. This induces maturation of secondary sexual characteristics and enables a pubertal growth spurt. Pubertal induction can be achieved by parenteral, transdermal, or oral replacement of sex steroid hormones (see Chap. 36).

In Germany, only the intramuscular application of testosterone enanthate (TE), a depot testosterone preparation in ester form, is approved by the authorities for pubertal induction before the age of 18 years.

In addition, i.m. injections of human chorionic gonadotropin **(hCG)** are approved for pubertal induction in males with HH (see Chap. 38).

In previously testosterone-naïve subjects, the use of increasing hormone doses is recommended, in order to imitate physiological puberty (Ranke and Dörr 2009; AWMF guideline Delayed puberty and hypogonadism 2011).

Testosterone enanthate (TE), is administered at a starting dose of 50 mg i.m. at 4-weekly intervals. Every 6–9(−12) months, the dose is increased by 50–100 mg until the adult replacement dose of 250 mg i.m. is reached. Thereafter, the injection interval can be changed to a 3-weekly administration (see Table 14.1).

In adolescents with pubertal arrest, higher initial hormone doses are used, depending on the degree of previous spontaneous virilization.

Testosterone gel preparations are not approved for use in adolescents below 18 years of age.

Table 14.1 Dosing regimens for pubertal induction with testosterone

Hormone preparations	Application	Dose	Approved indications
Testosterone enanthate (TE)	i.m. injection at 3–4 weekly intervals	1st–6th month: 50 mg/month i.m. 7th–12th month: 100 mg/month i.m. 13th–24th month: 150–200 mg/month i.m from 25th month onwards: 250 mg/month i.m, if necessary, reduction of the injection interval to 3 weeks	Testosterone replacement for pubertal induction or for permanent substitution in case of – hypergonadotropic hypogonadism – hypogonadotropic hypogonadism
Testosterone gel	Transdermal (off-label in adolescents <18 years) Daily application in the morning	Initial dose for pubertal induction in testosterone-naïve boys: 1st–6th month: 10 mg/day 7th–12th month: 20–25 mg/day 13th–24th month: 40 mg/day from 25th month: 50 mg/day target dose: 50 mg/day adult dose: 50 (−75) mg/day	Testosterone replacement for men aged ≥18 years with – hypergonadotropic hypogonadism – hypogonadotropic hypogonadism

Nevertheless, it is possible to use testosterone gel **off-label** for puberty induction. For recommended doses in testosterone-naïve adolescents, see Table 14.1.

The gel is applied every morning to hairless areas of the skin (forearms, upper arms, shoulders, and/or abdomen). This administration modality mimics the physiological circadian rhythm. In contrast to depot testosterone preparations, nocturnal serum testosterone concentrations do not exceed the physiological range, making this transdermal treatment modality optimal for replacement.

Oral testosterone undecanoate may also be used **off-label** for pubertal induction at a dose of 40–160 mg/d p.o. (Schmidt et al. 1998; Lawaetz et al. 2015). However, due to the variable bioavailability of the preparation, this treatment modality has not been implemented in the AWMF guideline recommendations in Germany (AWMF Guidelines Delayed Puberty and Hypogonadism 2011).

After reaching late pubertal maturity, the depot preparation **testosterone undecanoate**, which is injected i.m. every 12 weeks, may be used for permanent testosterone substitution.

Until recently, testosterone replacement has been the standard of care in the treatment of delayed puberty. This included all causes of pubertal failure, thus also absent puberty due to congenital hypogonadotropic hypogonadism (CHH).
However, in contrast to testosterone, gonadotropin substitution with hCG and FSH offers the advantage of inducing testicular maturation in males with CHH: the testes undergo pubertal growth and the young man becomes fertile (see Chaps. 12 and 38**).**
Therefore, the previous standard is now increasingly being replaced by gonadotropin substitution in prepubertal adolescents with HH (Barrio et al. 1999; Rohayem et al. 2017).

In CDGP, pubertal onset occurs spontaneously at the latest by age 20. The indication for hormonal treatment of "late bloomers" is confined to subjects suffering from psychological distress caused by lack of maturity and/or short stature (Houchin and Rogol 1998).

If there is clinical or hormonal evidence that pubertal development is just beginning, hormonal therapeutic measures should be avoided. Reassurance that development will start spontaneously will suffice in most cases.

Otherwise, puberty can be triggered by testosterone priming with 3–6 x 50 (−100) mg testosterone-enanthate i.m. every 4 weeks.

As mentioned above, this procedure is also recommended for differential diagnostic clarification in case of uncertainty, whether pubertal failure is permanent or not.

Key Points

- The term "delayed puberty" includes both absent and arrested puberty.
- Pubertal delay can originate from hypogonadotropic or hypergonadotropic disorders of the hypothalamic-pituitary-gonadal (HPG) axis.
- Differentiation of hypothalamic pubertal failure (CHH) from constitutional delay of growth and puberty (CDGP) is challenging.
- Hormone replacement regimens are available for the treatment of delayed puberty, in hypogonadotropic disorders allowing not only for initiation of failed virilization, but also for induction of fertility.

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Pituitary Hypogonadism, Hyperprolactinemia, and Gonadotropin-Producing Tumors

15

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Abstract

Pituitary-induced hypogonadism may be hereditary or acquired. In most cases, adenomas are the cause of pituitary-induced hypogonadism. Adenomas can be treated with medication and/or neurosurgery. This often results in the restoration of function of at least some hormonal axes. In general, in cases of pituitary insufficiency that cannot be treated causally, the hormone at the end of the signal chain is substituted, in men this is testosterone. Subsequently, the administration of thyroid hormones, cortisol, and, if necessary, growth hormone must be considered. If the neurohypophysis fails, ADH is administered. An exception to the otherwise usual testosterone administration is when progeny is desired. Here, testicular function with sufficient testosterone production and spermatogenesis must be induced or maintained by administration of gonadotropins.

15.1 Pituitary-Induced Hypopituitarism

15.1.1 Etiology and Pathogenesis

The most common causes of **hypopituitarism** are **tumors (gonadotropin-, prolactin-, growth hormone-, TSH-, ACTH-secreting** or **hormone-inactive pituitary adenomas**) or **metastases of the pituitary gland and in the pituitary stalk,** as well as conditions following **surgery** and **radiotherapy** in the pituitary region. Trauma, infection, hemochromatosis, and vascular causes can also lead to pituitary insufficiency.

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Endocrine failure is expected when more than 75% of the pituitary tissue is destroyed. Disturbances of testicular function due to failure of **LH** and **FSH secretion** are usually the first sign of acquired insufficiency. The failure of the other pituitary hormones (**TSH, ACTH**, **STH**) also causes disorders of thyroid and adrenocortical function and growth. Failure of the neurohypophysis additionally results in diabetes insipidus (**panhypopituitarism**).

15.1.2 Clinic

Clinical symptoms depend on the time of onset of the insufficiency (see Chap 5, Table 5.1). In **prepubertal pituitary insufficiency**, there are **changes in body proportions** characteristic of **androgen deficiency**, but there is no typical eunuchoid tall stature due to **growth hormone deficiency**. In addition to the other androgen deficiency symptoms, clinical symptoms are characterized by signs of **hypothyroidism and adrenocortical dysfunction** and possibly **diabetes insipidus.** Puberty fails to take place, or there is delayed and incomplete pubertal development. In the case of **insufficiency occurring after puberty**, in addition to thyroid and adrenal cortical insufficiency and possibly diabetes insipidus, the typical postpubertal androgen deficiency symptoms occur (see Chap. 5 Table 5.1).

15.1.3 Diagnostics

Diagnosis is established by **MRI** (if necessary with gadolinium contrast) of the sellar region, examination of the **visual field,** and determination of the **basal hormones** in serum (LH, FSH, prolactin, TSH, ACTH, STH, thyroid hormones, cortisol, IGF-1) and the lack of stimulability of pituitary hormones in the combined pituitary function test (100 µg GnRH, 200 µg TRH [thyrotropin-releasing hormone], 100 µg GRH [growth hormone-releasing hormone], 100 µg CRH [corticotropin-releasing hormone] intravenously; blood samples to be taken before and 30, 60, and 90 min after administration of the releasing hormones(Müller et al. 2003). When a large pituitary tumor is suspected, pituitary function tests are relatively contraindicated because of possible complications (acute tumor necrosis). In the future, small pituitary tumors may be detectable by machine learning of 1 mm thin-section MRI images. This makes sense because even very small tumors may already show clinically significant endocrine activity (Lee et al. 2021). It also appears to be possible to directly infer histopathology on ultra-high field 7 Tesla MRI images (Yao et al. 2020).

15.1.4 Therapy

In the case of a **pituitary tumor** as the cause of insufficiency, **neurosurgical removal** is the goal, which is nowadays preferably performed transsphenoidally, sparing the healthy pituitary tissue. After surgery, regeneration of the remaining pituitary tissue is possible. Some tumor types can also be irradiated primarily. **Prolactinomas** (both microprolactinomas and macroprolactinomas) are **primarily treated with medication**.

Until pituitary function is restored, if neurosurgery is not possible or the pituitary tissue is completely destroyed, the hormones (cortisol, thyroid hormones, testosterone, possibly ADH) must be substituted. Before puberty or in the case of boys who have not reached adolescence, growth hormone is also administered. **Therapy of infertility** is performed by administration of hCG/FSH (see Chap. "Hypothalamic hypogonadotropic hypogonadism"). Compared with gonadotropin therapy for IHH, the success rate in terms of pregnancy occurrence is higher in patients with hypopituitarism, especially when pituitary insufficiency occurs after puberty has passed (Liu et al. 2009; Dwyer et al. 2015; Rohayem et al. 2017). Once spermatogenesis has been physiologically induced, ensuing attempts are likely to be easier. **1.**

15.1.5 Hypopituitarism in Hereditary Disposition

Congenital pituitary insufficiency is rare but can be caused by mutations in transcription factor genes. These factors play an important role in the development of the pituitary gland. While a gene defect in the transcription factor POU1FI (formerly Pit-1) results in pituitary failure of growth hormone, prolactin, and TSH and thus does **not** result in hypogonadism, a defect in the gene for the transcription factor PROP1 additionally affects the gonadotropins LH and FSH. PROP1 mutations are the most common cause of **congenital panhypopituitarism** (Mehta and Dattani 2008). The disease is inherited in an autosomal recessive manner. The clinical presentation is variable. It is determined by short stature and hypothyroidism in the first years of life. Gonadotropin deficiency appears to develop with age only, as many patients undergo spontaneous pubertal development (Deladoey et al. 1999; Flück et al. 1998). In adults, hypogonadism is then a constant symptom (Rosenbloom et al. 1999). The familial presence of hypopituitarism and prolactin deficiency are indicative of a mutation in the PROP1 gene. A number of other transcription factors with associated clinical pictures have been identified in recent years, such as somatic mutations in GNAS, USB8, PIK3CA, GPR101, and germline mutations in MEN1, cyclin-dependent kinase inhibitor genes, AIP, DICER1, PRKAR1A, PRKACA, SDH genes, and GPR101 (Mehta and Dattani 2008; Shaid and Korbonits 2017).

One of the most important differential diagnoses of pituitary insufficiency already manifested in childhood is **septo-optic dysplasia** (Willnow et al. 1996). This is a very

heterogeneous clinical picture with a variable phenotype of a combination of nervus opticus abnormalities, agenesis of the septum pellucidum, and deficiency of pituitary hormones. The prevalence is 6.3–10.9 per 100,000. Hypogonadotropic hypogonadism is not an obligatory symptom, but always occurs in combination with other pituitary failures in septo-optic dysplasia. Genes identified as genetic causes to date are HESX1, SOX2, and SOX3 (Kelberman and Dattani 2008).

A familial form of X-linked recessive **panhypopituitarism** is associated with intellectual deficiency or mild mental retardation (Lagerström-Fermér et al. 1997). The cause is thought to be an increased gene dosage due to an interstitial duplication in the Xq26.1-q27.3 region containing the SOX3 gene (Solomon et al. 2004).

15.2 Isolated LH or FSH Deficiency

Isolated **deficiencies of LH and FSH** are extremely rare forms of hypogonadotropic hypogonadism, but in which only one of the two gonadotropins is absent. In **isolated LH deficiency (Pasqualini syndrome** 1950), (“**fertile eunuchs”**), there is a striking dissociation between symptoms of hypogonadism already present prepubertally and almost normal testicular size. Testicular histology shows qualitatively preserved, quantitatively reduced spermatogenesis with atrophy of Leydig cells. A genetic basis for this could represent a constitutional activation of the FSH receptor (Oduwole et al. 2018). Endocrinological studies showed decreased LH with normal serum FSH levels. A homozygous mutation of the GNRHR gene was detected in a patient with Pasqualini syndrome (Pitteloud et al. 2001). Administration of hCG resulted in normalization of testosterone serum levels and quantitatively normal spermatogenesis. Interestingly, after cessation of hCG therapy, the patient spontaneously showed normal testosterone levels without further therapy.

Inactivating mutations of the gene for the β-subunit of LH (LHβ gene) are very rare and to date only four patients with these mutations have been described (Lofrano-Porto et al. 2008 including the publications described in this paper). Patients with LHβ gene mutations (homozygous or compound heterozygous) have decreased testosterone levels, pubertas tarda, and small testicular volume. Serum FSH levels are elevated, whereas serum LH levels are undetectable to elevated, depending on whether the mutation affects the immunogenicity of the LH molecule in the assay system. Testicular histology shows spermatogenesis arrest, and Leydig cells cannot be detected. Long-term hCG therapy results in normal testicular volume, virilization, and induction of spermatogenesis.

Isolated FSH deficiency due to mutations of the FSHβ gene is also very rare (Lofrano-Porto et al. 2008 including publications described in *this* paper). All three patients described showed azoospermia. Two of the affected males had a normal pubertal course with normotensive to low normal testosterone serum levels, whereas the third patient had testosterone deficiency and no normal puberty. In rare cases, patients have also been found to have **isolated FSH deficiency** despite a normal FSHβ gene sequence. Affected patients have decreased spermatogenesis, and LH activity and thus androgenization is normal (Mantovani et al. 2003). The cause of this FSH deficiency is not known.

15.3 Hyperprolactinemia

15.3.1 Etiology and Pathogenesis

The peptide hormone prolactin, produced in the anterior pituitary, has no known physiological function in males. However, some prolactin activity in men is thought to be necessary to improve sexual function (erection duration and ejaculate volume) (Rastrelli et al. 2015). The causes of hyperprolactinemia in men are diverse (Fig. 15.1).

Schematic representation of possible causes of hyperprolactinemia. *a*: prolactin-producing adenoma; *b*: inhibition of transport of prolactin inhibitory factor (PIF, dopamine), e.g., by a suprasellar growing pituitary tumor; *c*: Destruction of PIF-forming hypothalamic nuclear areas, e.g., by tumor; *d*: drug inhibition of PIF secretion and action; *e*: increased secretion of hypothalamic prolactin-releasing factor (PRF).

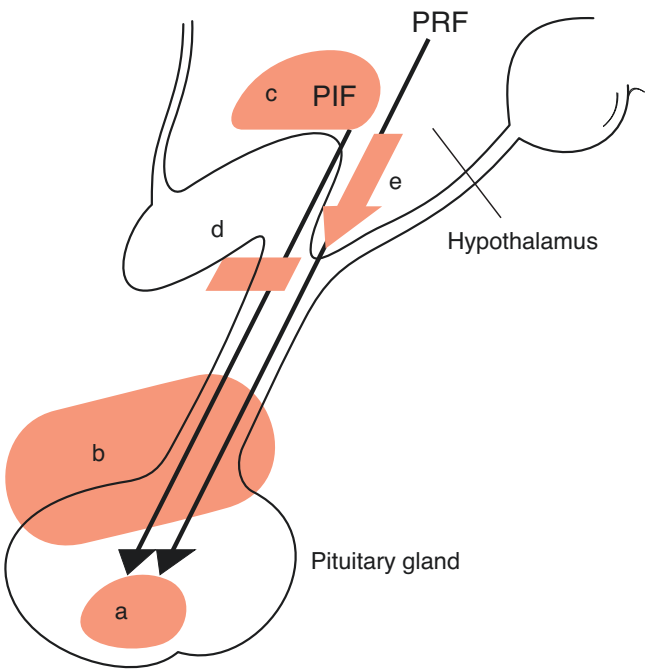


Fig. 15.1 The regulation of prolactin secretion

Mild elevations in prolactin levels can be triggered by **physical or psychological "stress."** In patients predisposed to this, the consultation situation or the announcement of a blood sample is often sufficient to trigger this reaction.

Prolactin-producing pituitary adenomas (prolactinomas) are the most common cause of organically induced hyperprolactinemia. Prolactinomas can be divided into **microprolactinomas** (with a diameter <10 mm) and **macroprolactinomas.** The latter are large, rapidly proliferative tumors that cross the borders of the sella and can lead to impairment of surrounding structures, especially the optic nerves.

Classification of prolactinomas should include not only the size of the adenoma but also the speed of growth, since macroprolactinomas also started as small, rapidly growing adenomas (von Werder 1995). The level of basal prolactin correlates roughly with the size of the adenoma. Malignant prolactinomas are extremely rare (Mancini et al. 2008). A familial clustering of prolactinomas has been described (Berezin and Karasik 1995; Melmed 2008), but in most cases prolactinomas occur sporadically.

Pituitary prolactin secretion is under an **inhibitory influence** of the hypothalamus mediated mainly by **dopamine** (Fig. 15.1). Lesions of the hypothalamus or the pituitary stalk therefore usually cause excessive prolactin secretion ("**inflammatory hyperprolactinemia**") (Melmed 2008). **Nonprolactin-secreting pituitary tumors, craniopharyngiomas,** and other processes occupying space close to the sella may be the cause of this inflammation hyperprolactinemia. In primary hypothalamic disorders, **increased stimulation of the pituitary by prolactin-releasing (releasing) factors** (Fig. 15.1) is also likely to occur, and a corresponding role is discussed for TRH, vasopressin, and VIP (vasoactive intestinal polypeptide) (Mancini et al. 2008). In inflammatory hyperprolactinemia, serum prolactin levels do not exceed 2000 mU/l in most cases; levels above 2000 mU/l are usually found only in macroprolactinomas (Karavitaki et al. 2006).

Hyperprolactinemias induced by **pharmaceuticals** are due to the dopamine antagonistic properties of these substances (e.g., butyrophenones, phenothiazines, imipramine, metoclopramide), whose interference with dopamine synthesis (e.g., α-methyldopa), depletion of dopamine stores (e.g., reserpine), or stimulation of prolactin synthesis and secretion (e.g., H2 blockers, estrogens). Other important causes of hyperprolactinemia include **chronic renal failure** and **hypothyroidism** (Schlechte 2003, 2007).

Hyperprolactinemia can lead to disturbances in male reproductive functions through several mechanisms. At the level of the hypothalamus, hyperprolactinemia may result in **impaired pulsatile GnRH release**. Consecutively, gonadal insufficiency with low testosterone levels and impaired spermatogenesis develops secondary to the deficiency of LH and FSH. Hyperprolactinemia is not necessarily the cause of a concomitant gonadotropin deficiency. Sellar and suprasellar space-occupying processes of any type can cause inflammatory hyperprolactinemia (Fig. 15.1) and at the same time interrupt GnRH influx to the gonadotropic pituitary cells.

The second, more clinically significant mechanism of impairment of testicular function by hyperprolactinemia is **displacement and destruction of gonadotropic pituitary cells** with suppression of LH and FSH levels by space-occupying macroprolactinoma (Fig. 15.2). A direct inhibitory effect of prolactin on the testis has not yet been demonstrated in humans.

15.3.2 Clinic

Clinically, hyperprolactinemia in males may be manifested by signs of **androgen deficiency** and **infertility**. Some of the patients primarily complain of disturbances in libido and erection function, and gynecomastia and galactorrhea rarely occur. Some men with definite hyperprolactinemia are also completely asymptomatic and symptom-free. Space-occupying processes in the area of the sella may cause additional symptoms, e.g., headache or visual field defects, depending on their localization and size.

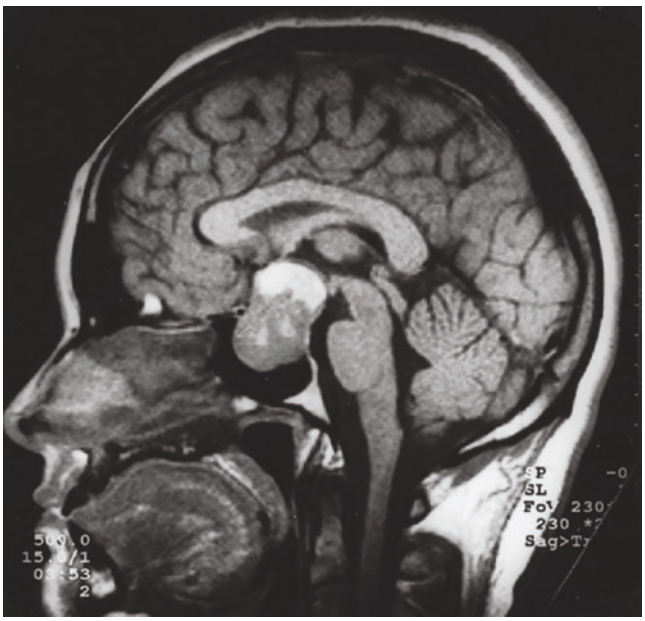


Fig. 15.2 A pituitary adenoma visualized by MRI

15.3.3 Diagnosis

Diagnosis is made on the basis of **elevated prolactin serum levels**. Basal hormone levels (LH, FSH, TSH, ACTH, STH, thyroid hormones, cortisol, IGF-1) are measured to assess pituitary function, and a combined pituitary function test (GnRH, TRH, GRH, CRH) may be performed.

In stress-induced hyperprolactinemia, basal prolactin levels usually do not exceed twice the norm (<1000 mU/l). Very high levels (>5000 mU/l) are indicative of macroprolactinoma, but serum levels can be highly variable. In any case, if prolactin levels are repeatedly measured to be markedly elevated, **magnetic resonance imaging** of the hypothalamic/pituitary region is required for detection of prolactinoma or other space-occupying processes with possible inflammatory hyperprolactinemia. Similarly, if suprasellar extension of the tumor is suspected, a **visual field examination** is performed.

Stimulation tests of the pituitary gland are generally not suitable for the differential diagnosis of hyperprolactinemia (Schlechte 2003; Casanueva et al. 2006). It should be noted that high prolactin levels may also be due to a misleading measurement of the biologically practically inactive macroprolactin. If this is suspected, precipitation with polyethylene glycol and then remeasurement are useful (Biagetti et al. 2022).

15.3.4 Therapy

Stress-induced hyperprolactinemia does not require treatment. For therapy of prolactinoma, **dopamine agonists** are primarily used. The longest experience is with bromocriptine (Pravidel, Kirim et al.) (Mancini et al. 2008). Therapy should be started with low late evening doses (1.25 mg/d) because of the hypotensive effects of bromocriptine. The dose is slowly increased and spread throughout the day (morning–noon–evening) until normalization of prolactin levels has occurred (Fig. 15.3). The decrease in prolactin levels under adequate therapy is accompanied by a reduction in the size of the prolactinoma (Bevan et al. 1992). In terms of side effects, blood pressure drop, fatigue, headache, or gastrointestinal disturbances may occur at the beginning of therapy; in the long-term, headache, dry nasal mucosa, psychiatric changes, and digital vasospasm during cold exposure are sometimes observed (von Werder 1995; Mancini et al. 2008).

Plot of prolactin serum concentration under dopamine agonist medication (Pravidel) in a patient with macroprolactinoma. After initial suppression of prolactin levels by high doses of Pravidel, the dose can be slowly reduced if prolactin suppression persists.

Because of high efficacy and better tolerability compared with bromocriptine, the long-acting **dopamine agonist cabergoline** (Dostinex, et al.) is now most commonly used (De Rosa et al. 1998; Cannavo et al. 1999; Gillam et al. 2006). Therapy is usually started at 0.25–0.5 mg once a week and increased monthly until prolactin levels normalize (Casanueva et al. 2006). Under high-dose cabergoline therapy of patients with PD (>3 mg cabergoline per day), damage and insufficiency of

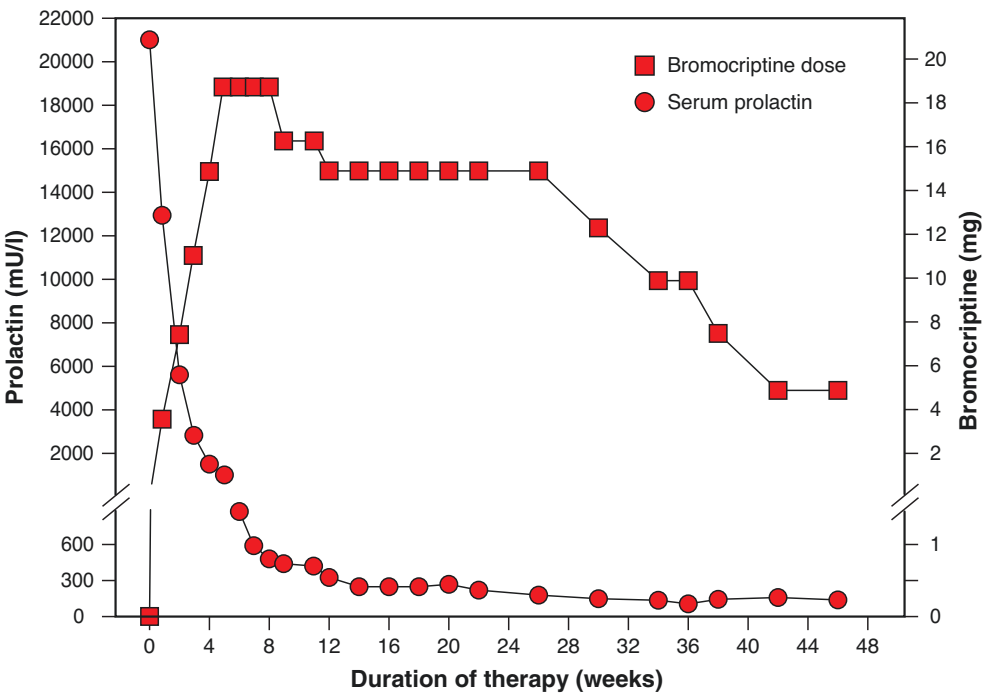


Fig. 15.3 Reaction of prolactin levels in serum during therapy with bromocriptin

the heart valves have been described as a potential side effect (Zanettini et al. 2007). Under the usual effective dose of 0.25–3 mg/week in patients with prolactinoma, these changes were not seen in most studies (Kars et al. 2008; Herring et al. 2009), but at higher doses, increased valvular fibrosis has also been described in patients with prolactinoma (Colao et al. 2008; Nachtigall 2017). In view of the relationship, which cannot yet be conclusively assessed, it is currently recommended that echocardiographic examinations be performed in patients with a prolactinoma before and during long-term therapy with cabergoline (Kars et al. 2008; Sherlock et al. 2009).

In case of side effects of bromocriptine or cabergoline, **quinagolide** (Norprolac), **lisuride** (Dopergin), or **metergoline** (Liserdol) are therapeutically available as alternatives. In principle, all dopamine receptor agonists can lead to addictive and compulsive behavioral disturbances, and this should be considered especially at the start of therapy (Castinetti et al. 2021).

In patients with microprolactinomas or macroprolactinomas who no longer have tumor tissue detectable by MRI while on long-term dopamine agonist therapy, a discontinuation of therapy can be attempted after 2 to 3 years of therapy (Colao et al. 2003; Gillam et al. 2006; Casanueva et al. 2006). After discontinuation of therapy, regular checkups with determination of prolactin serum concentrations are recommended, especially during the first year (Gillam et al. 2006; Schlechte 2007). Permanent normalization of prolactin without tumor regrowth is seen with varying frequency depending on the study and initial findings, but can be expected in approximately 30–50% of treated patients with original macroprolactinoma (Gillam et al. 2006; Schlechte 2007). Nonresponse especially to cabergoline is very rare and in most cases is the indication for neurosurgical intervention. Malignant or particularly fast-growing prolactinomas can then be (pre)treated with temozolomide if necessary (Maiter 2019).

The indication for **surgical extirpation of prolactinomas**, usually performed transsphenoidally, is now only given in less than 10% of patients, given the efficacy of drug treatments, and especially when drug therapy is not effective (Casanueva et al. 2006; Gillam et al. 2006; Mancini et al. 2008; Haider et al. 2022). However, the primary indication for neurosurgical intervention or neuroradiotherapy is inflammatory hyperprolactinemia induced by other space-occupying processes (Fig. 15.1).

Under effective therapy with dopamine agonists, the normal LH pulsation pattern soon reestablishes and testosterone levels rise (Inder and Jang 2022). A nevertheless **persisting androgen deficiency** must be **adequately** treated (see Chap. 36). When starting drug therapy for hyperprolactinemia, a wait-and-see approach can be adopted initially or transdermal or oral androgen therapy can be started, depending on the severity of the androgen deficiency symptoms in order to impair pituitary function as little as possible. If hypogonadism persists despite therapy or primary neurosurgical or neuroradiologic intervention with failure of pituitary function, adequate testosterone substitution therapy (see Chap. 21) is required in addition to possible thyroid hormone and cortisol substitution. In the case of a current desire for parenthood, hCG plus FSH therapy is given if gonadotropin suppression persists.

15.4 Gonadotropin-Producing Tumors

Histologically confirmed gonadotropin-producing pituitary adenomas are among the common pituitary tumors, accounting for approximately 25% (Saeger et al. 2007). Only in rare cases do these tumors secrete intact LH and/or FSH and may lead to pubertas praecox, supraphysiological testosterone levels or macro-orchy (Chaidarun and Klibanski 2002). In most cases, these tumors become clinically conspicuous only by a marked increase in size and associated neurological symptoms with visual field defects (Jaffe 2006).

The standard therapy for gonadotropin-producing macroadenomas (diameter ≥ 1 cm) is transsphenoidal surgery (Lüdecke et al. 2003; Jaffe 2006). Due to the usually slow growth of microadenomas (diameter < 1 cm), waiting, with regular endocrinological and MRI controls, is justified in the absence of clinical symptoms (Jaffe 2006). Radiotherapy is indicated only in special cases such as residual tumors or recurrences after transsphenoidal surgery (Chaidarun and Klibanski 2002; Lüdecke et al. 2003; Jaffe 2006; Melmed 2008). Relatively successful drug therapy attempts can be made with temozolomide, as in cabergoline-refractory prolactinoma, but also with somatostatin receptor ligands, peptide receptor radionuclide therapy, and mammalian target of rapamycin (mTOR) inhibitors (Ilie and Raverot 2020). In addition, immunotherapies are being discussed (Dai et al. 2020).

Key Points

- Pituitary insufficiencies can be hereditary or acquired.
- In most cases, adenomas are the cause. Adenomas can be treated with drugs and/or neurosurgery, after which functional capacity of at least some hormonal axes is often achieved.
- Generally, in cases of pituitary insufficiency that cannot be treated causally, the hormone at the end of the signal chain is substituted; in the case of andrology, this is testosterone. In a broader sense, administration of thyroid hormone, cortisol and possibly also growth hormone must then be considered. If the neurohypophysis fails, administration of ADH is added.
- An exception to testosterone administration occurs in the case of a desire for parenthood; in this case, testicular function in the sense of testosterone production and spermatogenesis must be maintained by the administration of gonadotropins.

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Part IV

Clinics in Andrology: Primary Hypogonadism (Disorders at the Testicular Level)

Anorchia and Polyorchidism

16

Eberhard Nieschlag

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Abstract

Anorchia is defined as absence of *Anlage* or loss of one or both testicles in genetically male individuals. Accordingly, a distinction is made between congenital and acquired anorchia. Congenital anorchia is a rare event with a prevalence of 1:20,000 newborns. The diagnosis of bilateral anorchia is based on the hCG test and the determination of AMH. Acquired anorchia can be the result of torsion, trauma, infection, failed surgery, or castration. Testosterone substitution is the means of choice to induce or maintain androgenization. After castration for medical reasons (e.g., prostate carcinoma) or for socio-cultural reasons, a testosterone substitution would contradict the purpose of castration. In **polyorchidism**, more than two testicles are found, most commonly triorchia, but the occurrence of five testicles has also been described. Polyorchidism usually remains asymptomatic and is discovered by chance, e.g. by sonography or MRT which can also confirm suspicious findings.

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16.1 Anorchia

Anorchia is present when, in genetically male individuals, testicular tissue or residuals of such tissue are completely absent on one or both sides. This differentiates anorchia from complete or partial testicular atrophy (e.g., after torsion, severe orchitis), where, histologically at least, degenerated remains of a male gonad can be detected. A distinction must be made between congenital and acquired anorchia.

16.1.1 Congenital Anorchia

16.1.1.1 Prevalence and Pathophysiology

Congenital **double-sided Anorchia** occurs in only one in 20,000 men. **One-sided anorchia** is about four times more common. Among 8518 patients at the EAA Andrology Center in Tartu/Estonia, there were two cases with bilateral and five with unilateral anorchia (Punab et al. 2016).

Mainly vascular and genetic disorders, intrauterine infections, trauma, or teratogenic noxae are discussed as causes for intrauterine loss of one or both testicles. A suspected abnormality in the sex-determining region of the Y chromosome, the SRY gene, could not be confirmed in several studies (Lobocarro et al. 1993; Vinci et al. 2004; Brauner et al. 2011). Mutations in other genes, which are responsible for the development and descensus of the testis, could not be

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clearly identified, e.g., *NR5A1*, *INSL3*, *MAMLD1*, and *LGR8* (Vinci et al. 2004; Brauner et al. 2011). Of 24 cases only one case could detect a mutation in the *SF1* gene (Philibert et al. 2007). Currently, intrauterine testicular torsion and previously unidentified genetic factors are favored as possible causes.

The anatomical findings in bilateral congenital anorchia can be directly derived from the embryonic development of the male genital system. The testis, which develops from the indifferent gonadal system in about the sixth week of pregnancy, initially secretes only anti-Müller hormone (AMH), not until a later stage does it also produce testosterone. If the loss of testicular tissue occurs before testosterone production begins, the Müller ducts have regressed, but the androgen-dependent differentiation of Wolff's ducts and the masculinization of the urogenital sinus and the external genitals have not yet occurred. If, on the other hand, the primary testes perish after having secreted testosterone for a certain time, the androgen-dependent target tissues of the urogenital tract are more or less completely differentiated in the male direction (Josso et al. 2006).

16.1.1.2 Diagnostics

Individuals with **bilateral congenital anorchia**, in whom the testes still produced AMH but no more testosterone, show the picture of a **46,XY disorder of sexual development (46XY DSD)** with female external genitals. Neither gonads nor derivatives of Müller ducts (tubes, uterus, upper third of the vagina) or Wolff's ducts (epididymis, vas deferens, seminal vesicles) are found. If the testicles have secreted testosterone during the embryonic period, the external genitals are male and the Wolffian derivatives are developed. A small penis (**micropenis**) may indicate reduced androgen-dependent growth in late fetal development.

If left untreated, patients with congenital bilateral anorexia will not reach puberty and the typical picture of **eunuchoidism** will develop. Since a single intact testis is functionally sufficient, congenital unilateral anorchia does not involve disorders of sex differentiation and pubertal development.

In patients with **congenital bilateral anorchia**, testicular tissue cannot be detected morphologically or endocrinologically. Serum **FSH** and **LH** levels are already elevated in children and are extremely high from pubertal age onward. **Testosterone** and **anti-Müller-hormone (AMH)**, on the other hand, are very low or immeasurable.

Cryptorchidism (see Chap. 17) must be excluded by differential diagnosis. The **hCG test** is used for differentiation (see Chap. 7). While an increase in serum testosterone can be measured in cryptorchidism, the values remain low in bilateral anorchia even after several days of stimulation (Jespersen et al. 2020). Furthermore, **AMH** must be measured, which is absent in anorchia and has a higher sensitivity than testosterone with the same diagnostic specificity.

If unilateral anorchia is suspected, imaging procedures (sonography, computer tomography, and magnetic resonance imaging) and, if necessary, exploratory surgery/laparoscopy must be used to ensure that no gonadal tissue is actually present, since non-descended and dysgenic gonads have a high rate of malignant degeneration.

16.1.1.3 Therapy

Unilateral congenital anorchia does not require therapy as long as testosterone production is sufficient. Whether or not the existing testis should be fixated by orchidopexy is controversially discussed (Szmer and Chrzan 2019).

In cases of **bilateral congenital anorchia**, testosterone replacement should be started in phenotypically male patients at the time of expected puberty (see Chap. 36). Testis prostheses can be inserted into the scrotum for cosmetic reasons (Hayon et al. 2020). The case of identical twins, one of whom donated a testis to his anorchid twin brother at the age of 30, caused a sensation. After transplantation, both had normal testosterone levels and sperm in the ejaculate (Silber 1978).

16.1.2 Acquired Anorchia

16.1.2.1 Accidental Testicular Loss

Testes can be lost as a result of **trauma**, **tumors**, severe **inflammation, torsion**, **surgical accidents** (e.g., **herniotomy**, **orchidopexy**), and **intended surgery** (e.g., because of a testosterone-dependent tumor such as prostate cancer) but also through self-mutilation. The loss of a testis is compensated by the remaining normal testis in terms of fertility and endocrine functions, and does not require any therapy. However, some authors argue in favor of removing testicular tissue remaining after treatment for torsion because in some unexplained manner the function of the intact testis may be negatively affected (perhaps by autoantibodies) (Arap et al. 2007).

In cases of acute bilateral testicular loss, it must be remembered that sperm can still be found in the ejaculate up to several weeks after the event, depending on the ejaculation frequency. To create a fertilization reserve, these sperm can be cryopreserved (Fig. 16.1) (see Chap. 42).

16.1.2.2 Diagnostics and Therapy

For the **clinical symptoms** of bilateral testicular loss, the time of loss of testicular function is of crucial importance. Before puberty, it leads to the typical picture of **eunuchoidism,** after puberty to the appearance of post-pubertal testosterone deficiency (see Chap. 5, Table 5.1). In the case of bilateral loss, testosterone must be substituted from the beginning of puberty or, in adults, from the time of loss in order to induce pubertal development or to maintain the various androgen-dependent functions (see Chap. 36). For psychological or cosmetic reasons, testicular prostheses can be implanted (Hayon et al. 2020).

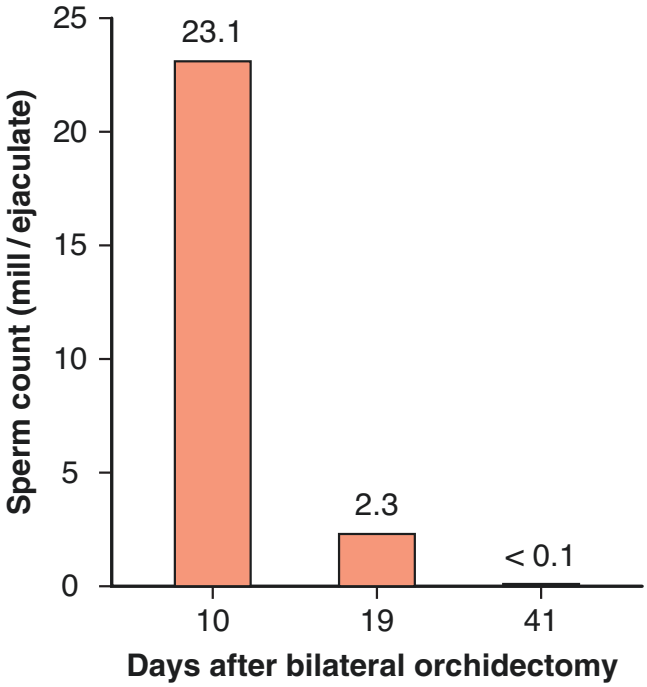


Fig. 16.1 Sperm in the ejaculate of a 15-year old boy following bilateral orchidectomy due to delayed discovery of testicular torsion. Although several ejaculations had taken place, even 10 days after surgery (when the patient was first seen by us), enough sperm were present to make cryopreservation worthwhile

16.1.2.3 Castration for Medical Reasons

If the testes are removed for the treatment of prostate cancer or sexual delinquency (see Law on Voluntary Castration BGB = German Civil Code I, 1143 of 15.08.1969, last amended 4.11.2016), the androgens must certainly not be substituted, as their elimination is a therapeutic principle in these cases. Between 1970 and 1980, about 400 men in the Federal Republic of Germany were voluntarily castrated on the basis of this law (Wille 1991). At present, the possibilities offered by this law are rarely used, and "chemical castration", e.g., with anti-androgens, has also been abandoned in favor of psycho-socially oriented treatment methods.

16.1.2.4 Socio-cultural Castration

Beyond medical indications, castration has been and is also performed for **socio-cultural reasons**. Almost all societies practicing polygamy employed castrates as guardians (eunuchs, harem guards), who also often gained political influence at the Chinese imperial court, for example. In China, castration for such purposes was usually performed on adult males. During these "operations," the testes as well as scrotum and penis (!) were removed, so that the operations were extremely painful and accompanied by so many complications that 25% of those treated did not survive the operation. Since lucrative positions at the imperial court could be achieved in this way, many candidates took this risk (Wagenseil 1933; Mitamura 1992; Wilson and Roehrborn 1999; Nieschlag and Nieschlag 2019). The last castrate of the empire, terminated by revolution in 1912, Sun Yaoting, died in 1996 at the age of 93.

Castration before puberty **maintains the high voice of a boy**, so that in the adult soprano or alto voices with the acoustic volume of an adult man are produced. Such high-pitched voices were much sought after by music-lovers. Prepubertal castrates belonged to the casts of the seventeenth and eighteenth century opera-houses and courts. In Vatican choirs, these voices could still be heard until the early twentieth century (Blume 2019).

The Romans recommended **castration as a treatment** for leprosy and epilepsy, in the seventeenth century it was prescribed for gout and insanity. Until the beginning of the twentieth century, castration was still performed in the USA to treat the mentally handicapped.

Castration as self-mutilation deriving from religious fanaticism was also observed from time immemorial. The church father Origenes (186–254) is a prominent example. Most recently, castration was practiced by the **Scoptic sect** founded in Russia in the eighteenth century. The largest group of castrates in the present day are the **Hijras** in India. They appear as professional congratulators at birth ceremonies and receive ample financial support in return. Their number is several thousand.

Patients with acquired anorchia could make an (involuntary) contribution to the question whether the **shorter life expectancy of men compared to women** could be explained by testosterone or the presence of testes, as is often claimed. Surprisingly few studies, however, have used this "model." A retrospective analysis of the life expectancy of inmates of an institution for the mentally handicapped in the USA comes to the conclusion that early castration may lead to higher life expectancy (Hamilton and Mestler 1969). In contrast, an analysis of the life data of prepubertal castrated and intact singers of the sixteenth to nineteenth century showed no difference in lifespan (Fig. 16.2) (Nieschlag et al. 1993). Since neither a study of disabled persons nor the historical analysis can be regarded as representative for today's general population, these studies have provided interesting food for thought, but the real question of the current worldwide **4.5 year shorter life expectancy** of men compared to women must still be considered as open. The analysis of the lifespan of 292 female opera singers who died between 1900 and 1995 showed that sopranos, being more highly estrogenized, live significantly longer than androgenized alto singers; this was interpreted to mean that same-sex hormones have a life-prolonging effect. Among the 505 male opera singers analyzed, however, basses showed only a tendency towards higher life expectancy compared to tenors (Nieschlag et al. 2003).

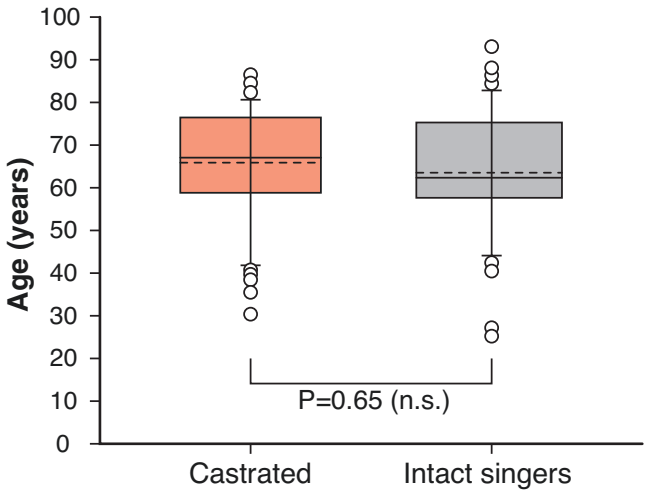


Fig. 16.2 Lifespan of castrated and intact singers from the sixteenth to nineteenth centuries. In each group, 50 singers with similar birthdates were selected. Box and whisker plot: solid line = median, dotted line = average. (Nieschlag et al. 1993)

16.2 Polyorchidism

16.2.1 Prevalence and Pathophysiology

Supernumerary testes are a rare finding. In the literature, little more than 200 cases have been described. In most cases, a third testicle (**triorchism**) is found on the left side. A transverse division or doubling over of the genital ridge caused by peritoneal strands in early embryonic development (4–6 weeks) is the most plausible pathophysiological explanation for polyorchidism. In addition to the testes, the duplication may also affect the epididymis and the vas deferens (O'Sullivan et al. 1995), so that the supernumerary testis may have its own common epididymis and vas deferens with the adjacent testis (type A) or be without an excretory duct (type B) (Leung 1988; Bergholz et al. 2007; Bergholz and Wenke 2009) (Fig. 16.3).

16.2.2 Diagnostics

Most patients have no symptoms, and the excess testicle is discovered by chance. Most cases are diagnosed at the age of 15–25 years (Avargues et al. 2015), but a 13-month old child with five testicles has also been described (Zahirian Moghadam et al. 2020). Fertility can be completely normal. However, the supernumerary testis may torquify or become malignant and require acute intervention. Most supernumerary testes are found in connection with positional anomalies, inguinal hernias, torsion, hydrocele, or tumors. A third testis with ducts may be the rare cause of persistent fertility after vasectomy (Hakami and Mosavy 1975).

Ultrasound and MRI imaging have significantly improved diagnostics and may eliminate the need for surgery for histological confirmation (Ogul et al. 2014)).

16.2.3 Therapy

The therapeutic procedure must be decided on a case-by-case basis. While tumor or torsion leading to necrosis require surgical removal, in other cases the supernumerary testis may remain in situ. However, these patients must be monitored regularly, including sonography and tumor markers, due to the risk of malignant degeneration.

16.2.4 Historical Aspects

In the medical literature, a case of polyorchidism was recorded in 1880 (Ahlfeld). However, there have been historical figures in the past to whom extraordinary strength and sexual activity have been attributed because of supernumerary testicles. For example, the Venetian Captain General Bartolomeo Colleoni (1400–1475) achieved special fame for his "virility" (*colleoni* means "testes" in the Italian colloquial language and is written today as *coglioni*). The triorchism is also symbolically represented in his coat of arms. In 1539, Landgrave Philipp of Hesse (1504–1567) received permission from Martin Luther to be married to Margarete von der Saale as an additional wife because of an alleged third testicle (Nieschlag and Nieschlag 2019).

Key Points

- Anorchia means non-investment or loss of one or both testicles in genetically male individuals, a distinction is made between congenital and acquired anorchia.
- Congenital anorchia is a rare event with a prevalence of 1:20,000 newborns.
- The diagnosis of bilateral anorchia is usually made between the ages of 15 and 25 and is confirmed by hCG test and determination of AMH.
- Acquired anorchia can be the result of torsion, trauma, infection, failed surgery, or castration.
- Testosterone substitution is the method of choice in bilateral anorchia to initiate or maintain androgenization.
- After castration for medical reasons (e.g., prostate carcinoma) or for socio-cultural reasons, testosterone therapy would defy the purpose of castration.
- In one of the rare cases of polyorchidism, more than two testicles are found, most often a triorchia, but the occurrence of five testicles has also been described.
- Polyorchidism usually remains without symptoms and is discovered by chance, e.g., by sonography or MRI, which can also confirm a suspicion.
- Polyorchidism usually remains asymptomatic and is discovered accidentally, e.g., by sonography or MRI, which can also confirm a suspicion.

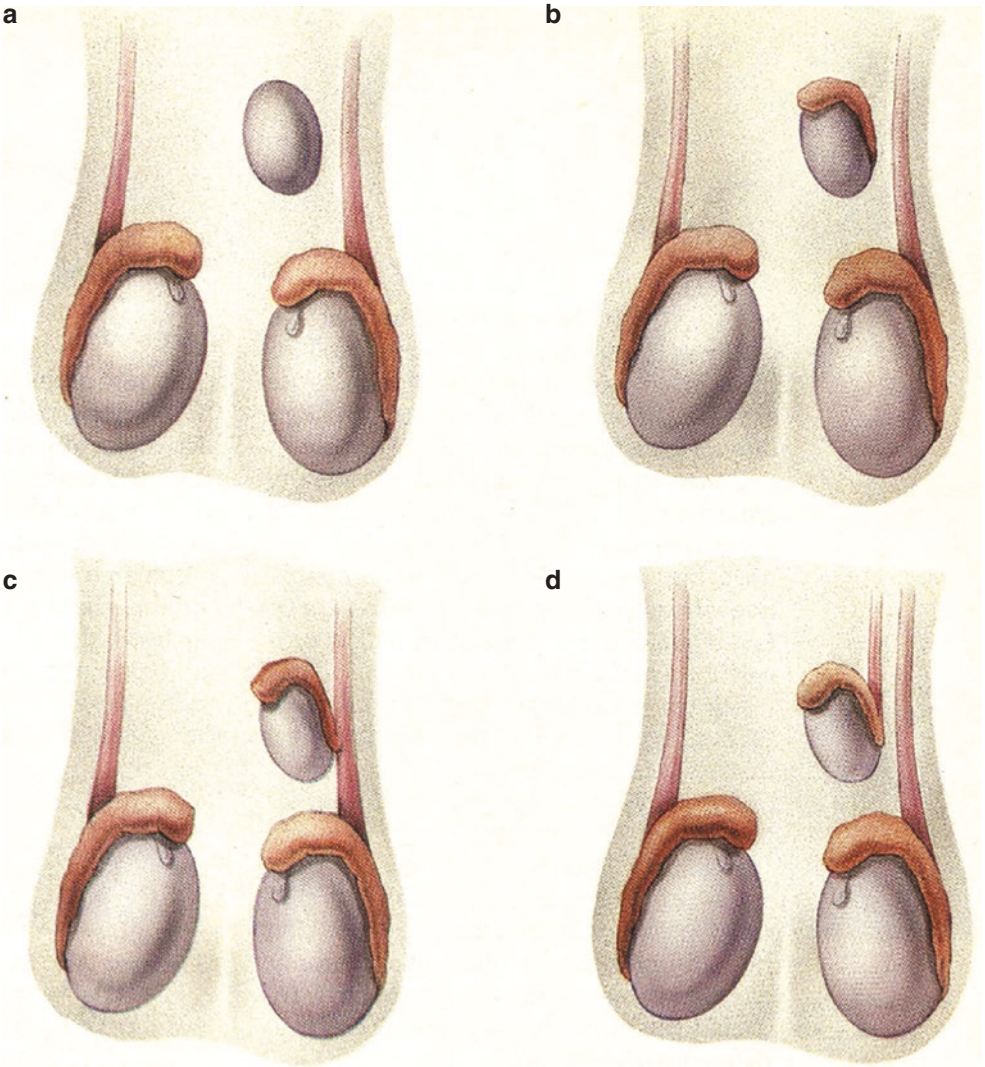


Fig. 16.3 (**a**–**d**) Various forms of polyorchidism (Leung 1988)

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Undescended Testes

17

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Abstract

Undescended testes (cryptorchidism) represent the most common malformation in boys: retractile, inguinal, or abdominal testes are present in 1–5% of male neonates born at term. During the first 9 months of life, some of these testes descend spontaneously as they continue to mature, reducing the prevalence of cryptorchidism at age 9 months to 1–1.5% without treatment. Among other factors, fetal androgen deficiency or insufficient androgen action may be at the origin of impaired testicular descent into the scrotum. Previously undescended testes may lead to fertility limitations in men and may cause endocrine testicular dysfunction. If treated too late, the undescended testis carries an increased risk of malignant transformation. The high-ambient temperature of testes located in an extra-scrotal location is held responsible for both increased apoptosis of the germ cells and for the increased risk of testicular germ cell tumors. Persistent undescended testes therefore require treatment. According to current guidelines, hormonal or surgical correction should be completed by the boy's first birthday. Early correction reduces the risk of testicular tumor development and may have beneficial effects on testicular endocrine and spermatogenic function in adolescence and adulthood.

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17.1 Epidemiology

Undescended testes (UDT) (cryptorchidism) at birth are a common cause of infertility in men. Undescended testes are present in **1–5% of all boys born at term** (Berkowitz et al.

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1993; Barthold et al. 2004); in these boys, the testes have not migrated properly from their original position near the renal "anlagen" into the scrotum during embryonic and fetal development. In **preterm male infants**, the prevalence of undescended testis may be as high as **45%**, depending on gestational age. Postnatal spontaneous descent of maldescended testes into the scrotum may occur during the first 6–9 months of life, i.e., during "**mini-puberty**"). After the 9th month of life, the prevalence of undescended testes is **1–1.5%** (Toppari and Kaleva 1999); in formerly preterm infants, the prevalence is **2%**.

Although a persistent non-scrotal testicular location represents an absolute indication for treatment, testicular position anomalies are nevertheless observed in adult men, even in Western European countries (Bradshaw et al. 2014; Hensel et al. 2015).

17.2 Physiology and Pathophysiology of Testicular Descent

17.2.1 Physiology

During embryonic and fetal development, descent of both testes occurs between the **7th and 38th week of gestation**. Between week 7 and 12 of gestation, the gonads migrate from the area of the renal "anlagen" through the abdomen to the inguinal canal. This is referred to as the "**transabdominal phase**"of testicular descent. The testes remain in the area above the groins until the 7th month of gestation. Around 25–28 weeks, the testes continue migration through the inguinal canals, reaching their definitive position in the scrotum between 35 and 40 weeks of gestation. This is referred to as the "**inguinoscrotal phase**"of testicular descent (Hutson et al. 2013).

Testicular migration is facilitated by the **genito-inguinal ligament (= gubernaculum)**. In addition, **hormones** are involved in the control of testicular descent. The gubernaculum consists of connective tissue linking the lower gonadal ligament to the inguinal region in the early fetus. From the 7th week onwards, the Leydig cell product, **insulin-like hormone 3 (INSL-3)**, induces a "swelling reaction" of the gubernaculum. In addition, the Sertoli cell product, **anti-Müllerian hormone (AMH)**, and the bioactive androgen 5-α-dihydrotestosterone (DHT) promote growth of the gubernaculum. The formation of both testicular hormones is stimulated by **human chorionic gonadotropin (hCG)** from the placenta until the end of the third month (10-12th week) of gestation. By week 12, the gubernaculum shortens, thereby pulling the testes, the ductus deferentes, and their vessels downwards, close to the later annulus inguinalis internus, thereby completing the "**transabdominal phase**" of testicular descent. **Testosterone and DHT** subsequently induce regression of the ligamentum suspensorium testis which is located cranially. At the same time, an outpouching of the peritoneum, the **processus vaginalis**, develops along the lower gonadal ligament. From week 9–14 onwards, fetal hypothalamic **gonadotropin-releasing hormone (GnRH)** induces fetal pituitary **LH secretion**, which in turn stimulates the secretion of testosterone by the fetal testes. From the 7th month (week 25–28) of gestation onwards, the genito-femoral nerve differentiates. The gubernaculum elongates further and migrates downwards. Under the influence of testosterone and DHT, and mediated by **calcitonin gene-related peptide (CGRP)**, the gubernaculum tightens, thereby relocating the testes through the inguinal canal, past the processus vaginalis, into the scrotum (Hughes and Acerini 2008; Hutson et al. 2009). During the first year of life, the upper part of the processus vaginalis obliterates. The lower part of it, the serous double-sheet structure of the tunica vaginalis remains present as a remnant of the bulging peritoneal cavity, ventral to the testes.

17.2.2 Pathophysiology

The causes of disordered testicular descent are complex and to this day largely unknown. **Fetal androgen deficiency** is thought to be in part responsible for incomplete testicular migration. This hypothesis arises from the observation that undescended testes are frequent in male neonates with **congenital hypogonadotropic hypogonadism (CHH)** (Pitteloud et al. 2002; Rohayem et al. 2016). In boys with CHH, there is a disturbance in fetal GnRH secretion or action with consequent intrauterine testosterone deficiency.

Inguinal or abdominal testicular locations are also regularly observed in chromosomally male individuals with an androgen receptor defect, i.e., **46,XY** individuals with **partial (PAIS) or with complete androgen insensitivity (CAIS)** (Hutson 1986).

Forty-six 46,XY individuals with 5-α-reductase deficiency and a resulting lack **of DHT** (Imperato-McGinley et al. 1974), as well as individuals with **testosterone biosynthesis defects** and with resulting undervirilization at birth are also regularly affected by cryptorchidism.

Finally, in subjects with chromosomal abnormalities, such as **Klinefelter syndrome, XX males (=XX testicular DSD) or gonosomal mosaicism, such as 46,XY/46,XX DSD**), undescended testes are observed more frequently (Hiort et al. 2005).

Isolated undescended testes, without other features of undervirilization, may also be considered a disorder of somato-sexual development (DSD).

Undescended testes are found in association with anatomic defects, such as weakness of the abdominal wall (e.g., in Prune-Belly syndrome), bladder exstrophy, omphalocele, or gastroschisis. However, it remains unclear whether there is a causal relationship here (Kaplan et al. 1986).

It has been suggested that undescended testes could be induced by **toxic** (nicotine, ethanol, and pesticides), mechanical, and neurological factors (Damgaard et al. 2008).

There are two hypotheses regarding the explanation for the **detrimental consequences of undescended testes on reproductive capacity and malignant degeneration** in affected individuals:

First, an **abnormally high-ambient temperature** of the testes when located inguinally (35–37 °C), compared to the temperature when located in the scrotum (33 °C), is believed to promote increased apoptosis of germ cells, thereby limiting fertility of affected individuals and increasing the risk of germ cell degeneration within the gonads. In a rat model, gene expression of testicular germ cells was shown to be temperature sensitive (Yadav et al. 2018).

Secondly, in boys with positional abnormalities of the testes, **inadequate gonadotropin stimulation** of the testes between 3 and 9 months of age, i.e., during "minipuberty" could impair the **transformation of gonocytes to A dark (Ad) spermatogonia** (Hadziselimovic et al. 1986; Huff et al. 2001). While some investigators observed reduced testosterone (and LH) which increases during the first 6 months of life in male infants with undescended testes (Job et al. 1987; Job et al. 1988; Pierik et al. 2009), other investigators measured normal LH, FSH, and testosterone levels (Barthold et al. 2004; Suomi et al. 2006; Cortes et al. 2016). Hadziselimovic postulated that treatment with an LHRH analogue after orchidopexy could improve fertility later in life (Hadziselimovic and Herzog 1997). Decreased Leydig cell numbers or **Leydig cell hypoplasia** in undescended testicles have been observed by one group (Gotoh et al. 1984).

17.3 Clinics

Unilateral testicular maldescent is five times more common than bilaterally undescended testes. Depending on the localization of the **undescended testis** (Fig. 17.1), a distinction is made between abdominal, inguinal, retractile, sliding, and ectopic testes.

- **The abdominal testis** (retentio testis abdominalis) is located right above the inguinal canal, either within the abdominal cavity or retroperitoneally. The abdominal testis is neither palpable nor visible. Therefore, the term **"cryptorchidism" (=hidden testis)** may also be applied.
- **The inguinal testis** (retentio testis inguinalis) is located in the inguinal canal and detectable by palpation of the groins (Fig. 17.2).
- **A retractile testis** (retentio testis praescrotalis) is located at the distal end of the inguinal canal. It can be pushed down into the scrotum, but slips back to its original position immediately after release. Retractile testicles require treatment.
- The retractile testis is to be distinguished from the **sliding testis** that lies predominantly in the scrotum if the environment is warm and relaxed. The testes ascend temporarily to the groin whenever the cremaster reflex is activated by cold or touch. This condition is normal and does not require therapeutic intervention.
- **Testicular ectopia** is present if a testis is situated outside the normal pathway of testicular descent. The condition results from an abnormal insertion of the gubernaculum. Depending on the location, the ectopy is classified as **ectopia femoralis, perinealis, praefascialis, penilis, or retrovesicalis**.
- The term "**cryptorchidism**"is—strictly speaking—used only for a non-palpable testes. However, in the English literature, it is also used as a synonym of "undescended testes." If the testes are non-palpable, there is either anorchia due to testicular atrophy during the embryonic or fetal period (**"vanishing testes"**) or the gonads are **hidden in the abdominal cavity**. Very rarely do cryptorchid testes have an **ectopic** location.

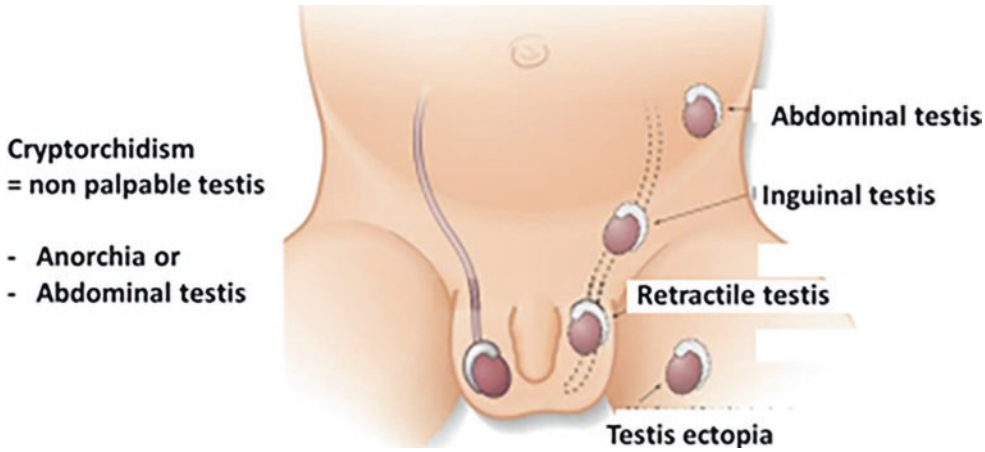


Fig. 17.1 Topography and nomenclature of maldescended testes



Fig. 17.2 A 32-year-old patient with bilateral inguinal testes. (Abb. aus 3. Auflage Abb. 13.3)

Primarily undescended testes are to be distinguished from **testes with secondary ascension after birth**. Secondary ascension occurs in 1–2% of boys; it results from inadequate longitudinal growth of the funiculus spermaticus, due to the persistence of tight connective tissue within the processus vaginalis (Clarnette et al. 1997). Importantly, these gonads are not primarily affected by dysgenesis.

17.4 Diagnostics

Undescended testes are diagnosed by **palpation** and additional **ultrasound investigation** of scrotal contents and groins.

In the infant, the examination is performed in a supine position. A warm environment is important to avoid activation of the cremaster reflex. Boys, adolescents, and adult males are examined in a standing position. The tension on the spermatic cord is investigated while attempting to pull the maldescended testis downwards into the scrotum. Testicular volumes are assessed by Prader orchidometry.

In the infant or toddler, testicular positions may be difficult to determine. It is therefore helpful to ask the parents for documentation of the observed testicular position when changing nappies or while bathing the child in the bathtub.

Ultrasonography is performed with a high-resolution (7–12 MHz) linear transducer (see Chap. 6). Abdominal testes cannot be visualized by ultrasound investigation. Imaging of abdominal testes can also be problematic when using magnetic resonance investigation (MRI) for imaging: the young child has to be sedated and intra-abdominal testicles are not always visible. That is why, in infants with true cryptorchidism, laparascopic exploration of the abdomen is preferred to assess the presence of testicular tissue. If indicated, orchidopexia can be performed at the same time.

Karyotyping is indicated in boys with additional signs of under-virilization, such as hypospadia or a small penile size ("micropenis"). Variations in sexual differentiation (DSD) with cryptorchidism include 46, XY DSD with undervilization or chromosomal DSD with the presence of cell lines containing a Y chromosome.

Determination of the Sertoli cell markers **inhibin B and AMH** in infants or pre-pubertal boys with cryptorchidism is helpful for exploring the presence of functionally active testicular tissue. If these markers are undetectable in serum, this may indicate that testes have regressed and disappeared during intrauterine development ("vanishing testes") (see Chap. 7). In this case, an additional **hCG test** is indicated, as it allows the detection of testosterone-producing gonadal tissue:

A serum sample is drawn between 8 and 10 a.m., followed by one intramuscular injection of hCG (5.000 IU/m^2 body surface). Further blood samples are drawn after 48 and 72 h. In all samples, serum testosterone concentrations are determined.

- If pre-pubertal testicular function is uncompromised, serum testosterone levels are expected to increase 10- to 20-fold from baseline after 72 (−96) h (McEachern et al. 2004).
- In the case of gonadal dysgenesis (e.g., due gonosomal mosaicism 46,XY/45,X0 or 46,XY/46,XX (see Chap. 31), hCG-testing enables exploration of the potential endocrine capacity of the pre-pubertal maldescended gonad.

Rarely, cryptorchidism is misdiagnosed as "vanishing testes" in pre-pubertal boys. However, during adolescence, spontaneous pubertal development will reveal that testicular tissue with residual endocrine function is present intra-abdominally or at another ectopic position.

17.5 Therapy

Relocation of maldescended testes to the scrotum may be achieved by either **surgical or hormonal treatment or both.** The current German guidelines recommend treatment of undescended testes before the end of the first year of life. As spontaneous descent is possible during the first months after birth, the period between the **6th and 12th month of life** represents the **optimal therapeutic window** for treatment. In pre-term infants, the chronologic age has to be corrected according to prematurity.

The European Society for Pediatric Urology Guidelines (Radmayr et al. 2017) recommend treatment of testicular positional anomalies **until 18 months of age**. Orchidopexia is considered as the therapeutic standard. The use of (neo)adjuvant hormonal therapy is recommended on an individualized basis, specifically in the case of bilaterally undescended testes.

The recommended age for treatment of undescended testes has been progressively revised downwards since the 1950s (Ritzén et al. 2007; Mathers et al. 2009; Bradshaw et al. 2014), with the intention to normalize the ambient temperature of the gonads in a timely manner and thereby favorably affect testicular function. Meanwhile, there is evidence that positioning the testes in the scrotum has beneficial effects on testicular growth, testicular germ cell count, and on the risk for testicular malignancies (Cortes et al. 2001). However, evidence for improvement of fertility outcomes in adulthood by timely treatment is still lacking (Hadziselimovic and Herzog 2001; Tasian et al. 2009).

17.5.1 Surgical Therapy

The success rates of surgical **orchiolysis**, tension-free positioning into the scrotum and **orchiopexia** depend on the initial testicular location (Taran and Elder 2006). The overall success rate of orchiopexia is ~90% (Docimo 1995). For testes situated distal to the external inguinal ring (incidence: 25%), the success rate is 92%; for inguinal testes (incidence: 69%) it is 87%; and for intra-abdominal testes (incidence: 6%) it is 74%.

If the testes are non-palpable and if there is laparascopic evidence of testes within the abdominal cavity, orchiolysis and orchiopexy are carried out in the same session. If necessary, the surgical procedure is performed in **two separate sessions**, according to **Fowler-Stephens** (Stephens 1988). If testicular atrophy is evidenced (incidence: 45%!), removal of the gonadal residual tissues, the epididymes, and the spermatic cords is indicated.

Microsurgical **auto-transplantation** of bilaterally high-abdominal testes is considered only at post-pubertal ages, in view of the small vascular diameters of pre-pubertal testes. Auto-transplantation is delicate and therefore preferentially performed in specialized centers using laparoscopically assisted techniques (LATA) (Bukowski et al. 1995). The success rates range around 86%. Anatomic changes are often present in males with abdominal testes (Han et al. 2004).

Surgery is always indicated in patients with abdominal testes and with additional inguinal hernias. The same is true for patients with ectopic testes or in conditions with previous surgery.

The most frequent **complication of surgery** is **testicular atrophy**. Atrophy occurs in 2%, of which 1% occur after ligation of the testicular artery (procedure according to Fowler-Stephens). In microvascular auto-transplantation, testicular atrophy is expected in 20–30%. Other complications include transection of the ileo-inguinal nerve, with hyposensitivity in the affected skin area, or accidental entrapment of the ileo-inguinal nerve into the fascia, resulting in pain, and injury of the ductus deferens with secondary occlusion. Secondary re-ascent of testes after orchidopexia is reported in 1–5%.

17.5.2 Hormone Therapy

According to current German guidelines, **hormone therapy** is recommended **only for retractile testes**. The Nordic countries have ceased using hormones for the treatment of undescended testes (Ritzén et al. 2007; Henna et al. 2004). The fear of **harmful effects of hCG** is based on the observation that hCG (or GnRH) and/or subsequent androgen deprivation results in increased germ cell apoptosis and reduces the number of spermatogonia/tubule, especially if hormonal treatment is performed after the first year of life (Dunkel et al. 1997; Heiskanen et al. 1996). In histological studies, hCG treatment was shown to increase the density of interstitial tissue and the density of blood vessels, in comparison to eutopic testes and in comparison with testes without hormonal treatment (Kaleva et al. 1996). In boys with undescended testes aged 1–3 years and treated with GnRH or hCG, the number of spermatogonia per tubule ("fertility index") was lower than in boys treated by surgery alone (Cortes et al. 2000). By contrast, in another study, the "fertility index" was higher in patients pre-treated with GnRH than in those untreated (Schwentner et al. 2005).

> As a consequence of the inconclusive evidence regarding harmfulness of hormonal treatment during infancy, in current guidelines, hormonal therapeutics are mentioned only as a "second line" treatment option.

Hormonal treatment of undescended testes is performed either by intra-nasal administration of GnRH or by intramuscular injection of hCG. The two therapeutic options can also be applied sequentially.

GnRH/Gonadorelin: 200-µg is administered per nostril three times daily for a period of 4 weeks.

Success rates range around 18–21% (Li et al. 2016).

hCG: 500 IU i.m. is administered once a week for 3(−5) weeks.

Success rates range around 19–25% (Pyörälä et al. 1995; Henna et al. 2004); 0–50% for unilaterally maldescended testes and 0–22% for bilaterally maldescended testes (Wei et al. 2018).

In boys aged 2–6 years, the hCG dosage is doubled (1000 IU hCG per injection); it is quadrupled (2000 IU hCG per injection) in boys over 6 years of age.

Hormone therapy is more successful in testes that have partially and spontaneously descended previously (Kaleva et al. 1996). Additional administration of FSH does not lead

to better therapeutic results regarding testicular position (Hoorweg-Nijman et al. 1994).

The **re-ascension rate** after successful hormone therapy is 24% (Pyörälä et al. 1995).

Possible **side effects of hormone therapy** are frequent spontaneous erections, penile growth, pain, and growth of pubic hair.

In neonates and infants with bilaterally undescended testes, combined with a **micropenis**, or in neonates with **additional neonatal hypoglycemia and/or prolonged jaundice** (as symptoms of congenital anterior pituitary insufficiency), hormone replacement should be given special consideration as a therapeutic option. A **central disturbance of the gonadotropic axis** is the most likely underlying cause; therefore, replacement of gonadotropins within the first months of life (i.e., during minipuberty) could be of benefit, not only with regard to the induction of spontaneous testicular descent, but also with regard to future fertility.

Combined gonadotropin substitution (rLH and rFSH) applied subcutaneously or via an insulin pump has proven very successful regarding spontaneous descent into the scrotum (Bougnères et al. 2008; Lambert and Bougneres 2016; Kohva et al. 2019), even for intraabdominal testes (Papadimitriou et al. 2019).

In **adolescents** with **congenital hypogonadotropic hypogonadism (CHH)** and with previously **untreated undescended testes**, combined gonadotropin replacement with hCG and rFSH, used with the goal of inducing pubertal maturation, can also initiate late spontaneous testicular descent (own observations).

17.6 Effects of Undescended Testes on Testicular Function in Adulthood

17.6.1 Testicular Size

Unilateral or bilateral testicular positional anomaly may result in inadequate growth of the affected testis during prepuberty and puberty, even if it has been treated successfully (Kollin et al. 2007; Kollin et al. 2012; Varela-Cives et al. 2015; Sadov et al. 2016).

At puberty, significant psychological distress may occur, resulting from small testes due to testicular atrophy, or in the case of anorchia due to the absence of testes in the scrotum. In these cases, implantation of **testicular prostheses** may provide cosmetic relief.

17.6.2 Endocrine Testicular Function

Undescended testes at birth may affect testicular Leydig cell function in adulthood. Enhanced LH serum concentrations with either normal or slightly decreased serum testosterone concentrations may be observed (**compensated or decompensated hyper-gonadotropic hypogonadism**) (Rohayem et al. 2017). Only endocrine **decompensatio**n of hypogonadism entails a lifelong need for testosterone supplementation. This is the case in boys with testicular atrophy or profound gonadal dysgenesis, where testosterone replacement has to be applied for induction of puberty.

17.6.3 Spermatogenesis and Paternity Rates

Undescended testes at birth have consequences on fertility: in men with one undescended testis, sperm counts in the ejaculate are reduced to the level of **oligo- or azoospermia** in 70%. In those with bilaterally undescended testes at birth, 90% have bad semen quality (Yavetz et al. 1992; D'Agostino et al. 1996; Cortes et al. 2001). **Paternity rates** in men with bilaterally undescended testes are reported to range around 65%, versus 88% in those with a unilaterally undescended testis and versus 93% in the control population (Lee and Coughlin 2001; Miller et al. 2001; Lee 2005).

17.6.4 Risk of Germ Cell Tumor

The **incidence of a testicular tumor** following positional anomalies of the testes is 1:2000 men (incidence in men without undescended testes: 1:10,000 men), which corresponds to a 5-fold (2.75- to 8-fold) increase of the risk for tumor development, even after previous successful treatment (Giwercman et al. 1987; Pettersson et al. 2007). Surprisingly, in 20%, the contralateral testis is affected by a testicular tumor. It has been shown that orchiopexia performed before 12 years of age may reduce the risk of malignant degeneration to 2- to 3-fold (compared to control population) (Pettersson et al. 2007; Cortes et al. 2001).

17.6.5 Therapeutic Options for Azoospermia

Men with azoospermia, with desire for biologic paternity, have the option to attempt surgical **retrieval of testicular sperm (TESE)**.. To date, there is no valid predictive marker that could pre-operatively indicate whether there are "islands" of intact spermatogenesis in the formerly undescended testes. Microsurgical testicular sperm extraction (m) TESE has been shown to be superior to classic

TESE. Combined with **intracytoplasmic sperm injection (ICSI**), it can enable biological paternity in 30–40% of men with non-obstructive azoospermia (Vloeberghs et al. 2015). However, in men with **untreated cryptorchidism** who display azoospermia and who undergo orchidopexy as adults, the chances to find testicular sperm by mTESE are minimal (Muncey et al. 2021).

Key Points

- The causes of undescended testes are complex and largely not understood.
- Long-term consequences of undescended testes include infertility due to oligo- or azoospermia, testicular tumor development, and impaired Leydig cell function. Endocrine testicular dysfunction may manifest as compensated hypogonadism. Decompensation of hypergonadotropic hypogonadism is rare and occurs as the consequence of testicular atrophy following surgery or of profound testicular dysgenesis; in these males, pubertal induction by testosterone substitution is indicated, followed by lifelong hormone replacement.
- Early correction of undescended testes may be achieved by hormonal or surgical means. It may reduce the risk of malignancy and may have a beneficial effect on endocrine and spermatogenic testicular function in adulthood.
- Currently, the therapeutic gold standard for undescended tested at birth is surgical orchidopexia before the end of the first year of life. As an exception, replacement of gonadotropins is very successful in inducing testicular descent in infants in whom gonadotropin deficiency is causative for the condition.

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Varicocele

18

Eberhard Nieschlag, Sabine Kliesch, and Hermann M. Behre

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Abstract

Varicocele is the enlargement, elongation, and varicose tortuosity of the pampiniform plexus in the scrotum. Approximately, 25–35% of infertile men have a clinical varicocele, but a similar prevalence is also found in fertile men. Varicocele can lead to decreased blood flow and volume depletion of the testis, as well as to elevation of scrotal temperature, oxidative stress, and increased DNA fragmentation, which are discussed as causes of infertility. Diagnosis must include a thorough physical and sonographic examination with Valsalva maneuver. Familial clustering of varicocele suggests genetic or epigenetic causes. Surgical and angiographic procedures aimed at occlusion of the spermatic vein are treatment modalities. Recent guidelines are cautious about interventional therapy. Treatment of subclinical varicocele is discouraged. If at all, treatment is most likely to be considered in men with clinical varicocele and abnormal ejaculate levels in the setting of otherwise unexplained couple infertility and adequate ovarian reserve in the female. Long-term fertility and paternity studies are lacking to assess the success of varicocele treatment in adolescents.

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18.1 Epidemiology

Varicocele is the widening, lengthening, and varicose-like tortuosity of the internal spermatic veins forming the **pampiniform plexus** in the scrotum. The prevalence is 25–35% in infertile men (Alsaikhan et al. 2016). However, since normally fertile men have a comparable prevalence of clinical varicocele of 33% (Lotti et al. 2020), a causal association between varicocele and infertility still remains a matter of debate.

The **prevalence of varicocele** is high, but the data vary considerably. This is partly due to the composition of the groups studied. Furthermore, despite all attempts at objectification, the diagnosis of varicocele is subject to subjective influences. This is especially the case with the less pronounced

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varicoceles I° and II°. In 3 million young men born between 1937 and 1945, 17% were found to have a varicocele when they were recruited for the German armed forces (Nöske and Weidner 1999). Using strict diagnostic criteria, a prevalence of 23.3% was found among sperm donors (Handelsman et al. 1984) and 25.5% among volunteers in clinical studies (Lemcke et al. 1996). A recent multicenter study by the European Academy of Andrology (EAA) demonstrated a prevalence of clinical varicocele of 33% in normal fertile men (Lotti et al. 2020). A recent meta-analysis concluded that the proportion of men with varicocele ranges from 25% to 35% in patients with fertility disorders (Alsaikhan et al. 2016). Thus, although varicocele is the most common pathologic finding after **idiopathic infertility** detected in men attending fertility consultations, this fails to analyze its significance and does not indicate anything about the value of the finding.

18.2 Pathophysiology

Varicose changes are favored by the elongated, free course of the testicular vein in the retroperitoneum without a supporting muscle pump, congenital vascular wall weaknesses or atony of the cremasteric muscle partially accompanying the spermatic vein. It was long thought that insufficiencies or aplasias of the valves of the spermatic vein might be the cause of varicocele formation. However, cadaver studies and angiographic results showed that the spermatic vein and pampiniform plexus of men without varicocele show no or hardly any valves (Ergün et al. 1996). Probably because of the hemodynamically less favorable confluence of the left spermatic vein with the renal vein—the right spermatic vein terminates directly in the inferior vena cava—a **varicocele is found on the left side in about 95%** of patients and rarely on the right or both sides. If the internal spermatic vein is compressed (e.g., by a renal tumor), the condition is referred to as a **secondary varicocele**.

Ultimately, it is not clear by what mechanism a varicocele affects fertility. A **reduced blood supply** to the affected testis due to increased venous pressure, leading to atrophy with typical **testicular volume reduction**, **oxidative stress,** or a **lack of removal or reflux of toxic substances** from the kidney are discussed.

Continuous measurements of **scrotal temperature** over 24 h have shown that a testis surrounded by a varicocele has a significantly higher temperature (35.6°C) than an unaffected testis (34.8°C) (Garolla et al. 2015). Since optimal spermatogenesis requires lower temperatures than the core body temperature, the testes are located in the scrotum and the elevated temperature may cause disruption of spermatogenesis. The thermal changes may explain the infertility but do not indicate the cause of varicocele. The higher sperm **DNA fragmentation index (DFI)** may also be considered a consequence of varicocele (Zhang et al. 2021).

The occasional **familial occurrence of varicocele** suggests that **genetic or epigenetic factors** may play a role in the causality of varicocele. Until recently, "elusive genes" were referred to in the context of varicocele (Santana and Esteves 2021). Nevertheless, three candidate genes have been identified in families with varicocele: *AAMP, SPINT*, and *MKI67* (Yang et al. 2021). These genes have been implicated in angiogenesis and support the hypothesis that the development of varicocele may have a genetic background (Santana et al. 2017).

18.3 Influence of Varicocele on Fertility

The causal **relationship between fertility and varicocele** is difficult to assess due to the complexity of the couple's fertility issues. The fact that a varicocele does not preclude fertility leads some investigators to see no association. However, mild fertility disorders in one partner may be compensated by particularly good reproductive functions in the other partner. Only when the disturbances coincide in both partners does the problem of infertility arise. Recent studies of fathers with and without varicocele show no effect of clinical varicocele on ejaculate levels (Lotti et al. 2020). Nor does age seem to have a significant effect—in our own patient population, no increase in the prevalence of varicocele with advancing age of infertility patients can be detected; the prevalence remains as high in patients over 50 years of age as in patients 30 years of age.

Thus, to date, there is no clear evidence that the varicocele itself reduces fertility, and the hypothesis that the varicocele is at most a cofactor for infertility in the context of other **genetic or molecular events** is gaining ground.

Overall then, uncertainty remains regarding the prevalence of varicocele in the normal population and in men attending fertility consultations.

18.4 Clinic

Infertility observed in varicocele manifests in the spermiogram by **oligo-**, **astheno-**, **or teratozoospermia** or a combination of these findings in variable expression (**OAT syndrome**), without proving a causal relationship. Varicoceles may also occur in patients with azoospermia. Varicoceles may also be associated with an increase in **FSH levels**, which indicates damage to the testicular germinal epithelium and is a poor prognostic sign. Because Leydig cell function may also be affected, some patients have **low testosterone** levels (Li et al. 2012). It should be emphasized that not all men with a varicocele present with limitations in ejaculatory parameters and fertility.

Some patients report feelings of pressure, swelling, and occasionally **pain in the affected testis or scrotum**, which may intensify after prolonged standing or sitting in an

unchanged position or with increased physical or athletic activity. The cut and fit of pants and underpants can cause pain. Patients with pain usually present with a grade III varicocele (see below) (Baek et al. 2019). Pain symptoms usually lead to earlier diagnosis and surgical therapy than infertility. Occasionally, a varicocele may become so large that it presents a **mechanical obstruction** and therefore requires treatment. It is not uncommon for patients to find large varicoceles cosmetically disturbing as well.

18.5 Diagnosis

Careful palpation of the pampiniform plexus on a standing patient is an important diagnostic measure. **Valsalva maneuver** can provoke filling of the pampiniform plexus. Varicoceles that have existed for a long time may also lead to a reduction in volume and consistency of the affected testis. According to palpation findings, varicocele is classified into **four degrees of severity**:

- **Subclinical varicocele:** not visible or palpable, but presentable with Doppler sonography.
- **Varicocele I°:** palpable dilatation of the pampiniform plexus only on Valsalva maneuver.
- **Varicocele II°:** clearly palpable dilatation of the pampiniform plexus.
- **Varicocele III°:** visible dilatation of the pampiniform plexus (Fig. 18.1).



Fig. 18.1 A 28-year-old patient with varicocele III°

While varicoceles III° are easy to detect clinically, the diagnosis of varicoceles of lesser severity is highly dependent on the experience of the examiner. In addition, palpation may be complicated by previous surgery, hydrocele, or a high-scrotal location of the testes. In this case, **Doppler sonographic** and **imaging sonography** are indispensable examination methods to ensure diagnosis. Duplex sonographic can objectify the widening of the diameter of varicose veins and reflux in the **Valsalva maneuver** (Pilatz et al. 2011). Similarly, the success of treatment can be assessed sonographically in the Valsalva maneuver (Cavallini et al. 2019; Bertolotto et al. 2021) (see Chap. 6).

18.6 Influence of Varicocele Therapy on Chances of Fertility

Because therapy for varicocele offers an outstanding example of **evidence-based medicine**, the historical development of the assessment of therapeutic effects is presented here. The focus is on what is judged to be the therapeutic criterion: **varicocele elimination**, **ejaculation parameters, pregnancy rates**, or **live births**.

Since the early 1950s, **ligation of the spermatic vein** in the presence of a varicocele has been an integral part of the therapeutic measures that the andrologist can offer for male fertility disorders. A review of 50 publications, including a total of 5471 patients, showed a mean pregnancy rate after ligation of the spermatic vein in varicocele of 36% with a range of variation from 0 to 50% (Mordel et al. 1990). The wide range of variation is probably explained by the very heterogeneous patient groups in terms of size, composition, and observation period—mostly without a control group. Overall, the larger, more representative studies are interpreted as indicating that surgery has a beneficial effect on fertility. Thus, treatment of varicocele formed an oasis of hope in the otherwise bleak landscape of ineffective drug therapy attempts for male infertility. This general attitude was not changed by the development of new angiographic techniques for occlusion of the spermatic vein.

Notwithstanding, little attempt was made to subject the invasive procedure to critical evaluation. However, some studies provided evidence that this was urgently needed. The first of these studies concluded that the **pregnancy rate** in varicocele with and without treatment was about the same if only the partner was adequately treated (Rodriguez-Rigau et al. 1978). Another study, but equally subject to the criticism of a lack of an adequate control group, reached similar conclusions (Vermeulen et al. 1986). However, randomization was performed in a third study, which again showed no better pregnancy rate after varicocele treatment than without treatment (Nilsson et al. 1979).

Against this background, several controlled studies of the effectiveness of therapy for varicocele have been conducted in the spirit of **evidence-based medicine**. These studies not only had the physical success of the intervention (**negative Valsalva maneuver**) as the target parameter, but also take into account the resulting **pregnancy rates** in particular, in addition to the **ejaculate parameters**. A small study of 20 infertile couples concluded that invasive treatment of varicocele significantly increased the chances of inducing pregnancy in the following year (Madgar et al. 1995). This study is part of a larger WHO multicenter study that yielded similar results. It included 238 couples and resulted in 35% pregnancies in the ligation group versus 17% in the untreated group. However, this study was not published in a peer-reviewed journal because of massive criticism of its design (Hargreave 1997), and its validity must be doubted. In contrast, we performed a unicenter study in which the same pregnancy rates resulted in the year following surgical (high ligation) or angiographic occlusion treatment as when patients with varicoceles were treated noninvasively (pregnancy rates of 29% in 62 couples versus 25.4% in 63 couples) (Fig. 18.2) (Nieschlag et al. 1998). No differences were found between the two groups in the duration of time until pregnancy occurred. However, regardless of group membership, the partners of patients who induced pregnancy were significantly younger than those whose partners did not become pregnant within 12 months (28.8 ± 0.6 vs 31.2 ± 0.3 years). Because comparable criteria were chosen for patient selection, the results of the two studies mentioned above initially appear contradictory. In contrast to the WHO study, in which the non-treated couples were left to their own devices for 1 year, the patients in our study presented every 3 months for an **examination and detailed consultation**, and the partner's reproductive functions were regularly checked by the gynecologist and—if necessary—optimized. This study suggests that regular and intensive examination, counseling, and follow-up of these couples may be as successful in achieving pregnancy as interventional treatment of varicocele.

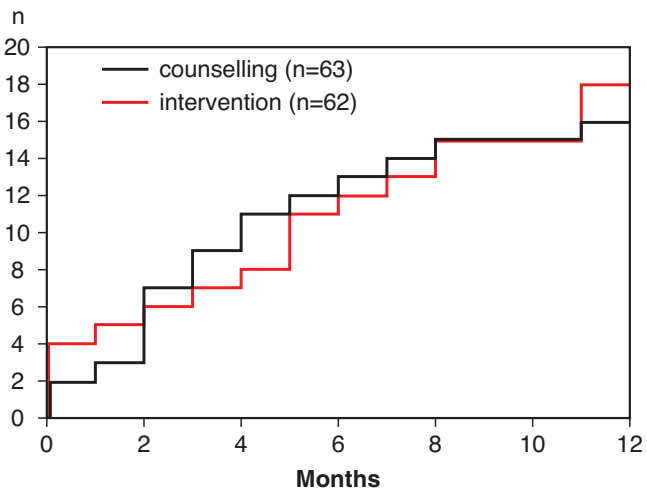


Fig. 18.2 Cumulative pregnancy rates over 12 months in 62 untreated and 63 surgically or angiographically treated patients with varicocele (after Nieschlag et al. 1998)

Subsequently, further studies (Grasso et al. 2000; Unal et al. 2001) on the therapy of varicocele were conducted under the auspices of the German Society of Urology (Krause et al. 2002), among others. None of these studies shows on its own a treatment effect with regard to an improvement in pregnancy rates.

In a meta-analysis, it was found that a total of 41 patients had to be treated to achieve an additional pregnancy (Kamischke and Nieschlag 2001). A meta-analysis including all previous peer-reviewed randomized trials of varicocele therapy concluded that the treatment of varicocele did not lead to an increase in pregnancy rates (Evers and Collins 2003). Similarly, ICSI treatment did not result in differences in patients with varicocele whether they were previously treated or not: it did not affect pregnancy rates (Pasqualotto et al. 2012).

Thus, the impact of varicocele therapy on **pregnancy rates** remained controversial. For example, a review focusing exclusively on surgical procedures and including not only two randomized but also three nonrandomized controlled trials in patients with higher-grade varicoceles and disorders of ejaculatory parameters finds a significant improvement in pregnancy rates in the operated group (Marmar et al. 2007).

However, taking into account all the controversies surrounding varicocele therapy, a **Cochrane Review** also states, "There is no evidence that treatment of varicoceles in men from couples with otherwise unexplained subfertility improves the couple's chance of conception" (Evers et al. 2008).

With adherence to strict study criteria and improvement in study quality, outcomes have also improved. For example, a further **Cochrane Review** from 2012 concluded that if occlusion therapy is used in the presence of a clinically manifest varicocele (grade III) and sub-fertile ejaculate parameters are present, seven patients need to be treated to achieve an additional pregnancy (NNT = number needed to treat) (Kroese et al. 2012).

The current **update of this Cochrane Review** concludes, "Based on the limited evidence, it remains uncertain whether any treatment (surgical or radiological) compared to no treatment in sub-fertile men may be of benefit on **live birth rates**; however, treatment may improve the chances for pregnancy. The evidence was also insufficient to determine whether surgical treatment was superior to radiological treatment. However, **microscopic sub-inguinal surgical treatment** probably improves **pregnancy rates** and reduces the risk of varicocele recurrence compared to other surgical treatments. High-quality, head-to-head comparative RCTs focusing on

live birth rate and also assessing adverse events and quality of life are warranted"“(Persad et al. 2021).

Further studies need to provide clarity and consider the **placebo effect** of physician action. Such studies may lead to the identification of subgroups in need of treatment. However, it will hardly be possible to conduct a **definitive study**, as it would have to include a sham-operated group in addition to an operated, a non-treated, and a placebo group, which is difficult to justify ethically because of the risk of surgery and anesthesia, but could certainly find supporters given the dimension of the problem (Horing and Miller 2002). Furthermore, it is becoming increasingly difficult to recruit patients for such a study, as they are unlikely to wait, given assisted fertilization procedures (Zini et al. 2008; Silber 2018).

In light of this, the recent **EAU Guidelines on Sexual and Reproductive Health** (Salonia et al. 2021) recommend:

- Do not treat men with varicocele and normal sperm parameters and men with subclinical varicocele.
- Treat men with clinical varicocele and abnormal sperm parameters in the presence of otherwise unexplained infertility in the couple and good ovarian reserve in the woman to increase fertility rates.
- Treatment of varicocele may be considered in men with increased DNA fragmentation after repeated failed assisted reproduction procedures.

The **European Academy of Andrology (EAA)** in its **Guideline on Management of Oligo-Astheno-Teratozoospermia** (Colpi et al. 2018) recommends a cautious approach given the paucity of evidence, advising only a nuanced discussion of potential therapy with the affected couple in cases of OAT and palpable varicocele. In addition, treatment of subclinical varicocele is rejected.

18.7 Treatment Procedures

If a varicocele is to be treated interventricularly, the following **minimum requirements** for an indication must be considered:

- unfulfilled desire to have a child for at least 1 year,
- varicocele confirmed by somatic and ultrasonographic examination,
- smaller testicular volume on the affected side,
- subnormal ejaculate parameters,
- FSH not elevated above normal range,
- largely clarified female reproductive functions without untreatable disorders.

Varicoceles that cause **pain in the scrotum** or form a **mechanical obstruction** due to their size may be cause for interventional treatment regardless of an existing desire for offspring.

Once the indication for therapy has been established, surgical or angiographic procedures are available for treatment. All of these procedures have the same goal of interrupting the main branch or branches of the spermatic vein and directing venous return to collaterals with sufficient venous valves.

Surgical procedures: The classic procedures require surgery whereby ligation of the internal spermatic vein is performed. While open surgical procedures were standard in the past, nowadays the laparoscopic approach and microsurgical procedures are preferred. Both high ligation from a skin incision medial to the left anterior iliac spine with the "highest" possible ligation of the spermatic vein near its junction with the renal vein and deep ligation from a left suprapubic incision with ligation of the veins underlying the spermatic fascia allow selective sparing of the testicular artery or the accompanying lymphatics. In a comparative randomized study of all three surgical procedures, increased sperm concentration and motility were found in the group with the microsurgical procedure, with no significant difference between the three procedures in pregnancy rates at 1 year (Al-Said et al. 2008). With regard to sperm parameters, a meta-analysis has shown that grade III varicoceles benefit most from treatment (Asafu-Adjei et al. 2020).

Surgical success can be demonstrated by flaccidity of the venous convolute palpable in the scrotum, a negative Valsalva maneuver, and the absence of reflux documentable by Doppler and ultrasonography. About 5% of operations do not produce this result, and recurrences may occur in about 10%. In the longer term, success can be recorded by increasing testicular volume, which can be accurately measured ultrasonographically, and improvement in ejaculate parameters. Simultaneous ligation of the lymphatic vessels running along the spermatic vein may result in hydrocele in about 5%. Other postoperative complications such as epididymitis or hematoma are rare at 1 to 3%. The lowest complication and recurrence rates are described for microsurgical varicocele ligation (Salonia et al. 2021).

Angiographic procedures: Interventional radiology procedures for occlusion (sclerotherapy or embolization) of the spermatic vein emerged from attempts to clarify causes of surgical failure by phlebography. In the late 1970s, **sclerotherapy** of the spermatic vein using hypertonic solution applied into the vein was first described. This was followed by angiographic occlusion of the spermatic vein by means of tissue glue polymerizing in situ or by balloons or coils inserted into the vein. Generally, the guiding catheter is inserted from the right femoral vein under local anesthesia. In a comparison between sclerotherapy and surgical procedures, both resulted in better sperm parameters, but the pregnancy rate was higher with sclerotherapy (28%) than with varicocelectomy (13%) (Mongioì et al. 2019).

The complications of surgical procedures observed due to impaired lymphatic drainage do not exist with angio-

graphic procedures. However, the sclerosant may cross over into the testicular or renal region, leading to thrombophlebitis, epididymitis, testicular atrophy, and renal capsulitis. These disadvantages do not exist with the tissue adhesive embolization procedure. Also, the recurrence rate is lower with embolization than with sclerotherapy. The main advantage of angiographic procedures is that they are performed on an outpatient basis, and there is no need for hospitalization and general anesthesia required for surgical procedures. The shorter time required to perform the procedure is perceived as advantageous by the otherwise healthy patient. However, they are associated with undesirable radiation exposure to the gonadal area in the usually young male, and recurrence rates are the highest in comparison (13%) and are particularly high in third-degree varicoceles. Thus, these procedures appear unsuitable in clinically relevant varicoceles nowadays (Ragab and Kliesch 2017).

18.8 Varicocele in Adolescents

Varicocele in the adolescent generally raises the question of the **prophylactic approach** with regard to later fertility in adulthood. As transverse and longitudinal observations show, varicoceles develop in parallel with testicular development with the onset of puberty. Before that, there is practically no varicocele. In 6200 Bulgarian boys aged 10–19 years, the incidence of varicocele averaged 7.9%, but was significantly higher in older boys than in younger ones (Kumanov et al. 2008). In 2470 Polish boys aged 10–20 years, first-degree varicoceles were found in 18%, second-degree in 12%, and third-degree in 5% (Niedzilski et al. 1997). With the completion of sexual development, the prevalence of varicocele reaches a plateau.

Prophylactic ligation or **occlusion** of the spermatic vein is advocated by several authors. Adequate studies to support this recommendation are lacking. A growth or volume of the affected testis lagging behind the contralateral testis has been documented as a sign of reduced supply and morphologic changes of the intratesticular vessels and the seminiferous epithelium as in adults with varicocele have been described. However, whether normalization of testicular volume and unrestricted fertility can be achieved by early therapy has not been demonstrated. In the short term, however, an increase in testicular volume in the sense of a catch-up growth and—if investigated at all—an improvement of sperm parameters can be observed (Laven et al. 1992). However, this phenomenon is also observed spontaneously (Chu et al. 2016). However, **recurrence rates** and **long-term fertility studies** are not known. To definitively answer the question of early prophylactic varicocele treatment, at least 10- to 15-year studies are needed, which investigators usually shy away from.

Under these circumstances, a more cautious approach is recommended primarily because long-term studies of subsequent fertility and paternity are lacking (Silay et al. 2019; Kliesch 2020). The finding of a varicocele should not automatically imply therapy. First, a careful somatic and physical diagnosis should be made. Particular attention should be paid to accurate recording of testicular volumes. As soon as possible, ejaculate parameters should be examined (it is usually more difficult to convince parents than sons of the necessity of this measure). This should be followed by an observation period of 1–2 years, during which the development of varicocele, testicular volumes and ejaculate and hormone parameters are checked. Only at the end of puberty can a decision be made as to whether **subnormal sperm parameters** are an expression of incomplete pubertal development or are due to the varicocele. If the varicocele is progressive, testicular volume is significantly decreased, ejaculate parameters remain subnormal, and **testosterone levels** do not increase, treatment can be considered (Chiba et al. 2016). A particularly large varicocele, which may present a mechanical obstacle, may prompt earlier indication. Treatment should be followed by **long-term follow-up** of physical examinations and laboratory parameters, approximately at annual intervals.

Key Points

- Varicocele is the enlargement, lengthening, and varicose-like tortuosity of the pampiniform plexus in the scrotum.
- Fertile and infertile men have a comparably high prevalence of clinical varicocele.
- Varicocele can lead to decreased blood flow and volume depletion of the testis, as well as elevation of scrotal temperature, oxidative stress, and increased DNA fragmentation, which are discussed as causes of infertility.
- Diagnosis must include a thorough physical and sonographic examination with Valsalva maneuver.
- Familial clustering of varicocele suggests genetic or epigenetic causes.
- Surgical and angiographic procedures aimed at occlusion of the spermatic vein are available for treatment.
- Recent recommendations are cautious about interventional therapy. Treatment of subclinical varicocele is not recommended. If at all, treatment is most likely to be considered in men with clinical varicocele and abnormal sperm parameters in the setting of otherwise unexplained couple infertility and good ovarian reserve in the female.
- To assess the success of treatment of varicocele in adolescents, long-term studies in terms of infertility and paternity are lacking.

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Orchitis

19

Hans-Christian Schuppe and Adrian Pilatz

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Abstract

Testicular inflammation may occur with acute symptoms or evolve as chronic asymptomatic disease. Among the acute forms, epididymo-orchitis due to local ascending bacterial infection is the most common, whereas isolated orchitis associated with systemic viral infection is considered to be rare. Complications include obstruction of the seminal ducts, post-infectious inflammatory reactions, testicular atrophy, and infertility. In addition, sterile orchitis can occur in systemic autoimmune diseases. Immune cell infiltrates frequently found in testicular biopsies of infertile males may be defined as low-grade, chronic asymptomatic testicular inflammation. The pathogenesis includes post-infectious or primarily pathogen-independent autoimmune responses. While treatment guidelines have been established for acute bacterial epididymo-orchits, treatment of post-infectious or sterile chronic testicular inflammatory reactions is predominantly empirical. In cases of persistent azoospermia, surgical sperm retrieval procedures are the only option.

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19.1 Epidemiology

Confirmed epidemiologic data on the incidence of testicular inflammation are not available. **Epididymo-orchitis** secondary to acute epididymitis is the most common, taking an incidence of epididymitis of 250–650 cases per 100,000 men per year into account (Nickel et al. 2005; Nicholson et al. 2010) (Chap. 26). In up to 90% of cases, acute epididymitis is associated with concomitant orchitis (Pilatz et al. 2019a). In contrast, **isolated clinically symptomatic orchitis** is considered rare. Based on submitted orchiectomy specimens from a pathology institute, early calculations indicated an incidence of 1.5/100,000, whereas more recent data from a prospective clinical study indicated 14/100,000 men per year (Mikuz 1978; Pilatz et al. 2019a). In addition to acute orchitis, a high rate of asymptomatic or **subclinical testicular inflamma-**

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tory reactions is to be expected, both primary chronic events and residuals after acute disease (Schuppe et al. 2008).

Although **infections and inflammation of the genital tract** are among the most frequent causes of male infertility with a prevalence of 10–15% (Tüttelmann and Nieschlag 2010; Olesen et al. 2017), no conclusions can be drawn from the available data regarding the occurrence of testicular inflammatory reactions. These remain unconsidered in the WHO criteria for the diagnosis of "**male accessory gland infections**" (MAGI), as is a differentiation between infectious and primary pathogen-independent processes (Chap. 26). While anamnestically only about 2–3% of patients evaluated for infertility report previous (epididymo)-orchitis, testicular biopsies from men with azoospermia show focal inflammatory reactions in about 25–30% of cases (Olesen et al. 2017; Fijak et al. 2018). Patients with asymptomatic inflammatory reactions in the testis and/or epididymis probably constitute a part of the large collective of men with so-called idiopathic infertility.

19.2 Pathophysiology

19.2.1 Basic Immunobiology of the Testis

The testis represents a so-called **immunologically privileged site** into which foreign tissue grafts can be introduced without causing rejection. Its physiological importance lies in the avoidance of immune system activation by specific antigens expressed by meiotic and post-meiotic germ cells in the testis with the onset of puberty (Fijak et al. 2018; Chaps. 26 and 28). Among the essential elements of this particular immune regulation is the sequestration of germ cell-specific neoantigens through "tight junctions" between neighboring Sertoli cells, the **blood–testis barrier** (Chap. 2). According to recent findings, antigenic material nevertheless enters the interstitium and thus comes into contact with immune cells; this allows the development of systemic tolerance (Tung et al. 2017; O'Donnell et al. 2021).

In this context, **antigen-presenting dendritic cells and regulatory T lymphocytes** play an important role, although they are present in very low numbers in the normal human testis (Duan et al. 2011; Klein et al. 2016). The most abundant cell type among testicular immune cells are resident macrophages, whose phenotype is likely shaped by the local microenvironment (Hedger 2011) (Chap. 26). **Macrophages**, with their pro-inflammatory properties, are involved in innate and adaptive immune responses, but under physiological conditions, a polarization towards anti-inflammatory functions, including anergy to inflammatory stimuli, predominates in the testis (Bhushan et al. 2020). The testicular milieu is determined by immunoregulatory and **immunosuppressive factors** (e.g., the cytokines TGFß and IL-10, activin, androgens, and others) provided by both resident immune cells and other somatic cells of the testis (Sertoli, Leydig, peritubular cells) (Fijak et al. 2018; Bhushan et al. 2020). Besides macrophages, **mast cells** are the second most abundant resident immune cells in the human testis and are also involved in tissue homeostasis as well as innate and adaptive immune responses (Mayerhofer et al. 2018).

Despite the immunosuppressive milieu, inflammatory responses can arise in the testis, as evidenced by animal models of bacterial epididymo-orchitis or experimental autoimmune orchitis (EAO) (Fijak et al. 2018; Klein et al. 2020). This is also true for the human testis, where both local and systemic infections as well as other factors lead to inflammatory processes with subsequent impairment of spermatogenesis, and more rarely, of androgen production (Schuppe et al. 2008). Under conditions of inflammation, non-resident immune cells are recruited and activated, apparently not subject to the control mechanisms of immune privilege (Bhushan et al. 2020). In the EAO model, regulatory T lymphocytes accumulate in the course of inflammation but do not prevent activation and proliferation of pathogenic autoreactive T effector cells (Lustig et al. 2020).

19.2.2 Etiopathogenesis and Classification of Testicular Inflammatory Reactions

Clinically, the term "orchitis" is associated primarily with infectious inflammation of the testis that is symptomatic in its acute stage, but a comprehensive classification of testicular inflammatory reactions should also include pathogen-independent causes (Table 19.1). In addition to etiologic factors, the classification of orchitis is based on histomorphologic criteria such as type, localization, and extent of inflammatory infiltrates or clinical course (acute, chronic, recurrent).

Bacteria play a major role as a **cause of inflammation** in the male genital tract (Table 19.1); irreversible damage may occur, particularly in the testis and epididymis, in view of the action of pathogens or pathogen components as well as inflammatory cells and their mediators (Schuppe et al. 2017; Chap. 26). The **ascension of bacterial pathogens from the urethra** via the prostate and the ductus deferens into the epididymis is particularly relevant. Through the epididymal duct, which is approximately 6 m long, the pathogens can eventually reach the testis, leading to epididymo-orchitis. The concept of ascension, which has been demonstrated in animal experiments, was confirmed clinically by comparative pathogen diagnostics from urine/urethra and epididymal aspirate/tissue (Pilatz et al. 2019a). In contrast, studies detecting bacteria in human testicular tissue yielded conflicting findings, e.g., for *Chlamydia (C.) trachomatis* in testicular biopsies from infertile men (Bryan et al. 2019; Pilatz

Table 19.1 Etiopathogenesis and classification of orchitis (modified from Schuppe et al. 2008; Fijak et al. 2018)

Etiologic factors		Pathomechanism	Clinical manifestation
Microorganisms	**Bacteria** Sexually transmitted infections (*Chlamydia trachomatis, Neisseria gonorrhea* and others); uropathogens (*E. coli, Enterobacteriaceae spp.* and others)	Intracanalicular ascending infection[a]	Epididymo-orchitis ("bacterial orchitis," formerly also called "non-specific orchitis")
	Mycobacterium (M.) tuberculosis, M. leprae, Treponema pallidum, Brucella spp.	Intracanalicular ascending and/or hematogenous infection[a]	Predominantly granulomatous orchitis (formerly also called "specific orchitis")[b]
	Viruses Mumps, Coxsackie, Epstein-Barr, influenza-, varicella-zoster-, human immunodeficiency virus, coronaviruses (SARS-CoV-1/-2) etc.	Hematogenous infection	Orchitis > epididymitis (formerly also called "concomitant orchitis")
	Fungi *Candida albicans*, *Histoplasma capsulatum* **Parasites** *Trichomonas vaginalis*, Schistosoma spp., filariasis	Intracanalicular ascending infection	Epididymitis > orchitis
Chemical noxae	Pharmaceuticals (e.g., immune checkpoint-inhibitors, amiodarone); heavy metals (e.g., mercury compounds)	Unknown	Orchitis; epididymitis
Physical factors	Genital trauma, vasectomy	Obstruction	Epididymitis > orchitis
Unknown	**Systemic autoimmune diseases** Behçet's disease, systemic lupus erythematodes, Schönlein-Henoch purpura and other systemic vasculitides, etc.	Autoimmune vasculitis	Orchitis > epididymitis
	Sarcoidosis	Unknown	Granulomatous orchitis
	Idiopathic	Autoimmune inflammation (?)	Granulomatous orchitis

[a] Predominant route of infection; locally also lymphogenic spread possible
[b] To be differentiated from primary chronic, "idiopathic" granulomatous orchitis

et al. 2019b). In systemic bacterial infections such as tuberculosis, both intracanalicular ascending and hematogenous spread are possible (Table 19.1).

Inflammation primarily affecting the testis is observed as a result of **hematogenous spread of systemic, mostly viral infections** in the sense of isolated orchitis, often without involvement of the epididymis (Table 19.1). A particular organotropy exists for the mumps virus, but the male genital tract also represents a possible reservoir for a variety of other viruses that induce viremia (Le Tortorec et al. 2020). Similarly, cytokines released in the course of systemic inflammation, as well as other pro-inflammatory mediators, can lead to impaired spermatogenesis and steroidogenesis (Fijak et al. 2018). Control mechanisms of the immune privilege may be permanently disturbed.

Both pathogen and host factors are important for disease progression and prognosis. For example, epididymo-orchitis following infection with α-hemolysin-producing *E. coli* strains is associated with an increased risk of permanent oligo- or azoospermia (Lang et al. 2013). Pathogen persistence and local adaptive immune responses have been discussed as a cause of **chronic inflammatory conditions** resulting from *C. trachomatis* infection (Mackern-Oberti et al. 2013). Animal data suggest that uropathogens induce an anti-viral rather than an antibacterial immune response and thus can progress to sterile testicular autoimmune responses with corresponding tissue damage even after their eradication by antibiotic therapy (Fijak et al. 2018).

Although **autoimmune orchitis** in males has not been established as a distinct clinical entity, pathogen-independent inflammatory reactions in the testis have been attributed to autoimmune responses, in addition to manifestations of systemic autoimmune diseases (Table 19.1). Histopathologically, the late phase of EAO in animal models and persistent inflammatory infiltrates as well as associated tubular damage after acute orchitis share similarities; this applies equally to chronic sterile inflammation of the human testis (Fijak et al. 2018). In accordance with their central role in the induction and regulation of specific immune responses, activated T lymphocytes are predominantly found in the infiltrates. In the context of inflammatory reactions and other disorders of testicular architecture, CD68+, inflammatory macrophages also migrate more frequently into the testicular tissue; in addition, the number of mast cells is significantly increased. Both in terms of their cellular composition and cytokine profiles, the immune cell infiltrates associated with germ cell neoplasia-in-situ (GCNIS) and germ cell tumors (Chaps. 11 and 24) largely differ from non-malignant disease (Klein et al. 2016).

An approach proposed from a rheumatologic perspective to diagnose autoimmune orchitis based solely on antisperm antibodies (ASA) (Silva et al. 2014) appears inappropriate.

The pathophysiological significance of sperm-specific autoimmune responses should be considered minor in the context of testicular inflammatory reactions (Schuppe et al. 2008). In a recent study, the detection of ASA in the ejaculate was associated with clinical sonographic signs of chronic epididymitis but not with a positive history of orchitis (Lotti et al. 2018) (Chap. 28). On the other hand, antibodies against autoantigens such as heat shock proteins and enzymes are found in the EAO animal model as well as in the serum of infertile men with focal testicular inflammatory reactions (Fijak et al. 2018).

19.3 Clinic and Diagnosis

Acute orchitis is characterized by **swelling of the testis**, usually unilateral, painful, and associated with redness and edema of the scrotal skin, which is frequently accompanied by general symptoms such as fever (Emerson et al. 2007). Because of the close anatomic and functional relationships between the affected structures, clinico-palpatory differentiation between orchitis, epididymitis, and epididymo-orchitis is often not possible. However, with **color-coded duplex sonography** as the gold standard, marked **hyperperfusion** can be visualized in the acute stage of orchitis (Fig. 19.1), whereas an absence of perfusion characterizes the most important **differential diagnosis**, **testicular torsion** (Altinkilic et al. 2013; Pilatz et al. 2019a). Painless swelling and increased consistency of the testis may occur in granulomatous inflammation, and differential diagnosis must also exclude testicular tumors (Chap. 24).

However, in the fertility consultation, patients with acute, painful inflammatory reactions in the testis and/or epididymis are an exception. The majority of patients with subacute or chronic courses are completely asymptomatic; low-grade intermittent pain in the history, tenderness on palpation, decrease in testicular volume and consistency, and thickened periorchium may be non-specific clinical symptoms (Wesselhoeft 1920).

Laboratory diagnostics include blood count and CRP, in cases of suspected mumps also amylase, and in cases of acute ascending infections a determination of serum PSA (detection of concomitant prostatitis). To assess endocrine testicular function, a **hormone status** should be obtained (basal: FSH, LH, total testosterone). If ejaculate collection is possible, the analysis should include inflammatory indicators and biochemical markers of accessory secretion in addition to basal parameters such as sperm concentration, motility, and morphology (Schuppe et al. 2017) (Chaps. 9 and 26). In the acute phase of mumps orchitis, the virus can also be isolated from the ejaculate (Jalal et al. 2004). **Microbiological examination of urine and ejaculate** is suitable for the detection of local infections (Fig. 19.2) (Chap. 26). Culture methods and culture-independent nucleic acid amplification techniques (NAT) are used for this purpose; in the case of negative results, a highly sensitive 16S rDNA analysis can also be performed (Pilatz et al. 2015). Serological tests (e.g., mumps serology), which can detect pathogen-specific antibodies and antigens, are used for the differential diagnosis of systemic infections; in addition, NAT are also used here. In cases of sterile testicular inflammation and suspected **autoimmune disease**, appropriate serological autoantibody diagnostics are required (Fig. 19.2). So far, asymptomatic inflammatory lesions can only be detected by testicular biopsy; specific markers for non-invasive diagnostics are not yet available (Fijak et al. 2018).

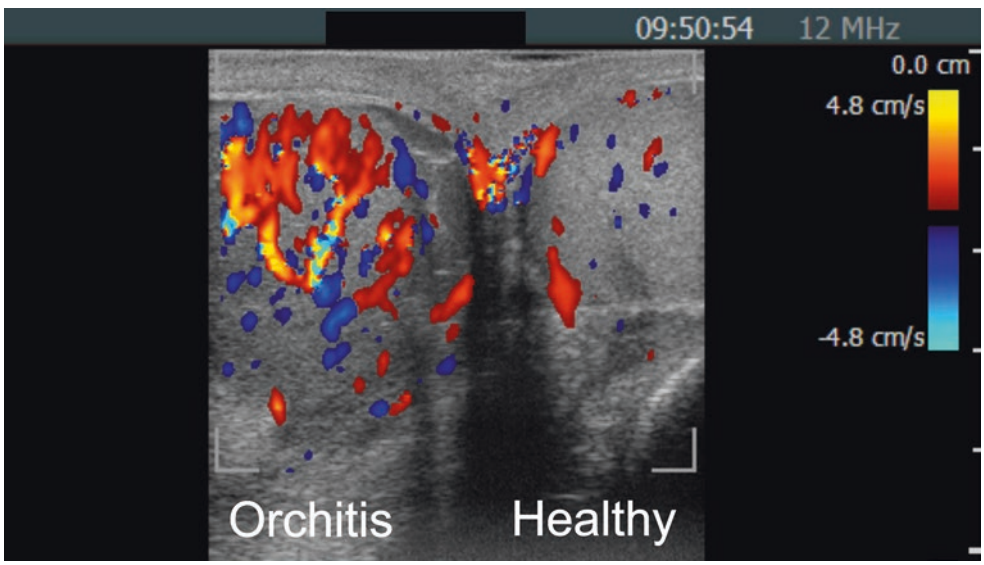


Fig. 19.1 Duplex sonography of both testes in cross-section; testis with isolated orchitis and massive hyperperfusion (left), compared with the healthy contralateral side (right); findings at the patient's initial presentation [from Pilatz et al. 2019a]

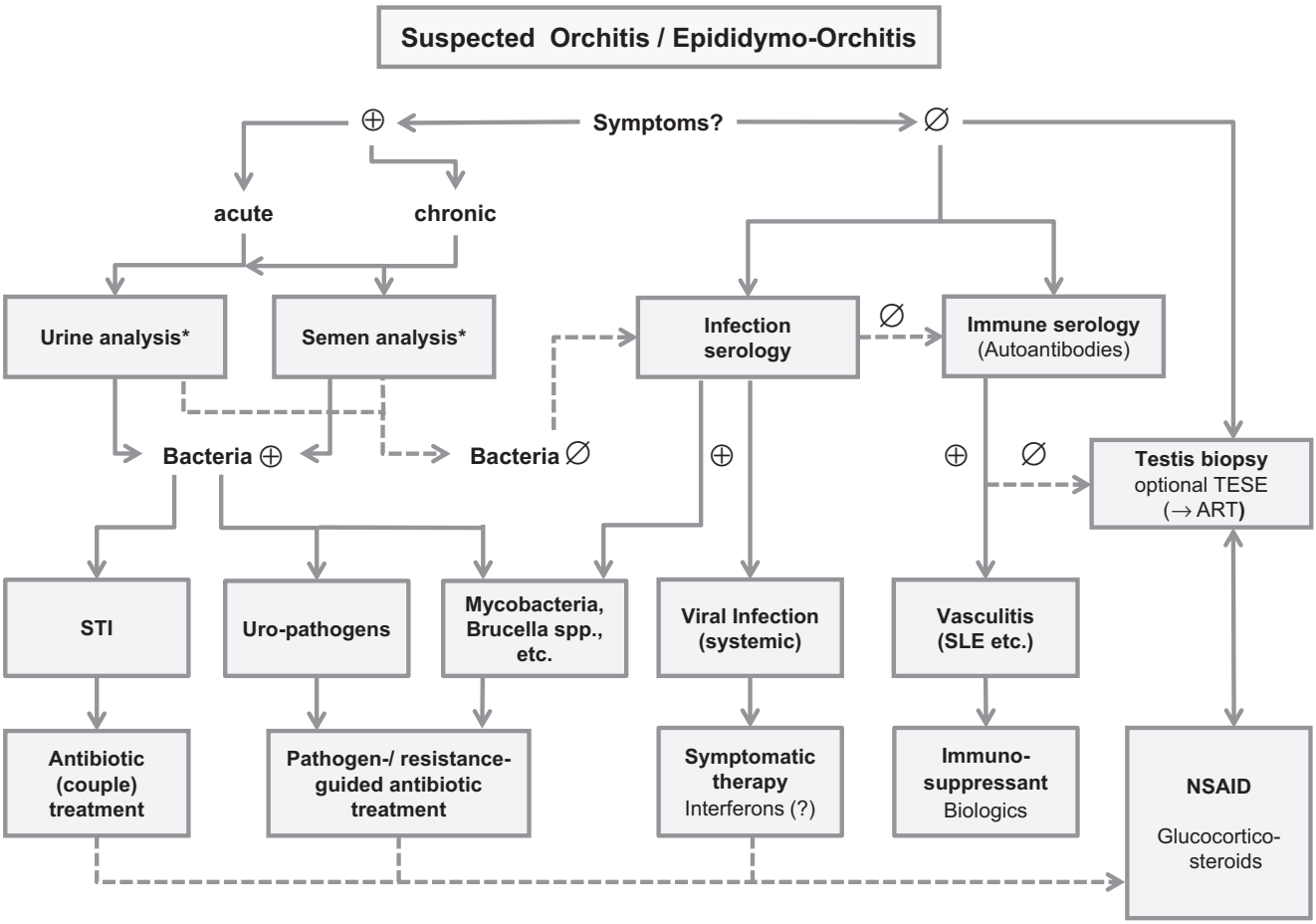


Fig. 19.2 Diagnostic-therapeutic algorithm for the care of patients with suspected orchitis or epididymo-orchitis. Not listed is orchiectomy, which is now only very rarely required (e.g., for idiopathic granulomatous orchitis or therapy-refractory fulminant epididymo-orchitis) (*Microbiology: culture, STI-PCR, 16S rDNA analysis; inflammatory markers: leukocytes, elastase, cytokines [IL-6, IL-8, and others]; in suspected cases, viral PCR) [from Pilatz et al. 2019a]

19.3.1 Pathogen-Induced Orchitis

In a mild clinical course of local bacterial infection with intra-canalicular spread of the pathogens, only the epididymis is affected, whereas epididymo-orchitis develops in case of a pronounced inflammation. Using modern molecular biology diagnostics, detection of a pathogen is successful in more than 80% of antibiotic-naïve patients (Pilatz et al. 2015). In younger patients, sexually transmitted infections (*C. trachomatis*, *Mycoplasma hominis/genitalum*, *Neisseria gonorrhea*) are frequently found; in older men, *Escherichia (E.) coli* and other *Enterobacteriaceae* predominate. However, the previous differentiation based on an age limit of 35 years appears to be of little use, as both uropathogens and sexually transmitted pathogens can regularly be found in young patients (Pilatz et al. 2015).

Very limited data are available on the effects of epididymitis/epididymo-orchitis on **semen quality** and fertility (Rusz et al. 2012) (Chap. 26). The acute phase showed **leukocytospermia** with significantly impaired sperm parameters (concentration, motility, morphology), which improved over a period of 3–6 months. Despite antibiotic treatment, 10% of patients had **persistent azoospermia** and another 30% had **oligozoospermia**, with no therapy effective against *C. trachomatis* in the majority of older studies. As an indication of an inflammation-related disorder of spermatogenesis, increasing serum FSH levels were found in some of the patients during the course of the disease (Osegbe 1991). On the other hand, a prospective study 3 months after acute unilateral disease and adequate antimicrobial therapy did not demonstrate a reduction in testicular volume on the affected side compared with the healthy contralateral testis (Pilatz et al. 2013). Various mechanisms have been described as the cause of reduced ejaculate quality. Thus, at the level of the epididymis, (partial) obstruction of the seminal ducts must be considered as an explanation for the quantitative reduction in sperm concentration.

Among systemic infections that primarily lead to orchitis as a result of hematogenous spread, **mumps virus** infection is the best known (Pilatz et al. 2019a). Data from before the availability of a vaccine show that mumps orchitis occurs in approximately 18% of those infected during or after puberty, usually about 5–10 days after the onset of **parotitis**, and in some cases without parotitis (Wesselhoeft 1920). Bilateral orchitis has been observed in approximately 10% of those infected (Ternavasio-de la Vega et al. 2010). Since introduction of the mumps–measles–rubella vaccine, the incidence of mumps orchitis has decreased dramatically. However, mumps orchitis can develop despite vaccination, although the clinical course appears to be milder (Patel et al. 2017; Pilatz et al. 2019a). Of andrological relevance is the **testicular atrophy** which may occur as most severe complication of acute orchitis, resulting in irreversible infertility. Methodologically heterogeneous studies from before mumps vaccinations reported post-infectious testicular atrophy in 55% of cases (Wesselhoeft 1920). In a follow-up study after experimental therapy with interferon-α2B in the acute phase of the disease, testicular biopsies from 38% of patients showed complete atrophy of the seminiferous tubules, and partial atrophy was found in 16% of cases (Yeniyol et al. 2000). A study from Mongolia reported an odds ratio of 3.4 for deterioration of ejaculate quality up to azoospermia after mumps orchitis (Bayasgalan et al. 2004); however, a broader epidemiologic database on the incidence of infertility after mumps orchitis is lacking.

In single case reports and smaller case series, orchitis has been reported in association with numerous other viral infections (Table 19.1) (Mikuz and Damjanov 1982; Fijak et al. 2018; Le Tortorec et al. 2020). Observations in previous SARS epidemics indicate that **corona viruses** can also cause orchitis (Xu et al. 2006). In the context of SARS-CoV-2 infections, some affected individuals reported testicular pain as a possible symptom of associated orchitis; autopsy specimens showed inflammatory infiltrates and damage to seminiferous tubules, even without viral detection in testicular tissue (Pan et al. 2020; Tian and Zhou 2021). In one case report, bilateral orchitis was described as a major symptom of COVID-19 disease (Bridwell et al. 2020). In contrast, **Zika virus** infection in humans does not appear to result in clinically symptomatic orchitis, whereas massive testicular damage has been observed in mouse models (Epelboin et al. 2017). However, systematic studies in patients with isolated orchitis using modern molecular biology diagnostics do not exist.

Histopathologically, bacterial acute orchitis is characterized by massive infiltration of the interstitium and seminiferous tubules with **neutrophilic granulocytes**, whereas viral orchitis is characterized by multifocal perivascular as well as peri- to intratubular **infiltrates with** neutrophilic granulocytes, **lymphocytes, plasma cells, and macrophages** (Schuppe and Bergmann 2013). The germinal epithelium of affected seminiferous tubules is largely disrupted, often leaving only single spermatogonia between preserved Sertoli cells in the basal compartment. The lamina propria of the tubules shows thickening and fibrosis up to the so-called tubular shadows (Fig. 19.3) (Chap. 11).

In individual cases, testicular biopsies performed 1–2 years after acute unilateral bacterial epididymo-orchitis showed significant damage to spermatogenesis as well as persistent inflammatory infiltrates, both in the ipsi- and con-

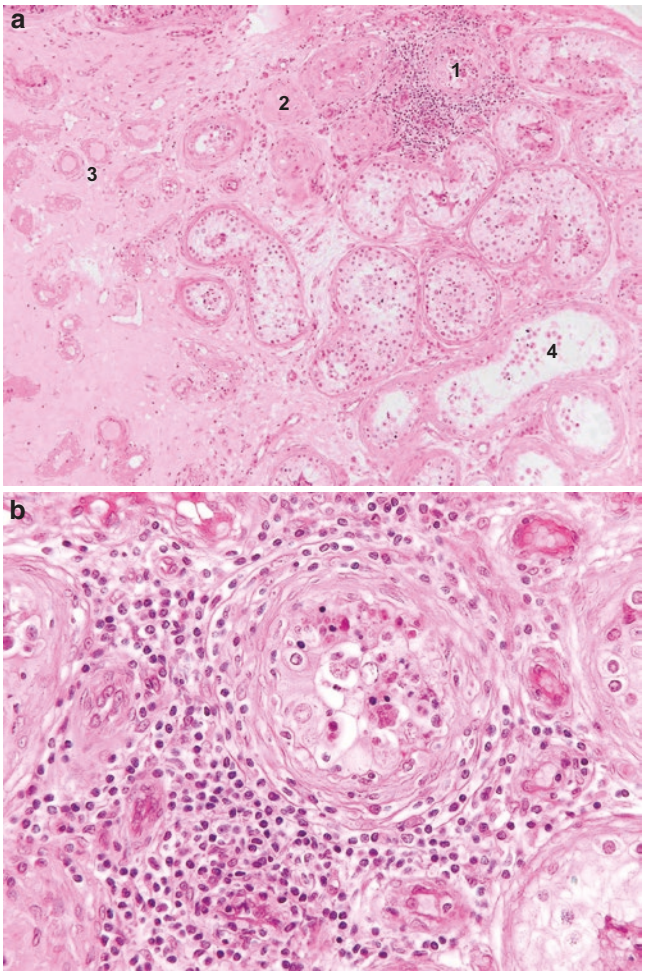


Fig. 19.3 Persistent testicular inflammatory reaction after mumps orchitis (asymptomatic, infertile patient, 28 years): (**a**) focal lymphocytic infiltrate (**1; b**); atrophy of seminiferous tubules to complete hyalinization (tubular shadows, "scars"; (**2, 3**), interstitial fibrosis (**3**); adjacent seminiferous tubules with preserved but damaged spermatogenesis (hypospermatogenesis); note flattened germinal epithelium with complete loss of the adluminal compartment in some tubules (**4**; cf. so-called aspermatogenesis in experimental autoimmune orchitis) (hematoxylin-eosin stain; ×10). (**b**) Area (**1**) at higher magnification; dense, peritubular-perivascular infiltration of lymphocytes that also invade the thickened lamina propria of seminiferous tubules; lamina propria shows a characteristic meshwork pattern; disruption of germinal epithelium with few remaining spermatogonia and Sertoli cells. (Hematoxylin-eosin stain; ×40). [from Schuppe and Bergmann 2013]

tralateral testis (Osegbe 1991). However, antibiotic therapy in this study did not adequately cover chlamydia. In addition to the chronification of acute epididymo-orchitis caused by STI or uropathogens, predominantly granulomatous orchitis that becomes chronic without treatment may occur as manifestation of **tuberculosis, lepromatous leprosy, syphilis, or brucellosis** and may also spread to the epididymis (Table 19.1) (Mikuz and Damjanov 1982).

Overall, the histopathologic pattern of chronic inflammatory lesions, such as after mumps orchitis, corresponds to "mixed atrophy" (Chap. 11) and exhibits morphologic characteristics of experimental autoimmune orchitis (Fig. 19.3; Fijak et al. 2018). Leydig cells are usually not destroyed, but in extensive, especially bilateral, orchitis, impairment of endocrine testicular function with corresponding testosterone deficiency may occur (Ternavasio-de la Vega et al. 2010).

19.3.2 Non-Pathogen-Related Inflammatory Reactions in the Testis

In older men, a primary chronic, painless **granulomatous orchitis** characterized by diffuse infiltration of the interstitium and seminiferous tubules with macrophages, plasma cells, lymphocytes, and single granulocytes has been observed and is probably due to a germ cell-specific autoimmune reaction (Mikuz and Damjanov 1982). Sterile granulomatous inflammatory reactions may furthermore occur as a rare manifestation of **sarcoidosis** in the testis (Chierigo et al. 2019). Non-pathogen-related processes also include systemic **vasculitis** and autoimmune diseases such as **systemic lupus erythematosus**, which affect the vasculature of the testis and epididymis and may be the only clinical manifestation in some circumstances (Mikuz and Damjanov 1982). Patients with autoimmune polyendocrinopathy type 1 (APS-1) develop testicular dysfunction as well as **sperm autoantibodies** in 30% of cases (Kisand and Peterson 2011). "Post-traumatic" testicular inflammatory reactions have been observed ipsi- and contralaterally after inguinal hernia surgery and have also been interpreted as autoimmune orchitis (Suominen 1995). Finally, **pharmaceuticals or other chemical substances** can trigger testicular inflammatory reactions (Table 19.1) (Keck et al. 1993; Dasu et al. 2019; Quach et al. 2019).

19.3.3 Asymptomatic Testicular Inflammatory Reactions

In the majority of patients consulting for fertility, a primary chronic, asymptomatic course of testicular inflammatory reactions can be assumed and the diagnosis can only be made by testicular biopsy (Schuppe et al. 2008). The high prevalence in men with azoospermia suggests that immunopathological reactions in the testis are induced not only by infections but also by other etiological factors (Table 19.1). In this regard, testicular inflammatory responses may be both a major cause and a cofactor or consequence of pre-existing organ damage (Fijak et al. 2018). Accordingly focal lymphocytic infiltrates were found in undescended testes of adult males in 44% of cases (Nistal et al. 2002). Histologically, peritubular-perivascular localized lymphocytic infiltrates are found, which are associated with characteristic changes in the seminiferous tubules (Fig. 19.3) (Schuppe and Bergmann 2013). Although the inflammatory infiltrates are mostly detectable only in a focal to multifocal extent in the testicular tissue, there is a significant correlation with the degree of damage to spermatogenesis and corresponding clinical and endocrinological parameters such as **testicular volume and serum FSH** (Fijak et al. 2018).

19.4 Therapy

Therapy of acute bacterial epididymo-orchitis initially consists of **empiric administration of antibiotics**, which should cover the most likely pathogens depending on sexual history and patient population (Pilatz et al. 2015; Bonkat et al. 2021) (Fig. 19.2). After **pathogen identification and antibiogram**, a change may be necessary in the course of treatment; in the case of a **sexually transmitted infection, the partner must also be examined and treated**. In acute disease, treatment failure is to be expected in only about 2.5% of cases when conservative treatment is performed in accordance with current guidelines (Bonkat et al. 2021).

As outlined above, persistent inflammatory reactions and corresponding fertility disorders can occur despite adequate antibiotic treatment of bacterial epididymo-orchitis. Consequently, a combination of **antibiotics** and **non-steroidal anti-inflammatory drugs** has been recommended for treatment (Haidl et al. 2019). However, controlled studies on antiphlogistic therapy of chronic testicular inflammation are not yet available, and the same applies to immunosuppression with glucocorticosteroids and the use of mast cell blockers. Experience with newer immunomodulators is not available (Fig. 19.2). Treatment of testicular involvement in systemic autoimmune disorders follows the respective disease-specific recommendations; for primary care, high-dose **glucocorticosteroids** are administered.

The preventive effect of treatment of acute mumps orchitis with interferon-α2B appears to be limited with respect to subsequent fertility problems (Yeniyol et al. 2000). Pilot studies on the preventive effect of down-regulation of spermatogenesis with gonadotropin-releasing hormone agonists in patients with acute orchitis or epididymo-orchitis were not

followed up. Therapeutic studies of other forms of virus-associated orchitis are not available.

The treatability of chronic (epididymo-)orchitis depends on the degree of damage to spermatogenesis. Considering the frequently encountered histological findings of "mixed atrophy" with focally and at least qualitatively preserved spermatogenesis up to elongated spermatids, the option of surgical sperm retrieval (testicular sperm extraction, TESE) remains an option for patients with a desire to have children in whom azoospermia persists despite pharmacological therapy (Chaps. 11, 40 and 42).

Key Points

- Frequent: Bacterial epididymo-orchitis secondary to pathogen ascension in acute epididymitis.
- Rare: Isolated orchitis as an acute symptomatic condition, mostly viral in origin.
- Possible: Sterile orchitis due to systemic autoimmune diseases and other pathogen-independent factors.
- Underestimated: Low-grade, chronic-asymptomatic inflammatory reactions in the testis as a cause or cofactor of fertility disorders; post-infectious, non-infectious or autoimmune; diagnosis so far only possible by testicular biopsy.
- Difficult: risk of permanent infertility after bacterial epididymo-orchitis despite adequate antibiotic therapy; lack of causal/preventive therapeutic approaches for the acute stage of virally caused orchitis; drug treatment of post-infectious or sterile chronic testicular inflammatory reactions mainly empirical.

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Disorders of Spermatogenesis and Spermiogenesis

20

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Abstract

Male infertility is often caused by congenital or acquired disorders of spermatogenesis and spermiogenesis; with impaired semen quality ranging from oligo- or oligoasthenoteratozoospermia (OAT) to non-obstructive azoospermia (NOA). While the etiology of OAT remains unclear in many cases, correlation of histopathological findings with cyto- or molecular genetic alterations allows an increasingly accurate differential diagnosis of NOA. Next-generation sequencing techniques have already identified more than 200 genes associated with disorders of spermatogenesis such as spermatogenic arrest or Sertoli cell-only phenotype. Monogenic causes can also be identified for specific structural sperm defects, which reflect disorders of spermiogenesis and can usually be identified by light microscopy. Causal therapeutic options do not exist for genetic disorders of spermatogenesis or spermiogenesis.

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20.1 Introduction

The general term **spermatogenesis** encompasses the entire process of germ cell formation in the seminiferous tubules of the testis, from spermatogonia to mature spermatozoa. It includes proliferation and differentiation of spermatogonia, meiotic divisions of spermatocytes, and transformation of haploid round spermatids to spermatozoa (Fietz and Bergmann 2017) (Chap. 2). The latter is called **spermiogenesis** and comprises the developmental stages from the second meiotic division to the release of the differentiated spermatids into the lumen of the germ tubules (spermiation). Disorders of this complex biological system are frequent causes of male subfertility or infertility. Deterioration of semen quality is ranging from oligo-(astheno-terato)zoospermia to non-obstructive azoospermia, the most severe form (Colpi et al. 2018; Jungwirth et al. 2019; Toth et al. 2019; Schlegel et al. 2021a). The etiology of these disorders of male fertility remain unclear in many cases. The use of molecular genetic testing methods such as whole exome sequencing (WES) is taking andrological diagnostics to a new level and helping to decipher the genetic basis of specific structural and/or functional defects in spermatogenesis or spermatozoa (Krenz et al. 2020; Houston et al. 2021). Regarding the various methods that can be used to detect cyto- and molecular genetic alterations, please refer to Chap. 8.

20.2 Oligoasthenoteratozoospermia

In most cases, male fertility disorders are associated with restrictions in semen quality, i.e., a reduction in sperm concentration or total number (oligozoospermia), reduced motility (asthenozoospermia) or an increased proportion of pathomorphic sperm (teratozoospermia) (WHO 2010; Chap. 9). The above findings may occur in isolation, but many patients with a pathological spermiogram have combined disorders. In andrology specialty consultations, the prevalence of oligoasthenoteratozoospermia (OAT) is approximately 50%, with 30% of patients found to have high-grade OAT with less than 5×10^6 sperm per ml (Tüttelmann et al. 2018).

> Results obtained from semen analysis, such as oligoasthenoteratozoospermia—also referred to as "OAT syndrome"—are merely descriptive laboratory findings and, strictly speaking, do not represent a diagnosis.

20.2.1 Etiopathogenesis

The possible causes of oligozoospermia or OAT are diverse (Jungwirth et al. 2019). Depending on their localization, disorders of the hypothalamic–pituitary system, impairments of spermatogenesis due to direct testicular damage, post-testicular disorders, and androgen receptor and enzyme defects can be distinguished (Table 20.1; Chap. 4).

Table 20.1 Possible causes of oligozoospermia[a]

	Clinical pictures (examples)	
Diagnostic categories	Congenital	Acquired
Primary testicular damage	Maldescensus testis Deletions of the Y-chromosome (AZFc) Klinefelter syndrome (mosaic forms) Anorchia (unilateral)	Infections/inflammation (Orchitis) Varicocele Trauma, torsion Malignant germ cell tumors, germ cell neoplasia in situ (GCNIS) Exogenous noxae (lifestyle factors, pharmaceuticals, occupational/environmental chemicals, physical factors)
Secondary testicular damage (hypothalamic-pituitary disorders)	Congenital hypogonadotropic hypogonadism, Kallmann syndrome (partial GnRH or gonadotropin deficiency) Hypopituitarism	Hypopituitarism (tumors, trauma, ischemia/hemorrhage, infections) Hyperprolactinemia Exogenous noxae (pharmaceuticals, etc.)
Mixed disorders of testicular function		General/systemic diseases (hemochromatosis, chronic renal insufficiency, liver diseases, obesity, etc.)
Post-testicular disorders		Infections/inflammation (epididymitis, prostatitis, prostato-vesiculitis) Disorders of emission/ejaculation (e.g. partial retrograde ejaculation)
Disorders of androgen action	Androgen receptor defect Androgen insensitivity (minimal or partial)	

[a] In the majority of cases combined findings, i.e. oligoasthenoteratozoospermia

In addition, diseases primarily not affecting the reproductive organs and exposure to exogenous noxae must be considered (Chaps. 34 and 35). With regard to the advances made in identification of genetic alterations associated with male infertility, a classification into genetic and non-genetic causes has also been proposed (Tournaye et al. 2017).

The most common causes of male fertility disorders—and, consequently, OAT—include (a history of) **cryptorchidism, genital tract infections and inflammation, and varicoceles** (Tüttelmann and Nieschlag 2009; Olesen et al. 2017). Frequently, however, there is a complex, multifactorial underlying etiopathogenesis that makes nosological categorization difficult. In at least 1/3 of cases, male fertility disorders are classified as idiopathic; for the clinical endpoint of oligozoospermia, these data even range up to 75% (Olesen et al. 2017; Punab et al. 2017; Tüttelmann et al. 2018).

20.2.2 Clinical and Diagnostic Findings

The majority of patients who present for andrological diagnostic work-up because of an unfulfilled wish for paternity are symptom-free. However, indicative symptoms and clinical findings may occur in association with the medical conditions listed in Table 20.1 (see respective specific Chaps. 12–35). Therefore, **general and specific andrological history and physical examination** are essential for the identification and diagnostic classification of possible causes of OAT (Colpi et al. 2018; Toth et al. 2019; Schlegel et al. 2021a) (Chap. 5).

In addition to the common causes of OAT mentioned above, such as a history of undescended testis, attention should be paid to the use of **medications**, including those without medical indication, **stimulants/narcotics** and other **lifestyle-related factors,** as well as potential **toxicants in the workplace** (Semet et al. 2017; Köhn and Schuppe 2021). Similarly, **febrile illness** that preceded the time of examination by 3–6 months should be inquired about.

Basic endocrinological diagnostics for differentiation of OAT include determination of FSH, LH, and testosterone in serum and are essential, especially when hypogonadism is suspected (Colpi et al. 2018). In the case of androgen deficiency symptoms, an extended hormone status with prolactin, estradiol, and sex hormone-binding globulin (SHBG; for the calculation of free testosterone from total testosterone and SHBG) should be performed; hormonal stimulation tests may also be required (Chap. 7).

Of central importance to the diagnosis of OAT is, of course, the **examination of the ejaculate**, which represents a complex mirror of various functions of the male reproductive system and their disorders (Colpi et al. 2018; WHO 2021) (Chap. 10). However, spermiogram variables such as sperm concentration/total number, motility, and morphology must be considered surrogate parameters for assessing male fertility; pathological results are not specific for underlying diseases or fertility disorders. In some cases, however, analysis of sperm morphology alone may provide clues to particular qualitative disorders of spermatogenesis (Coutton et al. 2015) (see Sect. 20.4).

Depending on the severity of OAT, **genetic diagnostics** and, if necessary, counseling are indicated (Toth et al. 2019). The prevalence of chromosomal abnormalities is increased in infertile males compared to the general population; it is approximately 5% with a sperm concentration below 5×10^6/ml and increases to 10–15% with azoospermia (Krausz and Riera-Escamilla 2018). Therefore, if sperm concentration is below 5 x 10^6/ml or total sperm count is below 10×10^6, **karyotype analysis** should be performed to detect **numerical or structural aberrations**. In addition, molecular genetic analysis can be used to confirm **deletions of the Y chromosome** (azoospermia factor, AZF; here, region AZFc affected) as the cause of high-grade OAT (Krausz et al. 2014; Toth et al. 2019) (Chap. 8).

20.2.3 Therapy

Similar to the diagnostic work-up, consideration of treatment options for OAT requires as far as possible accurate nosological classification of the underlying fertility disorder(s). Conditions amenable to causal drug treatment include the various forms of hypogonadotropic **hypogonadism** and **hyperprolactinemia**, as well as infections and inflammation of the male genital tract (Chaps. 26 and 38). Special attention should also be paid to **general/systemic diseases**, including lifestyle-related conditions such as **obesity**, that are associated with impaired reproductive health (Colpi et al. 2018; Toth et al. 2019; Schlegel et al. 2021a) (Chap. 34). Initiating, optimizing, or switching therapy, preferably **avoiding pharmaceuticals with potential adverse effects on male fertility**, can help improve ejaculate quality and thus the chances of pregnancy by natural conception. The same applies to the chances of success of **assisted reproductive procedures (ART)**, including the possibility of "downgrading" (Chap. 42). Counseling of patients with OAT also aims at avoiding relevant exogenous noxae as well as correcting lifestyle factors that should be considered as potential causes or co-factors of OAT in any treatment situation (Köhn and Schuppe 2021).

If a **varicocele** is diagnosed as the (main) cause of OAT, surgical treatment by ligation or microdissection or sclerotherapy can be considered (Chap. 40). **Testicular biopsy for testicular sperm extraction** (TESE, micro-TESE) is indicated only in exceptional cases, such as refrac-

tory necrozoospermia or a sperm count and quality in the ejaculate that is too low to perform IVF/ICSI (cryptozoospermia) (Tournaye et al. 1996; Alkandari et al. 2021). In contrast, in structural sperm defects characterized by a complete loss of motility with preserved vitality, there is no indication for surgical sperm retrieval (see Sect. 20.4).

The question of treatment options for patients with idiopathic OAT is a matter of ongoing debate. Study results and recommendations for pathophysiologically based pharmacotherapy with FSH, antiestrogens, aromatase inhibitors, and antioxidants are available, but the overall level of evidence is considered low (Colpi et al. 2018; Haidl et al. 2019). Therapeutic trials in idiopathic OAT are by definition considered empirical due to the lack of nosological classification of cases (Chap. 39).

20.3 Non-obstructive Azoospermia: Disorders of Spermatogenesis

20.3.1 Sertoli Cell-only Phenotype

The **Sertoli cell-only phenotype (SCO)** (often referred to as a "syndrome," which does not correspond to the term in its true sense) is characterized histopathologically by the absence of germ cells in the seminiferous tubules; this phenomenon may affect all seminiferous tubules or occur focally (Bergmann 2006; McLachlan et al. 2007) (Chap. 11).

SCO is not a nosological entity but a heterogeneous clinical entity that includes both **congenital and acquired forms**. Following the initial description by Del Castillo et al. (1947), the congenital complete SCO pattern is also called germinal or germ cell aplasia, in which biopsies show only SCO tubules. In congenital or primary SCO, migration of primordial germinal cells fails to occur for unexplained reasons, and Sertoli cell histology appears immature. In contrast, in secondary SCO, germ cell loss occurs later due to various causes (see Table 20.1). Here, the morphology of the Sertoli cells is mature and seminiferous tubules show obvious signs of hyalinization (Nistal and Paniagua 1997; Anniballo et al. 2000) (see Chap. 2). Known genetic causes of SCO include deletions in the long arm of the Y chromosome (Krausz et al. 2014) (see Sect. 20.3.5). Like oligozoospermia, the SCO pattern can also be caused by various endogenous and exogenous fertility-impairing factors, such as maldescensus testis, infections and inflammation, and chemical or physical noxae (see Table 20.1). At the molecular level, impaired Sertoli cell gene expression is thought to be associated with the development of SCO (Paduch et al. 2019). **Leydig cell dysfunction**, which can be observed in testicular dysgenesis, has also been described as a cause of the SCO phenotype, for example, due to dysfunction of the CYP19A1 and CYP17A1 enzymes (Adamczewska et al. 2020; Lardone et al. 2017; Lardone et al. 2018).

A SCO is the most common cause of **non-obstructive azoospermia (NOA),** with a prevalence estimated at 1% in the general male population and reported as high as approx. 10% in andrology consultations (Olesen et al. 2017; Tournaye et al. 2017).

20.3.2 Spermatogenic Arrest

Spermatogenic arrest, similar to SCO, is a histopathologically defined condition (Bergmann 2006; McLachlan et al. 2007) (Chap. 11). Interruption of maturation of spermatogonia to mature spermatozoa may occur at **different stages of spermatogenesis** and may be **homogeneous** in both testes or **heterogeneous** in both pattern and distribution. The disorder may be either genetically determined or due to acquired disruptive factors. Genetic causes include **chromosomal abnormalities** such as balanced translocations as well as **deletions of the Y chromosome** (Toth et al. 2019; Krausz et al. 2020). With the help of extended genetic diagnostics using exome or whole genome sequencing, monogenic causes for a **pre-meiotic or meiotic arrest** of spermatogenesis can be identified in a proportion of patients with NOA (Houston et al. 2021) (see Sect. 20.3.5). Acquired spermatogenic arrest may be related to general diseases; in addition, as in oligozoospermia, gonadotoxic or other factors must be taken into account as underlying causes (Tournaye et al. 2017) (see Table 20.1).

20.3.3 Clinic

As outlined above for patients with OAT, history and clinical findings may provide clues to possible causes of SCO. In this context, it is important to consider the clinical pictures and factors associated with primary testicular failure as well as general and systemic diseases (see Table 20.1 and Sect. 20.4). However, apart from the leading symptom of infertility, the majority of patients with SCO are asymptomatic.

While a complete SCO pattern is always associated with **azoospermia**, results of **semen analysis** in focal SCO can range from azoospermia to OAT of varying degrees. Testicular volume is decreased in the majority of patients with SCO but may also be in the lower normal range. **FSH** levels are usually elevated, with serum levels positively correlating with the severity of damage to spermatogenesis (Bergmann et al. 1994; Tüttelmann et al. 2011). Simultaneous determination of **inhibin B**, which is a secretory product of Sertoli cells and correlates negatively with the extent of germinal epithelial damage, can increase diagnostic confidence (von Eckardstein et al. 1999; Zhu et al. 2019). However, neither inhibin B alone

nor the combination with FSH can reliably predict the presence of focally preserved spermatogenesis up to elongated spermatids, and thus, the chances of success of testicular sperm extraction (TESE). Testosterone production in the Leydig cells of the testis may be undisturbed; though, in addition to the various forms of primary or mixed hypogonadism (see Table 20.1), an increased risk of **hypogonadal testosterone levels** must be expected in the context of male infertility (Bobjer et al. 2016; Corona et al. 2020; Ferlin et al. 2021).

Apart from their infertility, asymptomatic patients with complete arrest of spermatogenesis always present azoospermia. In the case of inhomogeneous or partial forms of a spermatogenic arrest, OAT can also occur, which is usually severe (Tüttelmann et al. 2018) (see Sect. 20.2). Testicular volumes and FSH and inhibin B serum levels values are within normal range in the majority of affected patients (Olesen et al. 2017). In contrast to obstructive azoospermia, semen analysis reveals normal values for biochemical markers of secretory function of the accessory glands as well as the epididymis (Tüttelmann et al. 2011; WHO 2021). However, the definitive differential diagnosis between spermatogenic arrest and obstructions of the seminal ducts close to the testis can often only be made surgically during scrotal exploration for testicular biopsy.

20.3.4 Histopathology

Histological evaluation of **testicular biopsies** allows accurate differential diagnosis of disorders of spermatogenesis, which, in the highly heterogeneous group of patients with NOA, range from **hypospermatogenesis** with signs of disorganization of the germinal epithelium and decreased number of elongated spermatids in the seminiferous tubules to **spermatogenic arrest** and **complete SCO** (Bergmann 2006) (Fig. 20.1; Chap. 11). However, etiologic classification of testicular damage based on these descriptive histopathological endpoints is not possible.

In approximately 1/3 of cases, histopathologic findings in NOA are heterogeneous, both within a testis and side-dependent (McLachlan et al. 2007). This is not only of diagnostic relevance, but especially important for the therapeutic goal of testicular biopsies in NOA, a TESE. Accordingly, both testes should always be biopsied and tissue samples taken from different sites in each case (Chap. 11). Histology has the greatest prognostic significance with regard to successful TESE and subsequent ICSI (Schulze et al. 1999; Tüttelmann et al. 2011).

With a prevalence of more than 30%, the SCO phenotype represents the most frequent histopathological diagnosis in NOA; a frequency of approximately 15% is reported for the complete, bilateral SCO (McLachlan et al. 2007; Schuppe et al. 2022). In the congenital or prepubertal acquired forms, the seminiferous tubules have reduced diameters; in addition to mature Sertoli cells, it is not uncommon to find tubules with immature Sertoli cells, up to the so-called immature seminiferous cords without tubular lumen (Nistal and Paniagua 1997; Anniballo et al. 2000; McLachlan et al. 2007) (Chap. 11). The lamina propria of secondary SCO tubules is thickened, and there is usually Leydig cell hyperplasia and increased numbers of macrophages and mast cells in the interstitium, the latter also in close association with the lamina propria (Meineke et al. 2000; Frungieri et al. 2002; Hauptman et al. 2021).

In contrast, the definition of focal or partial SCO is not uniform; germ cells are still found in a variable proportion of seminiferous tubules, even up to the stage of elongated spermatids. However, spermatogenesis is usually also severely impaired quantitatively and/or qualitatively in the affected tubules, resulting in azoospermia in the majority of patients (Bergmann 2006) (Chap. 11). In heterogeneous focal SCO, tubular diameters and pathological changes of the lamina propria can vary considerably, and completely atrophied tubules, the so-called **tubular shadows**, and interstitial fibrosis also occur. Mature Sertoli cells in seminiferous tubules with a preserved lumen usually show a highly prismatic arrangement with regular triangular nuclei and central nucleolus; pronounced vacuolization of the cytoplasm is taken as a sign of involution or dedifferentiation (Nistal and Paniagua 1997; Brehm et al. 2006). SCO tubules with strikingly flattened Sertoli cells may be associated with other signs of low-grade testicular inflammation (Schuppe and Bergmann 2013) (Chap. 19).

Histopathological findings in spermatogenic arrest can also vary widely. Classification is based on the level at which spermatogenesis is arrested, i.e., **the level of spermatogonia** (pre-meiotic arrest), **primary or secondary spermatocytes** (meiotic arrest), or **round spermatids** (Fietz and Bergmann 2017) (Fig. 20.1). In addition to a phenotypically homogeneous pattern in both testes, marked heterogeneity with discordant patterns and differences between each side also occur, including residual tubules with qualitatively preserved spermatogenesis up to single elongated spermatids (McLachlan et al. 2007). In association with the aforementioned disorders of spermatogenesis, **multinucleated spermatogonia or spermatocytes** can be observed, similar to the aging testis (Holstein et al. 1988). In testes with spermatogenic arrest, the so-called giant or **megalospermatocytes** are found more frequently; these oversized primary spermatocytes result from asynapsis of chromosomes during prophase I and thus lead to defective meiosis (Holstein et al. 1988; Johannisson et al. 2003). Data on the prevalence of spermatogenic arrest range up to 30% of testicular biopsy findings in the literature; in our own cohort of patients with NOA, it was 7% (Schuppe et al. 2022). Overall, spermatogenic arrest at the level of primary spermatocytes is most common.

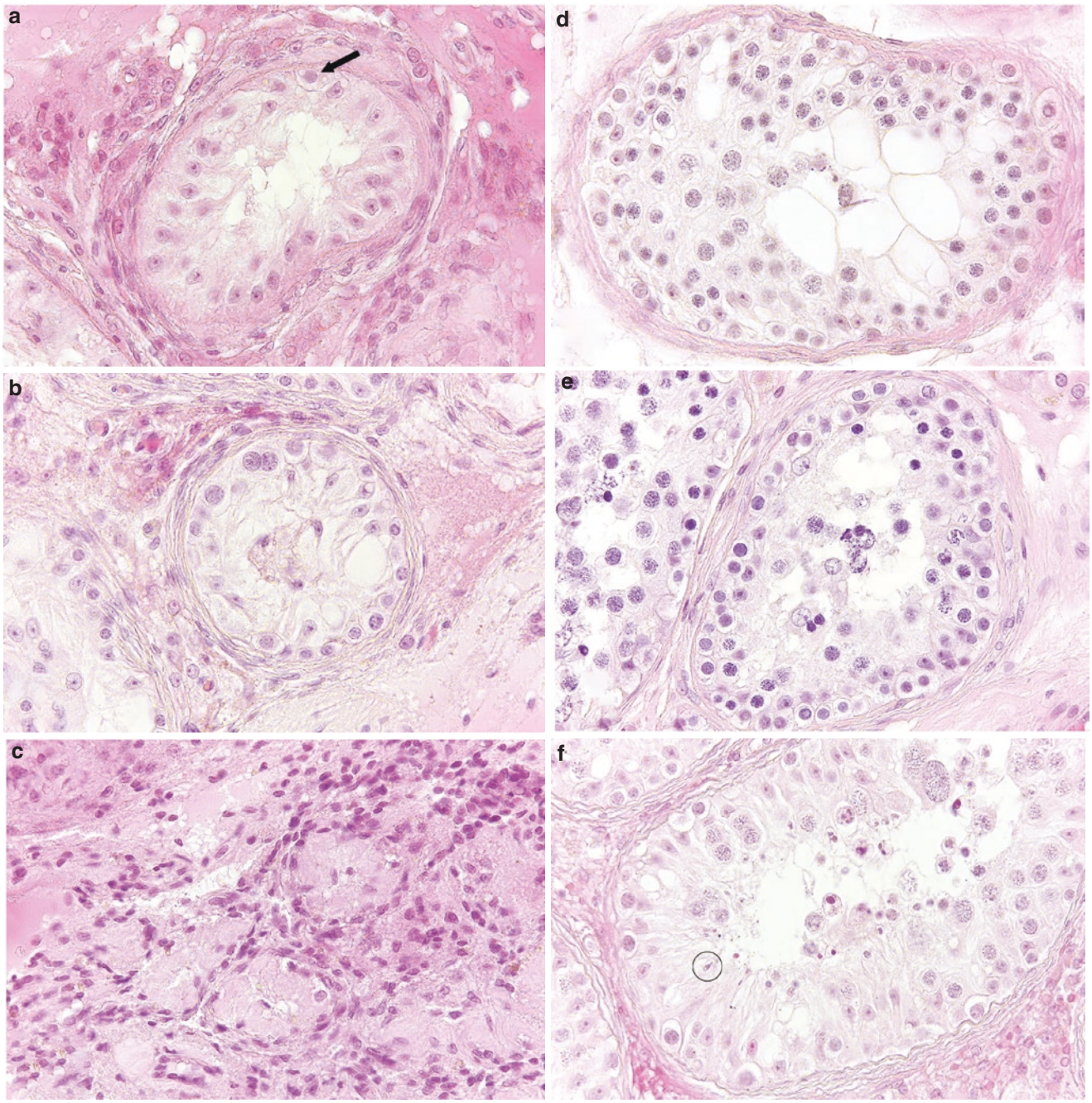


Fig. 20.1 Histopathological findings in disorders of spermatogenesis with defined genetic causes. (**a**–**c**) Patients with variants of the *DMRT1* gene; Sertoli cell-only phenotype with single remaining spermatogonia (→) (**a**); pre-meiotic arrest (level of spermatogonia) (**b**); complete tubular atrophy ("tubular shadow") with Leydig cell hyperplasia (**c**); (**d**, **e**) patients with meiotic arrest due to gene variants in *SHOC1* (**d**) and *M1AP* (**e**); (**f**) patient with AZFc deletion of the Y chromosome, with qualitatively preserved spermatogenesis up to single elongated spermatids (O) (hematoxylin-eosin; primary magnification ×40)

20.3.5 Genetic Causes

The most common genetic cause of spermatogenic failure, which predominantly results in NOA, is **Klinefelter syndrome**, a numerical chromosomal abnormality (karyotype 47,XXY; Chap. 21). Klinefelter syndrome is diagnosed in 10–15% of azoospermic males. Other chromosomal causes of disorders of spermatogenesis include: Y-isochromosome, Y-ring chromosome, **46,XX karyotype**, which is usually due to translocation of the SRY region, or balanced chromosomal translocations (Chaps. 22 and 23). In the latter, semen quality can vary widely, ranging from azoospermia to normozoo-

spermia. The testicular phenotype is similarly variable in men with **balanced translocation**, so that both SCO phenotype and meiotic arrest of spermatogenesis, hypospermatogenesis, or normal spermatogenesis may occur (Krausz and Riera-Escamilla 2018) (Chap. 11).

The second most common genetic cause of disorders of spermatogenesis are microdeletions of the so-called **azoospermia factors (AZF)** on the long arm of the Y chromosome (Chap. 23). Microdeletions of the AZF regions are found in 2–10% of all males with NOA, depending on selection criteria and population (Krausz et al. 2014). Of note, the three regions **AZFa**, **AZFb**, and **AZFc** are distinguished. In patients with a complete AZFa deletion, a SCO phenotype is found almost without exception. The testicular phenotype in males with complete AZFb deletion may be a SCO or meiotic arrest. In contrast, the testicular phenotype in males with complete AZFc deletion varies and can result in a SCO phenotype, meiotic arrest, or hypospermatogenesis (Fig. 20.1f). Severe oligozoospermia or cryptozoospermia are also seen in males with complete AZFc deletion (Krausz et al. 2014).

Through research and technical developments, particularly the introduction of **next-generation sequencing (NGS)** methods, more than 200 genes associated with disorders of spermatogenesis have now been described (Houston et al. 2021). However, to date, there is little clinical evidence for most of these genes available, so testing many of them is not yet useful in routine diagnostics. The level of evidence indicates how certain it is that **disease-relevant variants (mutations)** in a gene are associated with a particular phenotype. To determine the level of evidence, mainly the classification according to ClinGen is used (Riggs et al. 2020). Sufficient evidence exists based on a recent review article (Houston et al. 2021) that the genes *NR5A1, WT1, DMRT1*, *FANCA*, and *USP26* are associated with a SCO phenotype. The *AR, NR5A1, APOA1, CDC14A*, and *FSHR* genes are associated with pre-meiotic arrest, whereas the *AR, M1AP, TEX11, FANCM, MEI1, SYCP3, TEX15, CDC14A, DMRT1, FSHR, STAG3, TEX14*, and *XRCC2* genes are associated with arrest at the spermatocyte level. Detected variants are evaluated according to the American College of Medical Genetics and Genomics-Association for Molecular Pathology (ACMG-AMP) criteria and are mostly based solely on *in silico* predictions (Preston et al. 2022). A summary of genes associated with spermatogenesis and spermiogenic disorders including associated phenotypes can be found in Table 20.2.

In many cases, a clear **genotype-phenotype correlation** is not yet possible. For example, disease-relevant variants (mutations) in the *TEX15* gene have been described as causative for both a SCO phenotype and meiotic arrest (Okutman et al. 2015) or cryptozoospermia (Wang et al. 2018). Variants in the *M1AP* gene may be associated with azoospermia due to complete bilateral meiotic arrest as well as cryptozoospermia (Wyrwoll et al. 2020). A very broad phenotypic spectrum is associated with variants in the *NR5A1* gene, as severe phenotypes such as gonadal dysgenesis (46,XY differences in sex development, DSD) or adrenal dysfunction are possible, but isolated infertility with azoospermia due to a SCO can also occur (Domenice et al. 2016). In all cases, it has not yet been conclusively determined whether the variation in phenotypes is due to the interaction of multiple genes (di- or oligogenic as known, among others, in congenital hypogonadotropic hypogonadism, see Chap. 12), or to the nature of the variants in the respective gene (severe/mild influence on gene expression/protein).

Table 20.2 Selection of genes associated with disorders of spermatogenesis or spermiogenesis and associated phenotypes [adapted from Houston et al. 2021 and own data]

Phenotype	Gene
NOA: Sertoli cell-only-phenotype	*DMRT1, FANCA, NR5A1, USP26, WT1*
NOA: pre-meiotic arrest	*AR, CDC14A, NR5A1*
NOA: meiosis arrest	*ADAD2, AR, CDC14A, DMRT1, FANCM, GCNA, MEI1, M1AP, MSH4, MSH5, RAD21L1, RNF212, SHOC1, SPO11, STAG3, SYCE1, SYCP2, SYCP3, TERB1, TERB2, TEX11, TEX14, TEX15, XRCC2*
Macrozoospermia	*AURKC*
Globozoospermia	*DPY19L2*
Acephalic spermatozoa	*PMFBPI, SUN5, TSGA10*
Midpiece and flagellum defects (Multiple morphological abnormalities of the sperm flagella; MMAF)	*ARMC2, CCDC39, CCDC40, CDC14A, CEP290, CFAP43, CFAP44, CFAP65, CFAP69, CFAP91, CFAP251, DNAAF2, DNAAF4, DNAAF6, DNAH1, DNAH17, FSIP2, MNS1, LRRC6, PMFBP1, QRICH2, RSPH3, SEPTIN12, SPEF2, TTC29*

NOA non-obstructive azoospermia

Most of the genes mentioned above follow an autosomal recessive mode of inheritance (Houston et al. 2021). However, genes that are inherited autosomal-dominantly, such as *DMRT1* and *NR5A1*, or X-linked, such as *AR, TEX11*, or *USP26*, have also been described. De novo-mutations should also be considered as a cause of spermatogenic failure, however, testing of both parents is required for confirmation (Oud et al. 2022).

Detection of disease-relevant variants in any of the above listed genes can help to estimate the **chances of testicular sperm extraction (TESE).** Therefore, it is recommended to perform genetic testing before testicular biopsy. Although the number of cases per gene are still low and thus do not yet allow definitive statements, very low chances for a successful TESE must be assumed, when variants in most of the above-mentioned disease relevant genes are detected. Only in patients with variants in *M1AP, TEX15,* and *ZMYND15* have the recovery of sperm by testicular biopsy been described (Wyrwoll et al. 2022). The chances of success of IVF/ICSI using these spermatozoa are also still unclear.

Due to **the heterogeneity of disorders of spermatogenesis**, gene panel diagnostics are recommended. With this technique, many genes can be examined simultaneously. Ideally, a "virtual" panel testing is performed on the basis of an exome analysis. This enables re-evaluation of the data after some time with regard to new genes in the case of inconspicuous findings. According to the current AWMF guideline, molecular genetic analysis can be performed if a monogenic form of spermatogenic failure is suspected, although there are no specifications regarding the genes to be examined (Toth et al. 2019). This will change in the future with systematic investigation and genetic analysis of larger cohorts of patients with disorders of spermatogenesis.

20.3.6 Therapy

Causal therapeutic options, i.e., pharmacotherapy aiming at improvement of spermatogenesis, do not exist in the case of (complete) SCO or pre-meiotic and meiotic spermatogenic arrest. Spermatogenesis or maturation of immature germ cells in vitro is not yet possible (Chap. 43). Taking into account the genetic findings with a poor prognosis described above, the only remaining symptomatic therapeutic approach is a multi-locular bilateral testicular biopsy/TESE with the aim of IVF/ICSI using testicular spermatozoa (Chap. 23).

As with OAT, preoperative care of patients with NOA should include **elimination of relevant exogenous noxious agents, including lifestyle factors, and treatment of relevant general diseases** (see Sect. 20.2.3).

Evidence of infection and/or inflammation in the genital tract warrants **antibiotic and/or anti-inflammatory treatment** prior to surgical sperm retrieval (Chap. 26). In order to stimulate residual spermatogenesis that may still be present and thus increase the chances of successful TESE, the administration of antiestrogens or other pharmaceuticals such as aromatase inhibitors, human chorionic gonadotropin (HCG), and recombinant FSH has been suggested in NOA (Caroppo and Colpi 2021). However, general recommendations for preoperative use of these drugs prior to a planned testicular biopsy/TESE are currently not given due to a lack of sufficiently controlled studies (Schlegel et al. 2021b). Treatment must be classified as off-label (drug therapy for an unapproved indication), and adverse effects on spermatogenesis should also be taken into consideration (Fietz et al. 2020).

With the help of preoperative gene panel diagnostics as outlined above, frustrating drug therapy and surgical measures can be avoided in the future.

20.4 Specific Structural Sperm Defects: Disorders of Spermiogenesis

Analysis of stained ejaculate smear preparations shows a "mixed" pattern, i.e., **considerable morphological variability of spermatozoa**, even in fertile males. Normally shaped spermatozoa according to so-called strict criteria make up only a relatively small proportion (WHO 2021) (Chap. 9). On the other hand, the frequency of sperm with a defined pathomorphology and its degree reflect damage to spermatogenesis and spermiogenesis in the testis, but also disorders of epididymal function (Haidl and Schuppe 2006). In some patients, the majority of examined spermatozoa show **uniform structural defects in the head segments** and/or **flagella**, which are already visible by light microscopy (Fig. 20.2). A more precise ultrastructural characterization of such systematic morphological defects is possible by electron microscopy (Dadoune 1988; Holstein et al. 1988; Chemes and Rawe 2003). **Monogenic causes** have now been identified for several of these specific defects (Coutton et al. 2015; Houston et al. 2021) (Table 20.2).

20.4.1 Macrozoospermia

A special form of **teratozoospermia** is **macrozoospermia**, in which exclusively pathomorphic spermatozoa with too large, amorphous head segments, abnormal mid-pieces, and multiple flagella are found in the ejaculate, also referred to in the literature as "**large- headed multi-flagellar spermatozoa"**(Fig. 20.2b, b′). In the majority of patients, the complete absence of normally shaped spermatozoa is accompanied by a decrease in sperm concentration and motility (OAT). Ultrastructural studies showed an average three-fold increase in nuclear volume and 3.6 flagella per head segment (Escalier 1983). Fluorescence in situ hybridization can be used to demonstrate a correspondingly **high rate of polyploid** or **aneuploid spermatozoa** (Coutton et al. 2015).

The observation of a familial clustering of macrozoospermia in the North African region suggested a genetic cause. **Homozygous pathogenic variants in the Aurora kinase C** *(AURKC*) gene could be identified, and among the variants in the *AURKC* gene, the deletion c.144delC as the so-called "founder mutation" accounts for the largest proportion of approximately 85% (Dieterich et al. 2007; Ben Khelifa et al. 2012). The development of the phenotype of macrozoospermia is attributed to impaired chromosomal segregation and cytokinesis during meiosis, involving Aurora kinase C (AURKC) primarily expressed in the testis (Coutton et al. 2015). There is no division of tetraploid spermatocytes into

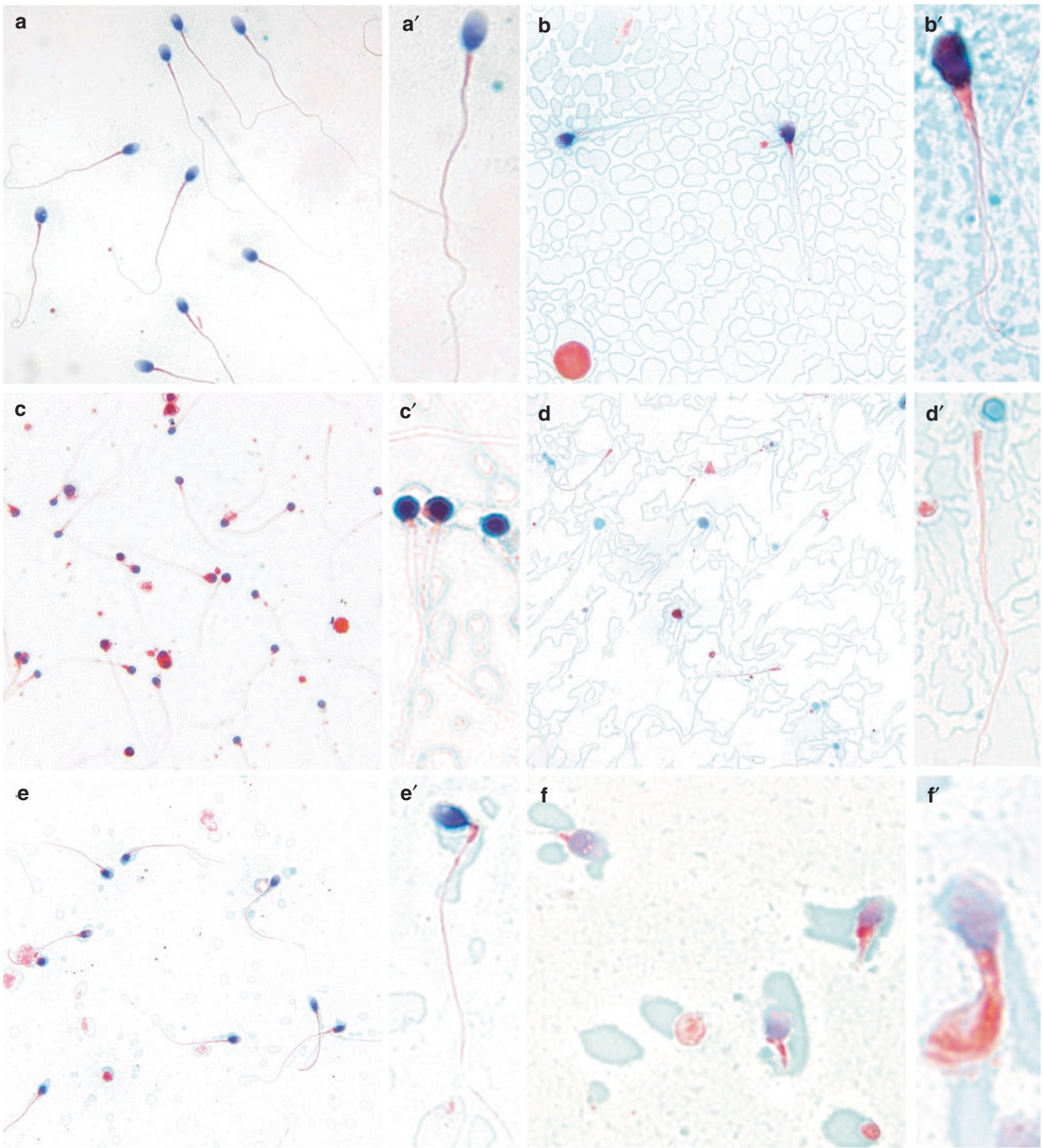


Fig. 20.2 Specific structural sperm defects. (**a, a′**) Normozoospermia; normal spermatozoon according to strict criteria (**a′**); (**b, b′**) macrozoospermia; (**c, c′**) globozoospermia; (**d, d′**) acephalic spermatozoa ("pinheads"); (**e, e′**) midpiece and flagellum defects in primary ciliary dyskinesia (**PCD**); (**f, f′**) rudimentary flagella ("stump tail") (smear preparations after Shorr staining; primary magnification ×100)

haploid spermatids, and the "fused" head segments form megaloforms with multiple flagella (Fig. 20.2b′). These mostly polyploid spermatozoa are not suitable for assisted fertilization by ICSI (Chelli et al. 2010).

20.4.2 Globozoospermia

Globozoospermia is a rare disorder of spermiogenesis in which the characteristic morphogenic differentiation of the

spermatid nucleus is disturbed and ejaculated spermatozoa show a **globular head shape without acrosome** (Schirren et al. 1971; Dam et al. 2007) (Fig. 20.2c, c′). Partial and complete forms of globozoospermia are found; in the latter, all spermatozoa exhibit the monomorphic defect (Coutton et al. 2015). The so-called pseudo-globozoospermia, in which round-appearing sperm heads with regular acrosomes are surrounded by residual cytoplasm, must be distinguished by electron microscopy.

The underlying cause of globozoospermia is a defect in the **complex biogenesis of the acrosome** from pro-acrosomal vesicles, which predominantly originate from the Golgi apparatus, fuse and interact with the spermatid nuclear membrane (Pleuger et al. 2020). The defectively formed acrosomal vesicles are taken up by the surrounding Sertoli cells.

Familial occurrence of globozoospermia and the establishment of knockout mouse models with a similar phenotype suggested a genetic basis early on. Mutations have since been described in a wide range of genes encoding proteins involved in acrosome formation (Coutton et al. 2015). Variants (mainly deletions) in the *DPY19L2* gene have been identified as the cause of globozoospermia in over 70% of cases, in addition to mutations in the *SPATA16* gene (Dam et al. 2007; Elinati et al. 2012; Oud et al. 2020).

Patients with complete globozoospermia usually have normal or subnormal sperm concentration and preserved motility; however, the spermatozoa cannot interact with the oocyte due to the lack of acrosome structures and enzymes, i.e., fertilization under natural conditions is impossible (Dam et al. 2007). The increased rate of sperm with fragmented DNA observed in globozoospermia is thought to be due to impaired chromatin condensation with pathological histone–protamine ratios (Dam et al. 2007; Faja et al. 2021).

20.4.3 Acephalic Spermatozoa

Acephalic, usually well-motile spermatozoa, are referred to as pinheads in the classification of sperm morphology in practice (WHO 2010) (Chap. 9) (Fig. 20.2d, d′). Such pathological forms can be observed sporadically in ejaculates of fertile males; in fertility disorders, their proportion may exceed 10% of spermatozoa. Rarely, **acephalic spermatozoa** occur as the predominant phenotype, i.e., a monosymptomatic defect, which has also been called "decapitation syndrome" (Haidl and Schuppe 2006). The underlying cause is a defective connection between the head segment and the neck region during spermatid differentiation. Ultrastructural studies showed either a lack of attachment of the capitulum as part of the junction to the so-called basal plate in the concave implantation fossa at the distal end of the nucleus or a dissociation of the proximal and distal centrioles (Chemes and Rawe 2003).

Molecular details of the complex connecting apparatus between sperm head and flagellum involving various proteins such as SUN5 and PMFBP1 have recently been studied in a knockout mouse model (Zhang et al. 2021). In humans, variants in the *PMFBPI, SUN5* as well as *TSGA10* genes can also cause a phenotype with acephalic spermatozoa (Houston et al. 2021).

20.4.4 Midpiece and Flagellum Defects

Many patients with monogenic forms of disorders of spermiogenesis have a combination of astheno- and teratozoospermia resulting from sperm structural defects in the midpiece and flagellum (Chemes and Rawe 2003). A normal sperm tail has **nine pairs of microtubules** arranged concentrically around a central pair (9 + 2). The tubules of each pair are interconnected to the neighboring doublet by two **dynein arms** and reach out to the central tubules via nine radial spokes; the axoneme is surrounded externally in the region of mid-piece and principal piece by **outer dense fibers** and a **fibrous sheath** (see Chaps. 3 and 9; Fig. 20.3). All individual components of this complex structure, in which more than 1000 proteins are involved, can be affected by defects and a corresponding loss of function. These are also summarized under the term "**multiple morphological abnormalities of the sperm flagella" (MMAF)** (Coutton et al. 2015). As a common feature of the phenotypically heterogeneous group of midpiece and flagellum defects severe impairment up to complete loss of motility with preserved normal sperm viability is observed. Abnormal, largely varying diameters along the midpiece and flagellum are found together with obvious membrane damage and disruption of the regular structures (Fig. 20.2e, e′); this may also include deficiency or complete absence of mitochondria localized in the midpiece. In addition, pathological changes in the length of the flagella are evident, ranging from missing end pieces to severe developmental disorders of spermatozoa resulting in a rudimentary arrangement of the flagellum due to dysplasia of the fibrous envelope (short or stump tail syndrome) (Stalf et al. 1995; Chemes and Rawe 2003) (Fig. 20.2f, f′).

In the absence of the dynein arms connecting the microtubules, there is complete sperm **immotility**. If, in addition to asthenoteratozoospermia, the patient also has abnormal ciliary function in the nose and lungs associated with impaired mucociliary clearance, respiratory tract infections, and bronchiectasis, the condition is referred to as **primary ciliary dyskinesia (PCD)**, formerly also called **immotile cilia syndrome** (Afzelius et al. 1975; Chemes and Rawe 2003; Sironen et al. 2020). A subtype of this rare condition is **Kartagener's syndrome**, in which situs inversus is found in combination with the aforementioned symptoms (Kartagener 1935). In addition to anomalies of the dynein arms, other

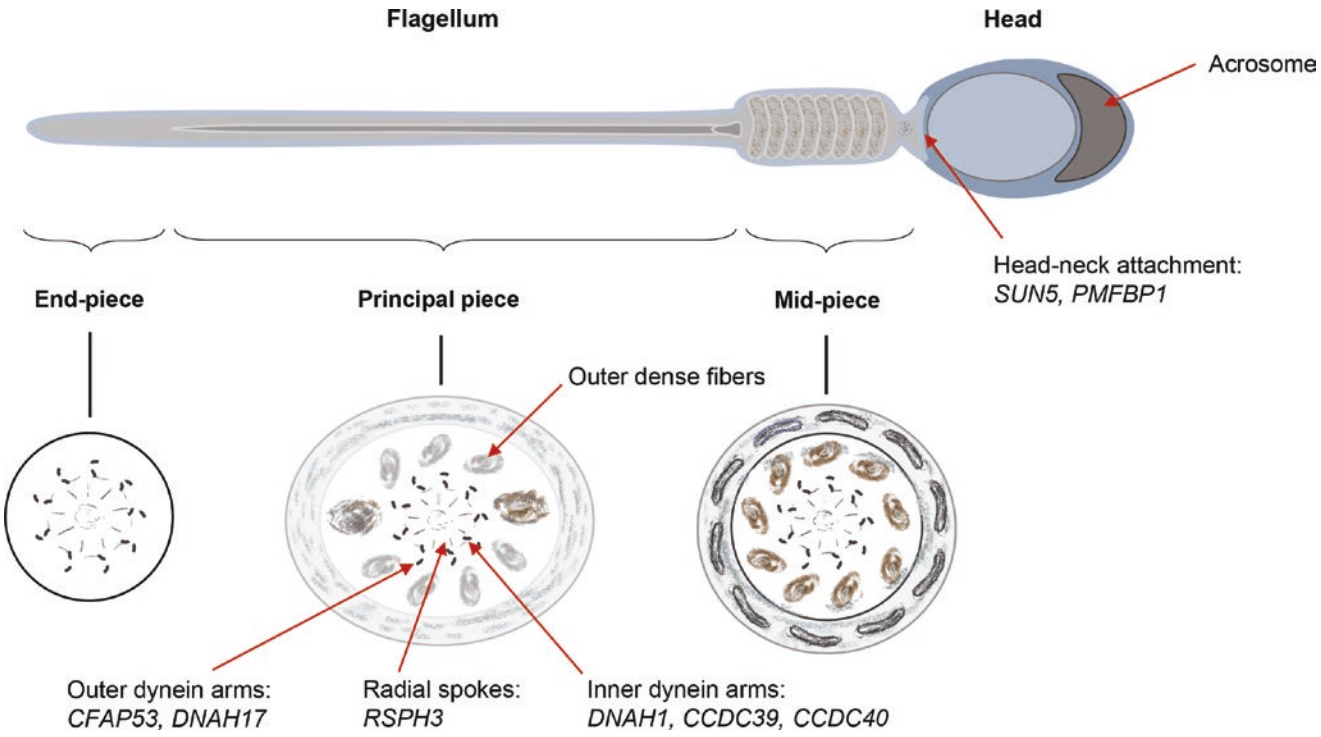


Fig. 20.3 Schematic cross-sectional view of a spermatozoon and its structural elements. The functions of proteins encoded by genes associated with astheno- or teratozoospermia are labeled; genes encoding proteins involved in acrosome formation include DPY19L2 (see Table 20.2)

ultrastructural defects of the sperm axoneme can occur in PCD (Coutton et al. 2015). For example, the **9 + 0 syndrome** is characterized by an absence of the centrally placed pair of microtubules (Baccetti et al. 1979).

Family studies provided early evidence that PCD, including Kartagener syndrome, is genetically determined; the disorder is inherited as an autosomal recessive trait in most cases. For example, genes encoding dynein proteins, such as *DNAH1* (dynein axonemal heavy chain I), have been linked to PCD and mutations in these genes have been described (Coutton et al. 2015). As mentioned above, some of the genes associated with an MMAF phenotype are expressed in organs other than the testis. The absence of the respective protein(s) may affect ciliary beating in the nose and lungs in addition to the beating of the flagellum. Various genes have now been described as associated with MMAF (Houston et al. 2021) (Table 20.2). The function of some of these genes is shown in Fig. 20.3.

20.4.5 Clinic and Diagnostics

The leading symptom of structural sperm defects is infertility, which may also be part of a syndromal clinical picture such as PCD. In patients with an MMAF phenotype, it is therefore advisable to specifically ask about complaints resulting from rhinitis, sinusitis, otitis media, recurrent or chronic bronchitis, and pneumonia when taking the patient's medical history. If bronchiectasis or situs inversus are already known, this can also be indicative.

Clinical andrological findings such as testicular volumes and basal hormone status are usually unremarkable in patients with specific structural sperm defects due to spermiogenesis disorders. If Kartagener syndrome is suspected, a chest radiograph should be included in the diagnostic work-up.

The central element of diagnosis is **ejaculate examination**, including accurate analysis of **stained smear preparations** (WHO 2021) (Chap. 9). Spermiograms typically show teratozoospermia or asthenoteratozoospermia, sometimes combined with oligozoospermia (Coutton et al. 2015). To differentiate between immotile and dead spermatozoa in the ejaculate, a vitality test such as eosin staining should be applied. When assessing sperm morphology, the monosymptomatic defects shown above can already be readily identified by light microscopy (Fig. 20.2). Detailed ultrastructural characterization of defects, especially in MMAF, is reserved for electron microscopic studies.

Genetic testing is gaining increasing diagnostic importance (see Chap. 8). In contrast to disorders of spermatogenesis, cytogenetic abnormalities are usually not found in patients with impaired spermiogenesis. Instead, more than 30 genes are now known to be associated with sperm

morphological abnormalities (Houston et al. 2021) (Table 20.2; Fig. 20.3). In this case, accurate phenotyping of sperm defects is even more important, as it opens the opportunity to targeted analysis of genes associated with them.

When monogenic causes of male infertility in conjunction with disorders of spermiogenesis are suspected, molecular genetic analysis can be performed prior to reproductive medical treatment according to the current AWMF guidelines on diagnostics and therapy before ART (Toth et al. 2019). There are currently no recommendations concerning the genes to be examined. As with the disorders of spermatogenesis presented above, **exome-based panel diagnostics** are recommended for patients with MMAF phenotype due to its heterogeneity (see Sect. 20.3.4).

20.4.6 Therapy

Causal therapy is not available for the structural sperm defects listed here. With the exception of macrozoospermia, the only remaining symptomatic treatment approach is **assisted fertilization by IVF/ICSI** (Chap. 42). However, in cases of complete globozoospermia, there is virtually no chance of success without **artificial oocyte activation**, and even with the use of calcium ionophore or other activators, fertilization rates after ICSI remain low (Chansel-Debordeaux et al. 2015). IVF/ICSI can also be performed in patients with immotile cilia; the HOS test or laser-assisted activation of membrane responsiveness are suitable for identifying and selecting vital sperm in the presence of absolute immotility (WHO 2021; Nordhoff 2015). Births of healthy children conceived in this manner have been reported (Gerber et al. 2008; Jayasena and Sironen 2021). Similarly, pregnancies have been observed after IVF/ICSI in patients with *SUN5* mutations and predominantly acephalic sperm, using motile sperm with pathomorphic but preserved head-neck junction (Fang et al. 2018).

Key Points

- The leading symptom of disorders of spermato- and spermiogenesis is infertility; corresponding limitations of semen quality in affected men range from oligo-(astheno terato) zoospermia to non-obstructive azoospermia.
- Multiple causes of oligoasthenoteratozoospermia include hypothalamic-pituitary disorders of endocrine regulation, direct testicular damage, and post-testicular disorders, each of which may be either congenital or acquired.
- Frequently, however, the etiology of male fertility disorders remains unclear.
- In non-obstructive azoospermia, testicular histology allows accurate differentiation of underlying disorders of spermatogenesis, the most serious of which include spermatogenic arrest and Sertoli cell-only phenotype.
- Structural sperm defects reflect disorders of spermiogenesis and can be diagnosed by light microscopy using stained ejaculate smear preparations; special attention is given to specific defects occurring in the majority of spermatozoa.
- Genetic diagnostics is becoming increasingly important; molecular genetic testing methods such as exome sequencing can be used to identify disease-relevant variants (mutations) associated with disorders of spermatogenesis or specific sperm defects.
- In cases of non-obstructive azoospermia due to homogeneous spermatogenic arrest or complete SCO phenotype, there are no causal therapeutic options; frustrating symptomatic therapy measures such as multi-locular testicular biopsies (TESE) may be avoided in the future by preoperative gene panel diagnostics.
- In case of systematic structural sperm defects the only remaining symptomatic treatment approach is assisted fertilization by IVF/ICSI, with the exception of macrozoospermia.

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Klinefelter Syndrome

21

Fabio Lanfranco, Lorenzo Marinelli,
and Eberhard Nieschlag

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Abstract

The Klinefelter syndrome (KS) is the most frequent form of male hypogonadism and of chromosome aneuploidy in humans. In the majority of cases, it is due to the congenital numerical chromosome aberration 47,XXY, with a reported prevalence of 0.1–0.2% in the general population and of up to 3.1% in infertile males. In adulthood, the syndrome is typically characterized by the constellation of small firm testes, infertility, and symptoms of androgen deficiency. Moreover, it is accompanied by a series of comorbidities, leading to more frequent hospitalization and contributing to a significant increase in mortality risk. Early recognition and hormonal treatment of KS can significantly improve the patients' quality of life and prevent serious consequences. Although KS patients are azoospermic or have only few sperm in the ejaculate, some may have isolated testicular foci with intact spermatogenesis so that sperm can be extracted and be used for intracytoplasmic sperm injection leading to pregnancies. Due to the extreme heterogeneity of the clinical phenotype, KS is vastly underdiagnosed, and greater knowledge among physicians as well as establishment of standard care in multidisciplinary centers and networks is mandatory.

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21.1 Introduction

The **Klinefelter syndrome (KS)** was first described in 1942 as an endocrine disorder characterized by small firm testes, gynecomastia, hypogonadism, and elevated levels of follicle-

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stimulating hormone (FSH) (Klinefelter et al. 1942). The genetic origin of the condition, namely a supernumerary X chromosome, was identified in 1959 (Jacobs and Strong 1959).

With a reported **prevalence of 0.1–0.2%** in the general population and of up to 3.1% in the infertile male population (Bojesen et al. 2003; Lanfranco et al. 2004; Kanakis and Nieschlag 2018), KS is the most frequent form of male hypogonadism and of chromosome aneuploidy in humans.

Due to extreme heterogeneity of the clinical phenotype, KS is largely underdiagnosed. However, KS is associated with a significantly higher morbidity rate compared to the male population as a whole, due to the involvement of almost all organ systems (Nieschlag 2013). This leads to a life expectancy 11.5 years below that of the entire male population (Bojesen et al. 2011).

Early recognition and hormonal treatment of KS patients can significantly improve the patients' quality of life and prevent serious consequences. Testosterone replacement corrects symptoms of androgen deficiency caused by the syndrome but has no positive effect on fertility. Nowadays, however, KS patients, including the non-mosaic type, may no longer be considered infertile a priori, as intracytoplasmic sperm injection (ICSI) offers the opportunity for procreation even when spermatozoa in the ejaculate are lacking.

21.2 Epidemiology

The prevalence of KS is 1–2/1000 according to the studies involving systematic screening of male newborns in the 1960–1970s (Maclean et al. 1964; Hamerton et al. 1975). The first investigations on postnatal prevalence were conducted in the USA, Canada, Europe (United Kingdom and Denmark), and Asia (Russia and Japan), and stated a prevalence ranging from 85 to 223 per 100,000 males (for a review, see Zavattaro et al. 2020). These data were confirmed by other studies (Swerdlow et al. 2005; Viuff et al. 2015). In particular, in Denmark, where data from solid newborn registries are available, a prenatal prevalence of KS ranging from 153 to 173 per 100,000 males has been estimated; this result is in line with the findings of a study performed in the early 90's, which found a postnatal prevalence (in liveborn males) of 152 per 100,000 males (Nielsen and Wohlert 1990; Bojesen et al. 2003; Gravholt et al. 2018).

These data imply that with the current diagnostic strategies more than two thirds of the actually existing **KS men may remain undiagnosed** throughout their lifespan (Nieschlag 2013). Abramsky and Chapple (1997) calculated that 10% of expected cases of KS boys were identified prenatally, 26% were diagnosed in childhood or adult life because of hypogonadism, gynecomastia, or infertility, while the remaining 64% were undiagnosed. More recent studies report different percentages regarding mean age at diagnosis (21% detected prenatally, 28% pre-adulthood, and 51% in adulthood) demonstrating the changes over time (Zavattaro et al. 2020).

Although the sensitivity and specificity of KS diagnosis have improved, it remains unclear whether still only a minority of all patients are properly diagnosed and most of them remain undetected despite high morbidity and mortality, and thus frequent contact with physicians (Nieschlag 2013).

21.3 Pathophysiology

About 80% of KS cases are due to the **congenital numerical chromosome aberration 47,XXY**; higher grade chromosome aneuploidies (48,XXXY; 48,XXYY; 49,XXXXY), 46,XY/47,XXY mosaicism, and structurally abnormal X chromosomes characterize the remaining 20% (Foresta et al. 1998; Kamischke et al. 2003; Kanakis and Nieschlag 2018). The true prevalence of mosaic forms may be underestimated because chromosomal mosaicism may be limited to testes, while the karyotype of peripheral leukocytes appears to be normal.

The aneuploidy in KS is the result of **non-disjunction** that can take place both in meiosis I and in meiosis II of **maternal oogenesis** or during meiosis I of **paternal spermatogenesis** (Fig. 21.1). The possibility that the supernumerary X chromosome in KS originates either from maternal or paternal gametogenesis seems to be almost equal. Less frequently (~3%), non-disjunction occurs during mitosis of the early post-zygotic divisions (Zitzmann et al. 2015).

Advanced maternal age is the only evidence-based risk factor for KS. In particular, maternal age >40 was associated with a 4-fold increase in the risk of conceiving a Klinefelter fetus as compared to maternal age <24 (Bojesen et al. 2003; Tüttelmann and Gromoll 2010; Mazzilli et al. 2018). The evidence relating advanced paternal age to sex chromosome aneuploidies is less robust (Fonseka and Griffin 2011).

In non-mosaic KS subjects, a **supernumerary sex chromosome** is most likely found in all cells, as demonstrated by the analysis of peripheral lymphocytes. This may lead to X-chromosomal gene overdosage originating from both genes located on the pseudo-autosomal regions at the very tips of each sex chromosome that regularly do not undergo lyonization, as well as from "escapee genes," dispersed all over the X-chromosome (Zitzmann and Rohayem 2020). The vast phenotypic spectrum encountered in KS results from a variety of further genetic mechanisms, among which

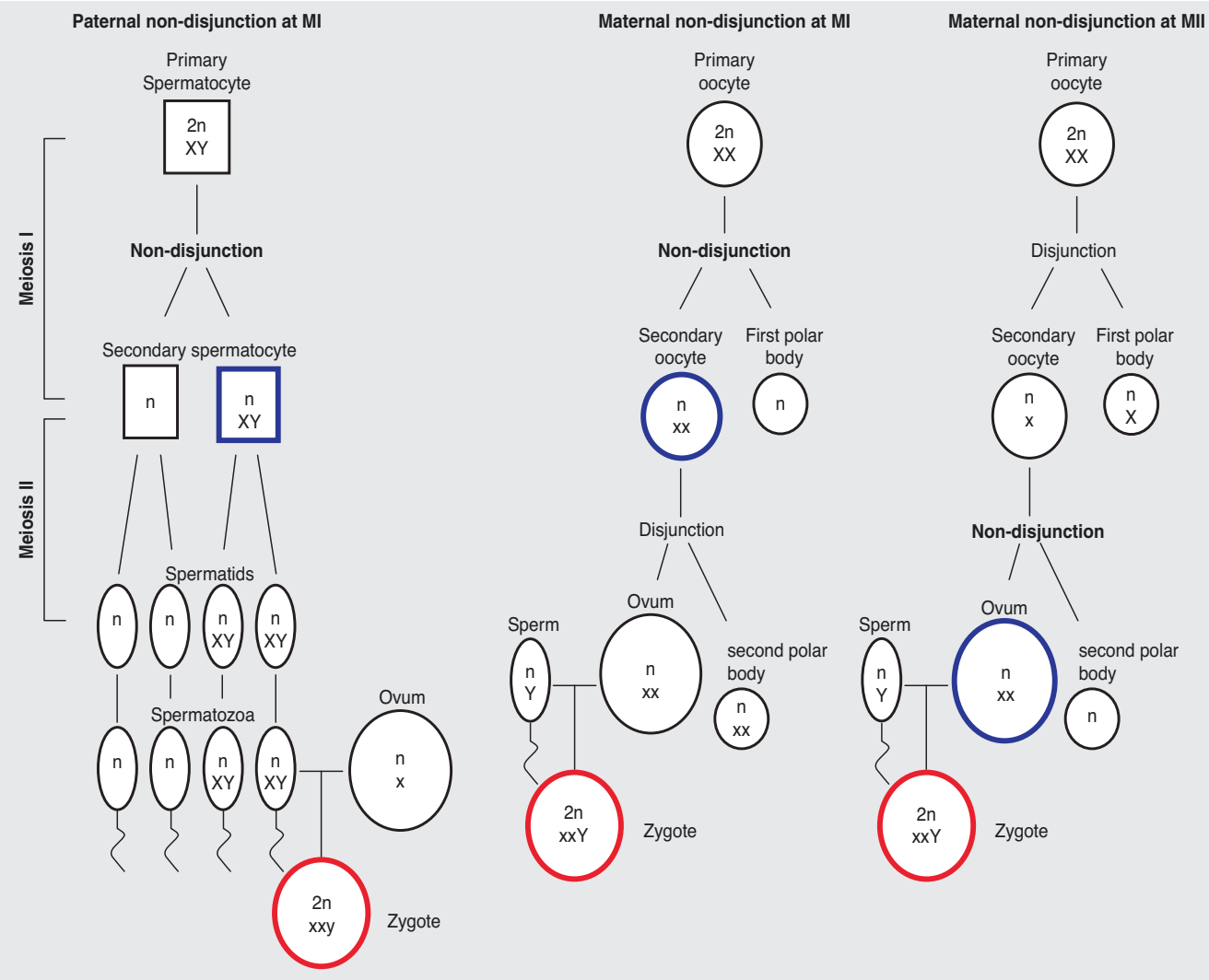


Fig. 21.1 Etiology of the Klinefelter syndrome: non-disjunction of the sex chromosomes in paternal or maternal meiosis I or in maternal meiosis II (modified from Lanfranco et al. 2004)

tissue mosaicism might be a potential contributor. In addition, differential expression of parental alleles, due to differential imprinting, differential methylation patterns, or other epigenetic regulatory mechanisms, may be involved, as well as differential expression of RNA and non-coding RNA and differential protein–protein interactions (Skakkebaek et al. 2018). Finally, the individual sensitivity of the human androgen receptor (AR) may modulate the phenotype (Zitzmann et al. 2004).

Although **primordial germ cells** are present in the testes of Klinefelter patients, they degenerate unusually fast, so that by the time of puberty, none or only few germ cells are still present (Aksglaede et al. 2006). Simultaneously, the **Leydig cells** become hyperplastic. However, since the testes remain very small because of the lack of spermatogenesis, the total number of Leydig cells remains low and different nuances of androgen deficit ensue.

21.4 Clinical Picture

The clinical picture of Klinefelter patients who come to medical observation varies according to age (Table 21.1) (Gravholt et al. 2018).

Prior to puberty, only discrete physical anomalies may be noticed. Some boys with KS show clinical signs of intrauterine hypogonadism such as cryptorchidism, reduced penile length, and testicular volume. Boys with KS rarely fulfil the criteria for **micro-penis** (<−2.5 cm length)), although one study found a prevalence of 17% (Davis et al. 2016).

Sexual development may be normal before puberty and includes initiation of normal pubertal changes and normal pituitary gonadal function (Kanakis and Nieschlag 2018). Some of the children have **learning difficulties** and their **verbal expressiveness** are limited.

After childhood, the syndrome is typically characterized by the constellation of small firm testes, infertility, and symptoms of androgen deficiency. The evolution of physical and psychological alterations in Klinefelter patients from childhood until adulthood has been exhaustively investigated by Ratcliffe (1999) and includes a tendency to central obesity, failure of normal testicular growth, development of gynecomastia in more than 50% of cases, delayed speech development, difficulty in learning to read, poorer mathematical ability, difficulties in relationships with peers and siblings, and a higher rate of psychiatric referral in comparison to cytogenetically normal subjects.

The **testicular volume** of an adult patient with a 47,XXY-karyotype is generally in the range of 1–3 ml, per testis, rarely over 4 ml (Fig. 21.2).

There is a great range of **variations in the degree of virilization** (Fig. 21.3). Because of initially still normal serum androgen levels, about 60% of patients have a penis of normal length. At the time of normal puberty, characteristic skeletal proportions begin to develop. The patients are often taller than average. In contrast to typical eunuchoid tall stature, the arm span seldom exceeds total body height; the legs, however, are remarkably longer than the trunk (lower height > upper height).

After the age of 25, about 70% of patients complain of **decreasing libido** and potency. Normal beard growth is present only in about one fifth of patients. The severity of testosterone deficiency can vary greatly; thus, the clinical phenotype can range from almost eugonadism to severe hypogonadism.

During puberty, nearly half of the patients develop painless bilateral **gynecomastia** of varying degrees. Gynecomastia may be associated with a slightly higher incidence of breast carcinoma than in men with normal karyotype who in total account for barely 1% of all breast cancers (Nieschlag 2013).

Apart from the apparent phenotypical aberrations, KS is accompanied by a series of **comorbidities** (Table 21.2) leading to more frequent hospitalization and contributing to a significant increase in mortality risk by almost 50% (Hazard ratio (HR) for all-cause mortality = 1.40–1.70).

Patients with KS seem to have an increased mortality ratio for all cancers, especially regarding **breast and lung cancer** as well as **non-Hodgkin lymphoma** (Swerdlow et al. 2005). **Mediastinal non-seminomatous germ cell tumors** are more common in KS patients, most commonly between the ages of 15 and 30 years. Interestingly, a lower mortality from **prostate cancer** has been reported (Swerdlow et al. 2005). **Osteoporosis** results in an increased incidence of bone fractures; femoral fractures are associated with a

Table 21.1 Main clinical findings in Klinefelter patients

Feature	Frequency (%)
Small firm testes (bi-testicular size <6 ml)	>95
Increased gonadotropin levels	>95
Azoospermia	>95
Maldescended testes/cryptorchidism	27–37
Decreased testosterone levels	63–85
Decreased facial hair	60–80
Decreased pubic hair	30–60
Increased height (leg length)	30

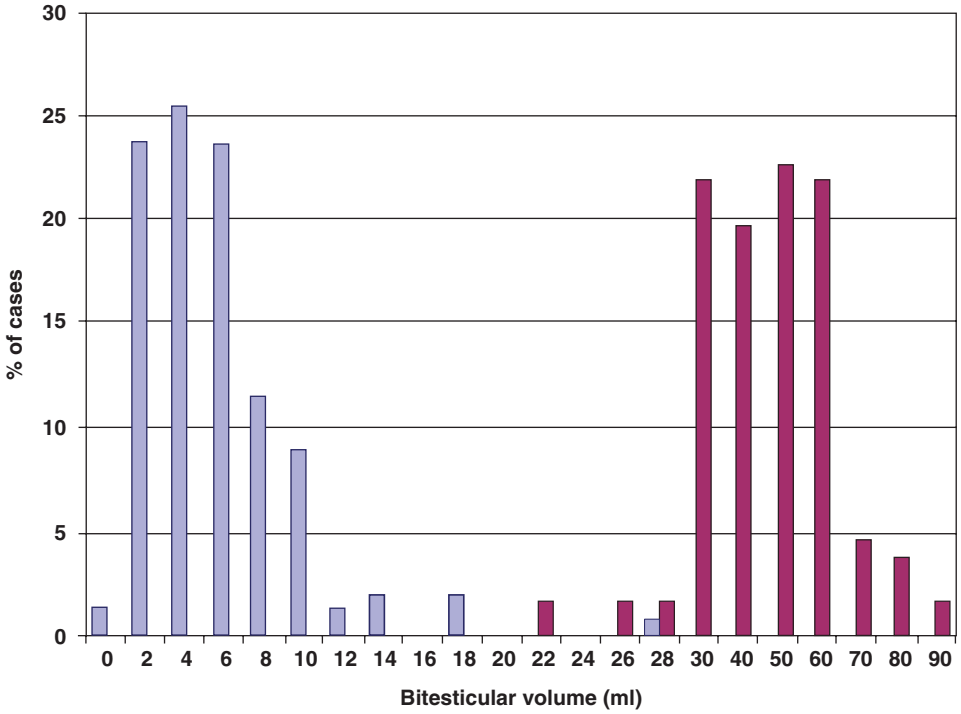


Fig. 21.2 Distribution of bi-testicular volume measured by ultrasonography in 160 Klinefelter patients (**blue**) and in 137 46,XY males (**red**) (Adapted from Lanfranco et al. 2004)



Fig. 21.3 A thirty-year old man with Klinefelter syndrome. Sparse virile hair pattern with horizontal pubic hair line, bilateral gynecomastia and bilateral testicular volumes of 2 ml each, venous varicosities of the legs

Table 21.2 Main comorbidities associated with Klinefelter syndrome, their incidence and their supposed etiopathogenesis

Comorbidities	Incidence (%)	Etiopathogenesis (based on the supernumerary X)
Type 2 diabetes mellitus	10	Prevalence of T2DM and MetS is higher in KS than in other forms of hypogonadism. Obesity precedes puberty
Metabolic syndrome	44	
Osteopenia	40	May be caused by several factors, e.g., low testosterone, long androgen receptor CAG repeats, low INSL3, low muscle mass or low vitamin D
Osteoporosis	10	
Hip fracture	7	
Verbal disorders	70–80	Abnormal structure of brain areas, executive and social dysfunction
Mental retardation	4.2	
Thrombosis	4.7	Combination of imbalance between thrombosis and hemostasis with increased platelet aggregation rate
Pulmonary embolism	2.3	
Maldescended testes	27	Possible relative androgen deficiency in the intrauterine milieu
Gynecomastia	38	Not present before puberty—associated with hypogonadism
Breast cancer	0.3	Independent of the increased gynecomastia rates
Mediastinal tumors	0.4	Not yet known

high-mortality rate. Osteoporosis in KS patients may be caused by a number of factors, e.g., low testosterone, long androgen receptor CAG repeats, low INSL3, low muscle mass, or low vitamin D. KS patients suffer from vascular diseases, particularly **varicose veins** and **thrombo-embolisms**, most commonly pulmonary embolisms.

Central obesity with reduced glucose tolerance is often observed and can lead to **metabolic syndrome and type 2 diabetes**. Epilepsy and other neurological and mental disorders occur significantly more frequently in KS patients (Swerdlow et al. 2005; Bojesen et al. 2006; Kanakis and Nieschlag 2018; Chang et al. 2020). There are reports on retinal dysfunction and impaired day vision/night vision in KS (Brand et al. 2017). Development of teeth may be affected, imposing as **taurodontism** (Giambersio et al. 2019) and an increased risk of caries has been reported (Belling et al. 2017). Also **autoimmune** disorders might be more frequent in KS. However, the reports on ophthalmologic, dental, and autoimmune diseases are based on small numbers of patients with KS and need validation (Zitzmann et al. 2020).

Whether testosterone deficiency alone is responsible for this **increased morbidity and mortality** is doubtful. One risk factor may be the usually reduced arterial diameter of KS patients, which leads to reduced perfusion of organ systems (Bojesen et al. 2011; Foresta et al. 2012). Finally, the increased mortality of KS individuals seems to be affected by their lower socio-economic achievements (Bojesen et al. 2011; Kanakis and Nieschlag 2018).

The **intellectual capacity** of KS subjects may be completely normal. Boys with KS may come to attention because

of deficits in very specific domains of cognition, mainly language and executive functions (involved in concept formation, problem solving, task switching, inhibitory processes, speeded responding and planning), which seem similar to those observed in cytogenetically normal dyslexic children (Ratcliffe 1999; Temple and Sanfilippo 2003). **Attention deficits** may arise and socialization causes problems. In younger boys with KS, a delay in speech development may be observed, whereas significant deficits in higher aspects of **expressive language** are more common in adolescents. In addition, it has been shown that KS is associated with difficulties in identifying and **verbalizing emotions** (Van et al. 2007). Often KS subjects fail to reach the level of achievement or professional niveau of their families (Ratcliffe 1999).

Observations on the clinical picture of KS have to be regarded with caution, since two thirds of all patients are not detected and, hence, not characterized. It must be speculated that the clinical picture observed is a biased one, showing only the extreme cases, while those men with more unobtrusive phenotypes lead "ordinary lives" except for perturbations of fertility due to meiotic malfunction. KS patients with chromosome mosaics may show very few clinical symptoms and the testes may be normal in size. Endocrine abnormalities are also less severe, and gynecomastia and azoospermia are less common (Lanfranco et al. 2004).

In poly X-KS, the phenotype progressively deviates from normal as the number of X chromosomes increases. In XXXY and XXXXY, the frequency of almost any somatic anomaly is increased compared to XXY and severe additional problems regarding psycho-motor development are present. Thus, these conditions may be considered as clinically different entities (Tartaglia et al. 2011; Gravholt et al. 2018).

21.5 Diagnosis

21.5.1 General Features

A suspected diagnosis can usually be based on the combination of typical clinical findings (Table 21.1). The most important of these are **very low testicular volume** (1–3 ml) and firm consistency of the testes. Symptoms described above of varying degrees provide additional indications. Of the general and clinical features, testicular volume appears to be the most sensitive parameter, showing the smallest overlap between KS and non-KS patients who are referred for medical assessment (Kamischke et al. 2003; Lanfranco et al. 2004) (Fig. 21.2).

While detection of an extra **Barr body** in cells from buccal mucosa has been used in the past to evidence the presence of the supernumerary X-chromosome, **karyotyping** performed in peripheral blood cells is currently the well-established standard procedure for diagnosing chromosomal aneuploidies (Zitzmann and Rohayem 2020; Zitzmann et al. 2020).

KS may be diagnosed **prenatally by cytogenetic evaluation** [conventional karyotyping or array-CGH (array-comparative genomic hybridization)] of chorion villus tissue or amniotic fluid. Massively parallel sequencing of circulating cell-free fetal DNA in maternal plasma has allowed for the development of **non-invasive prenatal testing (NIPT)**, which is widely used for the screening for autosomal trisomies. Data on sensitivity and specificity of the detection of sex chromosome aneuploidies (SCA) are sparse and there is a tendency towards increased false positive results. Importantly, NIPT is not a diagnostic test and **requires cytogenetic confirmation** (Gil et al. 2017).

The **large variety in the clinical phenotype** of KS patients can be explained in part, however. Some genetic aspects related to the phenotype remain undetermined. It involves the dosage effect and the expression/inactivation status of the X chromosome genes, the rate of androgen production, the presence of mosaicism, the number and the derivation (maternal or paternal) of supernumerary X chromosome/s, and the activity of the genes located in the pseudo-autosomal regions (PAR) of the sex chromosomes (Bonomi et al. 2017). In addition, in classic 47,XXY KS patients, polymorphisms of the androgen receptor play a role. Thus, patients with longer **CAG triplets** show more extreme symptoms of hypogonadism than patients with shorter CAG triplets. This gives rise to the assumption that patients with shorter CAG triplets tend to seek out centers of reproductive medicine because of involuntary childlessness while those with longer CAG repeats tend to visit an endocrinological center because of prevailing symptoms of androgen deficiency. The expression of specific symptoms may depend on the CAG triplets length such as gynecomastia, body height, penile length, hematocrit, as well as partnership and professional success (Zitzmann et al. 2004; Chang et al. 2015; Kanakis and Nieschlag 2018).

21.5.2 Endocrine Dysfunction

There is a paucity of data regarding sex steroid secretion in KS patients during childhood (Bojesen et al. 2014). The temporary surge in gonadotropins and testosterone observed in early infancy, also known as **"mini-puberty,"** is usually present with FSH levels peaking at 2–3 months of age, followed by a subsequent rapid decline. This pattern agrees with that observed in 46,XY males. A recent study showed that during mini-puberty of infants with KS, serum FSH and LH levels were significantly higher than in controls, as were inhibin B and testosterone. No significant differences were found in height, weight, testicular volume, and penile length (Spaziani et al. 2021).

During infancy and childhood, normal pre-pubescent serum concentrations of FSH, LH, testosterone, estradiol, anti-Müllerian hormone (AMH), inhibin B and insulin-like factor 3 (INSL3) have been demonstrated (Christiansen et al. 2003; Wikstrom et al. 2006). However, some studies have shown that boys with KS may already be androgen-deficient before puberty (Zeger et al. 2008).

Puberty in KS subjects seems to follow a pretty regular course. Adolescents with KS experience timely hypothalamic GnRH activation during puberty, with spontaneous pituitary LH and FSH secretion around 12 years of age. LH-stimulated testosterone secretion by Leydig cells induces **spontaneous pubertal virilization**. As a consequence, a pubertal Tanner stage IV–V is achieved in a high proportion of adolescents with KS, without any need for hormone replacement. These young males experience normal penile growth, pubertal body hair growth, beard growth, voice mutation, and awakening of libido with regular ejaculations (Zitzmann and Rohayem 2020). Nevertheless, **testosterone levels are usually suboptimal** to promote epiphyseal closure as in eugonadal boys, a fact that may contribute to the preponderance of tall stature among KS subjects and exacerbate the ratio between the trunk and the disproportionately long lower extremities (Chang et al. 2015).

Serum testosterone levels begin to decrease by 15 years of age, being reduced in about 80% of adult patients with 47,XXY karyotype. Not all men exhibit hypogonadal symptoms or low-serum testosterone levels. Hypogonadism may remain at a compensated state (with elevated serum concentrations of LH and yet normal testosterone levels) until the fifth decade of life (Lanfranco et al. 2004; Gravholt et al. 2018). Recent studies have suggested that the observed low-testosterone levels of KS are not the result of diminished production, but of defective release into the testicular bloodstream due to aberrations of the testicular vasculature (Tüttelmann et al. 2014) (Fig.21.4).

On average, **estradiol** is higher than in normal men. **Sex hormone binding globulin (SHBG)** serum concentrations are elevated, causing a further reduction of biologically active-free testosterone. The gonadotropins LH and FSH are usually elevated. **FSH** shows the best discrimination, and little overlap occurs with normal individuals, a consequence of the consistent damage of seminiferous tubules. **Inhibin B** levels are usually normal in prepubertal boys with KS but decrease significantly during late puberty (Christiansen et al. 2003). In adult KS men, serum inhibin B is undetectable due to tubular damage, serum **AMH** is lower than normal and lower **INSL3** levels have been described in comparison with normal subjects.

21.5.3 Disruption of Spermatogenesis

Practically all ejaculates from patients with 47,XXY karyotype show **azoospermia**. In some patients, few sperm or severe oligoasthenoteratospermia can be found, and exceptional cases of spontaneous paternity are reported in the lit-

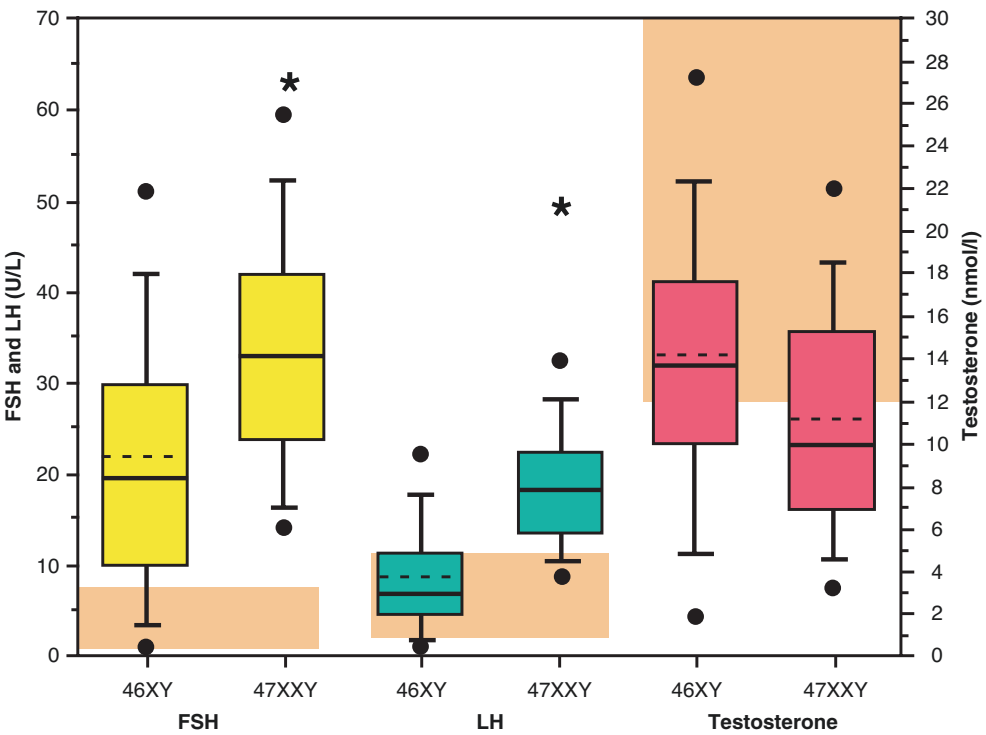


Fig. 21.4 Hormone concentrations in 228 Klinefelter patients and 224 infertile men with normal karyotype. The shaded areas represent the normal ranges of each hormone. Represented by box-and-whiskers plots, the asterisks indicate significant differences (Lanfranco et al. 2004)

erature. Among 29,336 karyotyped fathers, only one had a 47,XXY constellation (Samango-Sprouse et al. 2016).

Histologically, degeneration of seminiferous tubules with **Sertoli-cell-only syndrome (SCO)** is observed during puberty, followed by extensive hyalinization of the tubules. Foci containing intact spermatogenesis may exist in up to 50% of young KS males. In subjects with a history of undescended testes, the chances are reduced to around 30% (Ragab et al. 2018). Nevertheless, the chances to find **spermatozoa in semen** of KS adolescents only range around 7% (Lanfranco et al. 2004; Aksglaede et al. 2009; Rohayem et al. 2016), substantiating the rationale for surgical procedures to attempt **testicular sperm extraction (TESE** or **micro TESE)** for cryopreservation and future use in assisted reproductive techniques.

21.5.4 Genetic Counselling

If a KS is identified by chance during prenatal diagnosis **genetic counselling is urgently required** to inform the parents confronted with this dilemma about the extent of the disease (Meschede et al. 1998). The termination rate in KS pregnancies varies from 11.6% to 87.5% in different centers and countries. In certain societies, the way research has changed our understanding of the natural history and prognosis of this condition has led to a significant decrease in the rate of termination, whereas in other societies it still remains high, depending on local traditions, religious beliefs, and legislation (Lalatta and Tint 2013).

21.6 Clinical Management

Whereas KS is usually considered in all guidelines dealing with the management of hypogonadism, prior to 2021 no specific guidelines and recommendations to care for patients with KS were ever published. Since then, the European Academy of Andrology (EAA) has provided a list of suggestions and recommendations in order to correctly manage KS patients from the prenatal period to adulthood (Zitzmann et al. 2020).

The management of KS children warrants the collaboration of a **multidisciplinary team** consisting of pediatric endocrinologists as well as developmental and behavioral specialists, in order to ameliorate the developmental defects of early life (Kanakis and Nieschlag 2018; Zitzmann et al. 2020). Moreover an initial echocardiographic study should be performed to reveal congenital cardiovascular abnormalities (Salzano et al. 2016). As the boy transits to adulthood, follow-up should be performed by a specialized team consisting of adult endocrinologists/andrologists, cardiologists, geneticists, and occasionally psychologists to aid in facing the challenges of adult life (Kanakis and Nieschlag 2018; Zitzmann et al. 2020).

In adults with KS, **testosterone substitution (TRT)** should be used, as this is generally thought to increase quality of life and prevent long-term effects (Nieschlag 2013; see Chap. 36). Although there is evidence that TRT in KS is not as effective as in 46,XY hypogonadal males, at least regarding body proportions and BMD, the association of hypogonadism with both increased morbidity and mortality is well established and should be addressed accordingly (Khaw et al. 2007; Pasquali et al. 2013). Many benefits of TRT such as improvement in energy level/stamina, attention span, mood, measures of cardiometabolic health and general well-being are also observed in young KS patients (Davis et al. 2017). Moreover, it has been suggested that early TRT may have a beneficial effect on KS boys' developmental and behavioral issues without serious adverse effects (Samango-Sprouse et al. 2015; Davis et al. 2019) although TRT is not a "cure" for the neurodevelopmental deficits of KS that may exist due to the possible effects of the extra X chromosome on brain development (Nieschlag et al. 2016). Further randomized controlled trials are required to confirm possible positive short- and long-term effects of testosterone treatment in infants and pre-pubertal boys with KS (Mason et al. 2020). Current knowledge is not sufficient to support such a treatment (Zitzmann et al. 2020).

Since specific data regarding therapeutic targets are not available for KS patients, the general guidelines on TRT should be employed (Bhasin et al. 2018; see Chap. 36).

Testosterone therapy must be accompanied by **regular checkups** that include inquiries into wellbeing, strength, and sexual function and examination of general condition, red blood cell count and hematocrit, bone density, prostate, and prostate serum antigen (PSA) (Nieschlag 2013). In KS patients older than 50 years, guidelines on the treatment of late-onset hypogonadism should be followed (Bhasin et al. 2018; Salonia et al. 2019). All studies and experience indicate that substitution should not be withheld from KS patients, which is very often still the case. (Kanakis and Nieschlag 2018). However, fertility issues should be clarified **before initiation of testosterone treatment** (see below).

Apart from TRT, patients should be followed up for their related **comorbidities** (Zitzmann et al. 2020). Regarding cardiovascular morbidity, the initial evaluation has been proposed to include risk assessment for metabolic syndrome and thromboembolic disease as well as echocardiography focused on systolic and diastolic dysfunction. The subsequent follow-up should be based on the relevant findings (Salzano et al. 2016).

21.7 Fertility Issues

Infertility in men with KS has remained an untreatable disease for a long time. However, in the past 25 years, several data have emphasized that subjects with KS may benefit from assisted reproductive technology due to the presence of

residual foci with preserved spermatogenesis (Lanfranco et al. 2004; Aksglaede and Juul 2013). It is still unclear whether the residual spermatogenesis originates from 47,XXY spermatogonia or from euploid germ cells (Sciurano et al. 2009) and whether the chances to find foci with remnant intact spermatogenesis decrease with advancing age in KS (Bhasin and Oates 2020).

In 1996, successful sperm recovery from azoospermic men with KS by means of testicular sperm extraction (TESE) was reported for the first time (Tournaye et al. 1996). One year later, Palermo et al. (1998) documented the first **pregnancies in KS after TESE/ICSI**.

Surgical sperm retrieval has revealed spermatozoa in up to one-half of non-mosaic KS patients selectively referred to centers specialized in assisted reproduction techniques (Aksglaede and Juul 2013). No differences in terms of sperm retrieval have been found between classic TESE and micro-TESE (Corona et al. 2017). Although no direct comparison is available, the success rate of ICSI seems to be reduced in KS when compared to the general population (Corona et al. 2019).

So far, over 250 healthy live-born babies have been conceived after TESE combined with ICSI in couples including a 47,XXY father (Aksglaede and Juul 2013; Corona et al. 2017; Zhang et al. 2020). The specific predictors of this approach are, however, still conflicting. Hormonal parameters, including levels of FSH, inhibin B, testosterone, and estradiol, as well as testicular volume, seem not to be predictive factors for sperm recovery in males with KS (Aksglaede and Juul 2013). Some authors have emphasized that KS subjects of a younger age (below 35 years) have a better chance of positive TESE (Rohayem et al. 2015; for a review, see Corona et al. 2017). However, other authors have not confirmed these results (Plotton et al. 2015). In conclusion, although KS patients are usually azoospermic, their actual fertility chances are similar to those in subjects with non-obstructive azoospermia.

A normal karyotype is usually found in infants born after ICSI using sperm obtained from KS subjects, in agreement with the presence of high percentages of chromosomally normal spermatozoa in these men. However, in apparently non-mosaic KS individuals, the average incidence of sex chromosome disomy is 6.3% (range 0%–25%), while in 46,XY/47,XXY mosaics it is 2.5% (range 0%–7%) (Sarrate et al. 2005). In addition, aneuploidy rates for XX- and XY-disomies were also significantly higher with respect to controls and non-genetic oligozoospermic subjects (Selice et al. 2010). Moreover, an increased incidence of autosomal aneuploidies in spermatozoa from subjects with non-mosaic KS has also been demonstrated (Morel et al. 2003).

Altogether, even if conception in KS appears relatively safe and the risk of chromosomal abnormalities is similar to that reported in subjects without KS, it is under discussion whether or not **preimplantation genetic diagnosis (PGD)** should be offered to couples with KS who undergo successful TESE and ICSI to avoid transferring abnormal embryos.

Finally, as germ cell depletion is already evident in the testes of 47,XXY infants and rapidly progresses, cryopreservation of semen samples may preserve future fertility in those young KS men identified before the time at which they present with infertility (Nieschlag 2013). In fact, cryopreservation of semen samples in boys in early puberty or containing very low numbers of spermatozoa is possible and could be offered to appropriate candidates.

The recent EAA Guidelines (Zitzmann et al. 2020) place a high value on the recommendation to provide information on fertility status and treatment possibilities to patients with KS and their parents, while lower value is placed on the suggestion regarding semen collection and **TESE in adolescents**, because not all pubertal patients are psychologically ready to focus on fertility and because success rates are similar if these procedures are performed later. Information should be provided at a level appropriate to the patient's age and cognitive level.

Key Points

- KS is the most common sex chromosomal disorder in men.
- Clinical features are highly variable among patients with KS, although common characteristics are severely attenuated spermatogenesis and Leydig cell impairment, resulting in azoospermia and hypergonadotropic hypogonadism.
- In addition, men with KS have a higher risk of cardiovascular, metabolic, neurocognitive, and other comorbidities, which might explain increased morbidity/mortality in these subjects.
- Both genetic and epigenetic effects due to the supernumerary X chromosome as well as testosterone deficiency contribute to this pathological pattern.
- The majority of patients with KS are diagnosed during adulthood, but symptoms can already become obvious during infancy, childhood, or adolescence.
- Infants with KS usually present with a normal male phenotype, however, KS may be suspected in case of bilateral cryptorchidism and/or micro-penis.
- During childhood, speech and behavioral disturbances, excessive growth, and abnormal body proportions may lead to a suspicion of KS.
- Delayed puberty, poor testicular development, gynecomastia, excessive height, learning disabilities, and psychosocial problems are symptoms that should cause suspicion of KS.
- Preservation of the fertility potential, i.e., cryopreservation of spermatozoa from ejaculate or testicular tissue is an option now widely available.
- KS is vastly underdiagnosed and greater knowledge among physicians as well as establishment of standard care in multidisciplinary centers and networks is mandatory.

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XX Male and XYY Karyotype

22

Frank Tüttelmann and Eberhard Nieschlag

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Abstract

XX males with female karyotype (46,XX) are phenotypically male and have neither internal nor external female genital organs. The prevalence is much lower than Klinefelter syndrome, 1:10,000 to 1:20,000. Most XX males have a causative translocation of the *SRY* gene (80–90% SRY positive). XX men have a severe testicular development disorder and do not produce sperm, so that a testicular biopsy is not indicated if the patient wishes to have children. Testosterone deficiency is often present, requiring substitution. On the contrary, men with XYY karyotype are usually discovered incidentally, e.g., due to infertility, and can be treated with conventional or assisted reproduction procedures.

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22.1 XX Male

22.1.1 Definition and Epidemiology

XX males with **female karyotype (46,XX)** are phenotypically male and have neither internal nor external female genital organs. This variation has a prevalence of 1:10,000–1:20,000 male newborns.

A female karyotype, i.e., an XX constellation of sex chromosomes (karyotype 46,XX) is much rarer than Klinefelter syndrome (47,XXY; Chap. 21) in otherwise (largely) inconspicuous boys/men. Common designations are XX male, also 46,XX-DSD (Differences [formerly Disorders] of Sex Development), and De la Chapelle syndrome after the first describer (De la Chapelle et al. 1964). To be distinguished are other XX-DSD forms associated with intersexual genitalia, such as in severe congenital adrenal hyperplasia (CAH) with virilization (Chap. 31).

The diagnosis may be made prenatally as an incidental finding, in boys, for example, when puberty is absent or delayed, or most commonly in men with infertility. In a Chinese andrology center, 160 cases with a 46,XX constellation were detected among 183,342 patients, of whom a karyotype was performed in 85,352, giving an incidence of approximately 1 per 1000 infertile men (Chen et al. 2020). In an Iranian center, among 8114 patients with azoospermia or

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marked oligozoospermia, 57 were diagnosed with karyotype 46,XX (Mohammadpour Lashkari et al. 2017).

22.1.2 Genetics

In 80–90% of XX males, the seemingly paradoxical finding of female karyotype and male differentiated individual is explained by a translocation of Y chromosomal material including the *SRY* locus to one of the two X chromosomes (SRY positive). This translocation results from random crossing-over during germ cell formation of the father (Fig. 22.1). The region transferred to the X chromosome contains the ***SRY* (Sex Determining Region Y)** gene, which has also been referred to as the **"Testis Determining Factor" (TDF).** In fact, the *SRY* gene alone is sufficient to determine the bi-potent gonad toward the testis in an XX embryo.

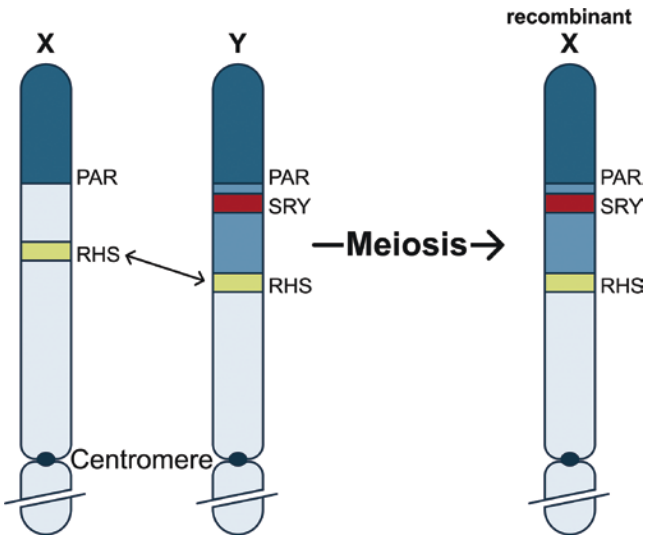


Fig. 22.1 Irregular meiotic recombination between X and Y chromosome leading to translocation of the testis-determining *SRY* gene to the X chromosome. This irregular exchange occurs during the meiosis of the father of the XX male. *PAR*: pseudo-autosomal region (identical sequences on X and Y); *SRY*: Sex Determining Region Y gene; RHS: Recombination Hot Spot (predilection site for irregular X-Y recombination). Light gray chromosome section = X chromosome-specific sequences; dark yellow-gray chromosome section = Y chromosome-specific sequences. ©MSc Nadja Rotte

Chromosomal analysis is supplemented with fluorescence in situ hybridization (FISH) of the *SRY* locus in the case of the surprising finding of 46,XX in a male sample (Fig. 22.2) to demonstrate the translocation.

In 10–20% of XX males, no *SRY* translocation is detected; these are termed **SRY-negative**. In these patients, the most common cause is found to be duplications in regulatory units upstream of the *SOX9* gene on chromosome 11. The *SOX9* gene (SRY-box transcription factor 9) is very closely linked to *SRY* in the cascade of sex determination, and an increased dose (due to duplication) appears to determine the gonad in the male direction even in the absence of *SRY*. Furthermore, if an XX male also has palmoplantar hyperkeratosis and possibly carcinoma, mutations in the **R-spondin 1 gene (*RSPO1*)** may be an extremely rare cause of XX-DSD (Parma et al. 2006). Currently available diagnostic possibilities mean that not all XX males can yet be causally clarified.

22.1.3 Clinic

Clinically, XX males are usually hardly distinguishable from Klinefelter patients and only the karyotype identifies the diagnosis. The testes are very small (1–2 ml) and firm and endocrine testicular function is often insufficient, with decreased testosterone serum levels and increased LH and FSH levels. Virilization may be lower than in Klinefelter men, especially in SRY-negative XX men. Feminine fat distribution is often found. Undescended testicles (usually bilateral) and gynecomastia are more common than in Klinefelter syndrome (Fig. 22.3). When testosterone falls below normal, there is decreased libido and erectile dysfunction.

In addition, abnormalities from the DSD spectrum such as hypospadias, as the mildest form, but also scrotum bifidum occur. The patients have normal intelligence. The body length is not only shorter than in Klinefelter patients, who are on average taller than 46,XY males, but is in the range of normal females (Vorona et al. 2007). This is likely due to the difference in copy number of the *SHOX* gene, which is located in the PAR region on X and Y chromosomes and is a major factor in length growth. Accordingly, Klinefelter

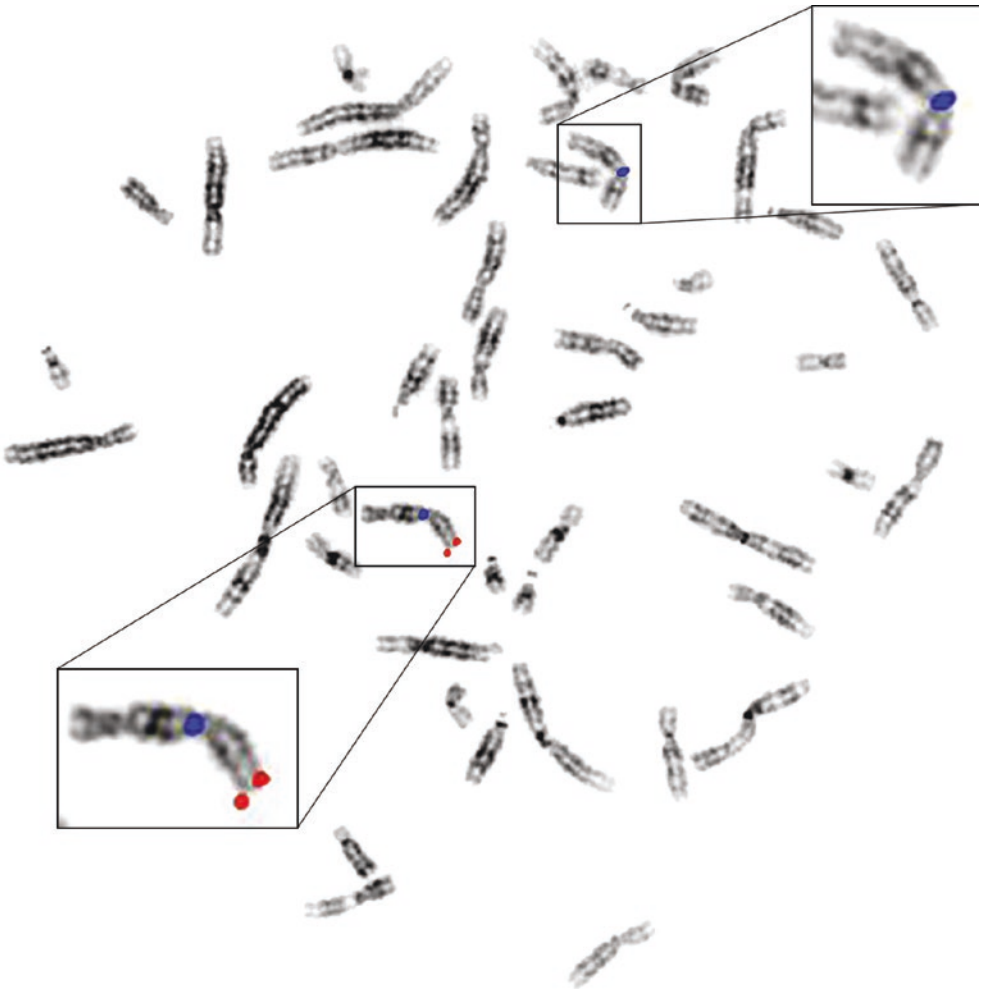


Fig. 22.2 Fluorescence in situ hybridization (FISH) in a man with azoospermia, karyotype 46,XX, and evidence of translocation of Y chromosomal material (red probe for SRY locus) to one of the X chromosomes (blue probe for X centromeres)

males have three *SHOX* copies and XX males as well as females have two. In the pre-pubertal period, cardiac abnormalities may lead to the diagnosis (Fechner et al. 1993). Diagnostically, an ultrasound of the abdomen is recommended to detect residual structures of the Müller ducts (Terribile et al. 2019).

Both SRY-positive and SRY-negative XX males lack the largest or all parts of the Y chromosome, including the AZF regions essential for spermatogenesis (Sect. 23.5). Accordingly, **XX males are infertile** due to a severe disorder of testicular anlage, which can range from a Sertoli cell-only (SCO) phenotype (Li et al. 2014) to gonadal dysgenesis. Testicular biopsy with an attempt at testicular sperm extraction (TESE) is not indicated (Terribile et al. 2019). If endocrine **hypogonadism** is present, which is not uncommon, **substitution therapy with testosterone** should be started (Chap. 36).

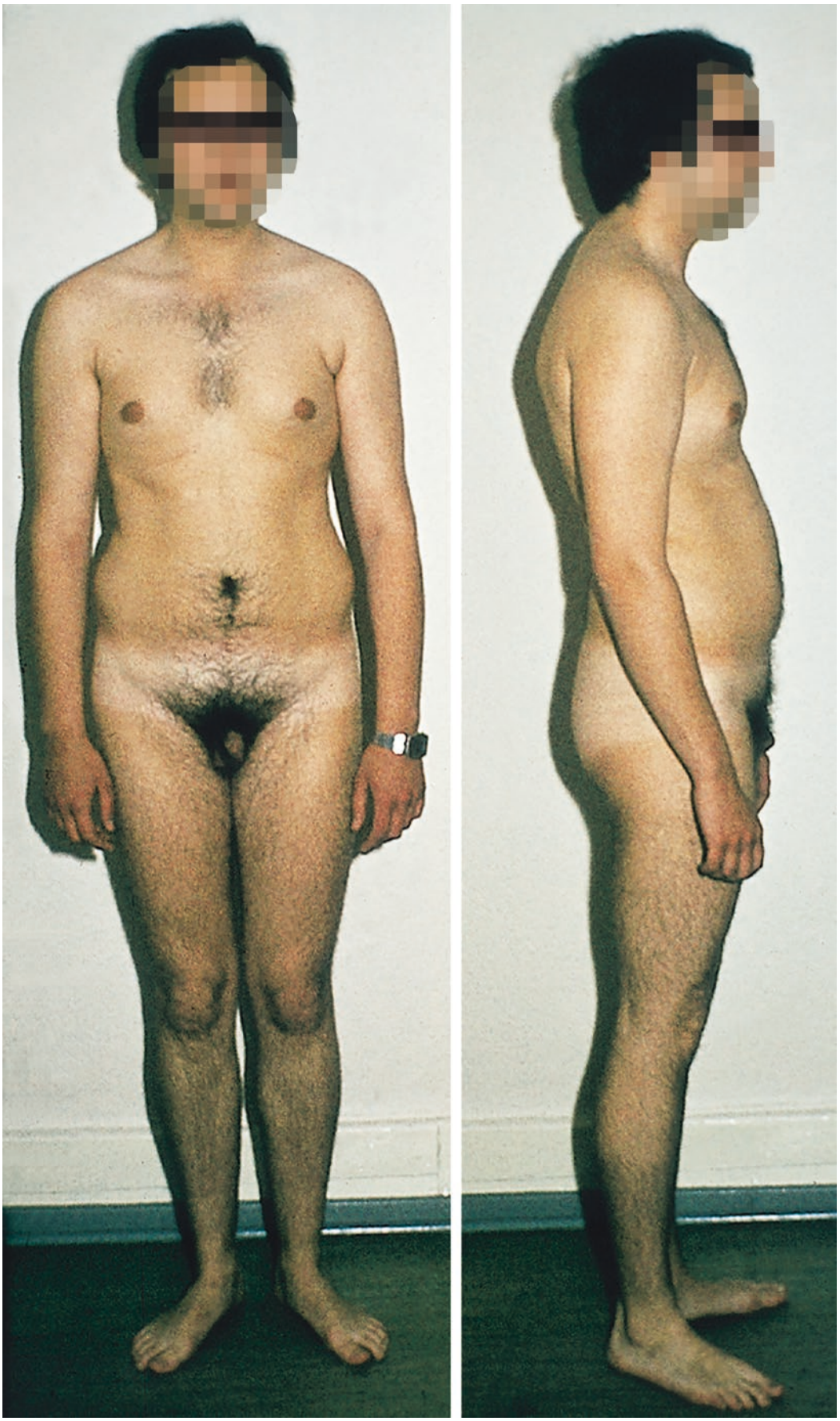


Fig. 22.3 A 27-year-old XX male

22.2 XYY Karyotype

Men with the 47,XYY karyotype are usually clinically unremarkable. The incidence is thought to be 1:1000 to 1:2000, but of these only 20% are diagnosed during their lifetime (Berglund et al. 2019). Considered as a group, their **height is 7 cm above the average of males** with a 46,XY chromosome set. Unlike patients with Klinefelter syndrome, these men are often **fertile**, although some have **oligozoospermia or azoospermia**, which may have another cause. In this respect, detection of the 47,XYY karyotype in an infertile male should not be uncritically equated with detection of the cause.

The IQ is within the normal range, but on average about 10 points below the level of chromosomally normal males. Approximately, one-third of affected individuals have autism **spectrum disorders (ASD)** and **behavioral disorders** (Joseph et al. 2018). Higher morbidity and hospitalization for congenital malformations, neurological and psychiatric disorders, and respiratory and urogenital diseases result in a **life expectancy about 10 years shorter** than in the general male population (Berglund et al. 2020; Stochholm et al. 2010).

Much attention has been paid to the observation that 47,XYY individuals appear to be more likely than other males to engage in **criminal activity**, most commonly property crimes, sexual offenses, and arson. However, socioeconomic stratification has put criminal behavior into perspective (Stochholm et al. 2012). It must be emphasized that the vast majority of males with a 47,XYY chromosome set exhibit completely normal behavior patterns and stigmatization based on karyotype must be avoided. Children and adolescents with the 47,XYY karyotype cause problems for their educators more often than others due to impulsivity, reduced frustration tolerance, and social maladjustment.

The cause of the XYY chromosome set is a **nondisjunction in the paternal meiosis**. The diagnosis is usually made as an incidental finding during karyotyping initiated for another reason. If a **spermatogenesis disorder** is present in a man with 47,XYY karyotype, the same treatment guidelines apply as for idiopathic male infertility and for assisted reproduction. The children of 47,XYY males almost always have a normal chromosome set. Prenatal karyotyping may be offered, especially if the pregnancy resulted from assisted reproduction.

Key Points

- XX males with female karyotype (46,XX) are phenotypically male and have neither internal nor external female genital organs.
- The prevalence is much lower than Klinefelter syndrome at 1:10,000 to 1:20,000.
- Most XX males have a causative translocation of the *SRY* gene (80–90% SRY positive).
- XX men have a severe testicular developmental disorder and do not produce sperm, so a testicular biopsy is not indicated in cases of desired paternity.
- Frequently, XX men have a testosterone deficiency that requires substitution.
- Men with XYY karyotype are usually discovered incidentally, e.g., due to infertility, which can be treated with conventional or assisted reproduction procedures.

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Structural Chromosomal Changes

23

Frank Tüttelmann and Albrecht Röpke

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Abstract

Both balanced and unbalanced structural chromosomal alterations can lead to fertility disorders. In oligozoospermia, especially balanced autosomal translocations play a role, and in azoospermia aberrations of the sex chromosomes may be causative. Structural chromosomal aberrations lead to significantly increased risks of miscarriage/stillbirth or birth of a mentally/physically handicapped child. Therefore, chromosome analyses are indicated in men with oligo- or azoospermia, but also generally in cases of infertility. The Y-chromosomal AZF deletions are of particular importance in cases of severe oligo- and azoospermia, since, on the one hand, they represent a cause and, on the other hand, they allow a prognosis about the chances of sperm retrieval in a testicular biopsy.

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23.1 Introduction

In addition to numerical chromosomal aberrations (e.g., Klinefelter syndrome, karyotype 47,XXY, Chap. 21), structural chromosomal aberrations form a separate category of partially disease-causing karyotypes with significance for clinical andrology. Structural alterations of the sex chromosomes (gonosomes) are distinguished from those of the non-sex chromosomes (autosomes). In addition, deletions, translocations, inversions, insertions, etc. are differentiated independently of the chromosomes involved. In the clinical evaluation of a structural chromosomal change, it is relevant, among other things, whether it is a balanced or unbalanced change. The latter is the case when genetic material is missing or in excess in the cell. For example, in the case of a deletion (piece loss), a chromosome segment is missing. In contrast, the set of all genetic sequences remains balanced, for example, in the case of a reciprocal translocation, i.e., an opposite piece exchange between two chromosomes.

Both unbalanced and balanced structural chromosomal alterations can lead to fertility disorders (in both males and females). However, most unbalanced chromosome sets, if at all compatible with survival, are often associated with serious impairment of general health. Severe congenital physical and mental disabilities are the rule. In this respect, unbalanced structural chromosomal findings play an important role in pediatrics and clinical genetics, but hardly in andrology. Marker chromosomes as well as deletions of the Y chro-

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mosome including AZF microdeletions are an exception— they can selectively affect fertility and are therefore also important in andrology.

23.2 Prevalence and Consequences

In contrast to Klinefelter syndrome, which is almost exclusively found amongst azoospermic men, the prevalence of structural chromosomal aberrations among men is not dependent on sperm quality when the couple is infertile. In fact, chromosomal abnormalities are found in 2–3% of these men, irrespective of sperm concentration and even with normal sperm count; the prevalence is thus increased up to 10-fold compared to the general population (Table 23.1). This justifies the indication for chromosome analysis in all men in the case of infertility of the couple, which has also found its way into the current AWMF guideline on "Diagnostics and therapy prior to assisted reproductive medical treatment" (DGGG 2019). Thus, in cases of etiologically unclear azoospermia and oligozoospermia but also normozoospermia and infertility of the couple, the indication for conventional chromosome analysis should be generous. In addition, recurrent miscarriages (habitual abortions) are a clear indication for chromosome analysis in both partners.

In cases of infertility or recurrent miscarriages, chromosome analysis should be performed in both partners regardless of other clinical findings (DGGG 2019).

Chromosomal abnormalities cannot be treated causally. Therefore, only symptomatic treatment procedures of assisted fertilization (Chap. 42) can be used in affected patients. It should be noted in particular that balanced parental chromosomal alterations significantly increase the risk of unbalanced chromosome sets in the fertilized egg, embryo, fetus, and child. These cause a reduced likelihood of pregnancy as well as an increased risk of miscarriage or stillbirth or the birth of a usually mentally and physically handicapped child. Genetic counseling to assess these risks and to explain options for prenatal or preimplantation genetic diagnosis (PND/PID) is obligatory, also in accordance with the Gene Diagnostics Act (GenDG).

Translocations, inversions, and, to some extent, also marker chromosomes are often familial chromosomal aberrations. Frequently, the infertile male is the first family member in whom the chromosomal aberration is detected. Often, a larger number of relatives, regardless of sex, can then be identified as carrying the same chromosomal aberration. Carriers of a structural chromosomal aberration have the above-mentioned significantly increased risks of miscarriage/stillbirth or birth of a mentally/physically handicapped child. Therefore, carrier testing must also be offered to family members, embedded in genetic counseling.

Table 23.1 Proportion of abnormal chromosome analyses in men with different sperm concentrations (Dul et al. 2010)

Sperm concentration	Prevalence of chromosomal abnormalities (95% confidence interval)
Azoospermia (n = 1599) (n = 1599)	15.4% (13.6–17.2)
Oligozoospermia ≤1 mill./ml. (n = 539)	3.0% (1.5–4.4)
Oligozoospermia >1–≤5 mill./ml (n = 475)	2.1% (0.8–3.4)
Oligozoospermia >5–≤10 mill./ml (n = 879)	3.5% (2.3–4.7)
Oligozoospermia >10–≤20 mill./ml (n = 808)	1.1% (0.4–1.8)
Normozoospermia >20 mill./ml (n = 729)	2.9% (1.7–4.1)
General population	0.3–0.5%

23.3 Structural Changes of the Autosomes

Structural alterations of the autosomes (chromosomes 1 to 22) may be associated with male fertility disorders. Autosomal structural alterations may interfere with meiotic pairing of chromosomes and thus impair spermatogenesis. In contrast to gonosomal aberrations, it is difficult to predict whether and to what extent a fertility disorder will occur in an individual case. The same autosomal aberration may have a serious effect on spermatogenesis in one patient and no or only a minor effect in another. Even among brothers with the same aberrant karyotype, ejaculate findings may differ significantly, and translocations may have been inherited from a normally fertile father to an infertile son. Thus, the relationship between structural autosomal chromosomal alterations cannot be causally established with certainty, but it can be stated solely on the basis of large case series that there is a significant association between these chromosomal alterations and male infertility (Table 23.1).

In interpreting karyotype analysis, chromosomal polymorphisms, such as small pericentric inversions at chromosome 9 or enlargement of pericentromeric heterochromatin at this chromosome, that have no disease value must be clearly distinguished from relevant aberrations. These known chromosomal polymorphisms are not mentioned in the karyotype formula.

In contrast, reciprocal and Robertsonian translocations, peri- and paracentric inversions, insertions, and marker chromosomes (small "extra chromosomes") may be of actual pathogenic significance (Fig. 23.1). In individual cases, no direct conclusion can be drawn from a detected chromosomal alteration to the ejaculate findings. Nevertheless, translocations, inversions, insertions, and marker chromosomes are found significantly more frequently among infer-

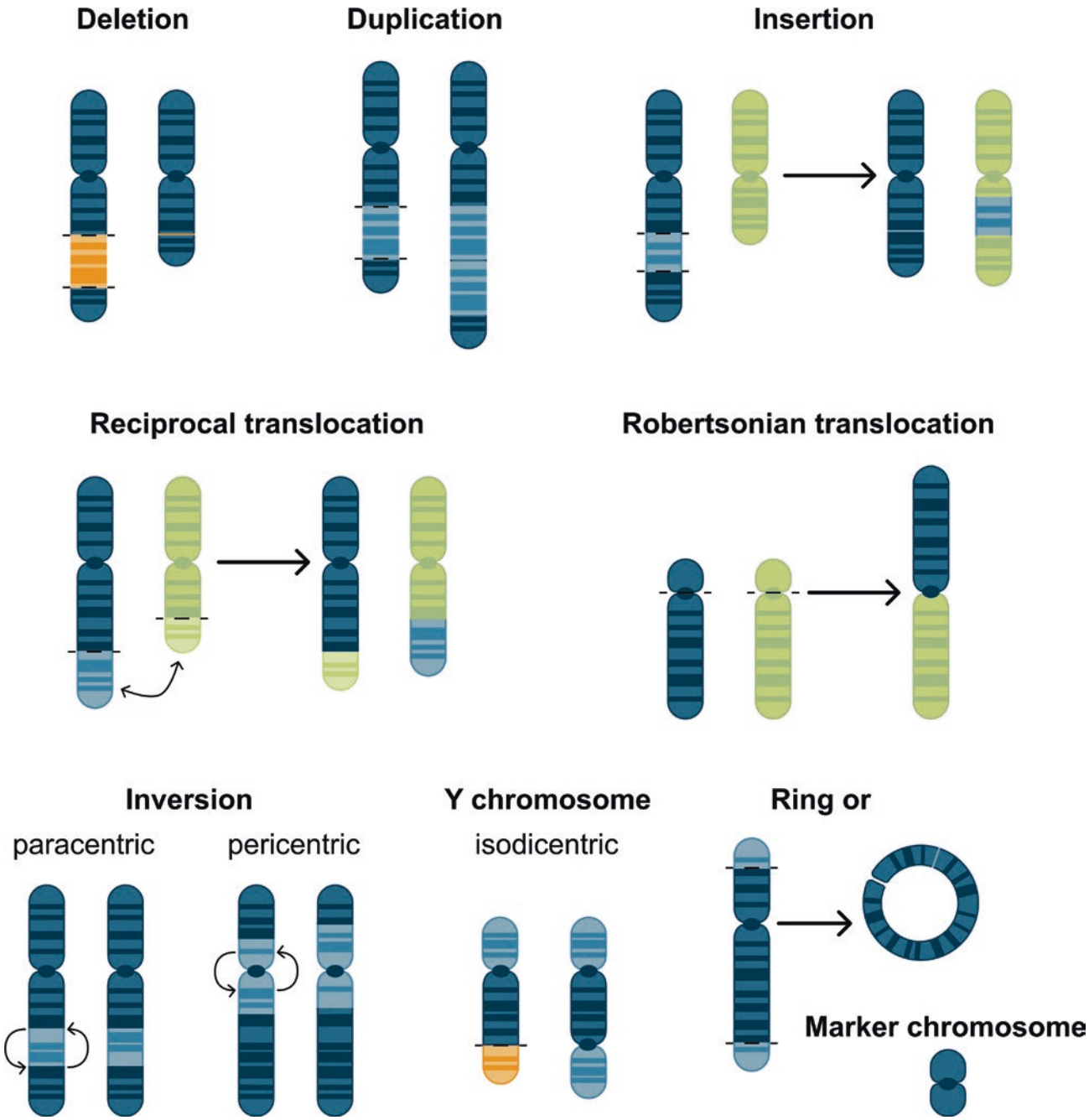


Fig. 23.1 Schematic figure of the most important structural chromosomal aberrations for andrology (© Nadja Rotte MSc)

tile males than among unselected newborns or in the general population. In contrast to numerical chromosomal alterations and structural aberrations of gonosomes, autosomal structural aberrations are more likely to be found in oligo- than azoospermia.

23.4 Structural Alterations of the Sex Chromosomes

An intact Y chromosome is essential for the normal structure and function of the male reproductive system. The *SRY* gene is located on the short arm, which determines the development of the embryonic bipotent gonad in the male testicular direction. In addition, the Y chromosome contains multiple, as yet, incompletely characterized segments responsible for the regular course of spermatogenesis. The long chromosomal arm of the Y chromosome is of particular importance in this regard. Microdeletions in the long arm of the Y chromosome are discussed separately in the following section.

Among the deletions of the Y chromosome to be discussed here first, which can be visualized by cytogenetic techniques (Chap. 8), those of the short and long arm must be distinguished. In case of loss of the entire short arm, the patient lacks the *SRY* gene. The cascade of embryonic sex development is thus already disturbed at the level of gonadal determination. Clinically, the result is a female phenotype similar to Turner syndrome with gonadal dysgenesis. If the deletion affects the long arm, the phenotype is male, and it depends on the size of the lost segment whether and to what extent spermatogenesis is impaired. On the long arm of the Y chromosome, a proximal euchromatic and a distal heterochromatic portion (chromosome band Yq12) are distinguished. The latter is variable in length from male to male and carries no active genes. Its loss has no consequences, but chromosome analysis reveals a smaller Y chromosome. If the deletion extends to the euchromatic region of the long arm (chromosome band Yq11), i.e., the region carrying active genes, this usually results in a severe disorder of spermatogenesis with azoospermia or severe oligozoospermia.

In addition to deletions, a number of other structural alterations of the Y chromosome are known (Fig. 23.1). Especially in the isodicentric Y chromosome, but also in other structurally altered Y chromosomes, mosaic states with a second 45,X cell line occur. The phenotype then depends on the distribution of the two cell lines. In the case of a continuous isochromosome Yp, a male phenotype is expected. In the case of a mosaic with a 45,X cell line, the genitalia may show a spectrum from male to intersex to female. Infertility is usually present.

Reciprocal translocations between the Y chromosome and one of the autosomes are rare. There is usually a severe spermatogenesis disorder, but some males with this karyotype are fertile. Translocations between X and Y chromosome occur in different variants, often the karyotype is unbalanced. The relationships between chromosome set and clinical presentation are complex. The phenotype can be male or female, fertility normal or impaired, physical and mental disabilities occur in some chromosomal variants of the X-Y translocation.

The X chromosome contains numerous genes essential for survival. Therefore, large deletions on this chromosome have a lethal effect in the male sex or are associated with severe diseases. The loss of smaller sections of the X chromosome can lead to the so-called microdeletion syndromes. For example, the deletion syndrome of the distal short arm of the X chromosome (Xp22 contiguous gene syndrome) is characterized by severe developmental disorders. Ichthyosis congenita, the skeletal dysplasia chondrodysplasia punctata, short stature, mental retardation as well as the Kallmann syndrome due to loss of the *KAL1* gene (Chap. 12) occur in variable combination.

23.5 Y-Chromosomal AZF Microdeletions

The long arm of the Y chromosome contains three separate regions essential for normal spermatogenesis. Loss of any of these loci, termed azoospermia factors (AZFa, AZFb, AZFc), due to a deletion occurring in the paternal germline results in severe fertility impairment (Vogt et al. 1996). The deleted regions are of submicroscopic size, so that they cannot be detected in conventional chromosome analysis and are referred to as Y-linked microdeletions. Complete sequencing of the Y chromosome has elucidated the mechanism of origin of AZF deletions: non-homologous recombination between repetitive sequences (palindromes) leads to loss of the intervening region (Noordam and Repping 2006) (Fig. 23.2). Complete, classical AZF deletions are clearly causal for azoospermia and, in some cases severe oligozoospermia, and do not occur in men with normal spermatogenesis. Therefore, in addition to chromosomal analysis, analysis for AZF deletions is part of routine diagnostics for azoospermia and severe oligozoospermia (Krausz et al. 2014).

In men with sperm concentrations $<1x10^6$/ml, analysis regarding AZF deletions should be performed; above this limit, deletions are extremely rare (DGGG 2019).

The prevalence of AZF deletions ranges up to more than 10% in men with non-obstructive azoospermia, depending on selection criteria, up to 5% in men with severe oligozoospermia, and is less than 1% in the totality of infertile men. The prevalence depends on ethnicity and Y haplogroup; for example, the prevalence in Germany and Sweden is lower than in Italy or other countries within and outside Europe (Simoni et al. 2008). AZFc deletions are the most common at about 70%, followed by AZFbc deletions, whereas AZFa and AZFb deletions are much less common (Fig. 23.3).

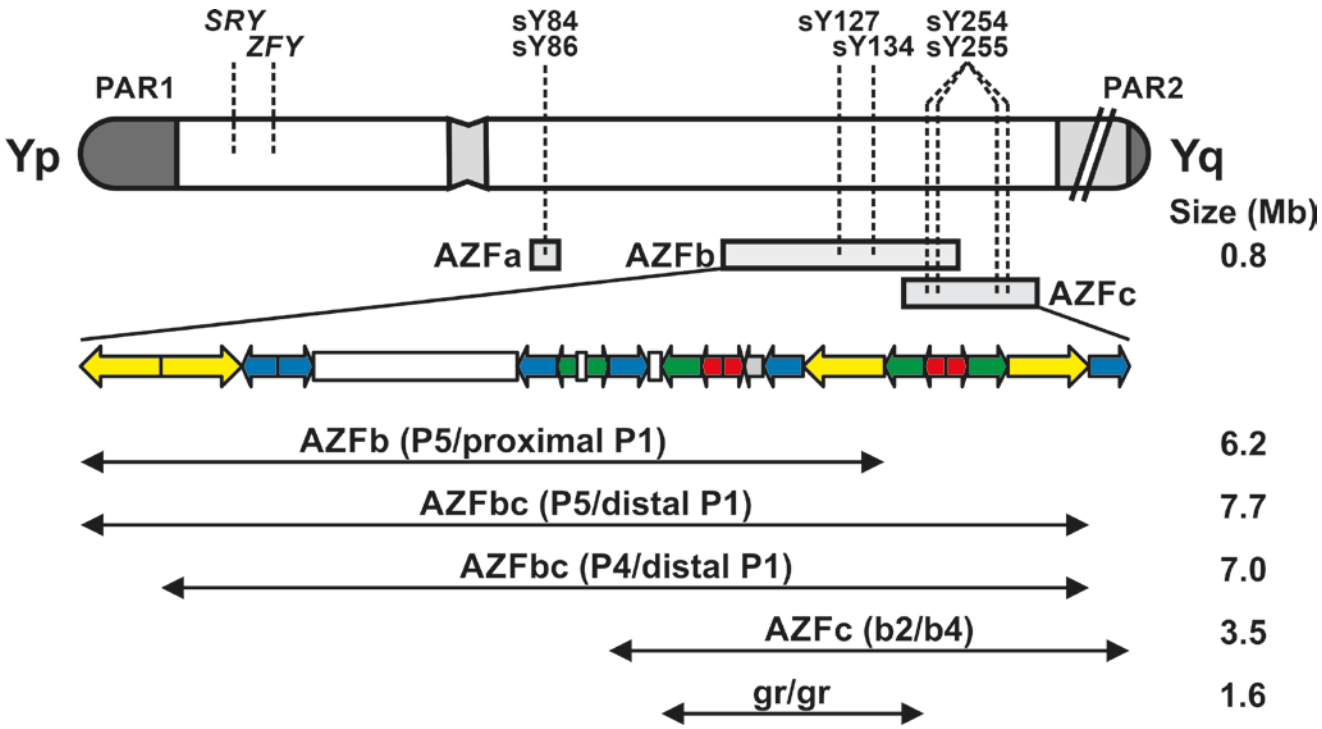


Fig. 23.2 Y chromosome showing classical AZFa, AZFb, and AZFc deletions, gr/gr deletion, standard sY markers in PCR analysis, and repetitive sequences (palindromes, highlighted in color) explaining the origin of these and others (b2/b3, gr/gr) by non-homologous recombination between similar regions (modified after Noordam and Repping 2006 and Krausz et al. 2014)

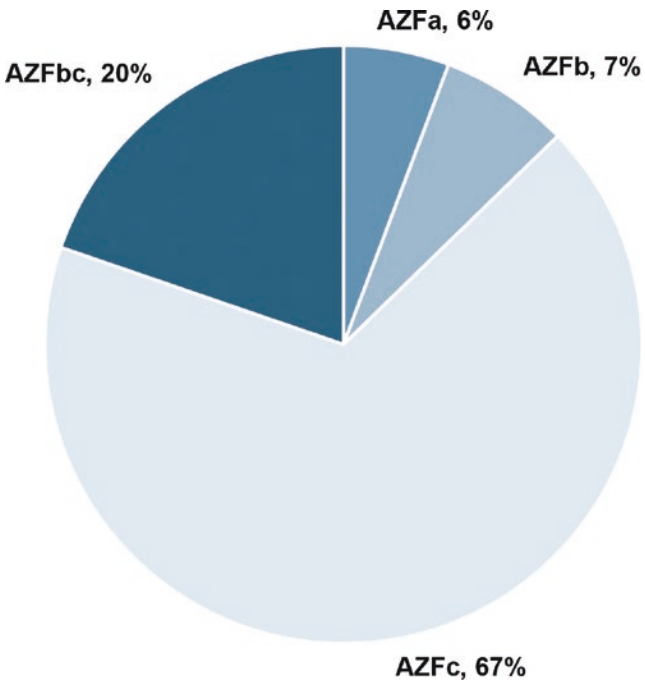


Fig. 23.3 Distribution of classical AZF deletions in 157 men of the Center for Reproductive Medicine and Andrology, University of Münster

Clinically, all men with a complete AZF deletion show a pronounced spermatogenesis disorder, whereas endocrine testicular function is mostly unaffected by the microdeletion. Loss of the complete AZFa region (less than 10% of all AZF deletions) leads to azoospermia and histologically to a Sertoli cell-only (SCO) phenotype in all patients described so far. Isolated cases with partial AZFa deletion, affecting only the *USP9Y* gene, have been described in men with normozoospermia. Patients with complete AZFb deletion also have azoospermia, but with more variable histologic phenotypes ranging from SCO to spermatogenesis residue. Rarely, elongated spermatozoa have also been found in testicular biopsy in men with AZFb deletions. In contrast, AZFc deletions result in a very heterogeneous picture in semen analysis and testicular histology, ranging from azoospermia due to SCO, arrest, or severe hypospermatogenesis to severe oligozoospermia (Fig. 23.4). Deletions of all three regions AZFa, AZFb, and AZFc are found in structurally altered Y chromosomes (Sect. 23.4 or in XX males, Chap. 22).

The diagnosis can only be made by specific molecular genetic testing. The classical test is based on the polymerase chain reaction (PCR) (Chap. 8), with the help of which the presence (amplifiability) of representatively selected marker sequences from the long arm of the Y chromosome is checked

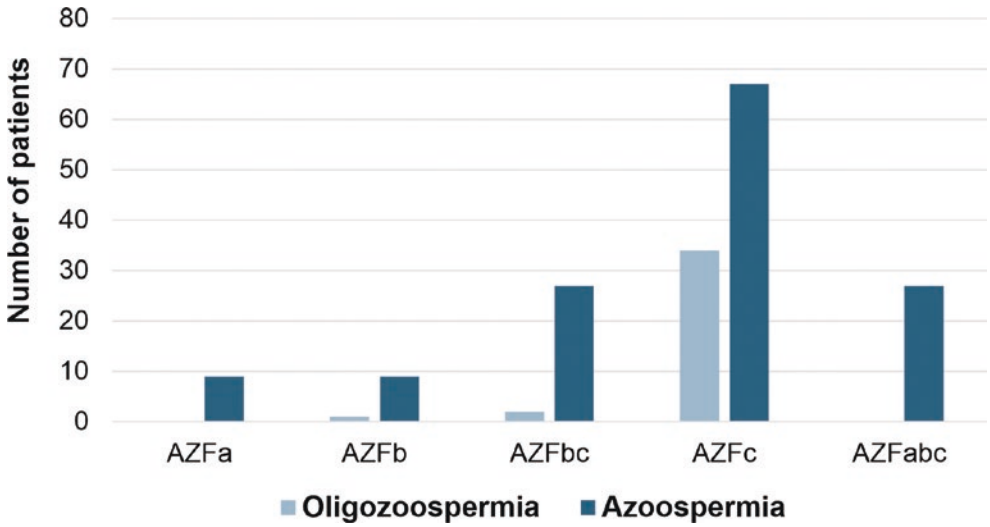


Fig. 23.4 Ejaculate findings in 176 men with Y-chromosomal deletions from the Center for Reproductive Medicine and Andrology, University of Münster

on the patient's DNA, e.g., sY254 and sY255 for the AZFc deletion. Meanwhile, other methods such as commercial kits, arrays, multiplex ligation-dependent probe amplification (MLPAs), and sequencing methods are sometimes used. The regularly updated Diagnostic Guidelines help with standardization and quality assurance (Krausz et al. 2014, https://www.emqn.org/2021 07 09). Regardless of the method, the analysis is generally useful as screening in all men with etiologically unclear severe spermatogenesis disorders, as those with and without AZF deletion cannot be clinically distinguished. The analysis consists of a basic screening and, if a deletion is detected, an extended examination follows, which validates the result and, at the same time, identifies the extent of the deletion (complete/incomplete). The prognostic statements mentioned here are valid only in the case of complete (classical) deletions.

Although isolated cases of spontaneous transmission of an AZFc deletion from father to son have been observed (Rolf et al. 2002; Zhu et al. 2010), complete AZF deletions are usually associated with infertility. Men with complete deletions of the AZFa, AZFb, or AZFbc regions also have virtually no prospect of obtaining sperm at testicular biopsy. In contrast, azoospermic men with complete AZFc deletion have up to 50% chance of finding sperm at testicular biopsy with TESE; however, success rates at ICSI appear to be low at <10%. An association with an increased rate of aneuploid sperm due to complete loss of the Y chromosome and associated risk for Turner syndrome or 45,X/46,XY mosaicism with variants of sex differentiation in offspring is conceivable. Due to the paucity of available data, this risk is still difficult to assess but can be classified as rather low. For these reasons, genetic counseling is necessary for men with AZF deletions, but especially before starting assisted reproduction, because all sons of an affected patient inherit his Y chromosome, therefore also the deletion, and thus male infertility as well.

Apart from the classical AZF deletions, other smaller deletions within the AZFc region have been described, including b2/b3 and gr/gr deletions. In contrast to the clear causal relationship between complete, classical AZFa, AZFb, and AZFc deletions, gr/gr deletions are found more frequently in infertile men but also (less frequently) in men with normal sperm counts. The statistically significant correlation makes gr/gr deletions a risk factor for poorer spermatogenesis/sperm concentration (Tüttelmann et al. 2007), but without any consequences for the affected man or therapy resulting from the detection. Therefore, there is currently no clear indication for the corresponding molecular genetic analysis in the context of fertility diagnostics.

Key Points

- Both balanced and unbalanced structural chromosomal alterations can lead to fertility disorders.
- In particular, balanced autosomal translocations play a role in oligozoospermia and sex chromosome alterations in azoospermia.
- Structural chromosomal alterations lead to significantly increased risks of miscarriage/stillbirth or birth of a mentally/physically handicapped child. Therefore, chromosome analyses are indicated in men with oligo- or azoospermia, but also generally in cases of infertility.
- The Y-linked AZF deletions are of particular importance in cases of severe oligo- and azoospermia, because, on the one hand, they represent a cause and, on the other hand, they allow a prognosis about the chances of sperm retrieval in a testicular biopsy.

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Testicular Tumors

24

Sabine Kliesch and Maria Schubert

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Abstract

A testicular tumor is one of the rare malignant diseases. Moreover, it is one of the few malignancies that can be cured in early stages and, in the majority of cases, in more advanced stages as well. In addition to germ cell tumors, which usually arise from germ cell neoplasia in situ, the rare Leydig cell and Sertoli cell tumors, which belong to the gonadal stromal tumors, are obligately endocrine active. In this chapter, the genesis, risk factors, diagnosis and subsequent therapy, and impact on fertility of germ cell and gonadal stromal tumors are presented.

24.1 Incidence

Overall in men, testicular tumors represent a rare disease. In 2018, 4159 new cases were reported to the German Registration Center for Cancer Data (RKI 2019), with 95% of testicular tumors representing malignant germ cell tumors, approximately 2–3% representing tumors of the stromal cells (Leydig cell tumors and Sertoli cell tumors), and 1–2% representing secondary tumors of other diseases (e.g., lymphoma metastases, leukemic infiltrates). To be distinguished from these are extra-testicular tumor diseases, such as rhabdomyosarcomas of the spermatic cord in childhood or benign tumors (rarely malignant) of the spermatic cord or epididymis (Kliesch 1998).

Malignant germ cell tumors (TGCT) represent the **most common malignancy in men**, accounting for 25% between the ages of 20 and 45. In industrialized countries, the incidence of TGCT has increased in recent decades and in

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Germany it is currently 10.2 per 100,000 men (RKI 2021). Bilateral testicular tumors are observed synchronously in 1% of men and metachronously (delayed) in up to 5%.

With the establishment of multimodal treatment concepts and the introduction of cisplatin into therapy, malignant germ cell tumors are among the predominantly **curable malignancies**. Survival probabilities are very high and patient prognosis depends on age, as well as histology, tumor stage, and quality of care (Kliesch et al. 2021a).

24.2 Risk Factors

The risk of suffering from a **malignant germ cell tumor** or of obligatory pre-cancer (germ cell neoplasia in situ, GCNIS [germ cell neoplasia in situ]) is significantly increased in men with **maldescensus testis** (3%), **contralateral testicular tumor** (5–6%), infertility, **extra-gonadal germ cell tumors** (50%), and **gonadal dysgenesis** (up to 30%) (Cools et al. 2006; Horwich et al. 2006; Raman et al. 2005). In addition, there is a significantly increased risk in infertility and a familial risk when relatives (brother, father, uncle) also have/had testicular tumors (see also current S3 guideline, Kliesch et al. 2021a).

24.3 Malignant Germ Cell Tumors (TGCT) and Infertility

Several factors are likely responsible for the increased incidence of infertility with one per 200 men. These tumors have their highest incidence at an age when involuntary childlessness also becomes evident. Positional abnormality of the testes can lead to both infertility and increased risk of testicular tumors. More than other primary non-testicular diseases, can lead to **fertility reduction**. Up to three-fourths of patients with germ cell tumors have significant limitations of sperm concentration (Petersen et al. 1999; Rives et al. 2012). **Normozoospermia** is detectable in only 25% of affected individuals at diagnosis and is thus much less common than in other malignancies (van der Kaaij et al. 2009; Williams et al. 2009).

Azoospermia is seen in 1 in 6 patients at the time of disease (Kliesch et al. 1996). Common pre-existing disorders of spermatogenesis and additional precipitating factors are likely causes.

Conversely, severe fertility disorders are associated with a significantly higher risk of GTCT and additional morbidities (Latif et al. 2017). Patients with severe oligozoospermia (<3 mill./ml), testicular atrophy, and inhomogeneous internal patterns of the testis are particularly at increased risk for testicular tumor (Giwercman et al. 1997). Microlithiasis is also an indicator of spermatogenesis disorder independent of malignancy risk (Rassam et al. 2020) and is considered an indicator of potential degeneration risk only in the context of additional risk factors (Kliesch et al. 2021a).

24.4 Germ Cell Neoplasia In Situ (GCNIS)

GCNIS is considered an obligatory precancerous condition of germ cell tumors. The term "germ cell neoplasia in situ (GCNIS)" has replaced the older term "testicular intraepithelial neoplasia (TIN)" (Moch et al. 2016). This refers to atypical, neoplastic germ cells that differ immunohistochemically and morphologically from normal spermatogonia and share several morphological and immunohistological similarities with embryonic germ cells (see Chap. 11). Niels Skakkebaek had first described carcinoma in situ as clinically relevant in 1972 (Skakkebæk 1972). Without therapeutic intervention, 70% of GCNIS-bearing testes progress to a malignant germ cell tumor after 7 years (Skakkebaek et al. 1987), so one must assume long-term development of a TGCT in all cases. Both seminomas and non-seminomas can develop from GCNIS (Table 24.1). Based on the current state of science, we assume that the potential of a GCNIS cell is already innate with the creation of the gonocytes. Which factors ultimately trigger the development of an invasive germ cell tumor from GCNIS has not yet been clarified. However, with regard to the development of the germ cell tumor from the GCNIS-bearing seminiferous tubules, it can be assumed that morphologically, the destruction of the intact tubule wall with increasing cell division of the GCNIS ultimately leads to invasion into the peritubular space and thereby to the further development of a germ cell tumor (Donner et al. 2004).

The **clinical and diagnostic picture** of GCNIS is inconsistent. The leading symptom may be an impairment of ejaculate parameters with an inhomogeneous internal pattern of the testis on ultrasound (microlithiasis testis) (see Chap. 6) (Fig. 24.1). Diagnosis of GCNIS is possible only by testicular biopsy (fixation in Stieve's or Bouin's solution, not in formalin) followed by histological and immunohistological (PlAP, M2A, c-kit, 43-9F, TRA-1-60) examination. Tubules with GCNIS are usually heterogeneous or segmentally distributed over the affected testis and few to all tubules may be affected. Therefore, it is now accepted standard to perform multiple biopsies to confirm diagnosis. Furthermore, testicular biopsy should be performed bilaterally or contralaterally

Table 24.1 Histological classification of testicular tumors according to WHO (simplified representation based on Moch et al. 2016)

Germ cell tumors arising from germ cell neoplasia in situ (GCNIS)
Non-invasive TGCT:
Germ cell neoplasia in situ (GCNIS).
Specific forms of intra-tubular germ cell neoplasia.
Tumors of one histological type (pure forms)
Seminomas
Seminoma with syncytiotrophoblastic cells.
Non-seminomatous TGCT Embryonal carcinomas
Yolk sac tumor of post-pubertal type
Trophoblastic tumors
Chorionic carcinoma
Trophoblastic tumors other than chorionic carcinoma
Teratoma of the post-pubertal type
Teratoma with development of somatic neoplasms.
Non-seminomatous TGCT of more than one histological type
Mixed germ cell tumors
TGCT not arising from germ cell neoplasia in situ
Spermatocytic tumor
Teratoma of pre-pubertal type (dermoid cyst, epidermoid cyst, monodermal teratoma)
Mixed tumor teratoma with yolk sac tumor of pre-pubertal type
Yolk sac tumor of pre-pubertal type
Germline/stroma tumors
Leydig cell tumor
Malignant Leydig cell tumor
Sertoli cell tumor
Malignant Sertoli cell tumor
Large cell calcifying Sertoli cell tumor
Granulosa cell tumor (adult or juvenile)
Mixed germ cell/germ line tumor
Gonadoblastoma
Para-testicular tumors
Adenomatoid tumors
Adenocarcinomas of the epididymis
Malignant mesotheliomas
Mesenchymal tumors of the spermatic cord and testicular adnexa
Secondary tumors of the testis

in the presence of a germ cell tumor (Kliesch et al. 2003; Dieckmann et al. 2007; Kliesch et al. 2021a).

Therapy of GCNIS depends, in particular, on the results of the testicular biopsy and the overall clinical constellation. Three constellations, in which, in principle, aspects of the desire to have children can also be included in the therapy decision, can be distinguished:

1. GCNIS unilateral with healthy contralateral testis
2. GCNIS in the single testis
3. GCNIS on both sides

Regarding (1): if there is unilateral involvement in the presence of a second and healthy testis, surgical **removal** of the affected testis is the safest but also the most radical method. Alternatively, active monitoring by scrotal testicular sonography can be performed and therapy is only performed in case of progression to a tumor manifestation, mostly sonographically detectable.

Regarding (2): if the only remaining testis is affected (e.g., after diagnosis and ablation of a germ cell tumor with contralateral GCNIS or in the case of a single testis due to a different genesis), local **radiotherapy** with 20 Gy (10 fractions of 2 Gy each) is the therapy of choice, since it allows preservation of endocrine testicular function with loss of tubular function in the vast majority of cases and eradicates GCNIS in 95% of cases (Dieckmann et al. 2013). To date reduction of the radiation dose had to be abandoned due to lack of therapeutic safety (Classen et al. 2003). Alternatively, close monitoring can also be performed in these cases.

Regarding (3): if both testes are affected with GCNIS, radiotherapy is primarily recommended. In case of simultaneous presence of bilateral germ cell tumors, depending on tumor size and localization, it is decided which testis will be radically removed (inguinal testicular ablation) and which will be treated by organ-preserving tumor enucleation in combination with subsequent radiotherapy (20 Gy) (Heidenreich et al. 2001; Kliesch et al. 2021a).

If chemotherapy of the germ cell tumor is performed because of metastases or adjuvantly, 50% of cases with GCNIS develop a manifest tumor. Therefore, additional radiotherapy of GCNIS should be given (with a time delay) (Kliesch et al. 2021a). Ablatio testis (loss of tubular and endocrine function) is indicated in cases of involvement of the only remaining testis, i.e., when a decrease in Leydig cell function has already occurred, in turn causing clinical hypogonadism requiring testosterone substitution. A wait-and-see approach, in the sense of active surveillance, is indicated in cases where there is a strong desire for progeny and residual spermatogenesis with corresponding ejaculate values suggest that spontaneous conception or active therapy with ART measures is possible (Kliesch et al. 1997; Petersen et al. 1999; Kliesch et al. 2021a). Due to the expected limitations of Leydig cell function during treatment in at least 30% of patients, monitoring of testosterone serum levels is recommended during follow-up.

Since all GCNIS therapies lead to sterility, the patient should be offered sperm cryopreservation in all cases and testicular sperm extraction for all patients with azoospermia (AWMF S2k Guideline on Fertility Preservation in Patients with Oncologic Diseases 2017; Kliesch et al. 2021a).

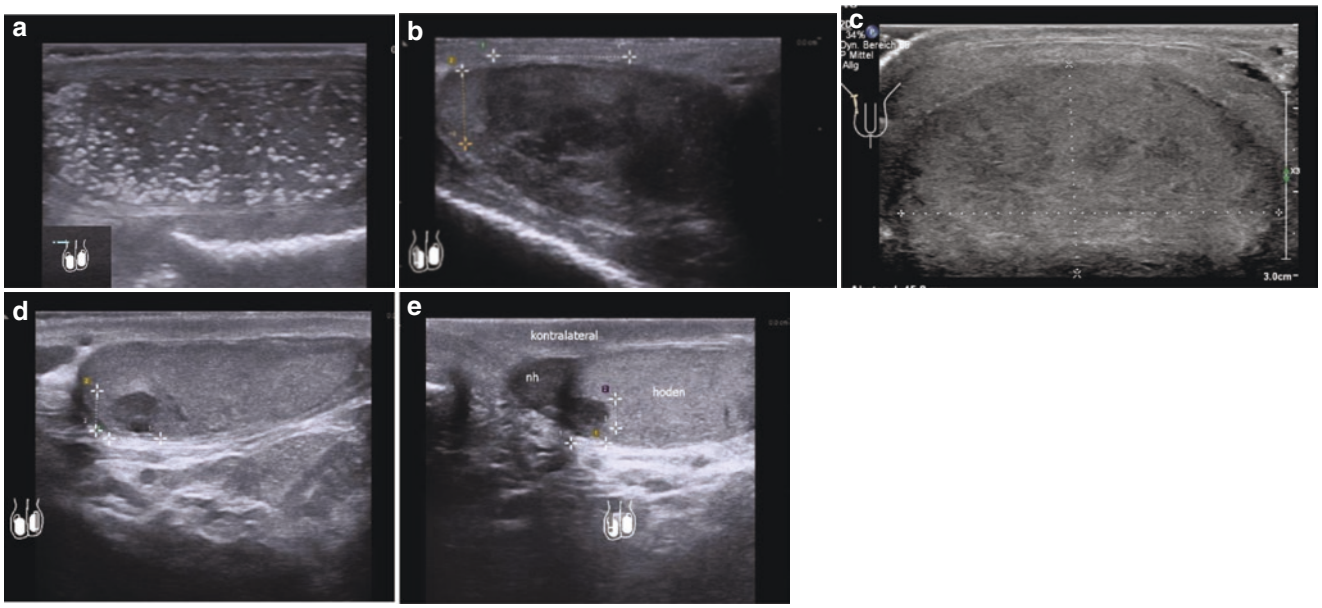


Fig. 24.1 Sonographic imaging of various testicular intrusions. Recorded with 7.5–12 MHz transducer at the Department of Clinical and Surgical Andrology in the Center for Reproductive Medicine and Andrology (CeRA), University of Münster, patient consent obtained. (**a**) Pronounced microlithiasis testis (starry-sky phenomenon). (**b**) Seminoma 15 x 8 mm in size at the right upper pole of the testis. (**c**) Non-seminoma (embryonal carcinoma) 45 × 25 mm in size, consuming almost the entire left testis. (**d**) Sertoli cell tumor 5.8 × 4.5 mm in size, left testis. (**e**) Leydig cell tumor 4 × 3.4 mm in size, right testis (same patient as **d**)

24.5 Germ Cell Tumors

GCNIS malignant cells can undergo transformational progression to both **seminomas** (except spermatocytic seminoma) and **non-seminomas** (Table 24.1). Seminomas and non-seminomas account for 93% of all testicular tumors. Proportionally, seminomas are the largest tumor entity at 60% with increasing incidence (Ruf et al. 2014), peaking in age in the fourth and fifth decades of life, while non-seminomas peak in the third decade of life. Almost all germ cell tumors and also GCNIS have an isochromosome on the short arm of chromosome 12 (iso-p12) as a specific chromosomal marker (Bosl and Motzer 1997).

- In clinical **stage I**, the germ cell tumor is limited to scrotal contents.
- In **stage II,** lymphogenic metastases are already present below the diaphragm and are smaller than 2 cm (IIA), between 2 and 5 cm (IIB) or larger than 5 cm (IIC, bulky disease).
- **Clinical stage III** is characterized by lymphogenic metastases above the diaphragm or extranodal, hematogenous metastases (Kliesch et al. 2021a).

24.5.1 Clinical Picture

The clinical presentation of seminomas and non-seminomas can be highly variable. The classic finding is a painless testicular enlargement. However, testicular pain occurs in 30% of patients, so the most common initial misdiagnosis is epididymitis (or orchitis). Tumor growth may cause a rapid decrease in ejaculate parameters associated with altered testicular consistency. Palpation findings—if the tumor size already indicates a palpation abnormality—are usually characterized by induration with or without a rough surface. Impaired ejaculate parameters range from azoospermia to oligoasthenoteratozoospermia of varying degrees. A sudden drop in ejaculate parameters should always prompt thorough testing. Retroperitoneal lymph node metastases may eventually lead to the development of a varicocele or abdominal discomfort. Approximately 10% of patients are primarily noticed by secondary symptoms (back pain, dyspnea/hemoptysis). Furthermore, 5% observe gynecomastia, which may be developed bilaterally or unilaterally, and is usually associated with elevated ß-hCG and a resulting imbalance of testosterone and estradiol serum levels (Kliesch 2004).

24.5.2 Diagnostics

In the event of appropriate clinical symptoms, **diagnosis** is made on the basis of **palpation** and **sonographic examination**. Palpation rarely yields evidence of an intra-testicular process in small early-stage tumors. Side-differentiated findings, changes in consistency, and testicular volume are cause for suspicion. Of the patients with testicular tumors detected in our outpatient clinic, only about **one-third had abnormal palpation findings**; in the rest, suspicion was based **solely**

on ultrasonography (see Chap. 6). Along with palpatory examination, sonographic examination occupies a prominent position in the diagnosis of testicular tumor. Testicular tumors are usually seen as low or high echo areas with inhomogeneous internal patterns. With the help of duplex sonography, perfusion of the tumors can also be visualized, depending on their size. MRI diagnostics are not superior to high-resolution sonography (min. 7.5 MHz) (Kliesch et al. 2021a).

The **tumor markers alpha-fetoprotein (AFP) and β-human chorionic gonadotropin (β-hCG) play a further important role in diagnosis and therapy.**

AFP and ß-hCG are elevated in about 60% of non-seminomas and only ß-hCG is elevated in about 20% of seminomas. Another tumor marker of relevance is **lactate dehydrogenase (LDH)**, a non-specific marker that correlates with the prognosis of metastatic stages and is therefore a useful adjunct to specific markers. **Serum placental alkaline phosphatase (PlAP)** plays no role (Kliesch et al. 2021a). A new serum tumor marker is miR-371a-3p; in clinical trials; it indicates the diagnosis of seminoma or non-seminoma other than teratoma with a sensitivity of 88.5% (Dieckmann et al. 2017).

24.5.3 Primary Therapy and Planning for Further Therapy

Diagnosis is confirmed in the course of the so-called **primary therapy by inguinal exposure** of the testis, if necessary with intraoperative examination of frozen sections, if the evaluation doubtful, **ablation of the testis follows**. The tumor-bearing testis is exposed via inguinal incision, the spermatic cord is interrupted by means of tourniquet during dissection, and the testis is excised. In the case of bilateral involvement, unilateral tumor enucleation can be performed for a singular tumor <2 cm and simultaneous ablation of the opposite side with the larger tumor finding. The procedure for a single testis is similarly differentiated: in the case of a singular tumor <2 cm, organ-preserving tumor enucleation is preferable to ablation of the testis if Leydig cell function is still preserved. Histological workup of the testicular ablate or enucleate in the course of this so-called primary therapy is of elementary importance for further therapy planning (Kliesch 2004). Patients with a testicular tumor and other risk factors should be advised to undergo a contralateral testicular biopsy to exclude GCNIS. In particular, the presence of testicular atrophy significantly increases the risk for GCNIS (Kliesch et al. 2021a).

During surgery, care should be taken to spare the genitofemoral nerve and adequate postsurgical pain management should be provided. Possibly, this contributes significantly to reduce the development of a so-called **phantom testis** syndrome. In particular, patients in whom the diagnosis of a testicular tumor is associated with testicular pain suffer from the sensation of phantom testicular pain (Pühse et al. 2010).

Post-operatively, tumor marker control and staging are performed by thoracic and abdominal computed tomography. Cranial CT and skeletal scintigraphy are only indicated if hematogenous metastasis has been detected in previous examinations.

In addition to the distinction between seminomas and non-seminomas, a clinical determination of disease stage is of particular importance for further therapy planning. The following evidenced-based therapy recommendations were summarized in the AWMF S3 Guideline on Diagnosis, Therapy and Follow-up of Germ Cell Tumors of the Testis, which first appeared in 2020 (AWMF Keimzelltumor 2020, Kliesch et al. 2021a, b):

- For **stage I seminoma** with or without risk factors (low risk or high risk), the standard therapy is active surveillance. Alternatively, depending on the constellation of findings, carboplatin monotherapy or radiotherapy of the infradiaphragmatic para-aortic lymph nodes (20 Gy) may be considered.
- In **stage IIA or IIB seminomas**, the infradiaphragmal para-aortic lymph nodes and the ipsilateral iliac lymph nodes are irradiated with 30 Gy (**stage IIA**) or 36 Gy (**stage IIB). In stage IIB**, cisplatin-based chemotherapy may alternatively be administered according to the PEB regimen (cisplatin, etoposide, and bleomycin). Modified radiation protocols to reduce toxicity are under clinical investigation.
- From **stage IIC and onward**, cisplatin-containing chemotherapy is used for seminoma. For patients in the good prognosis group according to IGCCCG (Prognosis Groups of the International Germ Cell Cancer Collaborative Group) with 3 cycles, and for intermediate prognosis with 4 cycles of PEB as standard therapy.
- A risk-adapted approach (surveillance) exists for **stage I non-seminomas: Low-risk tumors** (risk of metastasis 20%) for **high-risk tumors** (risk of metastasis 50%) polychemotherapy with one cycle of PEB, alternatively surveillance is recommended. Modified retroperitoneal lymphadenectomy may be an alternative if surveillance and/or chemotherapy are refused or not feasible.
- In **stage II A/B non-seminoma**, primary chemotherapy and subsequent residual tumor resection are performed for tumor marker-positive stage IIA (marker+) and stage

IIB (marker+ / marker-), or alternatively nerve-sparing lymphadenectomy with or without adjuvant chemotherapy for stage IIA (marker-).
- Patients with stage IIC or III non-seminomas are treated according to their prognostic criteria with 3 or 4 cycles of PEB as standard (see below).

For patients with advanced disease, treatment planning follows according to the International Germ Cell Cancer Collaborative Group classification (Table 24.2). Patients with good or intermediate prognosis receive three cycles of PEB. Patients with poor prognosis are treated with four cycles of PEB (cisplatin, etoposide, and bleomycin). Regarding recurrence therapy, chemotherapy protocols with PEI (cisplatin, etoposide, and ifosfamide) are used. In addition, in the salvage setting, high-dose chemotherapy protocols may be used.

Monitoring of residual tumors in metastatic seminoma is PET guided and further therapy is given only if PET-positive. Residual tumors in non-seminoma are completely resected regardless of the size of the residual lesion.

Table 24.2 Prognosis definition of the International Germ Cell Cancer Classification Group (adapted from (IGCCCG 1997, Kier et al. 2017)

Good prognosis (56% of patients) (5-year survival rate 86–95%)		
Non-seminoma	Testis/primary retroperitoneal tumor and	low markers
	"Low" markers and	– AFP <1000 ng/ml and
	No non-pulmonary visceral metastases	– hCG <1000 ng/ml (5000 IU/l) and
Seminoma	Any primary site and	– LDH <1.5-fold normal value
	Any marker elevation and	
	No non-pulmonary visceral metastases	
Intermediate prognosis (28% of patients) (5-year survival rate 72–85%)		
Non-seminoma	Testis/primary retroperitoneal tumor and	Intermediate markers
	"Intermediate" markers and	– AFP 1000–10,000 ng/ml or
	No non-pulmonary visceral metastases	– hCG 1000 – 10,000 ng/ml
Seminoma	Any primary site and	(5000–50,000 IU/l) or
	Any marker elevation and	– LDH 1.5–10[a] normal value
	Non-pulmonary visceral metastases (liver, skeletal, CNS, intestinal)	
Poor prognosis (16% of patients) (5-year survival rate 48–64%)		
Non-seminoma	Primary mediastinal germ cell tumor or	High markers
	Testis/retroperitoneal tumor with	– AFP >10,000 ng/ml or
	Non-pulmonary visceral metastases or high markers	– hCG >10,000 ng/ml (50,000 IU/l) or- LDH >10[a] normal value

24.5.4 Survival Data

Overall, the cure rate of germ cell tumors is excellent nowadays: the 5-year survival probability is 97.5% for seminomas and 94.9% for non-seminomas in Germany, regardless of stage.

In stage I for seminomas and non-seminomas, the tumor-specific 10-year survival probability is 99.7%. In metastatic stage, the 5-year survival probability depends on the prognosis group: good prognosis: 86–95%, intermediate: 72–85%, and poor prognosis: 48–64% (Kier et al. 2017).

Standardized and stage-appropriate therapy has a significant impact on the chances of cure. For this reason, there is an urgent recommendation to use the existing **national second opinion portal** for testicular germ cell tumors, which was developed by the GTCSG (German Testicular Cancer Study Group of the AUO) and further developed together with the DGU throughout Germany, in order to avoid under- and also overtherapy (https://ekonsil.org) (Schrader et al. 2010; Schrader et al. 2016; Zengerling et al. 2014). Advanced germ cell tumor patients should be treated in appropriately experienced centers to take advantage of the options of multimodal therapy (Kliesch et al. 2021b).

24.5.5 Influence of Germ Cell Tumors and Therapy on Spermatogenesis

Before orchiectomy, tumor enucleation, chemotherapy, testicular or retroperitoneal radiotherapy or retroperitoneal lymphadenectomy, patients with a potential desire to have children must be given the opportunity to cryopreserve sperm as a procreation reserve (AWMF S2k Guideline Fertility Preservation) (see Chap. 18). The indication must be certified in order to ensure that costs are covered by the patient's health insurance.

Cryopreservation of sperm before the start of therapy (from the ejaculate or, in the case of azoospermia, from testicular tissue) is the established option for a fertility reserve which, since July 1, 2021, has been reimbursable by the statutory health insurance funds for adolescents from puberty onward and for adults up to the age of 50 in Germany (GBA Cryo-RL 2021) (see Chap. "Cryopreservation"). During TGCT therapy, unilateral orchiectomy may already have led to a reduction in sperm concentration with an increase of FSH. If a nerve-sparing modified retroperitoneal lymphadenectomy is performed, older studies report **ejaculatory dysfunction** (especially **retrograde ejaculation**) in 7–10% of patients (Heidenreich et al. 2003). However, current follow-up data almost 14 years after completed treatment for stage I

non-seminomatous TGCT show retrograde ejaculation in 24% of patients treated with RLA (Hiester et al. 2021). With nerve-sparing retroperitoneal lymphadenectomy or residual tumor resection, rates of retrograde ejaculation or anejaculation are at least between 30% and 50%, and 100% with radical surgery. Radiation therapy (see Chap. 19) results in a mostly reversible disturbance of spermatogenesis (Hansen et al. 1990), depending on the amount of scattered radiation affecting the remaining testis (approximately 1.7 Gy without gonadal protection, approximately 0.5 Gy with gonadal protection). Standard cisplatin-based therapy for TGCT acutely results in at least temporary azoospermia with elevated FSH levels in almost all patients. As with radiotherapy, precise prediction of the potential long-term effects of chemotherapy on spermatogenesis is not possible because of the wide individual variation. Factors influencing this variation include baseline sperm levels and the duration of the follow-up interval, in addition to the dose of chemotherapy. In most patients, there is at least partial recovery of spermatogenesis during the second to fourth year after cessation of chemotherapy for the testicular tumor. Permanent azoospermia or severe oligozoospermia should be expected in patients receiving a total cumulative dose of cisplatin greater than 600 mg/m^2. As a result of ablation, radiation, and chemotherapy, there may be an increase in LH. However, testosterone levels remain within the normal range in most cases. In a total of about 25% of patients with TGCT, a (frequently subclinical) testosterone deficiency occurs in the course after completion of oncological therapy (Pühse et al. 2005). In the case of corresponding symptoms, this should be substituted with testosterone, as should a testosterone deficiency after bilateral orchiectomy (see Chap. 36) (Kliesch 2022).

24.6 Endocrine Active Testicular Tumors

In addition to germ cell tumors (TGCT), the rare Leydig cell tumors (LCT) and Sertoli cell tumors (SCT), which belong to the stromal tumors, are obligatory endocrine active.

Their proportion of testicular tumors is <10%, with LCT being the most common stromal tumor at 2%. The term "stroma" describes all non-epithelial parts of the gonads beyond the germinal epithelium (see also WHO classification 2016) (Loy and Linke 2003; Moch et al. 2016; Kliesch 2021; Kliesch et al. 2021b).

24.6.1 Leydig Cell Tumors

Leydig cell tumors (LCT) are benign neoplasms in >90% of cases. They are usually smaller than 5 cm and stand out as incidental findings on sonography. They are solid tumors that rarely occur bilaterally (3%) and have their peak age around 35 years of age. However, they are detectable pre-pubertally in 1 out of 5 patients with an age peak around 5 years of age (Kliesch et al. 2021b).

The causes for the development of Leydig cell tumors are not clearly understood. Activating mutations of the LH receptor in Leydig cell tumors have been described in both boys (Liu et al. 1999) and adults (Villares Fragoso et al. 1998) as well as possible alterations in the expression of a relaxin-like factor (Klonisch et al. 1999). Microarray analyses of fetal, neonatal, and adult Leydig cells have demonstrated differential gene expression patterns that may play a role in the differentiation of a normal Leydig cell into benign Leydig cell hyperplasia and in differentiation from malignant neoplasia (Lottrup et al. 2017).

Symptoms have age-specific characteristics. Before puberty, Leydig cell tumors are almost always benign (Freeman 1986). Before puberty, they mostly produce androgens, leading to **pseudo-pubertas praecox**, which leads to diagnosis in 2/3 of patients. Corresponding masses are detectable on sonography in all cases and elevated testosterone serum levels are usually detectable on blood tests (Luckie et al. 2019).

After puberty, LCT predominantly produce estrogens without necessarily leading to an increase in estrogen levels measurable in serum. Elevated serum estradiol levels (approximately 30% of affected individuals) are accompanied by low testosterone and compensatory elevated gonadotropin levels (Suardi et al. 2009). Therefore, clinical symptoms may include gynecomastia and disturbances in libido and/or erectile function.

In adult patients, feminization symptoms occur with the characteristic **symptom triad: gynecomastia**, **impotence, and testicular tumor** (see Chap. 32).

However, the symptoms can also be very discrete. Via feedback inhibition of hypothalamic/pituitary function by estrogens, atrophy of the contralateral testis and testicular tissue surrounding the tumor may occur. Spermatogenesis is arrested, and oligo- or azoospermia are found in the ejaculate. After tumor enucleation or removal of the affected testis (especially if the patient wishes to have a child, the organ-preserving tumor enucleation should be performed quickly), the symptoms usually disappear quickly.

Diagnostics and initial surgical therapy follow the recommendations of the current AWMF S3 Guideline for Diagnosis, Therapy, and Follow-up of Germ Cell Tumors (Kliesch et al. 2021b). Endocrinological diagnostics include LH, FSH, and testosterone, and can be supplemented by the determination of estradiol (in the presence of gynecomastia) and 17-OHP in the presence of a suspected adrenogenital syndrome (AGS, see below) (Kliesch 2021).

Testicular **ultrasound** confirms the mass, whereas complementary examination techniques (elastography, MRI of the testis) cannot differentiate between benign and malignant

tumors and are therefore dispensable (Pozza et al. 2016; Kliesch et al. 2021a).

Box:

It is important to **differentiate testicular adrenal residual tumors** (TART) in patients with adrenogenital syndrome (AGS), as they are **not an indication for surgical testicular** exposure (Engels et al. 2018; Rohayem et al. 2021). TART can be treated by effective therapy for AGS (Rohayem et al. 2021).

The options of **fertility protection** and **cryopreservation of spermatozoa** from ejaculate or testicular tissue (in case of azoospermia) should (= must) be offered before surgical therapy according to current guidelines (Kliesch et al. 2021a).

Therapeutically, rapid section-guided testicular tumor enucleation is preferred (tumors <2 cm) and only in clear cases of malignancy or in very large tumors ablatio testis is performed. Notably, even in children, no increased recurrence rate has been described after tumor enucleation (Miao et al. 2021; Bois et al. 2021).

LCT are indistinguishable from malignant TGCT on macroscopy and difficult to distinguish from malignant TGCT on microscopy. The diagnosis of LCT is ultimately confirmed by histopathology. The tumor nodule, which is usually smoothly circumscribed, may contain connective tissue septa and the cut surface may be variously colored: grayish-whitish to yellowish to mahogany-brown (Bremmer and Schweyer 2016). Reinke crystals are considered pathognomonic for LCT, but can only be visualized in one-third of tumors (Mesa et al. 2015).

There is a strong similarity to SCT and nodular Leydig cell hyperplasia. Leydig cell hyperplasia is frequently detectable in Klinefelter syndrome. Immunohistochemistry (ICH) successfully differentiates it from SCT by the usually absent detection of cytokeratin (epithelial marker). Inhibin is considered to be the most important marker in IHC, which is present in almost all SCTs and is only very rarely present in malignant SCTs. Other common markers are calretinin, vimentin, and melan-A (Loy and Linke 2003; Bremmer and Schweyer 2016).

Box:

A maximum of 0.8–10% of Leydig cell tumors degenerate malignantly, although malignancy cannot be determined with certainty based on the histology of the Leydig cell tumor.

In a total of five recent case studies, the incidence of metastatic LCT is 1.3% (two of 156 patients) (reviewed in Kliesch 2021). The definitive diagnosis of malignant LCT can only be made in the presence of metastases. Therefore, clinical staging by **CT/MRI abdomen** and **a CT thorax** should be performed after diagnosis of LCT, especially if malignancy criteria are present, according to the current S3 Guideline for Germ Cell Tumors (Kliesch et al. 2021b). If metastases are detected, surgical removal of the metastases is performed, as LCT are neither radio- nor chemosensitive (Kliesch et al. 2021b).

In principle, the **prognosis** for benign LCT can be classified as excellent. The data situation for malignant LCT is very poor. Ireland recorded only 22 malignant LCTs (0.8%) in a total of 2755 newly diagnosed malignant LCTs from 1994 to 2013, and the 5-year disease-specific survival was 100%. The 5-year recurrence-free survival was 93.3% of LCT patients due to local recurrence with organ-preserving tumor enucleation (Nason et al. 2018). In a recent study, approximately 7% of LCT are malignant with manifestation of metastasis within 5 years, predominantly in the retroperitoneum (60%), lung (38%), and liver (29%) (Fankhauser et al. 2020). Follow-up in patients with LCT should be adjusted based on data (Kliesch 2021).

24.6.2 Sertoli Cell Tumors

Sertoli cell tumors (SCT) are among the extremely rare stromal tumors of the testis and are found in adolescents (Chang et al. 1998), as well as adults. SCT arise from Sertoli cells and are almost always benign. Malignant progressions in no more than 1 in 10 patients occur in middle to older age, and the median age of onset is estimated at 43 years (Banerji et al. 2016). They may be associated with Peutz-Jeghers syndrome but also develop in patients with androgen insensitivity syndrome (de Souza et al. 2017) or in the context of the Carney complex (Young 2005). Peutz-Jeghers syndrome is associated with bilateral multilocular intra-tubular large cell hyalinizing SCT (Ulbright et al. 2007; Ham et al. 2013).

Histopathologically, SCT are divided into intra-tubular-large cell hyalinizing SCT, large cell calcifying SCT, and not otherwise specified (NOS) with the malignant SCT according to the 2016 WHO classification (Idrees et al. 2017).

Patients with SCT may show feminization symptoms and symptomatology like those with LCT in approximately 24% due to increased estrogen production (Gabrilove et al. 1980). Gynecomastia is present in approximately 16% of patients with benign SCT and in 60% of patients with malignant SCT. Therapy of choice is orchiectomy and, in the case of metastasis, surgical resection, since SCT, like LCT, are neither chemo- nor radiosensitive. Small tumors without malignancy criteria can be operated on in an organ-preserving manner, as can LCT (Kliesch et al. 2021a). The malignancy criteria are similar to those for LCT (overview in von Amsberg et al. 2021). Whether molecular pathological anal-

ysis of tumor tissue is helpful in the metastatic stage is not certain due to the rarity of tumors. Targetable genetic alterations could be identified that may, for example, justify therapy trials with mTOR inhibitors (Necchi et al. 2019).

Based on American data, the **prognosis** of malignant SCT is poor with only 77% overall survival in stage I after 5 years and in advanced stages with a median survival of less than two years (von Amsberg et al. 2021).

Key Points

- Malignant germ cell tumor (TGCT) is the most common malignancy in men, accounting for 25% between the ages of 20 and 45.
- More than other primary non-testicular diseases, TGCT can lead to reduced fertility.
- GCNIS (Germ Cell Neoplasia in Situ) is considered an obligatory precancerous condition of germ cell tumor.
- The malignant cells of GCNIS can develop transformationally into seminomas (except spermatocytic seminoma) as well as non-seminomas.
- Primary diagnosis includes a detailed medical history including risk factors, physical examination (including sonography of the testis), determination of the tumor markers AFP, ß-hCG and LDH, and imaging of the abdomen and thorax for staging.
- Diagnosis is confirmed in the course of the so-called primary therapy by inguinal exposure of the testis, if necessary with intraoperative frozen section examination, if the outcome is doubtful, and subsequent ablation of the testis.
- Cryopreservation of ejaculate for fertility preservation should precede testicular exposure or ablation of the testis.
- Depending on the tumor entity and tumor stage (and prognosis group), primary therapy is followed by surveillance, chemotherapy or radiotherapy. If necessary, RLA (or residual tumor resection) are required during the course.
- A stage-appropriate therapy has considerable influence on the chances of cure. For this reason, consulting the national second opinion portal for testicular germ cell tumors is strongly recommended.
- Leydig cell tumors are the most common stromal tumors, accounting for 2% of all testicular tumors.
- Depending on the time of tumor onset, the endocrine phenotype differs.
- Pre-surgical differential diagnosis, fertility protection, and organ-preserving surgery, if possible, are also of great importance in gonadal stromal tumors.

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Senescence and Late-Onset Hypogonadism

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Abstract

Thanks to increasing life expectancy with its concomitant number of aging men, medicine is forced to focus more strongly on the endocrine situation of this age group. In men, although there is no climacteric, there is a gradual decline in testosterone, and levels may well remain within the normal range of younger men. In some men, however, levels fall below normal. When this occurs in conjunction with characteristic symptoms, it may be genuine late-onset hypogonadism (LOH) or functional hypogonadism. In these cases, testosterone substitution may be considered and must be closely monitored.

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25.1 Physiology of Aging

The growing aging of the population and the resulting social, medical, and also economic consequences in most Western industrial nations are increasingly becoming the focus of public attention. For example, the **average life expectancy**

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in Germany has almost doubled in the last 100 years. This development is mainly due to improved hygiene, reduction in neonatal mortality, and more effective therapy and prophylaxis of acute diseases in adulthood. As a result of this development, the health, social, and psychological problems of the elderly play an increasingly important role in clinical practice and research. However, not only is life expectancy increasing, but due to changes in individual life planning and effective contraceptive options, the time of starting a family is increasingly being postponed to an older age, when the fertility of at least the female partner is already significantly reduced.

While there are hardly any specific diseases of old age, the number of simultaneously occurring diseases in old age is increasing. The increase in **multimorbidity in old age** poses problems in the treatment of individual diseases. Due to increased contraindications of certain surgical or drug treatment strategies, therapeutic options are not infrequently limited. The extent to which normal aging processes influence the onset and course of diseases and, conversely, pathological changes accelerate or inhibit the physiological aging process cannot yet be reliably determined. Aging is a normal physiological process. During the aging process, a number of morphological and functional changes occur in the human organism in all organs and tissues. A general tendency toward decreasing functional effectiveness and atrophy of various organs and systems characterizes the aging process.

25.2 Theories on Causes of Aging

There are numerous, often incoherent, theories about the aging process. It is estimated that about 20–30% of life expectancy is genetically determined.

While most **aging theories** attempt to explain the causes of the aging process, evolutionary theory explores the different evolutionary biology concepts. Central to the evolutionary theory of aging is the question of what opportunities there are to achieve the highest possible reproductive fitness under different environmental conditions, i.e., to be represented as frequently as possible in the gene pool of the next generation (Kirkwood 2008). Changes occurring during the aging of an individual are considered to be species-preserving meaningful changes, following Darwinian evolutionary principles. An individual high age per se is not the goal of a species in terms of evolutionary biology.

In contrast to this theory, there are various theories that try to explain the causes of the aging process. In this context, the longest possible individual life is regarded as the goal of each individual, and biological causes for the different life spans are investigated.

For example, **program theory** assumes that the aging process is genetically determined and that death is a form of planned self-extinction.

Located at the ends of all chromosomes are several thousand copies of a specific DNA sequence of six base pairs, called telomeres. With each cell division, the chromosomes of somatic cells lose about 200 base pairs at their ends. According to the **telomere theory**, the length of the telomeres limits the life span of somatic cells and thus of the entire human organism (Rossiello et al. 2022).

According to the **mutation theory**, increased spontaneous mutations in old age lead to functional and morphological changes that affect the whole organism. Alternatively, age-related perturbations in DNA repair mechanisms are postulated. In particular, mutations in mitochondrial DNA increase with age (Sharma and Sampath 2019).

According to the **waste product accumulation theory**, the accumulation of certain metabolic waste products (e.g., lipofuscin) damages cells and tissues and leads to aging processes. **Free radicals**, which are physiologically produced in oxidative metabolism, are also thought to be a cause of aging by damaging macromolecules.

According to the **autoimmunization theory**, the production of abnormal substances that are not recognized as endogenous by immunocompetent cells leads to the production of specific antibodies that produce irreversible cellular damage.

Physiological changes in the endocrine organs are also discussed as a cause of the aging process.

25.3 Sexuality in Advanced Age

In principle, both women and men in senescence continue to possess the ability to feel and also act out sexual feelings.

In aging men, however, changes can be observed in **the phases of the sexual response cycle**. The **arousal phase** and the attainment of the erectile phase are delayed, and the erectile phase until ejaculation is usually prolonged. Control over **ejaculation** is usually markedly improved with age. However, the duration of orgasm is shortened. The strength of the sperm expulsion decreases. Rapid loss of erection occurs after ejaculation. The **refractory period** to renewed arousal is prolonged. Nocturnal erections are significantly less frequent in older men than in younger men, and penile rigidity also decreases (Elliot 1990). Erectile dysfunction is found more often in older men. Diagnosis and treatment of **erectile dysfunction** are described in Chap. 30.

About 90% of men over age 60 report still being sexually active. In a group of healthy men over 80, over 60% reported still having sexual intercourse. The presence of a committed partnership plays a crucial role, because married men are

significantly more sexually active than men living alone (Morley et al. 1990).

Various pathophysiological causes may be the reason for the **reduced frequency of sexual intercourse** in old age. Increasing multimorbidity in advanced age, which often includes cardiovascular disease, hypertension, rheumatic diseases, renal disease, or diabetes mellitus, has an unfavorable effect on sexuality. Many medications such as antihypertensives and psychotropic drugs also have a negative impact on sexuality. A decrease in sexual activity is often also due to psychosocial reasons such as a serious illness of the partner or the absence of a partner. Another reason for a decrease in sexual activity that should not be neglected is the negative attitude of large parts of society toward sexuality in old age. Elderly people are often no longer considered to have a sexuality of their own. Children have no understanding of the sexual aspects of their parents' lives. The living habits in many old people's homes with strict regimentation often do not allow opportunities for sexual activity (Elliot 1990).

In advanced age, a reduced or insufficiently satisfying sexual life may also be responsible for psychosomatic disorders such as gastritis, colitis, constipation, or attacks of angina or dyspnea.

25.4 General Endocrine Changes in Advanced Age

A **reduction in performance** occurring in advanced age must not be dismissed as age-related and therefore not requiring therapy without further clarification. Endocrine causes of a decline in performance must also be considered in the diagnostic process. Due to the potential multimorbidity of sick people in old age and the often-altered clinical picture of a hormone deficit, an endocrine-related treatable cause of a decrease in performance is often not diagnosed.

An age-related decrease in **growth hormone** (hGH, STH) production is known. Daily hGH secretion reaches its maximum during puberty and decreases continuously thereafter. The hGH pulse rate (especially at night) and GHRH-induced hGH secretion also decrease with age. Reduced hGH levels are associated with a decrease in general performance, reduced muscle strength, and an increased tendency to obesity (higher body mass index). Also, cardiovascular-related mortality rates are significantly increased in patients with manifest hGH deficiency (Vance and Mauras 1999).

In prepubertal boys and hypogonadal men treated with testosterone or hCG, mean growth hormone levels, pulse amplitude, and serum IGF-1 levels increase. The low IGF-1 levels and the changes in growth hormone levels could be due to decreasing testosterone levels and relative hypogonadism. This possibility has not yet been adequately investigated.

Morning serum **cortisol** concentrations remain constant with age. There are also no significant changes in cortisol levels in chronically ill, alcoholic, or obese patients or patients with prostatic hyperplasia (Gray et al. 1991). However, the physiological cortisol drop in the late evening hours is lower with increasing age, and this relative hypercortisolism may be a reason for increasing insulin resistance in old age (Roelfsema et al. 2017). Older men with relatively high endogenous cortisol levels have reduced bone density and an increased incidence of fractures (Reynolds et al. 2005; Greendale et al. 1999). In many depressed patients, there is insensitivity of glucocorticoid receptors leading to a stress-induced enhanced cortisol rise; also, the significantly increased incidence of type 2 diabetes mellitus in depressed patients can be attributed to stress-induced hypercortisolism.

Dehydroepiandrosterone (DHEA) and **dehydroepiandrosterone sulfate** (DHEAS) are weak androgenic precursors of testosterone synthesis formed in the adrenal cortex. The highest plasma concentrations are reached in the 20th to 25th year of life. Thereafter, plasma levels decline steadily, so that by age 60 only one-third or less DHEA(S) can be measured (Herbert 1995). In patients with a history of coronary heart disease, significantly lower DHEAS plasma levels were found than in the age-matched control group. Also, correlations were found between lowered DHEA(S) levels and diabetes mellitus type 2, rheumatoid arthritis, some carcinoma diseases, and body fat distribution. However, whether plasma DHEA(S) levels decrease reactively in chronic disease or whether elevated DHEA levels exert a protective function is not known. A meta-analysis of the Cochrane database shows that for older men there appears to be no benefit in terms of cognitive function and mood from DHEA supplementation. However, it is emphasized that definitive statements can only be made after longer-term studies with sufficient case numbers (Grimley Evans et al. 2006).

The hormone **melatonin**, which is produced in the pineal organ (pineal gland), is responsible for the day/night rhythm. With age, the concentration of melatonin in serum decreases. Furthermore, melatonin concentration is lower in older people with insomnia than in people without sleep problems (Garfinkel et al. 1995). Whether melatonin has an influence on other endocrinological functions and whether the effectiveness of melatonin as an oxygen radical scavenger, which has been demonstrated in vitro and in animal models, has physiological relevance and thus an inhibitory influence on the aging process is controversial (Garfinkel and Berner 1998).

Diseases of the **thyroid gland** increase with age. The classic symptoms are often no longer as obvious and can lead to omission or misdiagnosis. With increasing age, there is a

very discrete increase in TSH levels per decade of life of about 0.3 mU/l, paralleled by a discrete decrease in triiodothyronine synthesis with a decrease in the T3/T4 quotient. In subclinical hypothyroidism, TSH is somewhat elevated, but peripheral thyroid hormones are normal. Substitution therapy in elderly patients should be reserved because of the increased rate of adverse side effects and is not indicated with certainty until TSH exceeds 10 mU/l (Iwen and Brabant 2020). The incidence of both hypo- and hyperthyroidism is lower in aging men than in women of the same age, but thyroid carcinoma is found comparatively more often in older men (Morganti et al. 2007). Testosterone and SHBG (sex hormone-binding globulin) serum levels are increased in patients with hyperthyroidism, but the level of free bioactive testosterone remains unchanged. Both hyperthyroidism and, in particular, hypothyroidism can lead to fertility problems in men (Krassas and Perros 2003).

Very often **diabetes mellitus type 2** is also a disease of old age. Hyperinsulinism is usually present, leading to a reduction in insulin receptors in the periphery. Erectile dysfunction and retrograde ejaculation based on diabetes mellitus are common and not necessarily dependent on the duration of the disease. Causes include both diabetogenic neuropathy and microangiopathy. Testosterone and SHBG serum levels of diabetic men tend to be lower than those of age-matched healthy men, and the rate of new-onset diabetes mellitus is also slightly increased in men with low testosterone levels (Kapoor et al. 2005). A meta-analysis has shown that the ejaculate volume of patients with diabetes mellitus is significantly lower than that of subjects without diabetes mellitus. Sperm motility decreases. However, the percentage of normally shaped sperm and total sperm count remains unchanged. FSH levels are slightly increased (Pergialiotis et al. 2016).

25.5 Reproductive Functions in Advanced Age

25.5.1 Sex Hormones in Advanced Age

A "**climacterium virile**" (male menopause), an abrupt age-related change in gonadal function comparable to menopause, does not exist in the male sex. Sudden loss of fertility does not occur. Older men without concomitant diseases may have comparable testosterone levels and testicular volumes compared to younger men.

For a long time, whether there is a general **decrease in testosterone production** independent of disease was discussed controversially. Due to the nonuniform selection of subjects (convalescents, nursing home inmates, healthy volunteers), the results of initial epidemiological studies were contradictory. After serum testosterone levels were reported to decrease with age in the 1960s, the view that testosterone levels were not lower in healthy older men than in control groups of young volunteers increased in the 1970s. More recent large-scale epidemiological studies have found a statistical mean **decrease in testosterone levels with age**, but with a considerable **range of dispersion** (Araujo et al. 2007; Wu et al. 2008).

Three different factors are responsible for the changes in testosterone serum levels in aging men. At the testicular level, the reserve capacity of Leydig cells decreases after stimulation. The decrease in testicular reserve capacity is accompanied by a decrease in the number of Leydig cells with increasing age (Neaves et al. 1985). After hCG stimulation, the serum testosterone increase is lower in older men. The LH increase after GnRH stimulation also shows a slight but significant decrease with age (Nieschlag et al. 1982).

Concomitant with decreasing testicular testosterone production, serum LH levels increase slightly with age. However, this increase is very small and older men with decreased testosterone levels often have normotensive serum LH levels. Also, the small LH increases may be explained, at least in part, by decreased plasma clearance.

The major portion of testosterone is bound to protein in serum. Only the free unbound testosterone is biologically active. With age, the level of **sex hormone-binding globulin (SHBG)** in serum increases, so that the proportion of bioactive free testosterone decreases. While in young healthy men an increase in SHBG leads to a consecutive increase in total testosterone, this increase is absent due to increasing testicular and pituitary–hypothalamic insufficiency (Kaufman and Vermeulen 2005).

The circadian rhythmicity of testosterone serum concentrations, with the highest values in the early morning, also persists in older men, albeit at a lower level and with lower amplitude (Diver et al. 2003). The proportion of men with serum testosterone concentrations in the hypogonadal range (i.e., <12 nmol/l) increases with age and is 20% in 60- to 80-year-olds and 33% in those over 80). However, a very different picture emerges for patients: In a large study of men (age > 45 years) attending a so-called "primary care center" in the USA, a proportion of hypogonadal men were found to be 40% (Mulligan et al. 2006).

Basal serum concentrations of **LH** (luteotropic hormone) and **FSH** (follicle-stimulating hormone) increase slightly between the ages of 40 and 70 and significantly thereafter. LH is released in a pulsatile manner, and the amplitude of pituitary LH pulses decreases with age, but the frequency and duration of pulsatile release increase with increased basal secretion (Liu et al. 2005).

A slight decrease in **prolactin levels** is observed with age. However, the age-related decrease in testosterone levels is hardly reflected in plasma estradiol levels, which is due to

the increase in aromatase activity with age and the age-related increase in fat mass. Free and bioavailable estradiol levels decrease slightly with age, as does the ratio of free testosterone to free estradiol, the latter indicating age-related increased aromatization of testosterone. Estradiol levels are highly significantly positively related to body fat mass and in particular to subcutaneous abdominal fat, but not to visceral fat (Vermeulen et al. 2002). With decreasing testosterone levels and decreasing SHBG, the increased influence of estrogens on the organism may be relevant, for example, in the development of gynecomastia (Morley et al. 1990). Relatively elevated estradiol levels in relation to testosterone have also been found in patients with benign prostatic hyperplasia (Gann et al. 1995; Suzuki et al. 1995). **Serum levels** of the testosterone metabolite **dihydrotestosterone** (DHT) do not decrease with age, but lower levels are found in chronically ill patients (see Chap. 34).

25.5.2 Testicular Morphology in Advanced Age

Interpretation of histological examination of the testes of elderly men is hampered by the virtual impossibility of obtaining testes or testicular biopsies from healthy subjects. Therefore, morphologic knowledge is based predominantly on autopsy findings and examinations of testes removed during orchidectomy for prostate cancer. In many cases, these testes do not show any differences compared to younger men. A general decrease in **testicular volume** cannot be observed (Handelsman and Staraj 1985). Completely normal morphology was found in 27 of 102 testes examined from men over 90 years of age (Schlüter 1978). However, in some older men, changes ranging from discrete abnormalities to complete atrophy are found. Normal adjacent atrophic tubules have also been observed in the same testes. When signs of atrophy and hyalinization were present, **arteriosclerotic changes in the vessels** could usually also be detected, and the extent of the degenerative changes correlated with signs of general arteriosclerosis in the affected individual. In some cases, a multilayered arrangement of **spermatogonia** was observed, which is rare in younger men. Some findings indicate that **spermatogenetic efficiency,** that is, the number of spermatozoa resulting from the division process of spermatogonia (which is in any case lower in humans than in most animal species), decreases with age (Johnson et al. 1990). Increasing degeneration of germ cells at all stages of spermatogenesis has been found in testicular preparations from older patients with prostate cancer. New degenerative forms of spermatogonia, spermatocytes, and spermatids not found in younger men are appearing (Holstein 1989). Also, the number of spermatogonia, spermatocytes, and Sertoli cells in the testis decreases with age (Dakouane et al. 2005). Histomorphometric studies of testes from accident victims showed that the single Leydig cell does not change in volume even at older ages. However, the **total number of Leydig cells decreases.**

25.5.3 Ejaculate Parameters of Older Men

Representative data from longitudinal studies on ejaculate changes are not available. In our own studies, the seminal parameters of both healthy grandfathers, i.e., men over 60 years of age who had proven their fertility in earlier life and men older than 50 years who visited our clinic because of unfulfilled desire to have children, were not significantly different from the respective control groups of younger men and fathers. A significant decrease was found only in the percentage of **progressively motile spermatozoa**. In the group of grandfathers, **sperm concentration a**ctually increased. These changes can possibly be explained by the longer w**aiting period**. In **sperm function tests** (hamster ovum penetration test), no differences could be found (Nieschlag et al. 1982).

The **ejaculate volume** in the group of older men of childbearing potential was significantly lower than in the comparison groups. **Fructose concentration** as a marker of seminal vesicle function decreased, but the other markers of accessory sex gland function did not change. Sperm production and ejaculate parameters decrease with **worsening health** and the ability to ejaculate comes to a halt in more serious diseases (Nieschlag and Michel 1986).

With increasing age, the frequency of morphologically normal-shaped sperm continuously decreases, a percentage decrease of 0.2–0.9% per year has been determined (Kidd et al. 2001; Kühnert and Nieschlag 2004). The percentage of progressively motile sperm decreases with increasing age in men with normozoospermia (Levitas et al. 2007). Less than half of all subjects over 45 years of age in a study of drug therapy for erectile dysfunction had normal ejaculate parameters according to WHO criteria; in the age group studied, 45–80 years, a significant decrease in the proportion of normally formed and progressively motile sperm, sperm count, and ejaculate volume was found with unchanged sperm concentration (Hellstrom et al. 2006).

Whether there is a relationship between decreasing testosterone production and reduced spermatogenesis is not known. In our own patient population, no relationship was found between testosterone serum levels and ejaculate parameters in fertility patients in whom manifest hypogonadism could be excluded. This may be interpreted as a sign of testosterone-independent decreased productivity of the testes and accessory sex glands with age. The age-related change in ejaculate parameters is accompanied by a significant increase in serum FSH level and a moderate but significant inhibin B serum level decrease (Mahmoud et al. 2003).

The incidence of **varicoceles** does not increase with increasing age of patients attending fertility consultations (Rolf et al. 1996). In contrast, andrologic disorders acquired in adulthood, such as infections of the vas deferens and sperm autoantibodies, are diagnosed somewhat more frequently in older patients (Rolf et al. 2002a).

Although in general **sperm count** does not decrease with age, acquired infections of the **vas deferens** may lead to partial or even complete obstruction of the vas deferens, so that in these patients, if adequate antibiotic therapy has not been initiated, an increasing decrease in ejaculate parameters cannot be excluded. This would mean that not age per se but prolonged **exposure to infections** could lead to deterioration of ejaculate parameters. Not only with increasing age, but also with increasing **BMI**, a tendency of decreasing sperm count is observed (Sermondade et al. 2013). Since BMI also increases with age, it cannot be excluded that the observed decrease in sperm count is partly due to the increased BMI with the common obesity-related cardiovascular risk factors of elderly patients; multivariate studies considering aging and BMI are not available. Medications such as analgesics or psychotropic drugs, as well as chronic stress, may also have an unfavorable effect on ejaculatory parameters (Soliman et al. 2022; Baran et al. 2015). In the more frequent polypharmacy of older men, pharmaco-induced influences, in addition to **multimorbidity**, may explain the somewhat poorer ejaculate parameters.

25.5.4 Fertility of Older Men

Pablo Picasso, Charly Chaplin, Anthony Quinn, Julio Iglesias, Klaus-Jürgen Wussow, Franz Beckenbauer, Bernie Ecclestone, Robert de Niro, and Johannes Rau are prominent examples of men who can still father children at a relatively **advanced age**. What all these examples have in common is the significantly younger age of the partner. The preserved ability to procreate is not only reserved for prominent personalities, but is also reflected in the statistics of the population as a whole, in which there are a respectable number of fathers of newborns over the age of 50, but hardly any mothers in this age group. Not only in Germany but also in Japan, a country with a completely different sociocultural and ethnic background, a relatively large number of older men have still fathered children.

In several large-scale retrospective and prospective epidemiological population studies of diverse populations, a slight but significant negative correlation between increased male age and couple fertility has been demonstrated (Kühnert and Nieschlag 2004).

Age-related changes in fertility among older men may have different causes. In addition to age per se, other factors such as decreasing frequency of sexual intercourse, increasing frequency of urogenital infections, increasing incidence of arterial circulatory disorders, or accumulation of toxic substances damaging spermatogenesis may be responsible for a decrease in fertility.

Information about the **reproductive capacity** of older men is also provided by figures from fertility clinics. Comparisons of the number of pregnancies achieved by men over 50 years of age with those achieved by men under 30 years of age whose wives were of comparable advanced age (37 ± vs. 34 ± years) at the time of conception show that pregnancy rates do not differ significantly. In contrast, men with younger partners are statistically more likely to father a child (Rolf et al. 1996).

Older men who desire children often also have older female partners whose reproductive functions are already impaired. Therefore, a study of **time-to-pregnancy (TTP)** in 638 couples in which the women were all under 25 years of age is particularly interesting: Until the men were 40 years old, TTP averaged about 7 months; above the age of 40, it increased threefold (Hassan and Killick 2003). In countries where IVF treatment with heterologous oocytes is approved, the fertilization potential of older men's sperm can be studied regardless of oocyte quality. In such heterologous IVF therapy, increased male age does not cause a change in the fertilization rate or implantation rate, but the rate of successfully terminated pregnancies decreases with an increased abortion rate when paternal age exceeds 50 years (Gallardo et al. 1996; Frattarelli et al. 2008). Also, in the context of intrauterine insemination treatment, which requires higher sperm quality, the success rate decreases with increasing paternal age (Kühnert and Nieschlag 2004; Halvaei et al. 2020).

Procreative ability decreases slightly with increasing paternal age. However, the fact of relatively infrequent paternity in men over 60 years of age mainly finds its rationale in the reduced or extinct conception capacity of the female partner or the deliberate refraining from having children.

If **fertility problems** occur in an older man of childbearing potential, they should be diagnosed and treated as in a younger patient.

Because of the man's age alone, appropriate **contraception** should not be abandoned if the partner is reproductively capable and there is no desire to have children.

25.5.5 Reproductive Risks of Increased Paternal Age

25.5.5.1 Abortions and Paternal Age

It is estimated that 30–70% of all pregnancies end in **miscarriage,** most of them unnoticed. Known risk factors include increased maternal age, previous miscarriages, high maternal alcohol consumption, maternal overweight, or underweight. Pregnancies induced by men older than 50 years have a nearly doubled risk of ending in miscarriage (Nybo Andersen et al. 2004). In another large-scale study, increased paternal age was found to be associated with an increased risk of miscarriage, particularly in women older than 30 years, with the highest risk in couples where both parents are already of advanced reproductive age (de la Rochebrochard and Thonneau 2002). The incidence of both early and late abortions increases steadily with increased paternal age (du Fossé et al. 2020).

25.5.5.2 Chromosomal Anomalies and Increased Paternal Age

An increased incidence of chromosomal anomalies is assured with increased **maternal age** (Nieschlag et al. 2000).

The main cause of **aneuploidy**, i.e., deviation from the normal chromosome set, is the **non-disjunction** of homologous chromosomes (maldistribution) during meiotic division of gametes. Meiotic divisions of the spermatocytes of the first order start in males after puberty. Non-disjunction during spermatogenesis is not a rare event. In one study, an abnormal chromosome number was found in 4.7% of spermatozoa from healthy males (Bordson and Leonardo 1991).

The frequency of numerical **chromosome abnormalities** in infertile men with reduced sperm quality is increased. While an age-related increase in the rate of numerical sex chromosome abnormalities has been found in sperm testing, an increased incidence of numerical chromosome abnormalities has not been documented for autosomes (Kühnert and Nieschlag 2004).

Trisomy 21 (Morbus Down) is one of the most common numerical chromosomal anomalies; increased maternal age has long been a known risk factor, and only 10% of cases are paternally transmitted. However, recent large studies showed a slightly increased risk of increased paternal age when maternal age was also increased (Kühnert and Nieschlag 2004).

Also, in the relatively common and thus clinically relevant **chromosomal aneuploidies** of trisomy 13, trisomy 18, Turner syndrome (– 45XO), and Klinefelter syndrome (47XXY), no relationship was found between paternal age and disease frequency (Bordson and Leonardo 1991; Luetjens et al. 2002).

There is evidence of increased **structural chromosomal abnormalities** in the sperm of older males. However, no accumulation of new-onset structural chromosomal abnormalities has been documented in living neonates or prenatally diagnosed fetuses (Kühnert and Nieschlag 2004). Inherited reciprocal translocations are reported to be clustered in children of older fathers.

25.5.5.3 Genetic Disorders and Increased Paternal Age

Genetic mutations are the result of errors in DNA replication. By the time of puberty, approximately 30 cell divisions result in a large pool of undifferentiated spermatogonia. These divide 23 times per year, so that the number of cell divisions undergone by a spermatozoon in a 20-year-old man is 200, in a 30-year-old man 430, and in a 45-year-old man 770 cell divisions (Crow 1997). With each division, *de novo* mutations can arise. Thus, it is understandable that mutations occur more frequently with age.

Late spermatids, like spermatozoa, do not have their own repair system. The effectiveness of antioxidant protective mechanisms in seminal plasma and spermatozoa decreases with age; spermatozoa from older males are thus more vulnerable to mutagens than spermatozoa from younger males (Tarin et al. 1998).

It is now generally accepted that **autosomal dominantly inherited diseases** such as achondroplasia, polyposis coli, basal cell nevi, Marfan syndrome, or Apert syndrome are associated with the increased age of the father. The incidence of other conditions, such as neurofibromatosis, retinoblastoma, or Sotos syndrome, increases only slightly with increasing paternal age (Crow 1997). About 5 percent of all heart defects in children whose fathers are older than 35 years are thought to be due to increased paternal age, this indicates that some heart defects are due to newly emerged dominant mutations (Crow 1997). Also, the incidence of psychiatric disorders such as **schizophrenia** or **autism** increases slightly in children of older fathers. It is generally assumed that paternal age-related de novo mutations are the cause. However, there is strong evidence that genetic risk factors shared by older fathers and their offspring may explain the correlation of paternal age and incidence of psychiatric disorders, although it is not clear why fathers with a genetic predisposition to psychiatric disorders, or even manifest psychiatric disorders, have a higher reproductive age (Gratten et al. 2016).

Thus, even though there is a somewhat increased incidence of certain genetic disorders in children of older men, the individual risk of such a new disorder is considered extremely low due to the rarity of these disorders.

While according to the guidelines for prenatal diagnosis of diseases and disease dispositions of the German Medical Association, the increased age of the pregnant woman is a reason for targeted, intensified prenatal diagnosis, **increased age of the father is not an explicit indication for intensified prenatal diagnosis** (Bachmann et al. 2007).

25.6 Late-Onset Hypogonadism

25.6.1 Definition

In the aging male, testosterone is also responsible not only for secondary sexual characteristics, libido, and potency, but also has an influence on mood and intellectual abilities, erythropoiesis, bone metabolism, protein anabolism and muscle mass, and fat distribution. Testosterone is important in the aging male with respect to both sexual life and vital functions. Clinically, a decrease in testosterone serum levels may be accompanied by a decrease in libido and potency, as well as fatigue, decrease in muscle mass, osteoporosis, and mild anemia. In most cases, a **decline in erectile strength** alone is due less to testosterone deficiency than to concomitant diseases such as arteriosclerosis or diabetes mellitus. In particular, neural and vascular organic changes in the penis and pelvis are causally involved in potency disorders.

25.6.2 Mortality and Testosterone Deficiency

The **mortality** of patients with decreased testosterone serum levels is significantly higher than the mortality of patients with normotensive testosterone serum levels, even when mortality due to the disease leading to the current hospitalization was not considered (Shores et al. 2006).

Prospective studies showed that the mortality of unselected older men with testosterone serum levels in the lowest quartile was significantly higher than that of the men with relatively high testosterone serum levels after accounting for other risk factors such as age, BMIs, concomitant diseases, blood pressure, or lipid metabolism parameters (Khaw et al. 2007; Laughlin et al. 2008). While the mortality of men with lowered testosterone levels was increased by 30% and 40%, respectively, in subsequent years, relatively high testosterone serum levels were not associated with reduced mortality.

Decreased free testosterone serum levels and increased free estradiol serum levels are independent risk parameters for peripheral arterial disease (Tivesten et al. 2007).

25.6.3 Symptoms of Late-Onset Hypogonadism

25.6.3.1 General Symptoms

In addition to decreased testosterone serum levels, corresponding clinical symptoms of androgen deficiency must be present for the diagnosis of **late-onset hypogonadism** (Nieschlag et al. 2005; Bhasin et al. 2006; Wang et al. 2008). The main symptom is reduced **libido**, but **erectile dysfunction**, **decreasing muscle mass** and strength, **increasing body fat**, a **decrease in bone density**, and a decrease in **general performance** and **depressed mood** are also indications of androgen deficiency. For diagnosis, at least one of these symptoms and a decreased testosterone serum level must be present (Kelleher et al. 2004; Zitzmann et al. 2006).

The symptomatology of late-onset hypogonadism obviously has symptom-specific thresholds of testosterone concentrations. Usually, the symptom complex begins with a loss of libido and drive, and as testosterone levels continue to decline, an accumulation of visceral fat, depression, and hot flashes are added. At the very end, there is the full-blown picture of hypogonadism including erectile dysfunction (Zitzmann et al. 2006).

During the medical examination, a transient androgen deficiency due to an intermittent acute illness must be excluded. Chronic diseases that can cause late-onset hypogonadism are diabetes mellitus, COPD, chronic arthritis, renal disease, and HIV with its consecutive sequelae.

25.6.3.2 Osteoporosis

Osteoporosis is a systemic skeletal disease caused by decreased bone mass and deteriorated microarchitecture of bone tissue, leading to increased fracture incidence. A distinction is made between **primary osteoporosis** without an identifiable cause and **secondary osteoporosis** as a result of another disease, or even therapy, although this distinction is often impossible to make with certainty. Primary osteoporosis is a diagnosis of exclusion. Hypogonadism is a generally recognized risk factor for osteoporosis, just as testosterone replacement is a recognized therapeutic modality for both prevention of osteoporosis and improvement of bone density in patients with manifest hypogonadism. In women, the incidence of osteoporosis increases markedly after the menopause and it is generally accepted that menopausal changes should be considered as the cause. On average, comparable osteoporotic changes are found approximately 10 years later in men than in women. In men, the incidence of hip fractures also increases significantly with decreasing testosterone and estradiol serum levels (Amin et al. 2006; Meier et al. 2008).

25.6.3.3 Metabolic Syndrome

In the last century, living conditions in industrialized nations have changed considerably: The level of physical activity has decreased, and at the same time, there is an oversupply of high-calorie, high-fat food. This results in an increasing prevalence of obesity, especially in the last two decades. The accumulation of visceral fat as an endocrine highly active organ poses a particular problem here, manifesting as a complex pathological entity with disturbances in blood pressure, lipid metabolism, and glucose tolerance and termed **metabolic syndrome**. Visceral fat secretes inflammatory cytokines, pro-coagulant substances, and angiotensin-aldosterone

system activating substances, among others. Thus, individuals showing metabolic syndrome have a threefold increased risk of clinically manifest cardiovascular events or stroke. The risk of developing **diabetes mellitus type 2** is significantly increased. The International Diabetes Federation has identified abdominal circumference as a measure of visceral adipose tissue as a central factor. In this context, an abdominal circumference of 94 cm has been reported as the cutoff value for European men.

In men, central obesity as a key component of metabolic syndrome is frequently associated with testosterone deficiency. Accordingly, longitudinal epidemiological studies show that testosterone deficiency is an independent predictor of the development of metabolic syndrome in men; conversely, metabolic syndrome also poses a risk for the development of testosterone deficiency. This apparently affects not only men with an elevated **body mass index (BMI)**, but also normal-weight individuals with a then elevated abdominal circumference (Laaksonen et al. 2004; Muller et al. 2005; Kupelian et al. 2006).

Pathophysiological correlates are shown in vitro in that mesenchymal pluripotent stem cells, which have the possibility of further development into either adipocytes or myocytes, take the path into the adipogenic lineage in the state of androgen deficiency, whereas testosterone administration promotes differentiation into muscle cells in a dose-dependent manner (Woodhouse et al. 2004). Accordingly, decreased visceral fat and increased muscle mass are found with testosterone administration in hypogonadal men, as evidenced by magnetic resonance imaging studies in a placebo-controlled trial (Allan et al. 2008).

It is therefore not surprising that testosterone administration also improves insulin sensitivity, as this is significantly dependent on both mitochondrial function within muscle cells and androgen receptor-mediated processes in fat cells.

25.6.3.4 Psychosomatic Aspects

Although the **complaints associated with androgen deficiency** are in part quite nonspecific and may also occur in various psychosomatic syndromes, screening of androgen levels is not part of the standard diagnostic repertoire in psychosomatics. On the other hand, male patients with nonspecific physical complaints often seek andrological clarification, and in about half of them, a testosterone deficit is present (Zitzmann 2006; Zitzmann et al. 2006). It is assumed that this is partly the same patient clientele, whose utilization behavior is controlled by the subjective disease concept of the patients and referring physicians. This would mean that a certain percentage of patients present in psychosomatic medicine with a testosterone deficiency comorbid or causal for the complaints, but which is usually not diagnosed and not treated. Similarly, it is likely that a certain percentage of andrologic patients present with **psychological disorders (depression, anxiety, and somatoform disorders)** that are also undiagnosed and untreated.

The following is a review of studies on associations between androgen deficiency and psychological symptomatology. Results are not always consistent, as different samples have been examined in cross-sectional epidemiological approaches and also in intervention studies. Most studies deal with the relationship between androgens and depressiveness. In most cases, a partial negative correlation is found between depression scores and testosterone levels. Hypogonadal men have significantly higher depression scores. While there are no significant differences for anxiety per se, hypogonadal men have higher scores than eugonadal men on corresponding scales for "somatic symptoms" (Wang et al. 1996; Seidman et al. 2001).

One explanation for why androgen testing in a normal population sometimes yields inconsistent results may be that the low androgen-depressant association is detectable only for certain subgroups in which the CNS–testosterone interaction is modulated by other factors. A study involving the **CAG repeat polymorphism** of the androgen receptor gene points in this direction: A Finnish group investigated the relationship CAG repeat length of the androgen receptor gene in a sample of 172 men aged 41–70 years. A positive association was found between CAG-RL length and depression; i.e., weaker androgen action was associated with a desire to be dead (r = 0.45), increased depressiveness (r = 0.23), anxiety (r = 0.15), and decrease in general well-being (r = 0.22) (Harkonen et al. 2003).

Physical symptoms mentioned in association with **androgen deficiency** include **sleep disturbances, hot flashes, physical weakness, increased fatigability, decrease in libido, and subjective deterioration of memory**. These are nonspecific physical complaints that, although they may be associated with a diagnosis of **depression** or **dysthymia,** also occur independently of marked mood deterioration in other psychosomatic disorders, e.g., anxiety disorders, neurasthenia, post-traumatic stress disorder, and the somatoform disorders. Associations are often found between lower testosterone levels and higher scores for anxiety, depression, and attention deficit disorders (Zitzmann 2006).

Somatoform disorders represent a special aspect: They are characterized by physical symptomatology that limits the patient and cannot be explained by organic findings, or not adequately; the patient is usually convinced of an organic genesis and demands further organ-medical examinations, which can put a considerable strain on the doctor–patient relationship and cause considerable costs in the health care system. Hypogonadal men have higher scores on the GHQ "Somatic Symptoms" scale than eugonadal men (Delhez et al. 2003).

A larger study of over 400 older men (over 50 years old) attending an andrology outpatient clinic showed that

psychosomatic complaints organize into **clusters** that show an inverse association with testosterone levels. In particular, lack of drive, loss of libido, and depressed mood are found simultaneously with hot flashes and general physical weakness in age-related hypogonadism (Zitzmann et al. 2006).

A meta-analysis of randomized trials reported moderate improvement in depressive symptoms in men treated with testosterone compared with men treated with placebo (Walther et al. 2019). In meta-analyses, antidepressants have shown no benefit in **moderate depression** to date, but have a high rate of side effects and significantly increased mortality, particularly in older patients (Maslej et al. 2017). It is not clear why the current European guideline does not recommend an albeit moderate but still relatively safe form of therapy (testosterone) for depression, but recommends the ineffective and obviously significantly more side effect-laden therapy with antidepressants (Corona et al. 2020).

25.6.3.5 Cardiovascular Risk

Epidemiological studies have shown that mortality is increased in men with low- or low-normal endogenous testosterone levels. Cardiovascular disease accounts for the greater proportion of deaths in men with low testosterone. Men with angiographically proven coronary artery disease have lower testosterone serum levels compared with control subjects. Also, mortality in hypogonadal untreated men after coronary angiography is significantly increased over the subsequent 7 years. In addition, the incidence of ischemic heart disease correlates with an elevated cortisol/testosterone quotient, i.e., a pro-inflammatory state (Smith et al. 2005). In particular, in very elderly men, the incidence of atrial fibrillation increases highly significantly with lowered testosterone serum levels (Magnani et al. 2014).

Cross-sectional studies in middle-aged men have found a positive association between serum **testosterone levels** and **plasma HDL cholesterol concentrations**. Lower testosterone levels in men are associated with higher levels of LDL cholesterol concentrations, triglycerides, and prothrombotic factors (Bhasin et al. 2022).

Hypogonadism is also an independent predictor of adverse disease outcome in patients with **chronic renal failure**, who already have more frequently decreased testosterone levels. One standard deviation (SD) decrease in total testosterone increases the risk of all-cause mortality by 27%, cardiovascular mortality by 100%, cardiovascular events by 20%, and infectious events by 41% (Li et al. 2022).

25.6.4 Hormone Replacement in Advanced Age

25.6.4.1 Testosterone Substitution

When clinical symptoms of latent or manifest testosterone deficiency are present and the morning serum testosterone level has fallen below the norm of 12.0 nmol/l for younger men, testosterone substitution should be considered. Substitution is performed according to international recommendations (Wang et al. 2008) and according to the principles and at the doses given in Chap. 36.

By analogy with the term "late-onset diabetes," the term "**late-onset hypogonadism**" was developed for the age-related decline in testosterone caused by a **combination of primary and secondary hypogonadism** (Zitzmann and Nieschlag 2003). This term was adopted by most **andrological and urological societies in their guidelines,** and the recommendations for treatment with **testosterone preparations** were also largely adopted. There was only ambiguity about the threshold above which **androgen substitution** was indicated in the case of corresponding clinical symptoms (Nieschlag 2019). Following the establishment of **transdermal testosterone therapy**, there was a massive increase in the number of prescriptions for testosterone preparations in older men, particularly in the USA. For example, from 2002 to 2013, the number of men treated with testosterone increased fivefold (Baillargeon et al. 2018). In most cases, physicians cited "testicular hypofunction" as the reason for the prescription. Analyses of billing data show that testosterone levels were not determined at all in one-third of patients (Nieschlag 2019).

In 2014, the U.S. Food and Drug Administration (FDA) tightened the professional information because of these increasingly uncritical prescriptions of testosterone preparations for older men (FDA 2015). Since then, manufacturers have had to explicitly point out potential **cardiac risks**. The preparations are only approved for the treatment of patients in whom testicular, pituitary, or brain dysfunction has been identified as the cause of hypogonadism. Thus, the vast majority of prescriptions are "off-label."

The FDA's concerns relate primarily to cardiovascular effects. Five retrospective observational studies and two meta-analyses were considered.

The results were far from conclusive. In two retrospective observational studies, testosterone treatment was associated with an increase in cardiovascular disease. In one study, there was a significant increase in the rate of myocardial infarction, stroke, and death (Vigen et al. 2013) In the other study, there was a significant increase in nonfatal myocardial infarctions in older men as early as 90 days after the initial prescription and also in younger men with preexisting heart disease (Finkle et al. 2014). The other retrospective studies showed no unfavorable or no effect of testosterone therapy on the cardiovascular endpoints studied (Baillargeon et al. 2014; Muraleedharan et al. 2013; Shores et al. 2012).

A meta-analysis of 27 randomized, placebo-controlled trials involving 2994 middle-aged and older patients found that testosterone therapy was associated with an increased risk of adverse cardiovascular events (odds ratio [OR] = 1.5, 95% CI: 1.1–2.1). This finding could not be confirmed in

more recent meta-analyses, in part with the same data set but supplemented with more recent studies (Corona et al. 2018). An independent review conducted by the European Medicines Agency also found **no consistent evidence of an increased risk of coronary heart disease** associated with testosterone treatment in hypogonadal men (Bhasin et al. 2022).

In 2020, the **European Academy of Andrology (EAA)**, in contrast to the American professional society, changed its guideline and questioned the treatment concept of late-onset hypogonadism with testosterone therapy in the presence of an appropriate clinic and lowered testosterone levels. The term **functional hypogonadism** was chosen to define testosterone deficiency secondary to comorbidities (Corona et al. 2020). Primarily, the underlying disease should be treated. Testosterone therapy may reliably improve overall sexual performance and, in particular, libido in men with hypogonadism, but the long-term clinical benefit and safety of testosterone therapy for functional hypogonadism were not established. Testosterone therapy for the treatment of chronic diseases, such as metabolic syndrome disorders, osteoporosis, or depression, is not recommended. **Overall, the evidence regarding testosterone therapy in older men with functional hypogonadism/late-onset hypogonadism and concomitant diseases is inconclusive**.

Testosterone significantly regulates sexual function, particularly libido and to a lesser extent erectile function. Evidence is clear that testosterone modestly increases sexual interest and activity—from flirtation to intercourse—in hypogonadal men with testosterone <9.4 nmol/L (<270 ng/dL) compared with placebo (Corona et al. 2020). Moreover, the extent of the effect was inversely related to baseline testosterone concentrations and proportional to the increase in testosterone concentrations during the study. Stronger effects were observed on libido and sexual activity than on erectile function. These data are consistent with recent meta-analyses of sexual function, which also showed that testosterone therapy is effective when testosterone levels are <10.4 nmol/L (<300 ng/dL) 50 or 12 nmol/L (350 ng/dL) and ineffective in men with testosterone levels >12 nmol/L (>350 ng/dL). In addition, the efficacy of testosterone therapy on erectile function was higher in men with severe hypogonadism (T < 8 nmol/L or 231 ng/dL) and lower in men with type 2 diabetes mellitus and obesity (Corona et al. 2020).

In men with **diabetes mellitus type 2**, a placebo-controlled trial of intramuscularly injected testosterone demonstrated significant improvement in insulin resistance and glycemic control, as well as a decrease in visceral fat and a reduction in dyslipidemia (Kapoor et al. 2006). This is supported by long-term observations of hypogonadal men who received testosterone undecanoate as an intramuscular long-acting depot for several years: Lipid metabolism and blood pressure profiles showed significant changes toward normalization of metabolism (Zitzmann and Nieschlag 2007).

In general, muscle mass and strength decrease in the aging male. Hypogonadal young patients usually also have reduced muscle strength, which can be improved by testosterone substitution. An improvement in muscle mass with a decrease in abdominal fat mass has also been noted in older men on testosterone replacement therapy (improvement in **lean body mass index**) (Allan et al. 2008). In healthy subjects, an increase in body weight and percent fat is measured with increasing age, which correlates with a decrease in testosterone levels. In contrast, no change in body weight and percent fat is measured in hypogonadal patients on adequate testosterone therapy, in whom there is thus no age-related decline in testosterone serum levels (Rolf et al. 2002b). As already mentioned, in men with **diabetes mellitus type 2**, under testosterone not only an improvement in diabetes control takes place, but also a decrease in visceral fat percentage and a reduction in dyslipidemia (Kapoor et al. 2006).

Initial results of testosterone replacement therapy in orthopedic patients with **knee replacements** yielded encouraging results. In autoimmune diseases, androgens may have a protective role. The incidence of most autoimmune diseases is also increased in the elderly, and in women, and also in hypogonadal men, it is equally significantly higher than in healthy men. (Baillargeon et al. 2016). In the context of diagnosis and therapy of both senile and secondary **osteoporosis** in association with other diseases, changes in androgen levels should be taken into account, and in the case of corresponding pathological values, a therapeutic attempt with androgens should be considered (Francis 1999). For example, in patients with osteoporosis associated with chronic obstructive pulmonary disease, testosterone therapy has been shown to significantly improve bone density under cortisol therapy (Reid et al. 1996).

In the first large placebo-controlled study over 36 months, **transdermal testosterone substitution** in older men resulted in an increase in muscle mass and bone density (Snyder et al. 1999a, 1999b). Subjects reported a subjective improvement in physical performance. A significant increase in bone density was measured particularly in older men with lowered basal testosterone levels, whereas only a marginal increase in bone density was observed in patients with normative testosterone levels before the start of therapy. Evidence that testosterone therapy reduces fracture risk has not been published.

At this time, long-term testosterone replacement therapy for **osteoporosis prophylaxis** cannot be recommended for isolated low testosterone alone without other clinical androgen deficiency symptoms and without other complaints.

It is evident that hypogonadism can have negative effects on **quality of life** and **mood.** Testosterone substitution is considered to improve these parameters (Zitzmann 2020).

However, if these psychological symptoms are the patient's only problems, the use of testosterone to improve quality of life and mood in men with hypogonadism is not currently strongly recommended. Studies of testosterone substitution and depressive-tinged mood confirm cross-sectional epidemiologic studies: 22 hypogonadal depressed men were treated with testosterone gel or placebo. Preexisting antidepressant treatment was continued. The men who received testosterone had significantly more pronounced improvements in depressive symptomatology on the Hamilton Depression Rating Scale than those men who received placebo.

A large, non-controlled trial of testosterone gel as a substitution agent showed significant **improvement in mood** in a larger collective of hypogonadal older men, as demonstrated by several psychometric scales (Wang et al. 1996).

To date, there are no randomized trials large enough or of sufficient duration to determine the effects of testosterone treatment on the incidence of major adverse cardiac events (MACE). The incidence of MACE reported in randomized trials of testosterone was low—even lower than would be expected given the age and comorbidities of the participants (Bhasin et al. 2022). A randomized trial of testosterone in older men (the TOM Trial) with mobility limitations was stopped early because cardiovascular events were more common in men receiving testosterone than in men receiving placebo (Basaria et al. 2010), heightening concerns about the cardiovascular safety of testosterone in frail older men. In contrast to many other testosterone studies in older men, which involved relatively healthy older men, participants in the TOM study had a high prevalence of chronic conditions such as heart disease, diabetes mellitus, obesity, hypertension, and hyperlipidemia. Men who were 75 years or older and those with high testosterone levels during treatment appeared to be at the greatest risk for cardiovascular events. The TOM trial was not designed to detect cardiovascular events; therefore, cardiovascular events were not a prespecified end point and were not recorded in a standardized manner or assessed prospectively. In addition, many of the cardiovascular events were not MACE.

The higher incidence of cardiovascular adverse events observed in the TOM trial in older men treated with testosterone was not replicated in two larger trials with longer durations that were recently published; in the TEAAM trial, the incidence of serious adverse cardiac events during the 3-year intervention was similar between groups. Similarly, in the TTrials trial, the number of MACE (myocardial infarction, stroke, or death related to cardiovascular disease) during 1 year of treatment was similar in both groups, with seven men in each group experiencing an event (Snyder et al. 2016). The number of MACE in the TEAAM and TTrials was too small to allow firm conclusions about the effects of testosterone treatment on MACE (Bhasin et al. 2022).

In hypogonadal men, when normotensive testosterone levels are achieved, testosterone therapy results in a lower incidence of atrial fibrillation (AF) compared with untreated hypogonadal men (Sharma et al. 2017).

To date, there is no evidence that **testosterone substitution** induces **prostate cancer**. The molecular processes by which androgens affect prostate growth are not well understood. Androgens are essential for normal prostate growth; in the absence of androgens, the prostate cannot maintain its function or size. The adult prostate does not grow beyond the corresponding prostate size of age-matched healthy subjects when exogenous testosterone is substituted (Behre et al. 1994). In a meta-analysis of 14 clinical trials involving over 2000 patients, testosterone replacement therapy was shown not to worsen the International Prostate Symptom Score (IPSS) in men treated for LOH. Under testosterone substitution in older men, the incidence of prostate cancer does not increase (Debruyne et al. 2017). However, manifest prostate carcinoma is promoted by testosterone in growth and is a contraindication to androgen substitution. Isolated cases of patients on testosterone substitution who developed prostate carcinoma in old age have been described (Rolf and Nieschlag 1998).

The prostate should therefore be monitored regularly by digital examination and PSA determination before and during substitution therapy. According to LOH recommendations (Wang et al. 2008), these examinations should be performed 3–6 months and 12 months after the start of testosterone substitution and then at annual intervals. Supplemental transrectal ultrasonography is optional.

Gynecomastia may occur especially with the use of short-acting injectable testosterone esters, and this is caused by the aromatization of testosterone to estrogens, especially at higher peak testosterone levels.

High doses of testosterone can lead to **polycythemia**, and elevated hematocrit levels are found relatively frequently when older men are substituted with testosterone enanthate (Sih et al. 1997; Calof et al. 2005); therefore, hematocrit and red cell count should be monitored regularly during treatment. To avoid the complications of polycythemia, a dose reduction, a switch to transdermal testosterone preparations, or at least a short-term interruption of treatment must then be made.

Contraindications to testosterone administration include a history of **prostate or breast cancer** (Bhasin et al. 2022). Benign prostatic hypertrophy (BPH) per se is not a contrain-

dication unless it is accompanied by severe symptoms as indicated by an IPSS symptom score greater than 21. Men with baseline hematocrit greater than 50%, severe untreated sleep apnea, or **congestive heart failure** with class III or IV symptoms should not receive testosterone (Corona et al. 2020). Testosterone suppresses spermatogenesis and should not be prescribed to men who plan to have a child in the near future.

Long-term randomized trials of the effects of testosterone treatment on the incidence of coronary artery disease (MACE) are needed and are particularly important because even small changes in incidence rates could have significant public health implications.

A large randomized, placebo-controlled trial is currently underway to investigate the effects of testosterone replacement therapy on the incidence of major cardiovascular events in men aged 45–80 years with low testosterone and one or more symptoms of testosterone deficiency who are at increased risk for **cardiovascular events** (The TRAVERSE Trial, NCT03518034). The intervention duration is up to 5 years in this study of over 6000 men. Efficacy outcomes include noted clinical fractures, remission of low-grade persistent depressive disorder (dysthymia), progression from prediabetes to diabetes, correction of anemia, and overall sexual activity, sexual desire, and erectile function. This randomized, placebo-controlled trial provides a historic opportunity to improve our understanding of the cardiovascular safety and long-term efficacy of testosterone replacement in middle-aged and older hypogonadal men (Bhasin et al. 2022).

COVID-19-affected men with an unfavorable disease course have lower testosterone serum levels than patients with a milder disease course, this is usually considered to be due to the acute more severe disease. One study examined hospitalization rates after COVID-19 infection in men with hypogonadism, eugonadal men, and hypogonadal men receiving testosterone substitution for at least 6 months (Dhindsa et al. 2022.) This study showed that men with hypogonadism were more than twice as likely to be hospitalized after COVID-19 infection, regardless of other known risk factors; patients were also significantly more likely to require intensive care. This increased risk was not observed in hypogonadal men who had been receiving adequate testosterone replacement therapy for at least 6 months at disease onset. This is a clear indication that the decreased testosterone level was causal for the unfavorable disease course and not reactively caused by the COVID-19 infection and that a preexisting testosterone substitution has a favorable influence on the disease course.

25.6.4.2 Other Hormones

As described at the beginning, changes in testosterone production are not the only endocrine changes in senescence

Table 25.1 Endocrine changes with age (related to the means of large populations, which have wide variances)

Testosterone (total)	Decrease
Free testosterone	Decrease
LH	Increase
FSH	Increase
SHBG	Increase
Prolactin	Decrease
Estradiol (E2)	Constant
Estrone (E1)	Constant
Dihydrotestosterone (DHT)	Constant
Cortisol	Constant
Growth hormone (GH)	Decrease
Dehydroepiandrosterone (DHEA)	Decrease
Melatonin	Decrease

(Table 25.1). In recent years, increased attention has been paid to the decreased secretion of **growth hormone** and **insulin-like growth factor-1 (IGF-1**). Reduced growth hormone levels in the older man are thought to be partly responsible for the change in body shape. Recombinant growth hormone has opened up the possibility of substitution. In 1990, a first controlled study showed that by administering growth hormone in older men with growth hormone levels below the normal value of young healthy men, an anabolic metabolic state with an increase in bone density of the lumbar spine and a reduction in fat in favor of protein could be achieved (Rudman et al. 1990). In further studies, the positive effects on body composition could be confirmed, but no improvement in muscle strength or maximum oxygen uptake under stress could be proven (Vance 2003; Lombardi et al. 2005). At the same time, however, decreased glucose tolerance and increased blood pressure were observed (Rudman et al. 1990). Therefore, there is a possibility that hypertension, diabetes mellitus, or cardiomegaly may develop with prolonged substitution therapy. In older controlled studies with currently relatively high doses of growth hormone, more than 10% of patients each reported peripheral edema or arthralgias (Bryant et al. 2002).

Since acromegalic patients have a significantly increased **incidence of colon polyps** and **colon carcinomas**, it cannot be excluded that growth hormone replacement therapy may not also induce carcinomas (Jenkins et al. 2006). Likewise, it has been shown that the incidence of **prostate carcinoma** is significantly increased in men with high serum IGF-1 concentrations. Patients with pituitary-induced growth hormone deficiency under substitution appear to have a decreased risk of carcinoma (Li et al. 2016). Substitution of children and young adults with human pituitary growth hormone may result in a slightly increased risk of carcinoma in adulthood (Swerdlow 2006). Children treated with recombinant growth hormone after malignancy due to growth hormone deficiency had a slightly increased risk of second neoplasia (Ergun-Longmire et al. 2006). However, an increased risk of carci-

noma has not been found for growth hormone therapy in constitutional short stature. Considering the potential risks, growth hormone substitution is not indicated at this time in older men with physiologically decreased growth hormone levels without pituitary insufficiency (Aguiar-Oliveira and Bartke 2019).

Results of controlled trials of substitution of older men with synthetic growth hormone analogs, superior growth hormone-releasing hormone (GHRH), or IGF-1 have not been published to date.

Substitution with **growth factors** or with IGF-1 must be considered experimental and, given the current state of knowledge, should be performed only in controlled trials.

In a first clinical controlled study, **DHEA substitution therapy** yielded an increase in IGF-1 levels, with 70% of patients reporting an improvement in well-being. Further studies demonstrated an activation of the immune system with an increase in the number of monocytes, activated T-lymphocytes, and natural killer cells in older men and postmenopausal women. However, another larger placebo-controlled study found no beneficial effects of DHEA administration (Flynn et al. 1999). While a slight improvement in lumbar bone density was obtained in healthy elderly women by oral DHEA substitution, no improvement was observed in elderly men (von Mühlen et al. 2008). No improvement in muscle strength or physical performance with DHEA therapy has been demonstrated in controlled studies (de Spiegeleer et al. 2018).

Further controlled studies are needed to verify the efficacy of DHEA therapy.

Evening **melatonin substitution** improves sleep quality in the elderly (Garfinkel et al. 1995; Valtonen et al. 2005). There is no verified evidence for the tumor-preventive potency of melatonin substitution, which is frequently discussed in the media. So far clinical studies have failed to demonstrate efficacy (Garfinkel and Berner 1998).

Some gynecologists advocate **estrogen substitution** also of the aging male (Umbreit 1993). Results of long-announced controlled trials are still pending. Physiologically conclusive evidence for such substitution therapy is not known. In controlled clinical trials with higher-dose estrogen preparations for the prevention of coronary heart disease and for the treatment of prostate carcinoma, increased thromboembolic and cardiovascular complications were diagnosed under estrogen therapy. Furthermore, especially in men with relatively low testosterone levels, elevated serum estrogen levels favor the development of benign prostatic hyperplasia (Gann et al. 1995; Suzuki et al. 1995). **Estrogen substitution in men is not justifiable given the current state of knowledge**.

Selective androgen receptor modulators (SARMs) are synthetic, mostly nonsteroidal testosterone receptor-binding substances. They act similarly to anabolic and androgenic steroids, acting primarily in anabolic tissues such as muscle, while affecting androgenic tissues (e.g., prostate) much less. The binding affinity to the androgen receptor is stronger than that of testosterone. Currently, clinical trials are ongoing for the treatment of sarcopenia, BPH, and prostate cancer. SARMs, although not approved, are marketed by Asian manufacturers in Europe and the United States. The World Anti-Doping Agency (WADA) added SARMs to the doping list as of January 1, 2008.

Treatment with **selective estrogen receptor modulators (SERMs)** or **aromatase inhibitors** is also occasionally proposed as an alternative to testosterone therapy (Krzastek and Smith 2020). No clinical studies are available for these preparations, and there is no approval of the preparations for the treatment of men with androgen deficiency; each treatment is thus also to be regarded as an individual attempt at a cure without scientific rationale.

25.7 Diseases of the Prostate in Advanced Age

25.7.1 Benign Prostatic Hyperplasia (BPH)

Within the last few years, the term **LUTS (Lower Urinary Tract Symptoms)** has gained increasing currency among experts but also in the lay press. According to the definition of the International Continence Society, the LUTS symptom complex includes storage symptoms, voiding symptoms, and symptoms following bladder emptying (Abrams et al. 2003). Causes may include enlargement of the prostate, inflammation or tumor of the urinary bladder or prostate, and stricture of the urethra in a normal-sized prostate. LUTS thus subsumes a symptom complex, whereas benign prostatic hyperplasia is understood to be hyperplasia of the periurethral mantle zone of the prostate with consecutive micturition obstruction.

Benign prostatic hyperplasia (BPH) is a common disease of the aging male. Worldwide, somewhat 15–22% of men aged 50–59, 15–33% of men aged 60–69, and between 25 to over 40% of men aged 70 to 79 have moderate-to-severe LUTS symptoms (Lepor 2004); in autopsy studies, 50% of men aged 51–60, 70% of men aged 61–70, and 90% of men aged 81–90 had histologic evidence of benign prostatic hyperplasia (McVary 2006). The causes of BPH remain unclear. Hormonal shifts with age are thought to be one cause. BPH is not observed in prepubertally castrated men. In the prostate, testosterone is irreversibly metabolized to dihydrotestosterone (DHT) by 5α-reductase. In men with 5α-reductase deficiency, the prostate remains vestigial

(Lepor 2004). However, whether elevated DHT levels in the prostate are a cause of BPH or a consequence of the enlarged prostate is not clear (Lepor 2004). Statistically significant increases in testosterone levels have not been found in patients with BPH. An increase in estrogens with decreasing androgen action has also been discussed as a cause (Gann et al. 1995; Suzuki et al. 1995).

It is also possible that genetic differences in the androgen receptor determine the predisposition to BPH. It is not known for certain whether men with a lower number of CAG triplets in the androgen receptor gene are more likely to develop BPH than men with a higher number of CAG triplets (Giovannucci et al. 1999; Kristal et al. 2010). Analysis of the number of CAG triplets to evaluate the individual risk of developing BPH does not appear to be useful. There is an inverse correlation of prostate growth with the number of CAG triplets in hypogonadal patients on testosterone therapy (Zitzmann et al. 2003).

The prostates of patients with acromegaly are significantly larger than those of age-matched healthy subjects despite secondary hypogonadism (Salvio et al. 2022). Normalization of growth hormone levels leads to a reduction in prostate volume. Growth hormone stimulates hepatic IgF-1 synthesis. An elevated quotient of IgF-1 and its binding protein IGFBP-3 are risk factors for the development of BPH, especially in obese men (Neuhouser et al. 2008). Growth hormone thus also has an influence on prostate growth.

The first **symptoms of BPH** are often pollakisuria, dysuria, and nocturia. However, complete emptying of the bladder is still possible (Harzmann et al. 1998). In the stage of retention, complete emptying of the bladder is no longer possible due to the flaccidity of the bladder muscles. In the stage of bladder insufficiency, the bladder muscles and innervation have adapted to this condition. The backflow into the ureters and kidneys threatens a gradual onset of renal insufficiency. It remains unclear whether BPH is a predisposing factor for prostate cancer.

The diagnosis of BPH is based on **clinical history** including **medication history**, physical examination with **rectal digital examination, transrectal sonography**, **prostate-specific antigen (PSA)** and serum creatinine determination, uroflowmetry, and cystoscopy. In the application of the globally accepted **International Prostate Symptom Score (IPSS**), patients can be categorized according to their symptoms. This score, with a total of 35 possible points, should guide therapy. Differential diagnosis should exclude prostatitis and prostate carcinoma in particular (Harzmann et al. 1998).

- In mild symptomatology of BPH (**IPSS score 0–7**), no therapy is necessary, but patients should be monitored regularly.
- In patients with moderately severe symptomatology (**–IPSS score 8–19)**, treatment with α-receptor blockers or 5α-reductase blockers is induced. 5αreductase blockers such as **finasteride** or **dutasteride,** unlike α-receptor blockers, are able to reduce prostate volume. 5α reductase blocker therapy is promising with an **IPSS score of 8–19** and a prostate volume greater than 40 ml (Madersbacher et al. 2004). The widely used therapy with phytotherapeutics for the treatment of BPH could not yet prove efficacy according to the criteria of evidence-based medicine and is not recommended (Madersbacher et al. 2008).
- In severe symptomatology (**IPSS score 20–35)** and after unsuccessful conservative therapy, surgical treatment is necessary. In addition to transurethral incision, especially indicated in smaller prostates, transurethral resection of the prostate is the treatment of choice; in very large prostate volumes, transvesical adenomectomy should be performed. Transurethral laser therapy is a relatively new minimally invasive treatment alternative, with promising results so far, especially in patients under oral anticoagulation or even patients who want to retain their ejaculatory ability benefit from this therapy (Madersbacher et al. 2004).

25.7.2 Prostate Carcinoma

Prostate carcinoma is also almost exclusively a disease of older age. In systematic autopsies, significantly more prostate carcinomas are found than are clinically manifested. Within the last few years, an increase in the incidence of prostate carcinoma has been observed in many countries with the increasing aging of the population, and prostate carcinomas have also been increasingly diagnosed in men younger than 60 years of age (Quinn and Babb 2002). According to the current guideline, it is recommended that interested men should receive open-ended advice from their physicians about the advantages and disadvantages of prostate cancer screening. If men wish to have early prostate cancer detection, the professional society recommends a PSA test from the age of 45 and, if desired, additional transrectal palpation (**Guideline Program Oncology 2021**). Imaging methods are not suitable as primary examinations for the early detection of prostate carcinoma.

For men at hereditary risk, screening is recommended from the age of 40. The intervals at which a man should have his PSA level determined again depend on the current PSA level and the age of the man.

As part of early detection, a prostate biopsy should be recommended in the presence of at least one of the following criteria:

- Controlled PSA level of ≥4 ng/ml at the initial screening consultation, taking into account influencing factors.
- Carcinoma-suspicious result following digital rectal examination.
- Conspicuous PSA increase (without change in the determination method).

Punch biopsy should be performed under transrectal sonographic control. Palpatory conspicuous areas should be additionally biopsied by palpation.

Ten to twelve tissue cylinders should be obtained during punch biopsy. Heidenreich et al. 2014).

Initial symptoms often resemble those of benign prostatic hyperplasia, but dysuria is more common and pollakiuria less common. Hematuria is also frequently noted. Often, the carcinoma also first becomes apparent through the symptoms of metastases, especially pain in the skeletal system in cases of bone metastases.

The **treatment strategy of prostate cancer** depends on the stage of disease at diagnosis, on the age and the general well-being of the patient, and on histological differentiation. In general, radical prostatectomy, orchidectomy, radiation therapy, chemotherapy, hormonal treatment with antiandrogens, and, today, preferably GnRH analogs are available modalities (Heidenreich et al. 2014).

The 5α-reductase inhibitors dutasteride and finasteride reduced the incidence of newly diagnosed prostate carcinoma by nearly 25% during the 4- or 7-year treatment period, but were shown to slightly increase the incidence of undifferentiated aggressively growing tumors (Andriole et al. 2010; Thompson et al. 2003). However, there is increasing evidence that highly malignant tumors could only be detected earlier in the finasteride treatment arm due to the reduction in prostate volume and suppression of low-malignant tumors (Lucia et al. 2007). It is unlikely that taking 5α-reductase inhibitors increases prostate cancer mortality in men taking them for BPH (Azoulay et al. 2015).

After verification of the diagnosis of prostate cancer and completion of staging, the next course of action is determined. The following treatment methods are currently used to treat prostate cancer:

- Wait and see
- Surgery
- Radiotherapy
- Hormone therapy
- Chemotherapy
- Immunotherapy
- Radionuclide therapy

Which therapy is used depends on whether the tumor is still confined to the prostate at the time of diagnosis or has already invaded surrounding tissue and metastasized to lymph nodes and other organ systems. Patients with localized prostate cancer who are candidates for curative treatment should be informed not only about treatment procedures such as radical prostatectomy and percutaneous radiotherapy and brachytherapy, but also about **active surveillance.** Age and comorbidity should be taken into account when determining the indication.

In the metastatic stage, wait and see is also an option for all patients, regardless of age, comorbidities, and tumor stage. If this strategy is chosen, the disease is not treated as long as no symptoms appear. This spares the patient the side effects of treatment for as long as possible. Only when pain occurs due to bone metastases, for example, does palliative therapy begin.

Tumors that are limited to the prostate (early stage) are treated curatively. This is usually done by surgical removal of the prostate or by **radiation therapy**.

If, at the time of diagnosis, the tumor has already spread to adjacent tissue and/or metastasized to adjacent lymph nodes (advanced stage), surgery or radiation is insufficient as a single therapy. In this case, systemic hormone therapy is also administered. This adjuvant therapy is still carried out with curative intent.

However, if distant metastases are already present, a cure can no longer be achieved, and treatment now has a palliative character. This is achieved by means of hormone or chemotherapy, targeted radiotherapy of bone metastases, and pain therapy.

25.8 Outlook

Many questions remain open regarding both reproductive and endocrine functions in men. Increased research efforts in the field of physiology of the aging male are necessary. An uncontrolled substitution with androgens or other hormones is not justifiable according to the current state of knowledge. Further studies, especially controlled long-term studies that examine the possible effects of substitution therapy across disciplines, are urgently needed in the near future. Whether optimized specific androgen substitution can be achieved by tissue-specific selective androgen receptor agonists (SARMs) currently in clinical trials remains to be seen.

Key Points

- Thanks to increasing life expectancy the increasing number of aging men puts the endocrine situation of this age group more and more in the focus of medicine.
- In men, although there is no climacteric, there is a gradual decline in testosterone, although levels may well remain within the normal range of younger men. In some men, however, levels fall below normal.
- When this occurs in conjunction with characteristic symptoms, it may be genuine late-onset hypogonadism (LOH) or functional hypogonadism.
- In these cases, testosterone substitution may be considered and must be closely monitored.

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Part V

Clinics in Andrology: Diseases of Seminal Ducts and Accessory Sex Organs

Infections and Inflammation of the Seminal Ducts and Accessory Sex Glands

26

Hans-Christian Schuppe, Adrian Pilatz, Andreas Meinhardt, and Hermann M. Behre

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Abstract

Infections and inflammation of the genital tract are among the most frequent causes of male infertility. Urethritis, prostatitis/prostato-vesiculitis, epididymitis/epididymo-orchitis, and orchitis are distinct entities. While acutely symptomatic clinical entities can be clearly diagnosed, the majority of patients in fertility consultations are asymptomatic and have subclinical or primarily chronic courses or only sequelae of a previous disease. Diagnosis is accordingly difficult and relies primarily on the detection of pathogens, elevated leukocyte counts, and/or inflammatory mediators in the ejaculate, prostatic secretions, and urine samples. If pathogenic bacteria are detected, antibiotic therapy appropriate to the pathogen and resistance is indicated, but this does not exclude the development of permanent fertility disorders. This is probably caused by persistent pathological immune responses in the genital tract.

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26.1 Immunological Basics

Immunologically, the **testis** and **epididymis** differ in several essential aspects (Fig. 26.1). For example, **macrophages**, as well as **mast cells**, are found in the interstitium of the testis and adjacent to or even within the multiple layers of **peritubular cells**, which, together with the basement membrane, prevent direct physical contact with the basally located **germ cells** and **Sertoli cells**. In contrast, in the human epididymis, leukocytes are localized in the epithelium, but also in the lumen, and thus can come into direct contact with spermatozoa. Moreover, there is as yet no strong evidence that the epididymis—like the testis—is among the few **immune-privileged organs** in which neo- or alloantigens are tolerated (Fijak et al. 2018) (Chap. 19). Accordingly, inflammatory or infectious stimuli, such as those encountered in acute bacterial **epididymo-orchitis**, generate a much lower proinflammatory immune response in the testis compared to the epididymis, where it is particularly observed in the cauda epididymidis (Hedger 2011; Michel et al. 2016) (Chap. 28). Differences between the two organs also relate to the presence of specific immune cell populations. In focal inflammatory infiltrates in testicular biopsies of infertile males, no or very few neutrophilic granulocytes are found, whereas this cell type represents the most abundant leukocyte subgroup in bacterial epididymitis (Schuppe and Bergmann 2013; Michel et al. 2016) (Fig. 26.1). At least in mice, a common feature is the regionally different distribution of leukocytes, which in the testis are particularly clustered in the comparatively small compartments of the rete testis as well as in the subcapsular region, whereas in the epididymis, the caput area stands out with the highest leukocyte density (Pleuger et al. 2020; Wang et al. 2021).

In principle, data on the immune system of the testis and epididymis in men are rather sparse and based primarily on immunohistochemical studies (Fijak et al. 2018). Therefore, in the following, findings collected from rodent testis and epididymis will also be used to describe mechanistic concepts. There are no robust human data on the immunophysiology of the ductus deferens or accessory glands, and likewise only very little information from animal models (Chan and Schlegel 2002; Hedger 2015).

26.1.1 Macrophages

Macrophages represent an immune cell group consisting of many subpopulations that can ingest foreign particles, invading bacteria, and cellular debris through their **phagocytotic activity**. They are found in all organs, where they play immunological and non-immunological roles in **organ homeostasis**. Similar to other organs, macrophages represent by far the most abundant leukocyte subpopulation in the healthy testis and epididymis (Fijak et al. 2018; Bhushan et al. 2020) (Chap. 19; Fig. 26.1). Heterogeneity is determined by differences in localization, organ-specific functions, surface markers and gene expression profiles, ontogenetic origin, and postnatal development. In the testis, macrophages are phenotypically characterized by differential expression of markers such as CD64, MHCII, CX3CR1, CD11b, as well as CD206, to name a few (Bhushan et al. 2020; Lokka et al. 2020; Wang et al. 2021). Based on localization, in the human testis, a **peritubular macrophage population** can be distinguished from an **interstitial** one. Data in mice suggest a role for the peritubular (CD206-MHCII+) population in regulating spermatogonial stem cell numbers, whereas the interstitial (CX3CR1 + MHCII−) subset is involved in the local regulation of steroidogenesis and vascularization (DeFalco et al. 2015; Lokka et al. 2020). Fetal depletion of all macrophages produces severe damage to the germinal epithelium in adult animals (Lokka et al. 2020).

The **phenotype of testicular macrophages** is characterized by a moderate ("sedated") **proinflammatory immune response** to appropriate stimuli. This is characterized by a comparatively lower secretion of proinflammatory mediators (TNFα, IL-1, NO), whose inflammatory influence is further controlled by a rapid onset of a strong **anti-inflammatory response** (IL-10). This should protect the sensitive germ cells from the toxic influence of high cytokine levels in the same way as Leydig cells, whose testosterone production is negatively regulated by TNFα (Bhushan et al. 2020). Thus, potential negative consequences in infertile men with hypospermatogenesis and Sertoli-cell-only phenotype are attributed to, among other factors, increased production of TNFα, IL-1α, and IL-1β in testicular macrophages (Loveland et al. 2017; Fijak et al. 2018; Lustig et al. 2020).

The characteristic phenotype of testicular macrophages is most likely shaped by the **local** microenvironment, which is characterized by high concentrations of **immunosuppressive molecules** such as androgens, prostaglandins, glucocorticoids, activins, as well as 25-hydroxy-cholesterol (Meinhardt et al. 2018). Data from mice show colonization of the testis and epididymis with macrophages in the fetal period, which then differentiate into subpopulations in their microdomains postnatally, at least in the testis, presumably due to close proximity to vasculature and Leydig cells or alternatively the peritubular cells (Mossadegh-Keller et al. 2017; Lokka et al. 2020; Wang et al. 2021).

In contrast to the human testis, epididymal data are scarce because microsurgical sperm retrieval from the epididymis does not involve biopsies and surgical removal of the organ is only rarely indicated (Chaps. 11 and 40). Macrophages also represent the most abundant immune cell population in the epididymis; in mice, their proportion is higher than in the testis. Macrophages in both organs differ markedly in their

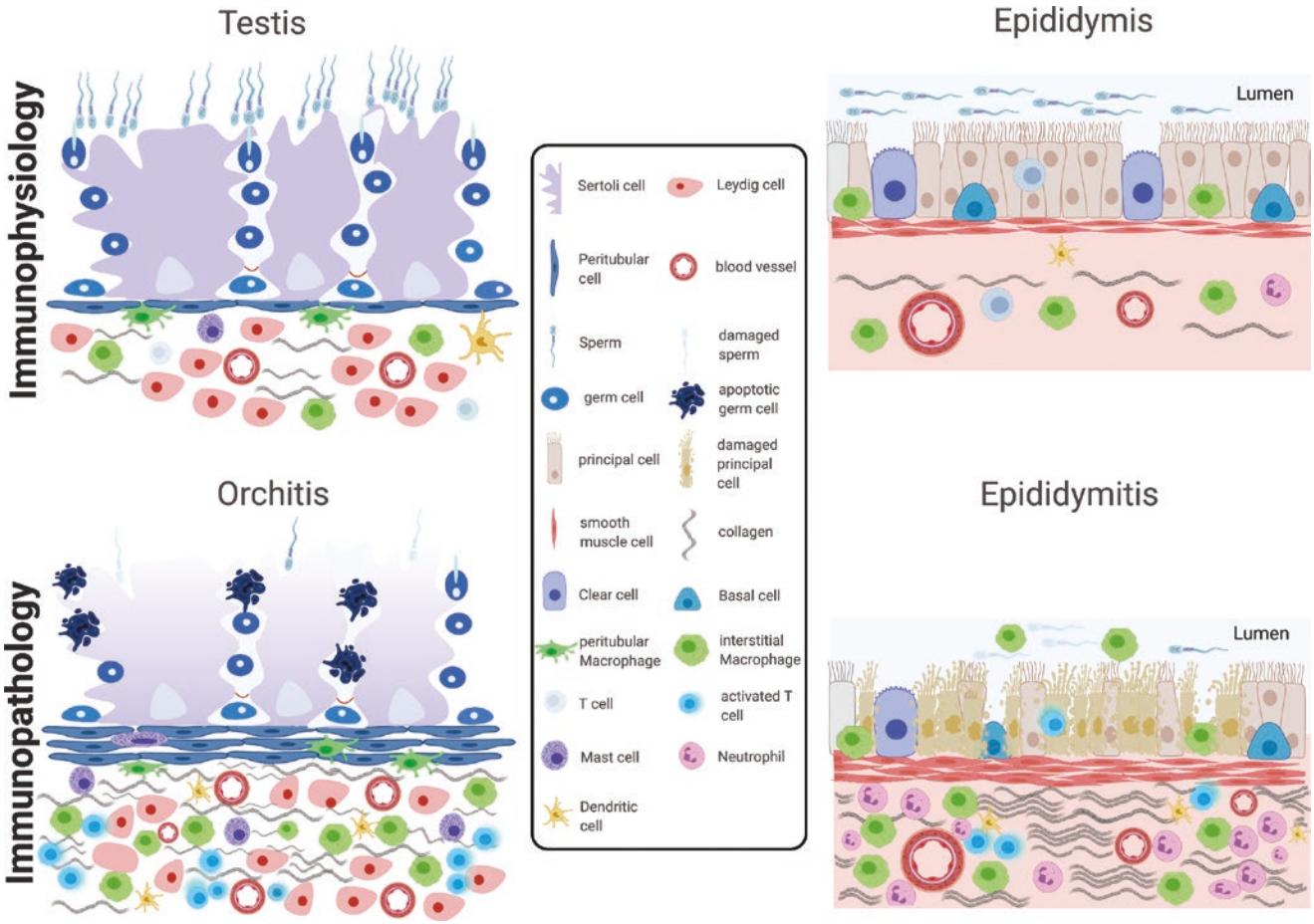


Fig. 26.1 Immune physiology and pathology in testis and epididymis. **Testis**: Under physiological conditions, the special immune regulation (immune privilege) is based on extensive sequestration of germ cell-specific neoantigens by "tight junctions" between neighboring Sertoli cells (blood–testis barrier) as well as an immunosuppressive milieu. Among the immune cells found only in the interstitium or peritubular, macrophages are most abundant and exhibit a polarization toward anti-inflammatory functions, including anergy to inflammatory stimuli. Resident mast cells are also involved in immune responses and tissue homeostasis. Antigen-presenting dendritic cells and regulatory T lymphocytes, present in small numbers, play an important role in the induction of systemic tolerance to defined germ cell antigens that bypass the blood–testis barrier by transcytosis. The testicular milieu is determined by immunoregulatory and immunosuppressive factors (e.g., TGFß, IL-10, activin, androgens, and others) provided by resident immune cells as well as other somatic cells of the testis (Sertoli cells, Leydig cells, peritubular cells). Despite the immunosuppressive milieu, inflammatory responses may develop in the testis, characterized by recruitment of activated immune cells and increased release of proinflammatory mediators (e.g., MCP-1, TNFα, IL-6, NO). As a result of disruption of the blood–testicular barrier, apoptosis and detachment of germ cells occur, with concomitant thickening and fibrosis of the lamina propria, and even complete atrophy of the seminiferous tubules; disruption of Leydig cell function is also possible. **Epididymis:** Immune cells are localized not only in the interstitium but also in the epithelium and may come into direct contact with spermatozoa. Macrophages (predominantly MHCII-) and dendritic cells play a central role in the regulation of immune responses against sperm antigens, as does the specific expression of immunomodulatory effector molecules (e.g., IDO, activin, defensins). T lymphocytes are present in small numbers. Inflammatory responses in the epididymis are accompanied by leukocyte recruitment, with interstitial infiltrates characteristically containing numerous neutrophilic granulocytes, whereas macrophages predominate in the lumen of the epididymal duct. In addition to elevated levels of proinflammatory cytokines, the inflammatory event is characterized by interstitial edema, dilatation of the ductal lumen, and destruction of epithelial integrity and function. In massive epididymitis, abscesses or granulomas may occur, particularly in the cauda epididymidis (see Sect. 26.4.1, Fig. 26.2); late sequelae include interstitial fibrosis and obstruction of the epididymal duct (created with Biorender.com)

gene expression profiles (Mendelsohn et al. 2020; Wang et al. 2021). As in the testis, a peritubular group can be morphologically differentiated in the epididymis from an interstitially located population classified by MHCII expression. In the mouse **caput epididymidis**, long, thin extensions extend from **intraepithelial CX3CR1+ macrophages** located between epithelial cells and reach the lumen of the epididymal duct; these extensions are not observed in the cauda (Da Silva et al. 2011; Battistone et al. 2020). The ability to perceive luminal factors has been associated with tolerance development to sperm neoantigens upon entry into the epididymis, but has not been experimentally proven. Findings from mice indicate a close functional coupling of epithelial cells with macrophages. For example, proton-secreting **clear**

cells, which establish the acidic environment of the epididymal fluid, release the chemokine CXCL-10 after an infectious stimulus and act both as sensor cells for bacterial colonization of the lumen and in the recruitment of additional macrophages (Battistone et al. 2019).

26.1.2 Dendritic Cells

Dendritic cells (DC) are **antigen-presenting cells** characterized by surface molecules such as **MHCII** as well as **co-stimulatory molecules** (e.g., B7). Basically, myeloid DC resembling monocytes (mDC) are distinguished from plasmacytoid dendritic cells (pDC), which in turn resemble plasma cells (Bhushan et al. 2020). Other subtypes are known but have not yet been described in male reproductive organs. In tissues, the dendritic extensions serve to increase the surface area and "monitor" the immediate environment. DC exert important functions in the immune system by phagocytizing extracellular components such as proteins or microorganisms and presenting them as peptide fragments to naive T cells after proteolytic processing as well as **migration to local lymph nodes** to induce specific cellular immune responses.

Whereas findings on DC in the testis are almost exclusively based on rodent models, suggesting an occurrence also in the human testis, at least some data are available for the human epididymis. In the normal epididymis, DC are exclusively of the immature type (CD1a+ DC, CD11c+ myeloid DC (mDC), and CD209+ DC) (Wang and Duan 2016). **Plasmacytoid DC** (CD123+) and CD83+ mature DC are found only in "chronic" epididymitis (Duan et al. 2016). Under these conditions, the population number of CD1a+, CD209+ immature mDC group also increases. Since the number of Th17+ CD4+−T cells was also increased, a role of epididymal DC in inflammation-related organ pathology can be hypothesized through recruitment of proinflammatory Th17+ cells (Duan et al. 2016) (Fig. 26.1).

26.1.3 Lymphocytes

Lymphocytes mediate **antigen-specific immune responses** to exogenous antigens and, in autoimmune responses, to endogenous antigens. In principle, T and B lymphocytes can be distinguished. Depending on the local microenvironment, T cells differentiate into lineages with effector or regulatory functions. With their activity, the latter determine whether antigen-specific immune responses are triggered or whether tolerance to the antigen is established (Hedger 2015). **T lymphocytes** differentiate mainly into T helper (Th) cells, cytotoxic T (Tc) cells, memory T cells, and **regulatory T cells.** Th cells secrete cytokines that control the differentiation of B cells into antibody-secreting plasma cells. Regulatory T cells—eponymously—significantly regulate the immune response.

CD8+ T lymphocytes are the dominant T cell population in the testis, epididymis, and vas deferens (Hedger 2015). In the testis of infertile patients, T cells are numerous in focal immune cell infiltrates. Here, the number of infiltrating cells—mostly CD4+ Th cells and CD8+ Tc—correlated with histologically detectable damage in the testis (germ cell loss, fibrosis) (Duan et al. 2011; Klein et al. 2016) (Chap. 19; Fig. 26.1). In the human **epididymal epithelium**, CD8+ T lymphocytes, also referred to as "Halo cells," are found in the epithelium, whereas MHCII-restricted **CD4+ T lymphocytes** appear to dominate in the **interstitium**. Their number increases towards the distal epididymis. Th17+ CD4+ T cells are found only in chronic epididymitis (Duan et al. 2016; Pleuger et al. 2020).

26.1.4 Mast Cells

Mast cells are tissue-resident immune cells with a heterogeneous phenotype found in the subtunical area of the human testis associated with blood vessels, and also distributed in the interstitial compartment. Mast cells derive from CD34+ hematopoietic progenitor cells that differentiate initially in the bone marrow and further locally in the target organ. The cells filled ("fattened") with characteristic granules have diverse roles in **innate and adaptive immune responses** as well as in **tissue homeostasis** and remodeling (Cildir et al. 2021). Depending on the mast cell type, the granules already contain preformed substances such as histamine, chymase, tryptase, and carboxypeptidase A, which are rapidly released after adequate stimuli. Other mediators such as TNFα, IL-6, and IL-1β are synthesized de novo only after mast cell activation and are secreted with a corresponding time delay. In the testis and epididymis, two tryptase-positive populations are known to be either chymase-positive or -negative. Mast cells can mediate steroid production in H1R- and H2R-positive Leydig cells through histamine, and furthermore influence **fibrotic tissue remodeling** through activation of matrix metalloproteinases and secretion of corresponding inhibitors (Mayerhofer et al. 2018) (Fig. 26.1).

26.2 Etiology and Pathogenesis

The main cause of **inflammation in the male genital tract** is represented by **local bacterial** infections that can ascend intracanalicularly via the urethra, involve the accessory glands, and reach the epididymis and testis via the ductus deferens (Weidner et al. 1999; Fijak et al. 2018). Relevant here are infections with typical **enterobacteria** (e.g., *E. coli*) as well as **sexually transmitted infections** (STI; e.g., *Chlamydia trachomatis*) (Mackern-Oberti et al. 2013;

Banyra et al. 2019) (see Sect. 26.4, Fig. 26.5). Recent studies of epididymitis indicate that the prevalence of STIs is not limited to patient groups younger than 35 years, as previously thought (Pilatz et al. 2015).

In systemic bacterial infections (e.g., tuberculosis, brucellosis), both intracanalicular ascension and hematogenous spread are possible, and rarely lymphogenous pathogen seeding or spread of disease to adjacent organs *per continuitatem* (Dean et al. 2012; Yadav et al. 2017) (see Table 19.1).

In addition to bacteria, **viruses** can also be detected in the genitourinary tract, both in localized and systemic infections (Le Tortorec et al. 2020). In this context, the induction of orchitis is of particular clinical relevance (see Chap. 19). In addition, fungi are rarely found as causative agents of ascending infections; among parasites, *Trichomonas vaginalis* is the most important STI to consider (Banyra et al. 2019).

In addition to infections, **post-infectious** and primarily **pathogen-independent autoimmune reactions** occur, as well as post-traumatic inflammatory reactions caused by sterile urinary reflux due to anatomical malformations or triggered iatrogenically (Fijak et al. 2018). Temporary or permanent impairment of male fertility can occur at different levels and via various mechanisms. With regard to the action of pathogens or pathogen components as well as inflammatory cells and their mediators, irreversible organ damage and corresponding consequences must be expected, particularly in the testis and epididymis (Wolff 1995; Schuppe et al. 2017) (Table 26.1; Chap. 19).

Table 26.1 Pathogenesis of male fertility disorders due to urogenital infections and inflammation (modified from Haidl et al. 2019)

• Infections of the urogenital organs
– Direct damage to sperm integrity and function by pathogenic agents or pathogen components
– Indirect damage to sperm by inflammation-associated molecules, e.g., proinflammatory cytokines or reactive oxygen species (ROS)
– Dysfunction of accessory sex glands
– Inflammation-induced obstruction of the seminal ducts, e.g., the epididymal duct
– Damage to spermatogenesis
• Direct effects of pathogenic agents or pathogen components
• Induction of cellular and humoral immune responses with (irreversible) disruption of specific local immune regulation in testis and/or epididymis
• Induction of a humoral immune response against spermatozoa, i.e., formation of antisperm antibodies
• pathogen-induced epigenetic changes
• Primary noninfectious (sterile) inflammatory responses
– Induction of autoimmune responses
– Chemical toxicant- or trauma-induced inflammatory responses
– Endpoints of organ or cell damage comparable with infectious or post-infectious lesions (see above)
• Formation of antisperm antibodies unrelated to urogenital infections/non-infectious inflammation

26.3 Clinical Entities

In infections and inflammation of the male genital tract, a nosological distinction must be made between **urethritis, prostatitis, vesiculitis** (prostato-vesiculitis), **epididymitis** (epididymo-orchitis), and **orchitis** (Weidner et al. 1999). Orchitis is discussed in detail in Chap. 19; the rare isolated inflammatory disorders of the ductus deferens or vesicular glands are not discussed here (Chan and Schlegel 2002). Inflammation of the skin of the external genitalia, such as balanitis or scrotal eczema, does not represent significant factors influencing male fertility (Chap. 29).

The acute clinical entities associated with corresponding symptoms can be clearly diagnosed and are regularly treated in urological emergency services, whereas in the fertility consultation, they occur only in exceptional cases. In the absence of clinical symptoms, diagnostic classification is based primarily on the detection of pathogens, elevated leukocyte counts, and/or inflammatory mediators in the ejaculate, prostatic secretions, and urine samples, and possibly also an impaired secretory function of the accessory sex glands (Schuppe et al. 2017).

26.3.1 Epididymitis

Acute epididymitis is a common disease of the male genital organs, with an incidence of 250–650 cases per 100,000 men per year (Nickel et al. 2005; Nicholson et al. 2010). The leading symptoms of **pain** and **swelling of the epididymis** manifest mostly unilaterally. In up to 90% of cases, there is secondary **testicular involvement**, i.e., concomitant orchitis (Chap. 19). **Chronic epididymitis** (epididymo-orchitis) can be assumed if the symptoms persist for >3 months (Nickel 2003; Çek et al. 2017).

Epididymitis is caused by **ascension of bacterial pathogens** from the urethra to the epididymis in more than 80% of cases, with **uropathogenic enterobacteria** such as *E. coli* or **STI pathogens** such as *C. trachomatis* being most frequently detected (Pilatz et al. 2015). Epidemiological data support the close pathogenetic association between epididymitis due to urethritis caused by STI pathogens and fertility problems, although the mechanisms have not yet been clarified in detail (Ness et al. 1997; Çek et al. 2017). Previous studies have described **leukocytospermia** during the acute phase of the disease, and also a progressive deterioration of semen quality in untreated patients (Dietz 1960). Under adequate antibiotic treatment of acute epididymitis, the initial deterioration of sperm concentration is reversible in the majority of cases over the course of 3–6 months; however, in a pooled analysis of available studies, approximately 10% of patients suffered **persistent azoospermia** and another 30% had **oligozoospermia** (Rusz et al. 2012). Proteomic analyses suggest that

despite pathogen-targeted antimicrobial therapy, qualitative changes in spermatozoa occur in addition to reductions in sperm concentration (Pilatz et al. 2014). **Virulence factors of the pathogens,** such as hemolysins, may also influence disease progression, with the latter particularly contributing to an acrosomal response of the spermatozoa that already occurs in the epididymis and is therefore premature (Lang et al. 2013; Khosravi et al. 2016).

Although epidemiological data on the **chronification of epididymitis** or epididymo-orchitis are lacking, permanent **damage to spermatogenesis** leading to testicular atrophy is considered a severe complication (Osegbe 1991; Fijak et al. 2018) (Chap. 19). On the other hand, an ultrasound study in 90 patients with acute epididymitis during follow-up over 3 months demonstrated that accompanying orchitis with initial testicular swelling does not result in testicular atrophy during its course, but that testicular volume merely returns to normal, when compared with the healthy contralateral side (Pilatz et al. 2013). These data suggest that inflammation-induced obstruction of the seminal ducts plays an important role as a cause of persistent deterioration of ejaculate quality. Extensive fibrosis of epididymal tissue with corresponding **obstruction of the epididymal duct** was found both in animal models of bacterial epididymo-orchitis and in histopathological specimens from patients with chronic epididymitis (Michel et al. 2016; Klein et al. 2020). Interestingly, the inflammatory infiltration and damage of the tissue is mostly limited to the **cauda epididymidis**. There is suggestive evidence that regionally different immunological conditions play a role here, leading to a stronger **antimicrobial immune response** in the cauda than in the more proximal epididymal sections (Pleuger et al. 2020). In addition, anatomically, functional septation between individual epididymal segments may at least temporarily limit pathogen ascension (Stammler et al. 2015). Preclinical data from a rodent model suggest that antibiotic therapy with levofloxacin in combination with a nonsteroidal anti-inflammatory drug largely prevented permanent **tissue damage such as fibrosis and obstruction** compared with antibiotic therapy alone (Klein et al. 2020). Human data on this are lacking, although in routine clinical practice, many patients are additionally treated with an anti-inflammatory drug due to pain symptoms alone (Pilatz et al. 2015). Moreover, it should also be noted that in many cases, chronic epididymitis is based on a noninfectious etiology (Çek et al. 2017).

26.3.2 Prostatitis

Prostatitis syndrome is a complex, **multifactorial condition** that occurs in younger men <50 years of age with a prevalence of approximately 12% (Nickel et al. 2001). According to the **National Institutes of Health (NIH) classification**, which is based on disease progression as well as pathogen and leukocyte detection in expressed prostatic secretions and urine after prostate massage, respectively, prostatitis is divided into four categories (Krieger et al. 1999):

I. acute bacterial prostatitis.
II. chronic bacterial prostatitis.
III. chronic (abacterial) prostatitis/chronic pelvic pain syndrome (category III A: inflammatory; category III B: non-inflammatory).
IV. asymptomatic prostatitis.

Acute bacterial prostatitis is characterized by severe obstructive and irritative lower urinary tract symptoms, pain in the prostate area, and acute bacterial urinary tract infection with general symptoms such as fever (Wagenlehner et al. 2009; Bonkat et al. 2021). In a complicated course, a prostatic abscess may develop; the suspected diagnosis can be confirmed by transrectal sonography.

While symptoms of **chronic prostatitis** such as pelvic pain, irritative voiding symptoms, and sexual dysfunction are common overall, the prevalence of **chronic bacterial infection** (NIH category II) among affected patients is only 5–10% (Weidner et al. 1991; Krieger et al. 2008; Lotti et al. 2014). The remaining symptomatic patients can be assumed to have **pelvic** inflammatory or non-inflammatory **pain syndrome** (NIH categories IIIA and IIIB, respectively), the etiology of which remains unclear. Asymptomatic prostatitis represents a histologic diagnosis in prostate biopsies or resectates in patients with benign prostatic hyperplasia or prostatic carcinoma (Wagenlehner et al. 2009).

The **pathogen spectrum** in chronic bacterial prostatitis corresponds to that of complicated urinary tract infections with **predominantly Gram-negative bacteria** (Wagenlehner et al. 2009). However, depending on the geographical region and patient population, a wide variety of STI pathogens and atypical bacteria may also be found (Skerk et al. 2004).

Diagnosis of chronic prostatitis can be difficult in individual cases due to nonspecific rectal palpation findings. By definition, chronic bacterial prostatitis is present when at least **tenfold higher bacterial counts are found in urine after prostatic massage** than in first-void urine; in 50% of cases, a significant bacterial count is also found in the ejaculate with the same pathogen (Weidner et al. 1999; Bartoletti et al. 2007) (see Sect. 26.4.2, Fig. 26.3). In addition, NIH categories II and IIIA disease is associated with elevated leukocyte counts in prostatic secretions and/or urine samples after prostatic massage and semen (Benelli et al. 2017). In the ejaculate, chronic bacterial prostatitis may be associated with distinctive signs of inflammation such as **leukocytospermia** and **elevated interleukin (IL)-8** levels (Penna et al. 2007), whereas the diagnostic value of classical biochemical

semen parameters is low (Marconi et al. 2009; see Sect. 26.3.5, Table 26.3).

In view of the prevalence of the disease even in younger men, the question arises as to the possible influence of chronic prostatitis on fertility (Weidner et al. 1999). However, the results of available studies are by no means consistent, with meta-analyses suggesting a negative **impact on sperm motility and morphology** (Rusz et al. 2012; Condorelli et al. 2017) (see Sect. 26.3.5, Table 26.3). On the other hand, the validity of basic semen analysis is limited, as illustrated by changes in sperm function, DNA, and chromatin integrity, and epigenetic markers of spermatozoa in patients with chronic prostatitis (Henkel et al. 2006; Schagdarsurengin et al. 2017; Berg et al. 2021).

26.3.3 Urethritis

Acute male urethritis is associated with **discharge**, itching or pain in the urethra, **dysuria**, and nonspecific micturition symptoms (**lower urinary tract symptoms; LUTS**); however, affected patients may remain asymptomatic (Wagenlehner et al. 2016; Bonkat et al. 2021). Infections are the most common cause of urethritis, but non-infectious factors should also be considered as triggers. However, no data are available on the incidence.

Acute infectious urethritis is predominantly caused by **STI pathogens,** whereby cases with evidence of *N. gonorhoeae* are distinguished from **non-gonococcal forms**. In the latter, *C. trachomatis* (11–50%), *Mycoplasma genitalium* (6–50%), *Ureaplasma urealyticum* (5–26%), and *Trichomonas vaginalis* (1–20%) are most frequently found, with adenoviruses (2–4%) being less common (Bonkat et al. 2021). *Ureaplasma* spp. should be differentiated, as *Ureaplasma parvum*, unlike *Ureaplasma urealyticum*, is considered non-pathogenic (Zhang et al. 2014). Coinfection with various STI pathogens is not uncommon (Horner et al. 2016).

Upon **physical examination,** redness of the glans penis and orificium urethrae externum may be present. The diagnosis of urethritis is based on the clinical and cytologic and microbiologic analysis of urethral swabs or first-void urine, although diagnosis from first-void urine is not inferior to the swab. In the absence of discharge, detection of **≥10 granulocytes per field of view** (at 400× magnification) in the sediment of first-void urine is considered pathognomonic (Bonkat et al. 2021) (see Sect. 26.4.2, Fig. 26.3). **Automated flow cytometry** represents a newer method for quantifying leukocytes and bacteria in urine (Tjagur et al. 2020). Microbiological pathogen identification is predominantly performed by STI-PCR, which is superior to cultural diagnostic methods (AWMF 2019).

Complications of the disease include the development of **chronic urethritis** with or without pathogen persistence, urethral strictures, and posterior urethral lesions resulting in impaired ejaculation (Rusz et al. 2012). There is an increased risk of fertility problems, particularly with ascending infections involving other compartments or organs of the genital tract (see Sects. 26.3.1 and 26.3.2).

26.3.4 Infectious and Inflammatory Obstruction of the Seminal Ducts

Follow-up observations after acute epididymitis indicate the risk of **azoospermia** due to **infectious inflammatory disease** (Rusz et al. 2012). Compared with obstructions of the epididymal duct or ductus deferens, occlusions of the ejaculatory ducts are rare (Diemer et al. 2011; Peng et al. 2012). With an overall prevalence of azoospermia in andrological consultations of 5–15%, **obstructive azoospermia** is present in 10–40% of cases, and the proportion of infectious/inflammatory occlusions ranges from 18 to 47% in respective studies (Dohle 2003; Han et al. 2016; Pilatz et al. 2016). The diagnostic differentiation of congenital forms of obstructive azoospermia (Chap. 27) from acquired infectious/inflammatory occlusions and their exact localization opens up surgical therapeutic options such as **vasoepididymostomy** in acquired disease (Diemer et al. 2011; Chen et al. 2016) (Chap. 40).

26.3.5 Asymptomatic Infections and Inflammation of the Genital Tract in Infertile Men

Infections and resulting inflammation of the genital tract are counted among the **most frequent causes of male fertility disorders** (Tüttelmann and Nieschlag 2010; Olesen et al. 2017; Boeri et al. 2020). However, the majority of affected patients are asymptomatic, which complicates diagnostic classification (Haidl et al. 2008). The latter is mainly based on laboratory findings such as pathogen detection and inflammatory markers in ejaculate, prostatic secretions, and urine samples. In the absence of compartment-specific diagnostic markers, pathological findings are usually grouped together under the term "**male accessory gland infection" (MAGI)** which neither allows for pathogenetic distinction between infection and primary non-pathogen-related inflammation, nor precise topographical/anatomocal localization of the pathology (Table 26.2) (Rowe et al. 2000). In contrast to the NIH classification of prostatitis, the MAGI criteria do not include any parameters for describing the acuity/course of the disease. Due to the lack of noninvasive diagnostic

Table 26.2 WHO criteria for the diagnosis of MAGI (Male Accessory Gland Infection)[a] (modified after Rowe et al. 2000)

Oligo- and/or astheno- and/or teratozoospermia[b]		
Plus		
Two positive findings from three categories (A–C) or two pathologic ejaculate findings (C)		
A	*Medical history*	Urinary tract infection, sexually transmitted infection (STI), epididymitis
	Clinical findings	Epididymis thickened, and/or tender; spermatic cord thickened; digito-rectal examination abnormal
B	*Urine after prostatic massage*	Abnormal urine (e.g., >10 leukocytes/high-power microscopic field[c]); evidence of *Chlamydia trachomatis*
C	*Ejaculate*	(Peroxidase positive) leukocytes $>10^6$/mL
		Culture with significant growth of pathogenic bacteria
		Detection of *Chlamydia trachomatis*
		Increased inflammatory markers, ROS[d]; pathologically altered biochemical parameters of seminal plasma

[a] Considering the lack of pathogenetic differentiation, defined by some authors as "Male Accessory Gland Inflammation" (Calogero et al. 2017)
[b] See Chap. 9 (WHO 2021)
[c] Magnification × 400
[d] *ROS* reactive oxygen species

markers, asymptomatic testicular inflammatory reactions are completely disregarded (Schuppe et al. 2008) (Chap. 19).

Data on the **prevalence of genital tract infections and inflammation** as a cause of male infertility mostly refer to the presence of MAGI, and are heterogeneous due to inconsistent diagnostic criteria. While frequencies of 10–20% are reported from andrology consultations, the proportion can exceed 30% in countries without adequate medical care (Pilatz et al. 2016). The observed regional differences suggest a direct link between the incidence of STI and secondary infertility (Mascarenhas et al. 2012; Chemaitelly et al. 2021; Paira et al. 2021).

Bacteriospermia does not necessarily reflect genital infection because urethral commensals are present in ejaculate samples (Schiefer 1998; Cottell et al. 2000; Koedooder et al. 2019) (see Sect. 26.4.2, Fig. 26.5). A cutoff value of $>10^3$ cfu/mL has been proposed for uropathogenic bacteria to define "significant" bacteriospermia (Weidner et al. 1999; Virecoulon et al. 2005). However, study results regarding an association with semen quality are conflicting (Farahani et al. 2021). For example, in a Canadian study of nearly 5000 subfertile men, an association between bacteriospermia (without testing for STI pathogens) and spermiogram variables was found only in the presence of concomitantly elevated leukocyte counts in the ejaculate (Domes et al. 2012). Other reports demonstrate a practice-relevant negative association between infection and sperm concentration and progressive motility (Ricci et al. 2018; Boeri et al. 2020). Given the prevalence of positive PCR findings for *C. trachomatis*, Mycoplasma, and Ureaplasma (see Sect. 26.4.2, Fig. 26.5), negative effects on semen quality and sperm function and integrity should also be noted for these pathogens (Eley et al. 2005; Gdoura et al. 2008; Huang et al. 2015; Ahmadi et al. 2017; Boeri et al. 2020). In infertile couples, an increased risk of unsuccessful IVF has been described in association with the detection of *Enterococcus faecalis* and/or *U. urealyticum/M. hominis* (Ricci et al. 2018). The extent to which alterations in the male genital tract or ejaculate microbiome contribute to the development of fertility disorders is the subject of current research (Mändar 2013; Koedooder et al. 2019; Lundy et al. 2021).

As mentioned previously, the **male genital tract is a reservoir for various viruses** (Bezold et al. 2007; Le Tortorec et al. 2020). However, in contrast to orchitis (epididymo-orchitis) in systemic viral infections (Chap. 19), the importance of viruses in the ejaculate as triggers of inflammatory reactions in the male genital tract and fertility disorders is still unclear. A prevalence of 10–36% has been reported for **human papillomavirus (HPV)** in ejaculates (Foresta et al. 2015). Here, the frequency of positive findings is significantly higher in men desiring procreation than in the general population, including those genotypes with high oncogenic potential (Moreno-Sepulveda and Rajmil 2021). Several studies suggest an association between HPV detection and deterioration of sperm motility and morphology, increased DNA fragmentation, positive antisperm antibody findings, and reduced natural conception rates, but the overall level of evidence is considered low (Muscianisi et al. 2021; Moreno-Sepulveda and Rajmil 2021).

The potential impact of **SARS-CoV-2 infection** is a subject of ongoing debate (Aitken 2022; Tian and Zhou 2021) (Chap. 19). Aside from a single early communication, there are no data demonstrating the detection of SARS-CoV-2 in the ejaculate following exposure to COVID-19; thus, based on current knowledge, sexual transmission appears unlikely (Patel et al. 2021). Studies with small numbers of cases have reported **impaired semen quality** as a result of SARS-CoV-2 infection, depending on the severity of the disease. In this context, nonspecific concomitant symptoms such as fever and their negative impact on spermatogenesis should be noted. On the other hand, sonographic signs of epididymitis were found as incidental findings in patients with moderate to severe disease (Carneiro et al. 2021).

Trichomonas vaginalis is not considered to be of major importance with regard to male fertility disorders, according to current knowledge; however, the possible role of men as carriers of this STI should be considered (Schuppe et al. 2017; Van Gerwen et al. 2021). Similarly, **fungal infections** are rarely diagnosed as a cause of inflammation-associated male fertility disorders.

Table 26.3 Possible changes of semen parameters in chronic inflammatory disorders of the male genital tract (modified from Haidl et al. 2008; Schuppe et al. 2017)

	Ejaculate parameters [lower limit or reference values][a]	Asymptomatic genital tract inflammation[b]	Chronic prostatitis (NIH II, III)
Basic semen analysis	Volume [1.4 mL]	→	(↓)
	pH [≥7.2]	→	(↑)
	Sperm concentration/total count [16 × 10^6/mL/ 39 × 10^6]	(↓)	→
	Motility [progressive: 30%]	↓	(↓)
	Morphology [Normal forms: 4%]	Membrane integrity of flagella (↓)	Hyperelongation of head segments (↑)
Sperm function/-integrity	Acrosome reaction	Pathological	Pathological
	DNA fragmentation	↑	↑
	Apoptosis	↑	?
Inflammatory markers in ejaculate	Leukocytes [<1 × 10^6/mL] (neutrophilic granulocytes, macrophages)	↑	↑
	Granulocyte elastase [<280 ng/mL]	↑	↑
	Proinflammatory cytokines (e.g., IL-6 [30 pg/mL], IL-8 [7000 pg/mL])	↑	↑
Accessory secretion	Zinc [≥2.4 µmol/ejaculate]	→	↓
	Fructose [≥13 µmol/ejaculate]	→	→
	α-Glucosidase [≥20 mU/ejaculate]	(↓)	→

[a] See WHO laboratory manual (WHO 2021); for granulocyte elastase and cytokines, see references in text
[b] Caused by infectious, post-infectious or primary sterile disorders; especially to be expected in chronic inflammatory reactions in the epididymis
→ Unchanged; ↑ increased; ↓ decreased; ? no data available

The pathological relevance of **leukocytospermia,** defined by the consensus-based WHO threshold for peroxidase-positive cells in the ejaculate of 10^6/mL, is controversial (Aitken and Baker 2013; WHO 2021). Some studies have shown a negative association between leukocyte count and sperm concentration, motility, and morphology in subfertile men (Henkel et al. 2021). A negative association was also found between the number of neutrophil granulocytes in stained semen smears and variables of the baseline spermiogram, as well as **sperm DNA integrity** (Domes et al. 2012). However, elevated seminal leukocyte counts do not allow prediction in terms of detection of relevant bacteria or viruses in the ejaculate (Bezold et al. 2007; Ventimiglia et al. 2020). Thus, the indication for microbiological diagnosis based on leukocytospermia alone, as recommended in **current guidelines** (Jungwirth et al. 2019; Toth et al. 2019; Schlegel et al. 2021), seems insufficient.

Infections or inflammation of the male genital tract can lead not only to impairment of sperm motility but also to disturbances of other essential sperm functions (see Table 26.3) (Henkel et al. 2006; Fraczek and Kurpisz 2015). Even below the WHO threshold of 10^6 leukocytes/mL, **oxidative damage** to sperm including **DNA fragmentation** is possible due to elevated levels of **reactive oxygen species (ROS)** in the ejaculate (Erenpreiss et al. 2002; Henkel et al. 2005; Lobascio et al. 2015). An association also exists between granulocyte elastase or cytokines in the ejaculate and sperm DNA integrity (Kopa et al. 2005). Macrophages (see Fig. 26.4) play a predominant role as a **source of pro-inflammatory cytokines**; in addition, an increased rate of apoptotic spermatozoa has been demonstrated (Pelliccione et al. 2009; Tremellen and Tunc 2010; Fathy et al. 2014). The pathophysiologically obvious link between infections and inflammatory processes in the genital tract and the formation of antisperm antibodies is controversially discussed (Francavilla and Barbonetti 2017) (Chap. 28).

In terms of reproductive medicine, the risk of **transmission to the female partner** must also be considered as a consequence of genital tract infections in men (AWMF 2019; Workowski et al. 2021). This applies not only to bacterial STI pathogens, which can for example adhere to spermatozoa as vectors, but also to viruses in the ejaculate. Special andrologic counseling should be provided to couples planning a pregnancy, who have been in areas at risk for **Zika virus infection**. Because of the potentially long persistence of the virus in the ejaculate for more than 6 months, it is recommended that men should not father a child for a correspondingly long period after their return from regions with risk for infection (Epelboin et al. 2017).

26.4 Diagnostics

26.4.1 Clinical Diagnostics and Imaging Techniques

When taking the **medical history**, attention is paid in particular to previous diseases such as STI and other urogenital and systemic infections, in addition to fertility-related aspects (Chap. 5) (Toth et al. 2019). Validated questionnaires such as

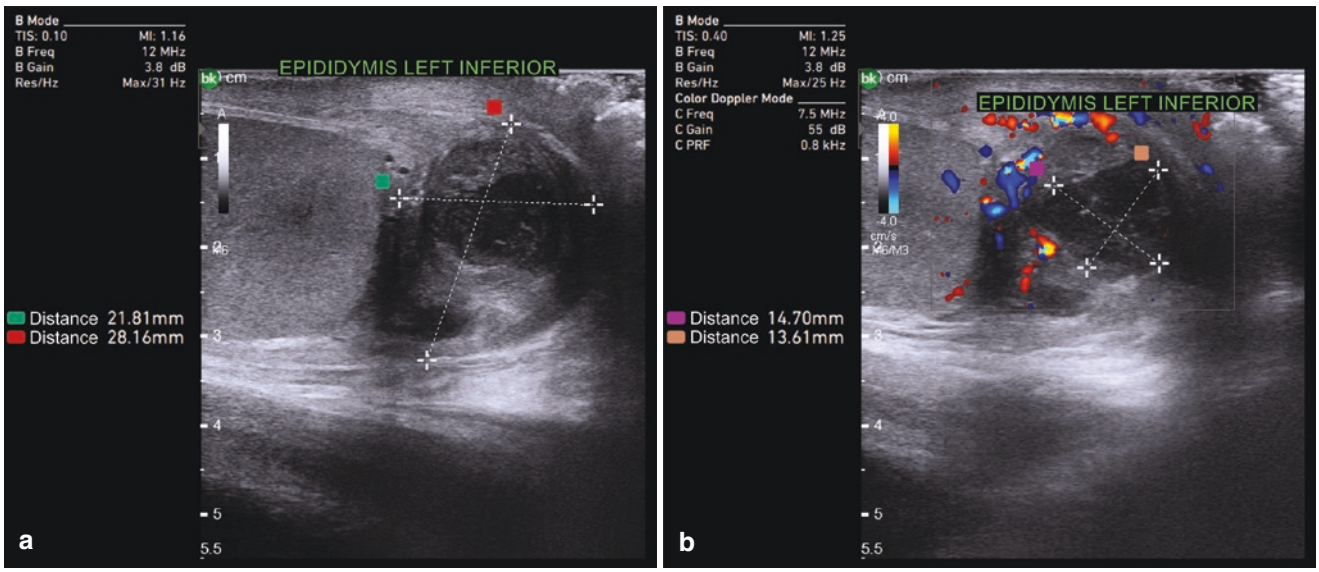


Fig. 26.2 Sonography (**a**) and color-coded duplex sonography (**b**) of the epididymis in acute epididymitis. The lower pole of the testis and the cauda epididymidis are shown; the cauda demonstrates massive infiltration with abscess formation of approximately 15 mm in diameter

the International Index of Erectile Function (IIEF-5), NIH Chronic Prostatitis Symptom Index (NIH-CPSI), or UPOINTS classification are available for the assessment of sexual dysfunction, prostatitis-associated symptoms, or LUTS, respectively (Rosen et al. 1997; Litwin et al. 1999; Magri et al. 2010). However, such questionnaires for symptom evaluation are by no means suitable or sufficient for diagnosis.

Clinical examination of the genital organs should be complemented by sonography of the scrotal contents if infection or inflammatory processes are suspected (Lotti and Maggi 2015) (Chap. 6) (Fig. 26.2). Color-coded duplex sonography can be used to visualize vascularization and perfusion of the organs (Pilatz et al. 2013). **Transrectal sonography** is essential when abscess formation along with prostatitis is suspected, but the relevance of the frequently encountered prostatic calcifications is questionable (Benelli et al. 2017). The EAA study in healthy fertile men offers appropriate reference values for clinical routine (Lotti et al. 2021). Sonographic criteria for the diagnosis of unilateral or bilateral MAGI with subgrouping into prostatitis, vesiculitis, and epididymitis have been published by some authors, but are not generally accepted and established (Calogero et al. 2017).

26.4.2 Laboratory Diagnostics

General laboratory tests such as **blood count** and determination of **CRP** and **serum PSA** (as an indication of concomitant prostatitis) are diagnostically helpful, especially in acute urogenital infections and inflammatory processes. A **hormone profile** should be obtained to assess endocrine testicular function (basal: FSH, LH, total testosterone) (Chap. 7).

A central component of diagnostics is the **examination of urogenital secretions** including the ejaculate (Figs. 26.3 and 26.6). For the localization of inflammation/infection in the male genital tract, especially in cases of suspected chronic prostatitis, the so-called 4-glass test has been established, a fractionated collection of initial urine, midstream urine, expressed prostatic secretions, and exprimate urine. As a simplified procedure, a 2-glass test with urine portions before and after prostatic massage has proven useful, if possible in combination with an immediately subsequent ejaculate collection (Wagenlehner et al. 2009) (Fig. 26.3). The diagnostic value of the 2-glass test is comparable to the 4-glass test (Nickel et al. 2006).

Semen analysis is performed according to WHO recommendations (WHO 2021) (Chap. 9). Just as for fertility assessment, sperm concentration/total count, motility, and morphology are only surrogate parameters; pathological changes are not specific with regard to infection/inflammation in the male genital tract. However, ejaculate volume, pH, viscosity/liquefaction, and biochemical parameters reflect the secretory function of accessory sex glands (see Sect. 26.3.5, Table 26.3). Sperm agglutination may be indicative of membrane-bound autoantibodies (Chap. 28).

Quantification of leukocytes as inflammatory markers in the ejaculate relies primarily on detection of peroxidase-positive granulocytes (WHO 2010, 2021). However, the currently used lower limit of 10^6 peroxidase-positive cells or leukocytes per mL of ejaculate is not evidence-based and is controversial concerning its significance for various clinical endpoints (Castellini et al. 2020; Henkel et al. 2021; Ventimiglia et al. 2020) (see Sect. 26.3.5).

Compared to the peroxidase method, immunocytochemical detection or flow cytometry of leukocytes has a much

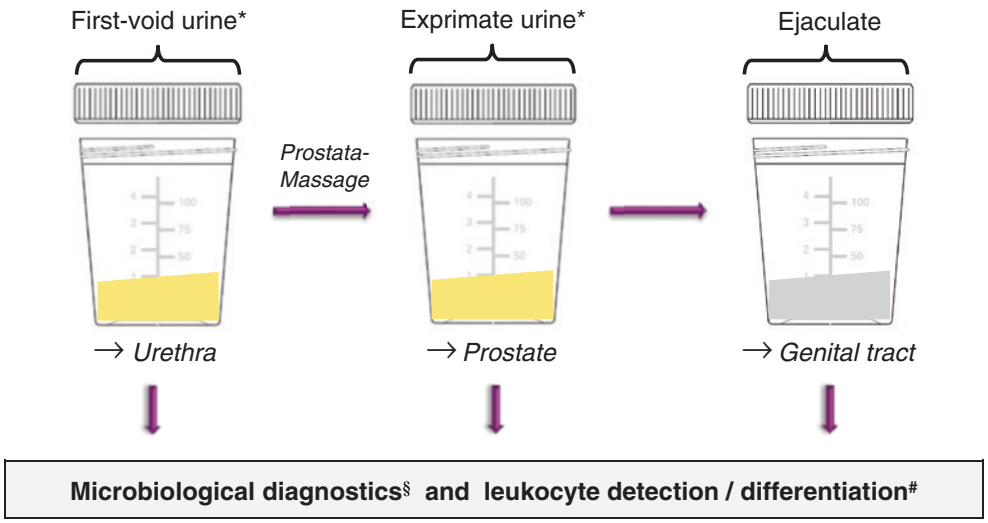


Fig. 26.3 Fractionated collection and examination of urogenital secretions: combination of the so-called 2-glass test with a subsequent semen analysis. (*approx. 5 mL; §tenfold higher CFU in exprimate urine versus first-void urine or bacteriospermia; #first-void urine: ≥ 10 leukocytes/high-power microscopic field in sediment [magnification × 400] → urethritis; #exprimate urine: ≥ 10 leukocytes/μL → prostatitis; #ejaculate: >10^6 leukocytes/mL [peroxidase-positive granulocytes; reference limits for macrophages, lymphocytes, etc., not available]); modified from Schuppe et al. (2017)

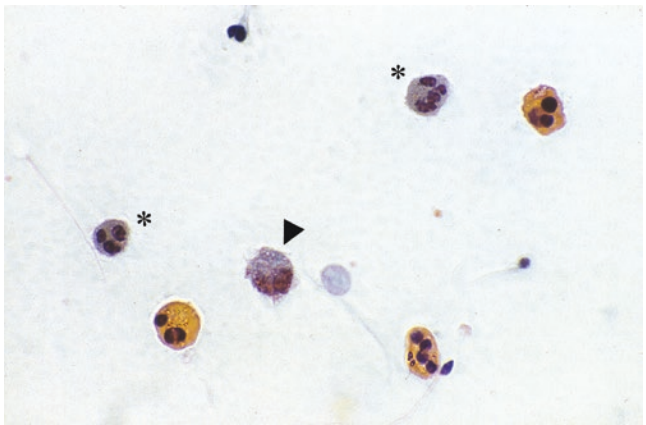


Fig. 26.4 Leukocytes in the ejaculate: neutrophilic granulocytes (*) and a macrophage (►) next to immature germ cells (multinucleated spermatids) (Papanicolaou stain of a smear preparation, oil immersion, primary magnification ×100). From Haidl and Schuppe (2006)

higher sensitivity and allows **differentiation of leukocyte subpopulations** in the ejaculate as well as conclusions about their function (Ricci et al. 2000; Fathy et al. 2014). In practice, assessment of stained smear preparations of an ejaculate (e.g., modified Papanicolaou or Shorr stain; Fig. 26.4) also allows differentiation and quantification of leukocytes, with the detection of macrophages in particular indicating chronic inflammatory reactions in the epididymis (Haidl and Schuppe 2006; Domes et al. 2012; WHO 2021).

Both neutrophilic granulocytes and macrophages (Fig. 26.4) are capable of forming so-called extracellular traps (ETosis) for defense against pathogens as well as elimination of defective spermatozoa (Schulz et al. 2019; Zambrano et al. 2020). However, compartment-specific localization and differential diagnosis of inflammatory processes in the male genital tract is not yet possible even with these methods.

Due to the methodological limitations of leukocyte detection in semen, released mediators such as **granulocyte elastase** and **pro-inflammatory cytokines** or **chemokines** serve as additional indicators of inflammation (Schuppe et al. 2017). The complex profiles of these immune mediators, also in semen, can be determined using multiplex enzyme immunoassays; available assay kits can be adapted for seminal plasma testing (Pilatz et al. 2017). Leukocytes also constitute the major source of **ROS**, which can be measured using chemiluminescence-based assays (Aitken and Baker 2013; WHO 2021). In addition, a point-of-care method is available to determine the oxidation–reduction potential in ejaculates (Agarwal et al. 2016). Characterization of sperm DNA fragmentation represents another level of cellular damage associated with leukocytospermia or oxidative stress, although its prognostic value with regard to fertilisation and pregnancy is still controversial (WHO 2021).

In cases of suspected urogenital infections, **microbiological examination** of ejaculate and urine samples as well as secretions or smears is essential (Fig. 26.3). Appropriate specimen collection, transport, and processing within 2–4 h of collection are critical (Podbielski et al. 2011). Preanalytical errors include failure to clean the urethral orifice and its surroundings; microbiological examination of ejaculate should only be performed after prior micturition (WHO 2021).

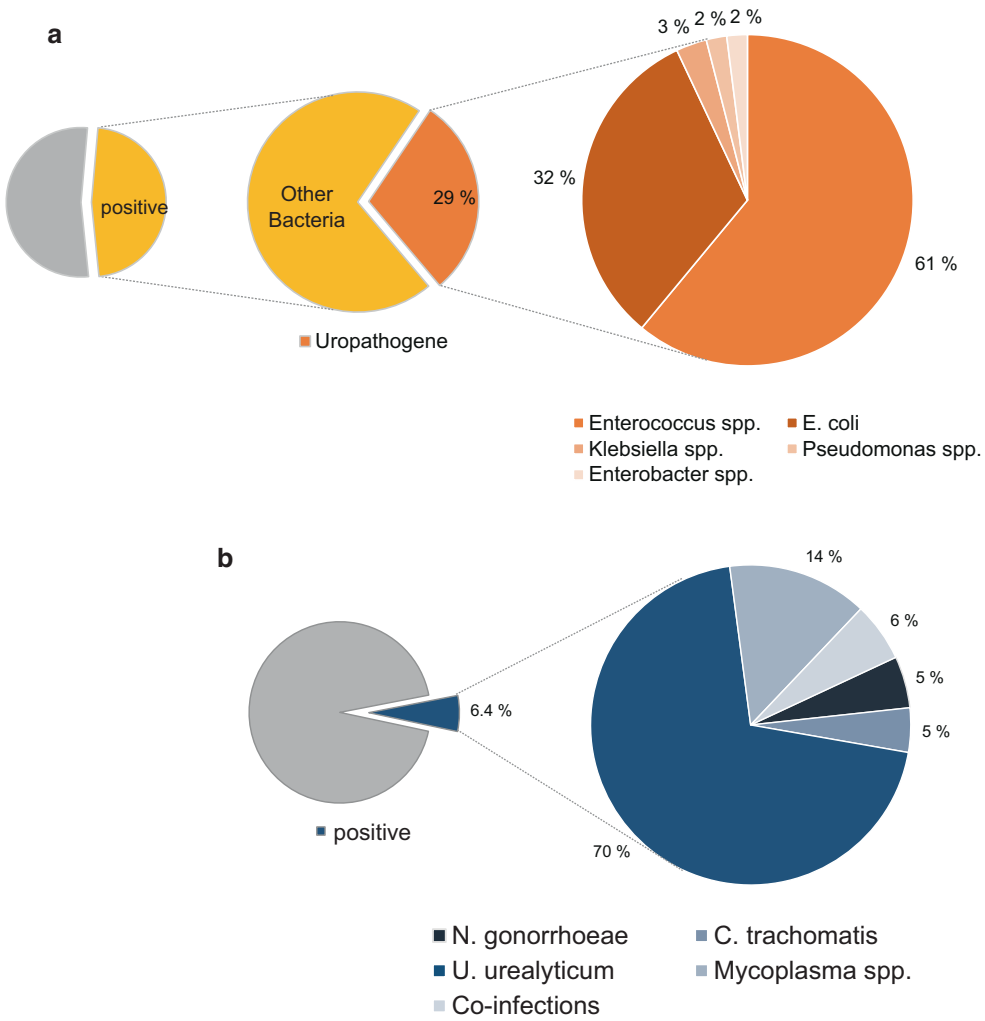


Fig. 26.5 Bacteriological findings in semen samples of patients from andrology consultations (n = 2080; presentation for couple infertility: n = 1322): (**a**) Results according to standard culture methods; bacteria classified as non-pathogenic include coagulase-negative staphylococci, *Streptococcus spp, Corynebacterium* spp., and others ("Other Bacteria"); (**b**) Results of a PCR-based panel for STI (modified from Falker 2018)

Taking into account the preanalytical requirements, only up to 50% of conventionally examined urogenital specimens are culture-negative even in asymptomatic men (Cottell et al. 2000). Bacteria of the physiological flora of the urethra are frequently found, e.g., *S. epidermidis* and streptococci of the viridans group, and are usually classified as non-pathogenic. In contrast, facultative pathogenic bacteria such as enterobacteria (e.g., *E. coli, Klebsiella* spp., *Proteus* spp.), enterococci, ureaplasmas, mycoplasmas, and *S. saprophyticus*, as well as obligatory pathogenic bacteria such as *N. gonorrhoeae* and *C. trachomatis* can cause infection (Schiefer 1998) (Fig. 26.5).

For the detection of pathogens in urogenital secretions, a **combination of culture and nucleic acid amplification techniques (NAT)** is recommended (Schuppe et al. 2017). Cultural methods can be used to identify rapidly growing bacteria (Podbielski et al. 2011). In order to evaluate the etiological relevance, the bacterial burden must be estimated by the number of colony-forming units (cfu) per mL in each case. Culture-independent nucleic acid amplification (NAT) techniques are used to detect bacteria that are difficult to culture and very sensitive (e.g., STI pathogens) (Gimenes et al. 2014). If results are negative, a universal bacterial PCR can be used additionally, which refers to the highly conserved *16S rDNA* gene in all bacteria as target sequence. With the aid of subsequent sequencing, 99% of human pathogenic bacteria can be detected (Podbielski et al. 2011; Pilatz et al. 2015).

26.5 Therapy

Therapeutic goals are **reduction or eradication of pathogenic bacteria** in the ejaculate or prostatic secretions, **normalization of inflammatory parameters**, and **improvement of impaired sperm parameters** (Schuppe et al. 2017; Haidl et al. 2019). However, no guidelines are yet available for the treatment of asymptomatic genital tract infections and/or inflammation of unknown acuity in cases of male infertility. The use of **antibiotics** is based on recommendations formulated primarily for acute or symptomatic disease and should be applied appropriately (Bonkat et al. 2021) (Fig. 26.6). If

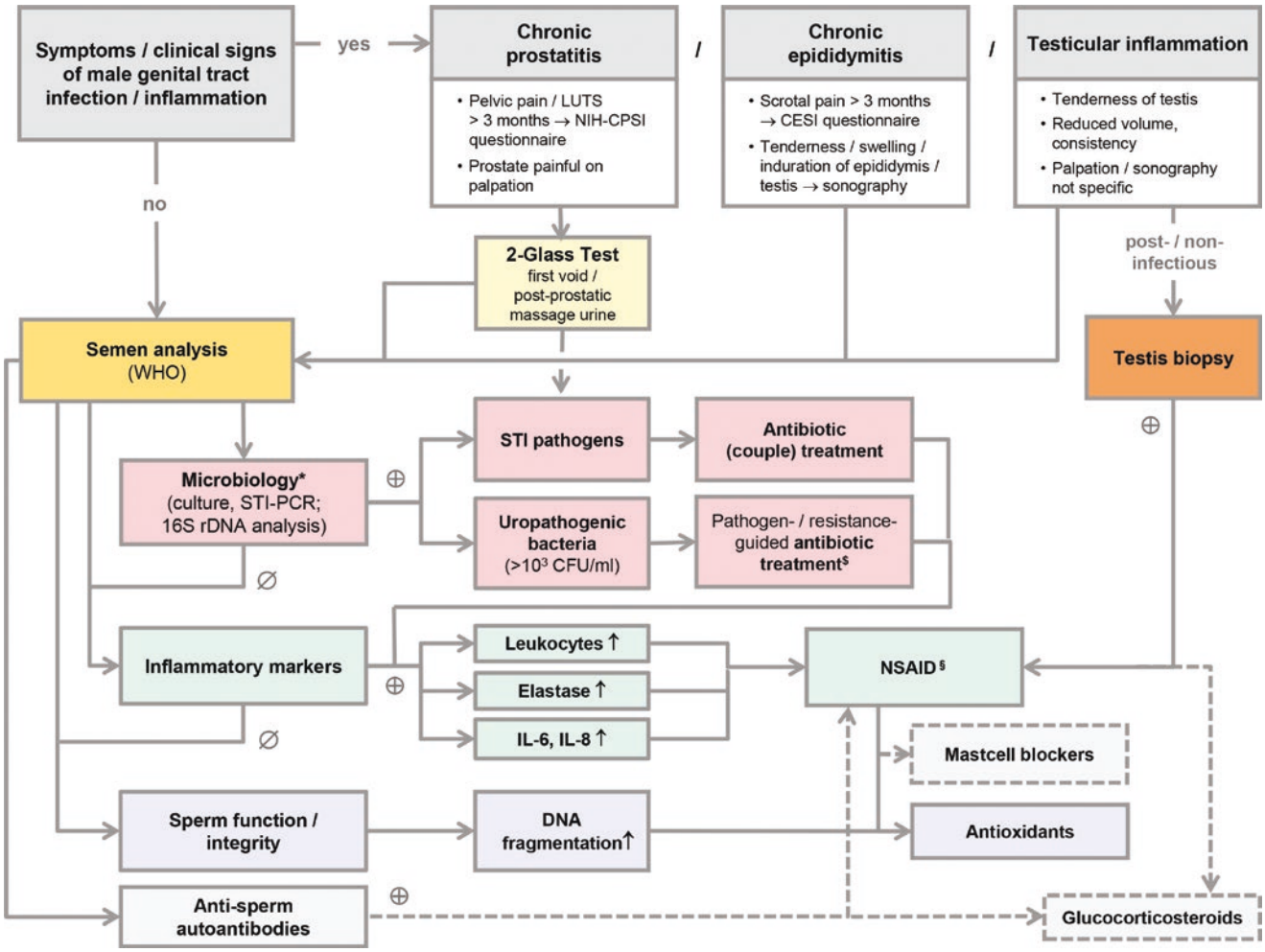


Fig. 26.6 Diagnostic-therapeutic algorithm for patients with suspected genital tract infections and/or inflammation (modified from Haidl et al. 2019). Rarely required surgical therapeutic measures (e.g., orchiectomy for therapy-refractory fulminant epididymo-orchitis) are not included. *to be supplemented by viral PCR in suspected cases. §in patients without symptoms of (chronic) prostatitis or epididymitis after positive confirmatory examination with significant bacterial count (consensus-based threshold >10^3 cfu/mL); duration of therapy depending on pathogen/indication and antibiotic preparation. §e.g., diclofenac [100–150 mg/day] or equivalent preparations including cyclooxygenase 2 inhibitors; duration of therapy 3–6 weeks. NIH-CPSI, NIH Chronic Prostatitis Symptom Index; CESI, Chronic Epididymitis Symptom Index; NSAID, nonsteroidal anti-inflammatory drugs

STIs are detected, **guideline-based antimicrobial therapy** involving the partner is mandatory (cycle-adapted administration in females or safe contraception to avoid negative effects of antibiotics on an occurring pregnancy; AWMF 2019; Workowski et al. 2021). When uropathogens are detected in the ejaculate of asymptomatic patients, the microbiological findings should first be confirmed in a follow-up examination, and the diagnostic procedure should be extended to include a 2-glass test if necessary (Schuppe et al. 2017) (Figs. 26.3 and 26.6). The indication for antibiotic therapy is based on the bacterial count (consensus-based threshold >10^3 cfu/mL). If chronic bacterial prostatitis or symptomatic epididymitis is present, the respective current guidelines must be observed; this applies particularly in the case of necessary antibiotic therapy of patients with acute symptoms (Bonkat et al. 2021). Prescription of fluoroquinolones, which are preferred as initial therapy due to their proven local tissue penetration and the expected pathogen spectrum, requires a strict indication; warnings about serious side effects must be observed (FDA 2018). In the case of STI pathogen persistence in the STI PCR despite guideline-compliant antibiotic therapy, it may be useful to send a sample to the respective national reference center where **antibiotic resistance** at the molecular biological level can be determined.

A probationary use of antibiotics, e.g., in the case of a nonsignificant bacterial count or isolated signs of inflammation in the ejaculate, is not indicated. This also applies to untargeted antibiotic treatment before ART (de Geyter et al. 1994; Liversedge et al. 1996). When antibiotic administra-

tion is indicated according to the criteria outlined above, potential negative effects on spermatogenesis or sperm function should be considered, and follow-up semen analysis or ART should be recommended only after a therapy-free interval of several weeks (Samplaski and Nangia 2015; Schuppe et al. 2017).

Investigating epididymitis/epididymo-orchitis as an example, it becomes clear that the development of persistent sub- or infertility is unpredictable despite pathogen-eradicating acute antibiotic therapy (Rusz et al. 2012). Similarly, antibiotic treatment for MAGI does not necessarily result in improved conception rates (Haidl et al. 2019). Although negative effects of antibiotics such as sulfonamides or tetracyclines on spermatogenesis or sperm function have been described, irreversible consequences of therapy are unlikely here (Samplaski and Nangia 2015). Rather, induction of unfavorable cellular and humoral immune responses in the genital tract can be assumed (Fijak et al. 2018) (Table 26.1).

Thus, despite the diagnostic shortcomings, **anti-inflammatory treatment** can be considered when there are signs of inflammation in the ejaculate (without pathogens or after antibiotic therapy) (Haidl et al. 2019; Velez et al. 2021). For the administration of **nonsteroidal anti-inflammatory drugs**, some open studies have shown an improvement in semen quality with a decrease in leukocyte counts (Lackner et al. 2006; Gambera et al. 2007) (Fig. 26.6). Similar results are found for mast cell blockers (Oliva and Multigner 2006; Azadi et al. 2011). Weak evidence is available for the therapeutic efficacy of adjuvant medications such as **antioxidants** (Showell et al. 2014). If sperm autoantibodies are detected, assisted reproduction is now preferred over immunosuppression with glucocorticosteroids (Chap. 28).

(Re-)fertilizing interventions concern occlusions at the epididymal level and along the ductus deferens (microsurgical vasoepididymo- or vasovasostomy) as well as the central areas of the prostate (transurethral resection of the ductus ejaculatorius) (Diemer et al. 2011) (Chap. 40). In cases of nonobstructive azoospermia due to epididymo-orchitis or orchitis, the only remaining option is multifocal **testicular biopsy for testicular sperm extraction** (Chap. 11).

Key Points

- There is a high prevalence of infectious and/or inflammatory diseases of the male genital tract.
- Etiologically, infections with STI as well as typical uropathogens are most relevant; however, primary pathogen-independent inflammation can also occur.
- Diagnosis is complicated by asymptomatic, primary chronic courses and the multifactorial etiology of male fertility disorders.
- Microbiological pathogen detection and leukocytes and/or elevated inflammatory mediators in ejaculate, prostatic secretions, and urine samples are important indicators of infections and inflammation of the seminal ducts and accessory sex glands.
- The predictive value of leukocytospermia is limited with respect to the detection of relevant bacteria or viruses in the ejaculate.
- Organ/compartment-specific noninvasive diagnostic markers are not yet available.
- In acute, symptomatic infections, immediate antibiotic therapy is indicated in accordance with current guidelines.
- In asymptomatic patients with fertility problems, antibiotic treatment should be given only when pathogenic bacteria are detected and the bacterial count is significant/confirmed.
- Probationary use of antibiotics is not indicated (e.g., when apathogenic bacteria are detected, bacterial counts are low, isolated signs of inflammation, or untargeted before ART).
- Nonsteroidal anti-inflammatory drugs may be administered if there are signs of inflammation in the ejaculate (without pathogens or after antibiotic therapy).
- There is a risk of irreversible infertility despite adequate drug therapy, probably due to the induction of persistent immunopathological reactions.

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Obstructions of the Seminal Ducts, Cystic Fibrosis, and Congenital Aplasia of the Ductus Deferens

27

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Abstract

Obstructions of the seminal ducts affect epididymis, ductus deferens, and ductus ejaculatorius. Bilateral obstruction causes azoospermia with usually normal testicular volume, FSH, and inhibin B. Causes of obstruction are varied and include congenital disease, infection, trauma, intentional or accidental surgical sequelae, and functional disorders. Surgical sperm retrieval followed by assisted reproduction may be offered in cases of an unfulfilled desire for a child with no causal therapeutic option. Cystic fibrosis is associated with obstructive azoospermia in most affected men. Congenital bilateral aplasia of the ductus deferens (CBAVD) is also genetic in most cases. In both conditions, genetic counseling and diagnostic testing of the couple is strongly recommended before performing assisted reproduction procedures.

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27.1 Obstruction of the Seminal Ducts

27.1.1 Etiology and Pathogenesis

Obstructions of the draining seminal ducts involve the epididymis, ductus deferens, and ductus ejaculatorius (Table 27.1). In addition to cystic fibrosis, congenital bilateral aplasia of the ductus deferens, and bilateral ductus ejaculatorius obstruction, which are presented separately in this chapter because of their partly genetically determined pathogenesis, the primary cause of obstruction is acute or chronic epididymitis or inflammation of the seminal vesicles and prostate gland (cf. "Infections and inflammations of the vas deferens and accessory sex glands," Table 27.1). Iatrogenically caused obstructions can be the result of herniotomies (especially in childhood) or surgical interventions in the region of the ejaculatory duct and also the result of—obsolete—vasography of the vas deferens with irritative contrast media. Procedures of sperm collection from the epididymis [**PESA** (percutaneous epididymal sperm aspiration), **MESA** (microsurgical epididymal sperm aspiration)], or accidentally performed epididymal incisions as well as epididymectomy may lead to obstruction of the draining seminal ducts. Acquired obstruction ultimately includes the condition following **bilateral vasectomy** (Table 27.1). Causes of functional obstruction of the vas deferens may also include local or systemic neurological dysfunction due to neurological disease, trauma, surgery, or drug side effects (Salonia et al. 2022).

Alterations in the area of the ductus ejaculatorii can be the cause of obstruction of the vas deferens. These include utricular cysts or other intraprostatic cysts that can lead to confluence of the ductus ejaculatorii with the cyst or external obstruction of the ductus ejaculatorii (Fig. 27.1), or post-infectious stenosis and cystic dilatation of the ductus ejaculatorius itself (Salonia et al. 2022).

Table 27.1 Causes of anatomic obstruction of the seminal ducts

Seminal ducts	Causes of obstruction
Epididymis	– Present or past acute or chronic epididymitis
	– Trauma
	– Epididymal incision/biopsy, epididymectomy
	– After PESA (percutaneous epididymal sperm aspiration), possibly also MESA (microsurgical epididymal sperm aspiration)
	– Aplasia/hypoplasia of the ductus deferens and distal epididymis
Ductus deferens	– Vasectomy
	– Herniotomy or other surgery with accidental ligation of the ductus deferens
	– Vasography with irritative damage to the draining seminal ducts
	– Aplasia/hypoplasia of the ductus deferens and distal epididymis
	– Trauma
Ductus ejaculatorius	– Congenital cysts (utricular cyst), prostatic cysts, seminal vesicle cysts
	– Post-infectious (seminal vesicles, prostate)
	– Aplasia/hypoplasia
	– Trauma
	– Post-operative

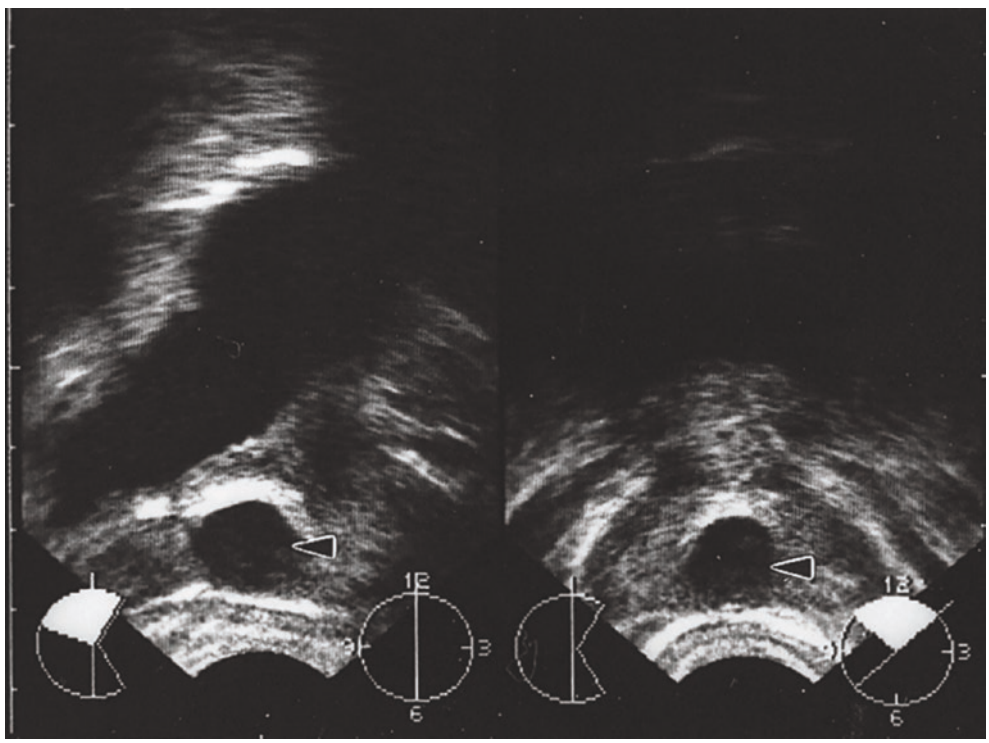


Fig. 27.1 Large intraprostatic cyst (black arrow) with secondary obstruction of the ductus ejaculatorii visualized by transrectal sonography

27.1.2 Clinic

Bilateral complete obstruction of the seminal duct leads to **azoospermia** and consequent infertility. **Unilateral obstruction** may be completely compensated by the opposite side and thus remain asymptomatic if the testis and the draining seminal ducts are intact contralaterally. **Partial obstruction** on either side may result in, e.g., significant reduction in sperm count, depending on the degree of severity.

If the obstruction is proximal to the ductus ejaculatorii, the **ejaculate volume** usually remains normal because most of the seminal fluid comes from the seminal vesicles and prostate. In distal occlusion, parvisemia (ejaculate volume < 1.4 mL) is often found.

27.1.3 Diagnostics

Differentially, occlusive azoospermia must always be considered in the presence of **normal testicular volume, normal FSH**, normal **inhibin B**, and **azoospermia**. However, it must be noted that if late maturational arrest of spermatogenesis is the cause of non-obstructive azoospermia, testicular volume, FSH, and inhibin B may also be normal. Palpation of scrotal contents can detect congestion-related dilatation and induration of the epididymis, as can aplasia or hypoplasia of the vas deferens. Imaging **sonography** of the scrotal organs allows visualization of changes in the epididymis that must be considered as a cause of obstruction (see Chap. 6) (Salonia et al. 2022). By means of transrectal sonography, changes in the area of the prostate and seminal vesicles (infectious changes, anomalies, cysts in the area of the ductus ejaculatorii) can be visualized (see Chap. 6).

The **marker substances for epididymis (α-glucosidase), seminal vesicles (fructose)**, and **prostate (zinc)** determined in seminal plasma are helpful in differential diagnosis (see Chap. 9 "Semen Analysis"). In bilateral obstruction, the marker substances secreted above the lesion are undetectable in the ejaculate, but the markers of the organs distal to the lesion are normal. In cases of unilateral and partial obstruction, differential diagnosis can be difficult. Prior to microsurgical reanastomosis, the obligatory performance of a bilateral testicular biopsy to assess spermatogenesis is obsolete. However, if spermatogenesis arrest is suspected, which may be accompanied by normal testicular volume, FSH, and inhibin B levels, testicular exposure to attempt reanastomosis should always be performed together with intraoperative testing for sperm. In addition, the quality of spermatogenesis should be evaluated by testicular biopsy, unless genetic alterations have already been identified in advance, which indicate meiosis arrest and make successful surgery unlikely (Wyrwoll et al. 2022).

27.1.4 Therapy

In cases of obstruction of the draining seminal ducts and clinically probable or histologically confirmed at least qualitatively normal spermatogenesis, **vasoepididymostomy** (for obstructions in the epididymis) or **vasovasostomy** (e.g., after vasectomy) may be considered, depending on the location of the obstruction (Schlegel et al. 2021b). Success rates regarding restoration of patency depend on the primary cause of the occlusion, its duration, and the surgical technique. The patient must be informed that in some circumstances, even if the obstruction is successfully removed, fertility may remain reduced due to anti-sperm antibodies (see Chap. 28), and reocclusion may also occur. However, the value of sperm autoantibodies remains controversial.

In principle, **microsurgical procedures** should be used because of higher patency rates in epididymo- or vasovasostomy. If possible, these should be performed in specialized centers, since the success rates increase significantly with the number of microsurgical procedures per year and surgical team.

In cases of ejaculatory duct obstruction, **transurethral resection** of the occluded duct or cysts can lead to improvements in ejaculatory parameters and pregnancies, although these are relatively rarely performed today due to frequent reocclusion (Schlegel et al. 2021b).

If patency of the draining seminal ducts cannot be achieved or if a primary microsurgical intervention does not appear to be reasonable due to the anatomical conditions, the procedures of direct sperm extraction from testicular tissue (**TESE**, testicular sperm extraction)) and sperm retrieval from the epididymis (MESA) with respective cryopreservation in combination with procedures of **assisted reproduction (ICSI**, intracytoplasmic sperm injection) are offered as an alternative (see Chap. 41). Sperm collection in cases of obstruction by direct fine-needle biopsy or percutaneous sperm aspiration from the testis or epididymis is not primarily recommended due to the unclear anatomical conditions with these procedures, the greater trauma and the lower success rate.

27.2 Cystic Fibrosis

27.2.1 Etiology and Pathogenesis

Cystic fibrosis (**CF; cystic fibrosis**) is one of the most common autosomal recessive inherited **diseases** in Central Europe. Approximately, 1 in 2500 children is affected. Initially, cystic fibrosis was practically only of importance in pediatrics, but today almost 60% of patients are over 18 years of age. The life

expectancy of a newborn with cystic fibrosis is 57 years in 2021 (Mukoviszidose et al. 2022). In the meantime, more and more patients are reaching adulthood due to improved therapy options. While symptomatic therapies and antibiotic treatment of intermittent and chronic infections were initially the mainstay, there are now CFTR modulators that improve the function of the CFTR channel. For example, the triple combination of tezacaftor, elexacaftor (both correctors), and ivacaftor (potentiator) have also been approved in Europe since August 2020. For CF patients aged 6 years and older with the p. Phe508del mutation in at least one gene copy (this affects 85% of CF patients), significant symptomatic improvement can be achieved (Mukoviszidose et al. 2022; Savant et al. 2022). Whereas in the past, the question of **fertility** and having one's own children was rarely raised in view of the chronic morbidity and low life expectancy, this has now become more important in the clinical care of patients with cystic fibrosis.

The disease is caused by **mutations in the CFTR gene**. CFTR stands for "*Cystic Fibrosis Transmembrane Conductance Regulator*," which refers to the function of the gene product as a membrane-bound ion channel. With a length of approximately 230,000 base pairs and 27 exons, it is a large hereditary unit. Since the cloning of the CFTR gene in 1989, more than 2000 different mutations have been described. In central European patients, the mutation c.1521_1523delCTT, p.Phe508del (former name F508del or ΔF508) accounts for about 70% of CF alleles. This is a deletion of three base pairs, and the resulting dysfunctional CFTR protein lacks one phenylalanine. As a result, spatial folding is impaired, limiting the protein's ability to function (Schmitt-Grohé et al. 2019). 10–15% of CF alleles are represented by about 10 other mutation types, all other mutations are very rare. The frequency of the different mutations varies depending on the patients' region of origin. The size of the CFTR gene and the heterogeneity of the mutations mean that only about 85% of CF alleles are detected in routine molecular genetic diagnostics. Sequencing of the CFTR gene is possible if the question is appropriate. There is a limited correlation between clinical phenotype and the type of mutations present in the CFTR gene. Apparently, other genes have a modulatory effect on the CFTR gene, its transcripts, or its protein product, which explain sometimes marked clinical variability of the clinical picture despite an identical genotype (Schmitt-Grohé et al. 2019). The genes TGFß-1 and EDNRA are particularly worth mentioning here with regard to the development of the vas deferens.

27.2.2 Clinic and Diagnostics

The CFTR protein is highly expressed in **airway epithelia**, where it acts as a regulator of electrolyte transport. If the protein is absent or its function is impaired by mutation, abnormally viscous bronchial mucus is produced. This leads to obstruction and bacterial colonization of the airways, resulting in long-term chronic progressive limitation of lung function with secondary right heart overload. In addition to lung involvement, which determines the course of the disease, 85% of patients with cystic fibrosis have **pancreatic insufficiency**. A severe complication is meconium ileus of the CF-ill newborn.

The most important laboratory diagnostic criterion for cystic fibrosis was the **increase of chloride ion concentration in sweat**. One stimulates sweat secretion by local pilocarpiniontophoresis. The measurement must be performed under standard conditions and should be reserved for experienced laboratories because of the susceptibility of the test to interference. A chloride concentration in the sweat of more than 60 mmol/L in two independent measurements is considered to be indicative of CF, values of 40 to 60 mmol/L are in the diagnostic "gray area." Since 2016, the determination of immunoreactive trypsin in heel blood has been established nationwide in Germany as part of newborn screening and thus provides the first indication of a suspected CF if the value is above the cut-off of the ≥99.8th percentile value. This is followed by molecular genetic testing for the most common CFTR gene mutations (Schmitt-Grohé et al. 2019).

Most male patients with cystic fibrosis have **obstructive azoospermia**. The cause is usually bilateral malformation of the vas deferens and distal epididymis (Savant et al. 2022), although the exact location of the obstruction cannot be demonstrated in all patients.

In typical constellations of findings, the **ductus deferens** is either not detectable in its intrascrotal course or has degenerated to a cord-like structure without a lumen. **Corpus** and **cauda epididymidis** are usually also aplastic or hypoplastic. Obstruction results in marked distension of the **head of the epididymis**, which, unlike the more distal sperm-draining structures, is not a derivative of Wolff's ducts. The **testicular parenchyma** is intact in most men with CF. In some cases, non-specific histologic changes are found that may be due to obstruction or general health impairment of the patient. Sonographically, calcifications, small cystic changes, or echo-deficient areas are sometimes observed in the testicular parenchyma. Typically, **abnormalities of the seminal vesicles** are associated with bilateral vas deferens and epididymal aplasia. Anatomic findings in this area vary from patient to patient. Aplasia or hypoplasia, but also obstruction, dilatation, and cystic degeneration of the glandulae seminales are observed. Malposition or dysfunction of the seminal vesicles explains the **parvisemia** that is almost always present.

In a small group of male CF patients, there is normal or only gradually reduced fertility. In these cases, the general course of the disease is also milder. In most cases, there is no pancreatic insufficiency, and pulmonary functional impairment develops relatively slowly. The genotype of these patients is often characterized by compound heterozygosity for CFTR mutations (e.g., p.Phe508del and a mutation from classes IV to VI) (Savant et al. 2022).

The andrologic diagnostic approach in men with cystic fibrosis is essentially the same as in other forms of obstructive azoospermia. Particularly important in ejaculate examination is measurement of volume, pH, and levels of **fructose and neutral α-glucosidase** or alternative markers of seminal vesicle or epididymal function. Abnormalities of the ductus deferentes and epididymis may be detectable by **scrotal palpation** or **testicular sonography**, but normal palpation findings and a normal ultrasound finding are not uncommon. **Transrectal ultrasound** is suitable for evaluating the ampullary portion of the deferent ducts, the ejaculatory duct, and the seminal vesicles (see Chap. 6). If assisted reproduction procedures are planned, a **testicular biopsy** for testicular sperm extraction (TESE) with cryopreservation should be performed before starting treatment.

Since cystic fibrosis follows the autosomal recessive mode of inheritance, the disease can be repeated in the patient's children only if the partner is also affected or heterozygous. While the former is a rarity, silent transmission for CF is common in central European populations. A heterozygote frequency of about 4–5% is assumed. Therefore, a **mutation analysis of the CFTR gene in the partner** is essential. If no mutation is found in the partner with today's routine methods and no CF cases have occurred in her family, only a small residual risk in the order of about 0.5% remains which is comparable to the risk in the general population. This recurrence probability can be modified by sequencing the CFTR gene in the partner. On the other hand, there is an up to 50% recurrence risk for cystic fibrosis if a CFTR mutation is detected. The above risk figures apply in this form only if the families of both partners are from Germany. For patients with a different ethnic background, the risk calculation must be modified because the spectrum of mutations in the CFTR gene varies greatly from region to region (Savant et al. 2022).

27.2.3 Therapy

It is strongly recommended to offer couples with a partner suffering from cystic fibrosis **human genetic counseling** and, if necessary, diagnostics before initiating reproductive medical treatment (Schlegel et al. 2021a). The potentially serious health consequences for the offspring of the couple as well as therapeutic options are discussed, and also **prenatal diagnostic** and **preimplantation diagnostic options**. If the couple wishes therapy to fulfill their desire for a child despite a significant risk of recurrence, this must be respected as an autonomous decision by the patient (Meschede et al. 1997).

Surgical reconstruction of the vas deferens is not possible in azoospermic patients with cystic fibrosis. Therefore, **assisted reproduction** with testicular or epididymal sperm is the only way to help these men fulfill their desire to have a child. If applicable, sperm can be aspirated from the remaining portion of the epididymis using microsurgical techniques or obtained directly from testicular parenchymal tissue (**MESA** or **TESE** technique). The spermatozoa obtained in this way are used for **intracytoplasmic sperm injection (ICSI)** (see Chap. 41).

27.3 Congenital Aplasia of the Ductus Deferens (CBAVD)

27.3.1 Etiology and Pathogenesis

Congenital bilateral aplasia of the ductus deferens occurs as an isolated malformation or as a partial manifestation of cystic fibrosis. For a long time, "isolated" ductus deferens aplasia was genetically considered to be only a minimal form of cystic fibrosis and, like it, caused by mutations in the CFTR gene. Mutations in the ADGRG2 gene on the X chromosome are now also known to be another cause of bilateral ductus deferens aplasia (Patat et al. 2016).

The acronym **CBAVD** (congenital bilateral absence of the vas deferens) has become common to describe "isolated" congenital bilateral aplasia of the ductus deferens. Approximately 2% of men with azoospermia receive a diagnosis of CBAVD.

The molecular pathology of bilateral vas deferens aplasia in the setting of CFTR mutations is complex and can only be outlined here. Globally, mutations of the CFTR gene are divided into "strong" and "weak" (mutation classes I–VI, Schmitt-Grohé et al. 2019). The former, in a state of homozygosity, cause full-blown cystic fibrosis, whereas the latter cause only a milder clinical picture, e.g., in terms of chronic bronchitis or pancreatitis. In addition to such strong and weak mutations, a polymorphism in intron 8 of the CFTR gene plays an important role in the pathogenesis of CBAVD. This gene segment contains a sequence that may consist of 5, 7, or 9 thymidine building blocks. In addition, a polymorphic TG repeat region of 11, 12, or 13 TG repeats is still adjacent. The combination of a longer TG repeat number with a 5 T allele has significance as a mild mutation because the splicing of

CFTR mRNA is disrupted (Savant et al. 2022). This results in a high percentage of mRNA that lacks exon 9 and therefore does not give rise to active CFTR protein.

Simplistically, it can be said that patients with full-blown **cystic fibrosis** usually have two severe mutations (one on each gene copy). In contrast, the most common finding in CBAVD is the combination of a strong mutation with a weak mutation or a strong mutation with a 5 T allele with a corresponding long TG polymorphism.

Using a powerful molecular analysis strategy, at least one mutation in the CFTR gene is found in approximately 80% of men with bilateral aplasia of the vas deferens. From both molecular genetic family studies and detailed clinical investigations in CBAVD patients, it can be deduced that approximately 20% of cases of CBAVD are not caused by mutations in the CFTR gene, but by mutations in the ADGRG2 gene, among others (Kirchhoff 2018). In patients with concomitant **renal anomalies** (unilateral aplasia, dystopic location, horseshoe kidney), CFTR mutations are almost never detectable (Kirchhoff 2018; Salonia et al. 2022). The same is true for men who have CBAVD but in whom both seminal vesicles and the ampullary portion of the vas deferens appear normal on transrectal ultrasound. Bilateral ductus deferens aplasia is thus etiologically heterogeneous.

27.3.2 Clinic and Diagnosis

The **anatomic abnormalities** in the genital tract and **ejaculate findings** in CBAVD are identical to those in cystic fibrosis. Patients with azoospermia and a CFTR mutation have significantly lower ejaculate volume, pH as well as α-glucosidase and fructose concentration in the ejaculate compared with fertile men or men with azoospermia without a CFTR mutation (Fig. 27.2) (von Eckardstein et al. 2000; Tüttelmann et al. 2011). As mentioned above, a definite clinical diagnosis of CBAVD is often not possible before testicular biopsy. Similarly, the clinical spectrum in men with CBAVD is broad (Rudnik-Schöneborn et al. 2021).

Abnormalities of the urinary system are clustered in CBAVD and suggest the non-CFTR mutation variant of the clinical picture. **Ultrasound examination of the kidneys** should be part of the routine diagnostic program in men with ductus deferens aplasia, especially in patients without previously identified CFTR mutations (Salonia et al. 2022).

27.3.3 Therapy

As in cystic fibrosis, **human genetic counseling** should precede treatment by assisted reproduction in CBAVD. Again, analysis of the CFTR gene in both partners is crucial. The assessment of the risk of CF for possible

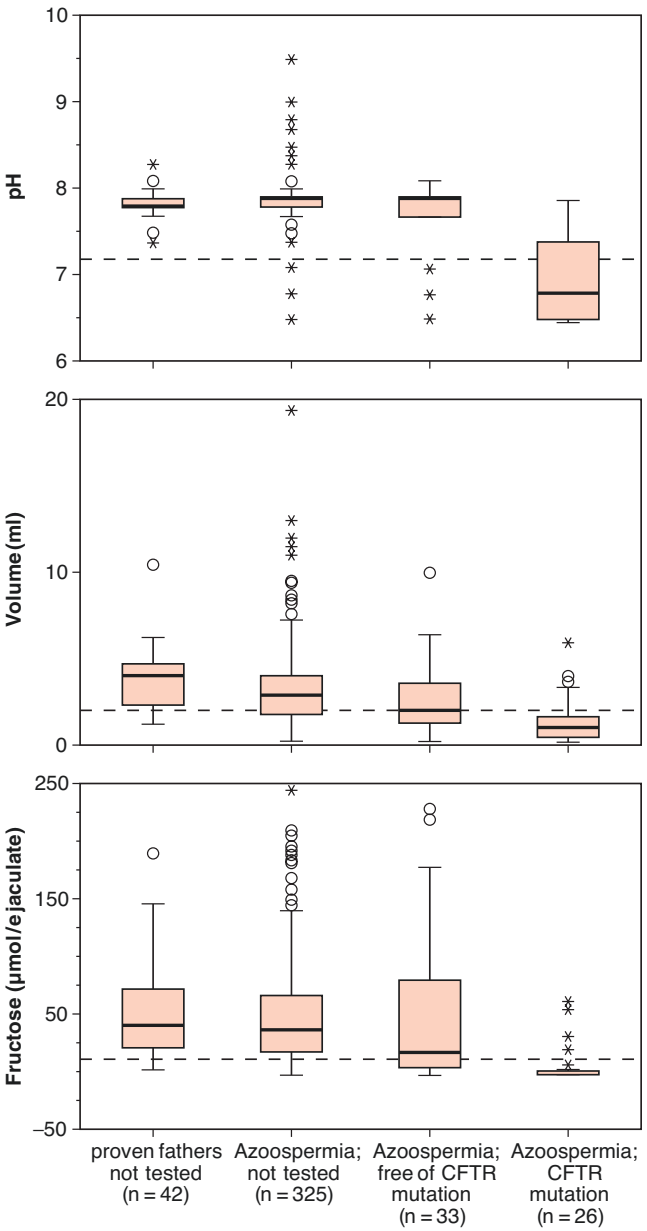


Fig. 27.2 PH (upper drawing), volume (middle drawing), and fructose concentration (lower drawing) of ejaculate in 42 fathers, 325 azoospermic men in whom mutations in the CFTR gene were not investigated, 33 men with azoospermia without CFTR mutation, and 26 men with CFTR mutation (von Eckardstein et al. 2000)

children depends on the probability of detection of CFTR mutations by the chosen analysis and on the family history/ethnicity of the partners. Mutations in the ADGRG2 gene as a cause of CBAVD do not lead to a "direct" risk of recurrence in offspring, but only again in male offspring of daughters.

For patients with CBAVD and desire for children, surgical sperm retrieval using TESE (in same cases in combination with MESA) and cryopreservation of the samples followed by ICSI is the therapeutic procedure of choice (Schlegel et al. 2021b) (see Chap. 41).

27.3.4 Unilateral Aplasia of the Ductus Deferens

Unilateral aplasia of the ductus deferens is in principle compatible with normal male fertility and probably often remains unnoticed throughout life. Sometimes the diagnosis is made on the occasion of a vasectomy as an incidental finding. However, unilateral vas deferens aplasia is also occasionally found in men with impaired ejaculation or azoospermia. The correct term here should actually be unilateral "intrascrotal" vas deferens aplasia, since the intra-abdominal portions of the ductus deferens elude palpatory evaluation. Indeed, patients with unilateral aplastic ductus deferens may have more distal obstruction of the vas deferens contralaterally. In this particular subgroup (unilateral palpatory aplasia, azoospermia, contralateral distal obstruction), CFTR mutations can be detected in the majority of patients. In contrast, such mutations are rarely found in men without additional distal obstruction of the contralateral duct.

Patients without CFTR mutations and unilateral aplasia of the ductus deferens often present with urinary tract anomalies, most notably unilateral renal agenesis, which likely have another underlying genetic cause (Salonia et al. 2022).

27.3.5 Bilateral Obstruction of the Ejaculatory Ducts

The short intra-prostatic segment of the ductus deferens beyond the confluence of the seminal vesicles is also known as the ductus ejaculatorius. Bilateral obstruction in this area results in azoospermia and parvisemia with low pH and low fructose content of the ejaculate. In the absence of detectable CBAVD, this constellation of findings is virtually conclusive of **bilateral ductus ejaculatorius obstruction**, which can also be visualized by transrectal **sonography** (see Chap. 6). Non-genetic causes of obstruction include trauma, iatrogenic injury, infection, tumorous space-occupying lesions, prostatic calcifications, and cysts (Salonia et al. 2022). Some patients have symptoms reminiscent of acute or chronic prostatitis, and pain on ejaculation or hematospermia is occasionally reported. The surgical approach depends on the individual anatomical findings.

In some men with isolated bilateral ductus ejaculatorius obstruction, none of the above causes can be demonstrated. In this case, it is probably a constitutional pre-dispositional disorder, which is underlined by the detection of mutations in the CFTR gene in most of these patients (Meschede et al. 1997). Thus, this is also a minimal form of cystic fibrosis limited to the male genital tract. With regard to genetic diagnosis and counseling, the same procedure should be followed as for CBAVD.

Therapeutically, transurethral surgery for desobliteration can be attempted. If surgery fails or, depending on the individual constellation, also primarily, sperm collection is possible by means of TESE and if applicable MESA with cryopreservation of the samples and consecutive intracytoplasmic sperm injection (ICSI) may be considered (see chapter "Assisted Reproduction").

27.4 Young's Syndrome

Young's syndrome is characterized by a combination of obstructive azoospermia and chronic sinubronchial symptoms and bronchiectasis. It is a clinical picture that should be distinguished clinically and etiologically from cystic fibrosis and congenital duct aplasia, which present similarly (Handelsman et al. 1984; Cihanbeylerden and Kurt 2022). It is as yet unclear whether the very rare Young syndrome is a disease caused by genetic or exogenous factors.

Key Points

- Obstructions of the seminal ducts can involve the epididymis, the ductus deferens, and/or the ductus ejaculatorius.
- Bilateral complete obstruction of the seminal ducts results in infertility with azoospermia with usually normal testicular volume, FSH, and inhibin B.
- The ejaculate markers α-glucosidase and/or fructose are decreased depending on the localization of the obstruction.
- Causes of obstruction are varied and include congenital disease, infection, trauma, intentional or accidental surgical sequelae, or functional disorders.
- In the case of an unfulfilled desire for a child without the surgical possibility of restoring the patency of the draining seminal ducts, surgical sperm collection from the testis or, if necessary, epididymis, in combination with cryopreservation and with subsequent assisted reproduction can be offered with a high success rate.
- Cystic fibrosis as one of the most common autosomal recessive genetic diseases in Central Europe is associated with obstructive azoospermia in most affected men.
- Congenital bilateral aplasia of the ductus deferens (CBAVD) as a cause of obstructive azoospermia is also genetic in most cases.
- In cases of cystic fibrosis or CBAVD, genetic counselling and diagnosis of the couple is strongly recommended before performing assisted reproduction procedures.

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Immunologically Induced Infertility

28

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Abstract

In addition to other immunopathological processes in the male genital tract, antisperm antibodies (ASA) can lead to immunologically induced infertility. Here, the spontaneous conception probability depends on the proportion of sperm carrying ASA. The main pathomechanisms of ASA-induced infertility include impaired sperm ability to penetrate the cervical mucus, impaired sperm capacitation and acrosome response, and impaired sperm binding to the zona pellucida of the oocyte. The detection of IgG or IgA autoantibodies bound to spermatozoa is performed according to WHO recommendations in the context of ejaculate analysis in the direct MAR or immunobead test. Since corticosteroid therapy shows only questionable therapeutic success, methods of assisted reproduction can be used to overcome male infertility associated with ASA.

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28.1 Definition

In the clinical nomenclature, fertility disorders resulting from the formation of antisperm antibodies (ASA) are termed as "immunologically induced infertility.". ASA are detectable in serum, freely in seminal plasma and directly bound to spermatozoa; IgG and IgA antibodies are virtually exclusively found in ejaculate.

In addition to ASA, other immunopathological processes in the male genital tract can lead to infertility, particularly infections and non-infectious or autoimmune inflammation of the testes and/or epididymis. Accordingly, the term "immunologically induced infertility" should be defined more broadly according to current understanding (Fijak et al. 2018) (see Chaps. 19 and 26).

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28.2 Epidemiology

Data on the incidence of ASA vary considerably depending on the selection of men studied, the detection method used, and the setting of cutoffs for positive results (Francavilla and Barbonetti 2017). The majority of studies refer to membrane-bound IgG-type ASA in the MAR test (see below) with detection rates ranging from approximately 1–15%. Patients investigated for **couple infertility** showed an ASA prevalence based on ≥50% positive sperm in the MAR test of 3.4–6.6%, whereas in fertile men the frequency is approximately 1% (Barbonetti et al. 2019; Lotti et al. 2018). After vasectomy, more than 50% of affected individuals present with ASA (Adams and Wald 2009).

28.3 Etiology and Pathogenesis

Meiotic and post-meiotic male germ cells express surface antigens from puberty onward that are not present on somatic cells or pre-meiotic germ cells. Upon contact with immunocompetent cells, these antigens are recognized as "foreign to the body" and an **autoimmune response** may develop.

Normally, this is prevented by multiple, complementary mechanisms that together constitute **testicular immune privilege**. This refers to a marked tolerance at the local level to neoantigens. Here, it does not matter whether these occur on meiotic and post-meiotic germ cells or are introduced experimentally (in rodents) into the testis via xenografts or allografts (Fijak et al. 2018). Important mechanisms in the maintenance of immune privilege represent:

- the **blood–testis barrier** (see Chap. 2) is formed within the seminiferous tubules from specialized cell junctions between adjacent Sertoli cells. As a dynamic barrier, it thus prevents direct contact between interstitially localized immune cells and the majority of meiotic as well as all post-meiotic germ cells. Interestingly, recent studies indicate that, at least in rodents, certain germ cell-specific neoantigens can bypass the blood–testis barrier. This occurs by luminal phagocytosis of the residual bodies of elongated spermatids by Sertoli cells with subsequent transcytosis of the "cargo" through the cell and egress at the basal pole. This permanently exposes peritubular macrophages in particular to specific neoantigens, which is an essential prerequisite for the development of systemic tolerance (Tung et al. 2017). This is important to avoid autoimmune reactions that would otherwise occur in the epididymis or the seminal ducts, as these organs are not among the immune-privileged organs.
- the synthesis of **immunoregulatory and immunosuppressive factors** (e.g., galectin-1, activin, IL-10) by the somatic cells of the testis (Sertoli, Leydig, peritubular cells, testicular macrophages), the combined effect of which can trigger excessive immune reactions or curb the development of sterile inflammation (Fijak et al. 2018; Gao et al. 2016; Kaur et al. 2014; Mayer et al. 2016; Wijayarathna and de Kretser 2016).
- an **anti-inflammatory phenotype of testicular macrophages** and tolerogenic properties in dendritic cells (Wang and Duan 2016).

Despite these immunological protective mechanisms, ASA occur in many men as "natural," i.e., physiological manifestations without pathological impairment (Vazquez-Levin et al. 2014). The other form is "pathological" autoantibodies, the so-called because they can lead to fertility dysfunction by impeding sperm **motility** and their ability to penetrate the **cervical mucus** (Barbonetti et al. 2019). Furthermore, ASAs may interfere with the **acrosome response** and with sperm **binding to the zona pellucida** of the oocyte (Shibahara et al. 2020). It is important to emphasize here that ASAs rarely exhibit specificity to sperm epitopes, but are mostly directed against ubiquitously occurring proteins (e.g., chaperones) (Kurpisz et al. 2017). The classification of ASA-associated male fertility disorders in terms of a distinct autoimmune disease has not been established (Bohring and Krause 2003; Fijak et al. 2018). Neither "natural" nor "pathological" ASA lead to autoimmune pathologies in other organs. However, **cross-reactivity of pathogen-specific antibodies** with sperm antigens has been reported as a cause of an association between infections and male infertility, e.g., for *Helicobacter pylori* (Moretti et al. 2015). Increased ASA prevalence has also been reported in association with the detection of human papillomavirus (HPV) in ejaculate (Garolla et al. 2013). In an Australian study, *Chlamydia trachomatis*-specific DNA and membrane proteins were found in biopsies from infertile men in 16% (fresh) to 45% (fixed) of samples. In some patients, findings correlated with the presence of *Chlamydia*-specific antibodies in serum, although cross-reactivity of these cohorts with sperm antigens for *Chlamydia* has not yet been reported (Bryan et al. 2019).

The exact mode of origin of ASA is unclear. Epididymal involvement in the formation of ASA is most likely due to its close association with **male reproductive tract obstruction** (Fijak et al. 2018; Lotti et al. 2018). Possible mechanisms include increased intraductal pressure and chronic phagocytosis of spermatozoa, which may trigger an autoimmune response to antigens that are normally tolerated or weakly immunogenic. Mouse experiments suggest a role for CD4 + CD25+ regulatory T cells, whose normal function in preventing autoimmunological processes may be inhibited (Rival et al. 2013).

Although ASA are also found as "natural" autoantibodies in otherwise fertile and healthy men, a clinicopathologic association has been documented primarily with **male reproductive tract obstruction** (Marconi et al. 2009). In vasectomized men, there is a high and increasing rate of positive serum ASA findings with increasing time interval after vasectomy, reaching 60–70% 5 months after surgery (Adams and Wald 2009). An increased risk of ASA induction has also been described in patients with varicoceles (Bozhedomov et al. 2014). A pathophysiologically obvious link between infections and inflammatory processes in the genital tract (see Chaps. 19 and 26) and the formation of ASA are controversially discussed (Francavilla and Barbonetti 2017; Marconi et al. 2009). In a recent study, detection of IgG-ASA in the MAR test was associated with clinical sonographic signs of chronic **epididymitis** as well as a positive epididymitis history (Lotti et al. 2018). In the vast majority of cases, the cause for the occurrence of ASA remains unclear.

28.4 Clinical Aspects

The clinical correlate for the presence of ASA is otherwise unexplained infertility in which normozoospermia or isolated astheno(terato)zoospermia may be observed. The spontaneous conception probability depends on the proportion of sperm carrying ASA (Abshagen et al. 1998 [see Fig. 28.1]). In couples with a 100% positive male MAR test, the rate of live births after **spontaneous conception** was significantly reduced compared with those with a 50–99% positive MAR test (Barbonetti et al. 2020).

While the functional relevance of ASA for fertility was not considered certain in previous reviews (Mazumdar and Levine 1998), more recent studies demonstrate a negative association between positive ASA findings in the MAR test and **progressive motility** as well as **sperm count** in the ejaculate (Barbonetti et al. 2019; Verón et al. 2016). Among the main pathomechanisms of ASA-related infertility is the impairment of the ability of sperm ASA to penetrate the cervical mucus (Francavilla and Barbonetti 2017). In this context, **IgA autoantibodies** are considered to be of particular functional importance (Abshagen et al. 1998; Leushuis et al. 2009; World Health Organization 2010). Patients with a 100% positive MAR test had an increased prevalence of a pathologic or negative **post-coital partner test** (Barbonetti et al. 2019). Furthermore, ASA may interfere with **sperm capacitation** and **acrosome response**, as well as with **sperm binding to the zona pellucida** of the oocyte (Vickram et al. 2019).

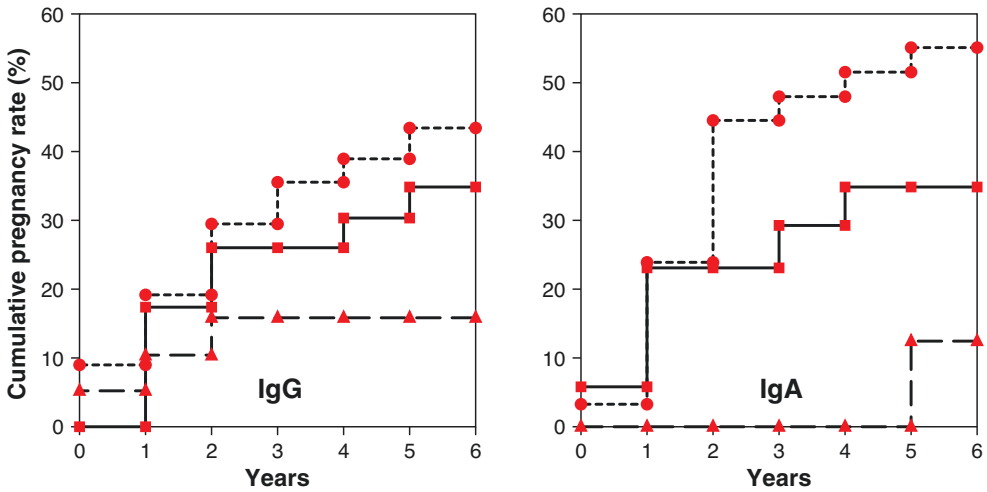


Fig. 28.1 Cumulative pregnancy rate in patients with anti-sperm antibodies. Circles, proportion of anti-sperm antibody coated sperm 10–49%: squares, 50–90%; triangles, >90%. Left panel, IgG anti-sperm antibodies; right panel, IgA anti-sperm antibodies (Abshagen et al. 1998)

Because of the high prevalence of ASA in vasectomized men, testing for antibodies in the ejaculate should be performed after **microsurgical reanastomosis** if pregnancy does not occur (see Chaps. 40 and 47).

28.5 Diagnostics

Sperm agglutination observed in native ejaculate may be a first indication of ASA, as may be a characteristic disturbance of sperm motility, the so-called shaking, in **periovulatory cervical secretions** (Gatimel et al. 2018; Shibahara et al. 2020; World Health Organization 2010). However, ASA may also be present in the absence of such phenomena. The detection of **IgG or IgA autoantibodies** bound to sperm is performed according to the WHO recommendations in the context of ejaculate analysis in the **direct MAR test** (Mixed Antiglobulin Reaction Test) or **immunobead test** (see Chap. 9). The diagnosis of the so-called immunologically induced infertility is made when 50% or more of motile sperm (progressive and non-progressive motile) have adherent particles (World Health Organization 2010). This cutoff value is consensus based. The extent to which the localization of antibody binding in the MAR assay has functional relevance has not been conclusively determined. In patients with 100% IgG-positive MAR test, a mixed pattern was predominantly found, i.e., particle binding to head segment and along the flagellum; IgA-ASA could be detected simultaneously (Barbonetti et al. 2019).

The **indirect MAR test** is used to detect unbound ASA in seminal plasma or serum, for example, when there is insufficient motile sperm in the ejaculate or azoospermia (WHO 2010). However, the significance is limited due to high interassay variability (Bohring and Krause 2002).

For the detection of unbound ASA, **enzyme immunoassays** have also been established in which biochemically undefined sperm extracts are used as antigen; with regard to the significance, the same limitations apply as for the indirect MAR test (Bohring and Krause 2003). A recently described biochip technique for the detection of ASA in serum is also based on sperm extracts as substrate (Xu et al. 2020).

In addition, ASA have also been determined by **flow cytometry**, which can simultaneously detect both the degree of IgG and IgA antibody binding on the sperm surface and differentiate vital and dead sperm (Räsanen et al. 1992). However, due to the complexity of the equipment, this method has not been widely used in practice.

However, the aforementioned test systems do not detect the antigen specificity of ASA, which is crucial for an understanding of the pathomechanisms of ASA-related infertility. The use of two-dimensional gel electrophoresis to separate sperm membrane proteins followed by immunoblotting and mass spectrophotometric identification of the addressed proteins is reserved for research (Bohring and Krause 2003).

Not every antibody detection may be equated with the diagnosis of immunological infertility. A so-called immunologically induced fertility disorder is only present if ASA lead to a significant disturbance of sperm function (e.g., mucus penetration, binding to the zona pellucida of the oocyte, acrosome reaction).

Antibodies against sperm in serum in males and females (also called **isoantibodies** in the female tract) have no significance for fertility and their determination can be omitted except for special questions (see above) (Shibahara et al. 2020). From a reproductive medicine point of view, it would be extremely useful to further clarify the clinical significance of ASA by **sperm-cervical mucus interaction diagnostics**, which unfortunately has hardly been performed in recent years (see Chap. 41).

28.6 Therapy

Immunosuppressive therapy with **corticosteroids** in immunologically induced infertility showed only inconsistent improvement in spontaneous pregnancy rates (Haidl et al. 2019). A long duration of treatment seems to be required for this therapy to be successful, which is associated with a high risk of corticosteroid-related side effects and is therefore rarely performed anymore.

Assisted reproduction methods can be used to overcome the disorders of sperm function and fertility associated with ASA (see Chap. 42). In couples with 100% IgG-ASA-positive sperm in the male MAR test, **intrauterine insemination (IUI)** was associated with a higher live birth rate than with a lower ASA percentage in a retrospective study (Barbonetti et al. 2020). A randomized controlled trial demonstrated that short-term supplemental administration of corticosteroids to men with ASA can significantly improve pregnancy rates with **in vitro fertilization (IVF)** (Taiyeb et al. 2017). The use of **intracytoplasmic sperm**

injection (ICSI) appears to reverse the negative effect of ASA on fertilization and pregnancy rates, eliminating the need for additional corticosteroid therapy in men (El-Sherbiny et al. 2021).

Key Points

- Fertility disorders resulting from the formation of ASA are clinically referred to as "immunologically induced infertility.".
- In addition to ASA, other immunopathologic processes in the male genital tract can lead to infertility.
- Patients evaluated for couple infertility show an ASA prevalence (≥ 50% positive sperm in the MAR test) of 3.4–6.6%, whereas in fertile men the frequency is approximately 1%.
- The spontaneous conception probability depends on the proportion of sperm carrying ASA.
- Among the major pathomechanisms of ASA-related infertility is impairment of the ability of sperm to penetrate the cervical mucus.
- ASAs may interfere with sperm capacitation and acrosome response, as well as with sperm binding to the zona pellucida of the oocyte.
- Detection of IgG or IgA autoantibodies bound to sperm is performed according to WHO recommendations in the context of ejaculate analysis in the direct MAR test (Mixed Antiglobulin Reaction Test) or immunobead test.
- Assisted reproduction methods can be used to overcome the disorders of sperm function and fertility associated with ASA.

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Andrologically Relevant Changes in the External Genitals

29

Frank-Michael Köhn

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Abstract

The andrological examination of the male includes an inspection of the external genitals. A variety of dermatologic changes may be noted. Not all skin changes have clinical relevance, but are nevertheless of importance in consultation because of inquiries from concerned patients. Papillae coronae glandis and heterotopic sebaceous glands are examples of normal variants. Most pigmented moles, angiokeratomas, fibromas, and angiomas do not require therapy.

In addition, inspection of the genital skin also offers the possibility of early diagnosis of malignant neoplasms (e.g., erythroplasia Queyrat), infectious diseases (e.g., condylomata acuminata), or generalized skin diseases (e.g., psoriasis vulgaris).

Since diseases of the external genitalia usually worry the affected patients, they can lead to impairment of sexual life, have serious consequences in the case of malignant changes, or, if infectious, can lead to infection of partners; also, in the context of reproductive medical procedures, andrologists should be familiar with their recognition and therapy.

29.1 Introduction

Diseases of the external genitalia may be **congenital malformations** or **acquired diseases** derived from deeper anatomical structures; they may occur in the context of generalized skin diseases or be caused by localized skin lesions. **Inflammatory and infectious** diseases as well as **benign** and **malignant neoplasms** must be differentiated. The burden of suffering is especially high when they lead to impairment or pain during sexual intercourse. Differential diagnosis of penile diseases includes infections, noninfectious balanitis/balanoposthitis, neoplastic diseases, trauma, papulosquamous or systemic diseases, drug reactions, allergic or irritant contact dermatitis, and various other diseases (English 3rd et al. 1997; Köhn 2016a; Köhn et al. 2016a, 2016b; Gabrielson et al. 2019).

Thus, abnormalities of the external genitalia, which are evident in the course of andrological examinations, can be classified as follows:

- Normal variants
- Benign neoplasms of the penis and scrotum
- Specific diseases of the glans penis and praeputium
- Infectious diseases
- Genital involvement in systemic dermatoses
- Malignant neoplasms
- Congenital malformations
- Induratio penis plastica

29.2 Skin Lesions Without Pathological Significance and Benign Neoplasms

29.2.1 Papillae Coronae Glandis (Pearly Penile Papules)

29.2.1.1 Introduction

Papillae coronae glandis occur with a frequency of about 20–50% in all men.

Synonyms are hirsuties papillaris penis, hirsutoid penile papillomas, and "pearly penile papules" (Bunker 2004; Köhn 2014a).

29.2.1.2 Clinic

At the proximal margin of the glans, before its transition into the sulcus coronarius, there is a single- or multiple-rowed fringe of whitish-red, fine, and quite regular nodules (1–3 mm) that appear partially vascularized (Fig. 29.1). Histologically, the skin lesions correspond to small **angiofibromas**. Human papilloma viruses are not detectable, so there is no danger of contagion.

29.2.1.3 Differential Diagnoses

The main differential diagnoses include condylomata acuminata and ectopic sebaceous glands. Dermatoscopy may be



Fig. 29.1 Pronounced papillae coronae glandis

helpful in differentiating between condylomata acuminata and papillae coronae glandis.

29.2.1.4 Therapy

Therapy is not necessary. If therapy is still desired for cosmetic reasons, successful approaches using an ablative laser or, alternatively, cryotherapy, curettage, or electrodessication have been described (Honigman et al. 2020).

29.2.1.5 Andrological Relevance

Educate the patient about the harmlessness of the changes. Diagnostic differentiation from condylomata acuminata.

29.2.2 Heterotopic Sebaceous Glands

29.2.2.1 Introduction

Free sebaceous glands, i.e., **sebaceous glands not associated with a hair follicle**, occur in various localizations of the skin. Synonyms are ectopic or heterotopic sebaceous glands (Bunker 2004; Köhn 2014b). They are found in more than 80% of people in the area of the labial red and buccal mucosa ("Fordyce's condition"). In addition, they occur in women on the labia minora and the inner sides of the labia majora. Free sebaceous glands in the area of the nipple are called "Montgomery nodes."



Fig. 29.2 Ectopic sebaceous glands

29.2.2.2 Clinic

On the penis, ectopic sebaceous glands occur preferentially on the inner prepuce. However, they are also detectable on the remaining penile skin, the scrotum, and very rarely, on the glans penis. Clinically, they appear as 1–3 mm large, yellowish, and non-irritant papules in larger numbers (Fig. 29.2). They are particularly visible when the penile skin is stretched or taut (e.g., during erection). In contrast to sebaceous gland hyperplasia in the facial region, free sebaceous glands on the penis develop at a younger age.

29.2.2.3 Differential Diagnoses

Milia, lichen nitidus, mollusca contagiosa, HPV-induced viral warts, papillae coronae glandis, lymphangioma circumscriptum, and multiple syringomas (Paolino et al. 2018).

29.2.2.4 Therapy

Therapy is not indicated. If therapy is desired for cosmetic reasons, successful approaches with small punch excisions have been described.

29.2.2.5 Andrologic Relevance

Educate the patient about harmlessness of the changes. Diagnostic differentiation from condylomata acuminata is usually sufficient.

29.2.3 Hemangiomas

29.2.3.1 Introduction

So-called senile angiomas occur most frequently in adults and are usually of no clinical significance. They are found in more than 50% of adults (Bunker 2004; Köhn 2014c).

In men presenting to a genital disease outpatient department of a university dermatology clinic, hemangiomas including angiokeratomas were **detectable in 4.5% of the genital area** (Michajłowski et al. 2012).

Synonyms are ruby spots, haemangioma senile, tardive hemangioma, and cherry angiomas.

29.2.3.2 Clinic

"Senile/eruptive" angiomas are usually intensely red-colored, flat raised nodules less than 5 mm in diameter (Fig. 29.3). They occur predominantly on the trunk and increase in size after the third decade of life. There is usually no association with internal disease. The histologic correlate is capillary vascular tangles without atypia.

29.2.3.3 Differential Diagnoses

Differential diagnoses are bright solitary angiokeratomas and small capillary or cavernous angiomas.

29.2.3.4 Therapy

Therapy is not indicated for the senile/eruptive angiomas. Exceptions can only be subjective impairment of the patient by the clinical findings in the genital area or ambiguities in the diagnosis requiring a histological diagnostic confirmation.

Therapeutically, laser therapy or electrocoagulation is used. Cryotherapy is performed less frequently.

29.2.3.5 Andrological Relevance

Informing the patient that the changes are harmless.



Fig. 29.3 Angioma senilis of the penile trunk

29.2.4 Penile Nevus Cell Nevi

29.2.4.1 Introduction

Melanocytic nevi ("moles") are circumscribed proliferations of melanocytes.

They may exist from birth (congenital melanocytic nevi) or develop later in life (acquired melanocytic nevi).

Melanocytic nevi occur in varying numbers in virtually everyone. There are few studies on the frequency of acquired **melanocytic nevi** in the genital region. They occur in approximately 9–10% of the white population (Michajłowski et al. 2012). They do not appear to be a significant risk for the development of malignant melanoma in most cases when the above frequency is compared with the rare occurrence of malignant melanoma on the penis.

Few melanomas of the glans penis or male urethra have been described in the literature. **Penile melanomas** account for only **1–1.5% of all malignant changes on the penis** and 0.15% of all melanomas. Penile melanomas most commonly involve the glans penis (55%), followed by the prepuce (28%), penile shaft (9%), and meatus urethrae (8%) (Baraziol et al. 2017; Papeš et al. 2014).

29.2.4.2 Clinic

Melanocytic nevi on the penis can vary in size from one to several millimeters or beyond (Bunker 2004; Hengge and Meurer 2005; Köhn 2014d). Their pigmentation shows a wide range from light brown to dark brown (Fig. 29.4). Atypical nevi appear to be very rare in this region.



Fig. 29.4 Nevus cell naevus on the penile trunk without evidence of malignancy

A special clinical manifestation in the genital region is the so-called divided nevus or "kissing nevus." This is a very rare congenital nevus (Godinho et al. 2017). In these cases, the circumscribed pigmented changes have developed at opposite sites (e.g., glans penis and inner foreskin leaf). This variant is due to the immigration of melanocytes before the separation of anatomical structures during the embryonic period. Malignant transformation appears to occur very rarely.

29.2.4.3 Differential Diagnoses

Differential diagnosis must include nevi coerulei ("blue nevi"), hemangiomas, malignant melanomas, syndromes with lentigines, and the melanotic spot of the penis. Reflected light microscopy and histological examination after removal of the skin lesions can confirm the diagnosis.

29.2.4.4 Therapy

In typical melanocytic nevus cell nevi, removal is usually not necessary. The patient should be informed and observe the skin lesion according to the **ABCDE** rule (**a**symmetry, **b**order, **c**olor, **d**iameter/dynamic, **e**levation). If necessary, a dermatoscopic image is recommended with the possibility of comparison during control examinations. In case of diagnostic uncertainty (e.g., suspicion of malignant melanoma), the pigmented skin lesion should be removed and examined histologically.

29.2.4.5 Andrological Relevance

Incidental finding during andrological examination. As a preventive measure, the patient should be advised to seek dermatological clarification in the event of changes. If malignancy is suspected, immediate presentation to a dermatologist.

29.2.5 Penile Lentiginosis

29.2.5.1 Introduction

Penile lentigines usually occur in early adulthood (fourth decade of life). They are benign, purely **macular, and pigmented skin lesions of varying color** intensity with a low growth tendency due to increased melanin production by melanocytes that appear morphologically normal and numerically normal or slightly increased (Lenane et al. 2000).

29.2.5.2 Clinic

Brown patches develop slowly (years to decades) on the glans penis, increasing in size, forming sharply demarcated bizarrely configured medium- and dark-brown mottled foci, confluent, and may also spread to the internal prepuce (Fig. 29.5). Rarely, previous trauma or irritation has been described. The course is benign; in atypical lentigo (initial melanoma in situ), the history is usually much shorter.



Fig. 29.5 Extensive penile lentiginosis. Trial biopsies were taken to exclude malignant melanoma

The epidermis shows irregular acanthosis with severe basal hyperpigmentation and typical melanocytes without nest-like aggregation.

29.2.5.3 Differential Diagnoses

Only rarely does the differential diagnosis of initial acral lentiginous malignant melanoma arise, which is also a rarity in people up to 30 years of age. In any case of doubt, a biopsy is indicated.

29.2.5.4 Therapy

If possible, avoidance of mutilating surgical procedures. Either regular control or superficial coagulation with diathermy or ruby laser.

29.2.5.5 Andrological Relevance

Mostly incidental finding in the course of andrological examination. Further dermatological clarification in case of unclear findings.

29.2.6 Fibroma Molle

29.2.6.1 Introduction

Fibromata molle are so-called **hamartomas**. Hamartomas are local tissue excesses, e.g., on the skin, as a result of embryonic maldevelopment. They usually remain benign.

Synonyms for fibroma molle are soft fibroma, fibroma pendulans, fibroepithelial polyp, or "skin tags."

They usually appear solitary or multiple only after puberty. They are most frequently observed in intertriginous areas such as axillae, groins or submammary, but also on the neck. There, they may be inflamed by irritation or become necrotic due to stem rotation.

Soft fibromas are significantly associated with **obesity, arterial hypertension**, dyslipidemia, and **insulin resistance** (Sari et al. 2010).

In obese patients, as well as in the general population, they are found in approximately 40–50%. Especially, in case of perianal involvement, the concomitant presence of Crohn's disease must be considered.

Regarding the occurrence of soft fibromas on the penis, there is only one study that could detect them in 7% of all examined men. The groin, scrotum, sulcus coronarius, and penile trunk were mainly affected (Bunker 2004, 4; Köhn 2014e; Michajłowski et al. 2012).

Histologically, soft fibromas contain varying proportions of epidermal, melanocytic, and vascular cells as well as adipose tissue.

29.2.6.2 Clinic

Soft fibroma appears as a skin-colored nodule with a usually wrinkled surface that is soft to palpation and pedunculated (Fig. 29.6). Malignant transformation is not known.

29.2.6.3 Differential Diagnoses

Because of the usually clear clinical appearance, the diagnosis is not difficult. Differential diagnoses include dermal or papillomatous nevi, neurofibromas, and more rarely condylomata acuminata.



Fig. 29.6 Fibroma molle. Differential diagnosis: papillomatous nevus cell nevus

29.2.6.4 Therapy

As long as there is no irritation of the skin lesion or diagnostic uncertainty, removal is not absolutely necessary.

Sometimes, however, affected men request this procedure because the fibroma is considered disturbing or unfounded uncertainty is expressed in the partnership regarding infectiousness.

In this case, superficial ablation is performed under local anesthesia. Suturing is often not necessary for smaller fibromas.

29.2.6.5 Andrological Relevance

None. Differential diagnostic differentiation from condylomata acuminata usually without problems.

29.2.7 Angiokeratomas

29.2.7.1 Introduction

Angiokeratomas in the genital area are found on the **penis and scrotal skin in up to approximately 50% of all men** (Cohen and Celano 2020). They result from ectasia of blood vessels in the stratum papillare and stratum reticulare with concomitant hyperkeratosis. If angiokeratomas occur without concomitant disease, they are more likely to be classified as a norm variant and not a relevant disease. This form of angiokeratoma typically develops in adulthood (Cohen and Celano 2020; Bunker 2004).

However, angiokeratomas can also occur in association with **Fabry disease (angioma corporis diffusum)** (Köhn 2016b). This systemic disease is rare overall; however, in the presence of Fabry disease, the occurrence of angiokeratomas is common, affecting approximately 50% of boys. In contrast to angiokeratomas without concomitant disease, they manifest in childhood and adolescence and preferentially affect the lower half of the body.

29.2.7.2 Clinic

Angiokeratomas of the scrotal skin, the penile trunk, and more rarely the glans penis appear as 2–5 mm large, livid-reddish, usually multiple, dark blue to black papules with a mostly smooth, but more rarely rough or keratotic surface (Fig. 29.7). If the penis is involved, angiokeratomas occur primarily on the glans penis (55.5%) and less commonly on the penile shaft (35%) and prepuce (5.5%) (Cohen and Celano 2020).

In angiokeratoma corporis diffusum (Fig. 29.8), patients may also suffer from a variety of other symptoms as a result of organ damage from storage disease.



Fig. 29.7 Typical scrotal angiokeratomas without pathologic value



Fig. 29.8 Multiple penile and scrotal angiokeratomas. Incidental finding on andrologic examination leading to the diagnosis of Fabry disease

29.2.7.3 Differential Diagnoses

Angiokeratomas in their typical multiple forms are usually easily recognized. In solitary angiokeratomas, differential diagnoses may include nevi, malignant melanoma, Kaposi's sarcoma, or bacillary angiomatosis. In this case, incident light microscopy is helpful.

29.2.7.4 Therapy

Angiokeratomas can be removed by laser therapy, cryotherapy, electrocautery, or excision if necessary.

29.2.7.5 Andrological Relevance

Usually none. Informing the patient that the changes are harmless. In very rare cases with pronounced findings, the discovery of these skin changes is also an opportunity for clarification of previously undiagnosed Fabry disease.



Fig. 29.9 Median raphe cyst

29.2.8 Median Raphe Cyst

29.2.8.1 Introduction

The external genitalia is formed during embryonic development by fusion of the paired urethral folds. A relic of this fusion zone is the raphe in the region of the penis and scrotal skin. As a consequence of **disturbances of folding/fusion**, so-called median raphe cysts may develop.

29.2.8.2 Clinic

Median raphe cysts (apocrine cystadenoma of penis) already occur in the first decade of life, but are more often diagnosed later. They present as skin-colored, bulging, spherical tumors, and usually up to about 1 cm in size (sometimes larger), in the area of the raphe between the orificium urethrae and the perineum (Fig. 29.9). They are usually asymptomatic; if located close to the urethral orifice (parameatal cyst), they may also cause dysuria at one time. Usually, they are solitary changes; rarely, multiple cysts are reported (Syed et al. 2019).

The epithelium may be glandular, epithelioid, or urethral in appearance.

Complications of these cysts may include superinfection.

29.2.8.3 Differential Diagnoses

Localization and clinical appearance usually permit definite diagnostic assignment. Perianal raphe cysts may be confused with hemorrhoids.

29.2.8.4 Therapy

Excision is indicated if symptoms are present or if the patient requests it.

29.2.8.5 Andrological Relevance

None. Mostly incidental finding. Reassurance of the patient.

29.3 Inflammatory Changes of the External Genitalia

29.3.1 Non-Venereal Coronary Lymphangitis or Coronary Phlebitis

29.3.1.1 Introduction

Non-venereal coronary furrow lymphangitis/coronary furrow phlebitis (Mondor's disease of the penis) is a blanched, stranded lymphangitis or phlebitis proximal to the glans penis that **usually occurs in younger men** (Köhn 2012).

29.3.1.2 Clinic

Clinically, the two conditions cannot be differentiated with certainty. Following forced sexual intercourse, but possibly also as an effect of other disorders (chlamydial urethritis, focal infections, chronic prostatitis), there is acute development of a sausage-shaped bulging cystic cord below the inner preputial leaflet that is vitreously translucent and transverse or oblique to the long axis of the penis (Fig. 29.10). More severe signs of inflammation are absent. Pain, however, may occur. Occasionally, mild edema of the internal preputial leaflet is found. Patients are concerned by the unusual change.

Limited data are available on the incidence of this condition. In men who visited a specialized outpatient clinic for sexually transmitted diseases, the diagnosis of coronary furrow phlebitis/lymphangitis was made in 1.4% (Kumar et al. 2005).

Spontaneous regression occurs in a few weeks. More severe inflammatory changes may occur after manipulations, even with ulceration.

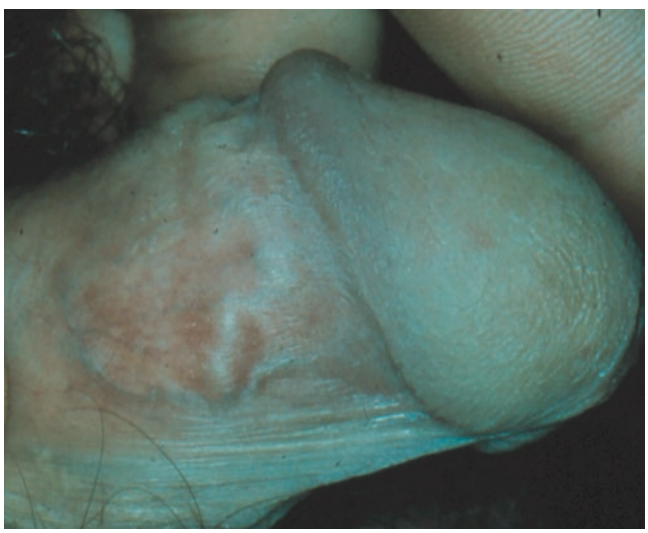


Fig. 29.10 Coronary furrow lymphangitis

29.3.1.3 Differential Diagnosis

Lymphangitis dorsalis penis as an accompanying symptom of primary syphilis I runs along the longitudinal axis of the penis and is much coarser. An examination for syphilis is advised.

29.3.1.4 Histopathology

Much more commonly than lymphangitis, there is obliterative endophlebitis with collagenization of the diseased vessel.

In rare cases, dilated lymphatic spaces with minor inflammation are found. In most cases, the site of obliterating phlebitis or lymphangitis is not reliably identified.

29.3.1.5 Therapy

Systemic therapy is usually not necessary; if internal treatment is necessary, nonsteroidal anti-inflammatory drugs such as acetylsalicylic acid, diclofenac, or indometacin are used.

Topical therapy is also usually not necessary because of spontaneous healing.

29.3.1.6 Andrological Relevance

Because of pain during sexual activity and sudden onset, patients are usually concerned. Therefore, informing the patient about the harmlessness and initiation of therapy (if needed) is the primary concerns. Long-term side effects are not expected.

29.3.2 Balanitis and Balanoposthitis

Balanoposthitis is an **inflammatory alteration of the glans (balanitis) and the inner preputial leaf (posthitis)** of various genesis. The most common causes are irritation (also iatrogenic, e.g., in the course of condyloma treatment, Fig. 29.11), infection with bacteria or fungi, or penile dermatoses such as lichen sclerosus et atrophicus, and less commonly, contact dermatitis (e.g., condoms, lubricants). Predisposing factors may include older age, congenital phimosis, preexisting penile dermatoses, poor or excessive hygiene ("overtreatment"), rubbing of clothing, metabolic disorders such as diabetes mellitus, or reduced immunity (Köhn 2016a). Balanoposthitis occurs much less frequently in circumcised men than in non-circumcised men. It is found in approximately 17% of men who attend a specialty outpatient clinic for genital skin disease (Shah 2017).



Fig. 29.11 Balanoposthitis associated with local therapy for condylomata acuminata (see proximal penile trunk)

Clinically, a distinction is made between **balanoposthitis acuta** and **balanoposthitis chronica**.

Causes of acute balanoposthitis include infection, acute exacerbation of penile dermatoses, trauma, noxious agents, smegma retention, and contact allergy. Iatrogenic triggers include local applications of podophyllotoxin, imiquimod, or green tea extract in the treatment of condylomata acuminata.

29.3.2.1 Andrological Relevance

Acute balanoposthitis usually requires immediate urologic or dermatologic evaluation. However, chronic balanoposthitis may be detected in the course of andrological examinations, which should then be further classified and treated. In the case of inflammatory phimosis or erosive skin changes, impairments may occur during sexual intercourse.

29.3.3 Fixed Drug Exanthema

After sensitization to a drug, fixed drug reactions manifest as solitary or multiple, sharply demarcated erythematous macules or plaques in which blisters may also develop (Fig. 29.12). Less commonly, ulcerations are observed. Typically, these changes **recur at the same site after use of the same drug** and heal within 2–3 weeks with postinflammatory hyperpigmentation. Substances that can cause fixed drug exanthema are as follows: tetracyclines, doxycycline, penicillin, phenolphthalein, sulfonamides, barbiturates, salicylates, dapsone, griseofulvin, carbamazepine, dimenhydrinate, metamizole, hydroxyzine hydrochloride, and colchicine (English 3rd et al. 1997; Köhn et al. 2016a).

29.3.3.1 Andrological Relevance

Fixed drug exanthema can occur in the setting of antibiotic therapy for seminal tract infections. Therefore, andrologists should be informed about this clinical picture and be able to classify it. In addition, patient education about recurrences when taking the same antibiotic must also be provided with other medication.



Fig. 29.12 Fixed drug exanthema on the penile trunk

29.4 Specific Diseases of the Skin of the Glans Penis and Praeputium

29.4.1 Balanoposthitis Chronica Circumscripta Benigna Plasmacellularis Zoon

29.4.1.1 Introduction

This balanoposthitis is a disease of unknown cause occurring mainly in **non-circumcised males in the fifth to eighth decade of life**. Irritative factors such as heat, trauma (friction), inadequate hygiene, or immunologic processes with IgE-mediated reactions are suspected triggers. Bacterial or viral causes have not yet been proven (Köhn et al. 2016a). This form of balanitis is not considered a precancerous

condition, although concomitant malignant changes of the skin have been reported in isolated cases (Wollina et al. 2017).

29.4.1.2 Clinic

Particularly on the dorsal side of the glans, but also on the inner foreskin blade, formation occurs of mirror-like plaques lying at the skin level and conspicuous by their brownish-red color without palpable increase in consistency (Fig. 29.13). Frequently, petechial punctate hemorrhages ("cayenne pepper spots") can be seen. Less commonly, the involvement may be multilocular or erosive. Subjectively, there may be mild burning or itching.

The course is chronic for years if left untreated. The prognosis is favorable in the absence of precancerous lesions.

The epidermis is markedly thinned in the area of the foci and shows discreet spongiosis and dyskeratosis with flattened basal keratinocytes. In the upper corium, dilated capillaries and plasma cellular inflammation with hemorrhages and hemosiderin deposition are found.

29.4.1.3 Differential Diagnoses

The most important differential diagnosis is Queyrat's erythroplasia, which is completely different in prognosis. Other differential diagnostic considerations include fixed drug reaction, contact dermatitis, psoriasis, lichen planus, or infectious forms of balanitis.

29.4.1.4 Therapy

Externally, glucocorticoid-containing topical preparations in a low-fat base and tacrolimus are used. The use of imiquimod for local therapy has also been reported. Good hygiene and keeping the preputial space dry (gauze strips) are supportive. Circumcision leads to improvement. Positive effects have also been described by treatment with CO2 or Erbium:YAG laser.

Although an infectious cause of balanitis plasmacellularis Zoon has not been demonstrated to date, there is evidence of response of this condition to local application of mupirocin (Cohen 2019).



Fig. 29.13 Balanoposthitis chronica circumscripta benigna plasmacellularis Zoon with involvement of the inner foreskin leaf alone

29.4.1.5 Andrologic Relevance

Especially in middle-aged and older men (e.g., when clarifying age-related hypogonadism), this disease may be an incidental finding during an andrologic examination. It requires differentiation from malignant changes of the penis and specific therapy.

29.4.2 Lichen Sclerosus Et Atrophicus

29.4.2.1 Introduction

Lichen sclerosus et atrophicus is **a chronic inflammatory connective tissue disease of the skin** that preferentially (>80%) affects the genital region. The cause of the disease is unclear; infectious and traumatic factors, autoimmune diseases, genetic factors, and hormonal factors are discussed (Fergus et al. 2020). Lichen sclerosus et atrophicus may be associated with diabetes mellitus, high body mass index, and coronary artery disease (Kirtschig et al. 2017).

29.4.2.2 Clinic

In women, lichen sclerosus et atrophicus is 5–10 times more common than in men. The peak of disease is biparental with a prepubertal peak, an increase in incidence after puberty, and decline after age 60. It mostly affects uncircumcised patients in middle age. The incidence in men is 0.07% (Kirtschig et al. 2017). Even in children, this skin disease is a common, although sometimes undiagnosed, phenomenon (Köhn and Möhrenschlager 2008; Köhn et al. 2016a).

Clinically, the disease presents with porcelain-like discolored, coarse, initially small-spotted foci that may eventually confluence to form larger areas with a parchment-like surface (Fig. 29.14). As the disease progresses, hemorrhages, erosions, and ulcerations may also occur. In males, adhesions between the glans penis and the internal foreskin sheet are the result. The orificium urethrae may become narrowed so that micturition and ejaculation are impeded. Initially, the skin lesions are asymptomatic; later, itching, burning, decreased sensitivity of the glans penis, and painfulness of erection or vaginal discomfort are reported. In males, there is scarring of the foreskin and secondary inflammatory changes due to tearing (rhagades) of the foreskin during erection and sexual intercourse.



Fig. 29.14 Lichen sclerosus et atrophicus with transition to spinocellular carcinoma

Always, in cases of genital involvement with lichen sclerosus et atrophicus, the entire integument, including the anal region, should be examined to detect extragenital foci of this disease.

29.4.2.3 Differential Diagnoses

Differential diagnoses of lichen sclerosus et atrophicus include vitiligo, postinflammatory hypopigmentation, scarring from trauma or surgery, or scarring pemphigoid.

29.4.2.4 Risks and Prognosis

Chronic progression of lichen sclerosus et atrophicus may result in **spinocellular carcinoma** in the genital foci. It has not yet been conclusively determined what the risk of this is. Recent studies suggest that it is much lower than originally thought (**up to 5%).** The risk for ano-genital carcinoma seems to be increased especially in familial cases of lichen sclerosus et atrophicus (Kirtschig et al. 2017).

29.4.2.5 Therapy

Therapeutically, circumcision should be considered early in men, as even skin lesions on the glans penis may show regression after circumcision. Local application of corticosteroid-containing (clobetasol) creams and also injections of glucocorticoids have been shown to be effective. Alternatively, externals with immunomodulators (pimecrolimus, tacrolimus) can be used as curative agents. Ablation of individual foci with CO_2 laser is possible. Positive effects have also been described by focused ultrasound (Kirtschig et al. 2017).

The testosterone-containing creams that were regularly recommended in the past are now considered obsolete.

29.4.2.6 Andrological Relevance

Lichen sclerosus et atrophicus may cause discomfort during sexual intercourse and ejaculation as a consequence of phimosis. Disease detected early in the course of an andrological examination may provide timely therapy and patient information about the (rare!) development of penile carcinoma.

29.4.3 Balanitis Erosiva Circinata (Circinate Balanitis)

29.4.3.1 Introduction

This is a **mostly chronic psoriasiform balanitis** that occurs spontaneously or is observed in combination with reactive arthritides (variant of peripheral spondylaorthritides) (Meier et al. 2020). It has also been previously characterized as part of Reiter's syndrome with the triad of urethritis, conjunctivitis, and arthritis. There is an association with Chlamydia trachomatis infections. Most patients are between 30 and 40 years of age. The incidence is 3.5 per 100,000 men under 50 years of age (Wu and Schwartz 2008).

29.4.3.2 Clinic

The disease usually begins with pinhead-sized, initially grayish-white macules in the preputial space that change to round, still small flesh-red erosions with gray margins (Fig. 29.15). These sharply demarcated foci enlarge on all sides and confluence. In this way, bizarre map-like eroded areas are formed, bordered on all sides externally by a whitish epithelial rim. The periurethral area of the glans remains mostly free. Painful itching or burning is not uncommon (Köhn 2012).

29.4.3.3 Therapy

For topical therapy of balanitis circinata, glucocorticoid-containing creams or tacrolimus are used temporarily. In erosive courses, desiccative measures are appropriate. Because of the association with psoriasis, cautious local therapy with creams containing calcipotriol may also be considered; however, because of the rarity of the disease, no extensive experience is yet available. The skin changes may also regress under systemic therapy of concomitant reactive arthritides (Meier et al. 2020).

29.4.3.4 Andrological Relevance

Balanitis circinata that is conspicuous during andrological examination should prompt further clarification of concomitant rheumatologic diseases. Only in the case of more pronounced inflammatory changes can it impair the patient's sexuality by causing discomfort. As a rule, however, patients are concerned about the visible changes and feel that their external perception is disturbed.



Fig. 29.15 Balanitis circinata

29.5 Genital Involvement in General Skin Diseases

29.5.1 Introduction

A large number of **inflammatory, allergic, genetically determined, or autoimmunological skin diseases** that also affect the rest of the skin can also affect the external male genitals. These include psoriasis vulgaris, atopic eczema, acne inversa, vitiligo, blistering diseases (pemphigus vulgaris, bullous pemphigoid), lichen ruber, or urticaria (hives) (English 3rd et al. 1997). As examples, changes caused by psoriasis vulgaris, atopic eczema, and acne inversa are discussed here (Köhn et al. 2015).

29.5.2 Psoriasis Vulgaris

29.5.2.1 Introduction

The prevalence of psoriasis is approximately 3% of the population (Elston 2021). In patients with hereditary predisposition, there is an inflammatory reaction mediated by cytokines (mainly interleukin-17A and 23, tumor necrosis factor alpha), accompanied by an excessive division rate and formation of keratinocytes as well as an invasion of neutrophil granulocytes (Grän et al. 2020). The disease is characterized by sharply demarcated erythema with whitish scaling, especially on the extensor sides of the extremities. However, the entire integument can be affected, including the scalp and genito-anal region (Fig. 29.16). In patients with generalized plaque psoriasis, the **genital region is involved in up to 40%** (da Silva et al. 2020).

The lifetime risk of developing arthritis is approximately 30% of patients affected by psoriasis (Ogdie et al. 2020).

Psoriasis may be associated with metabolic comorbidities (obesity, smoking, arterial hypertension, diabetes mellitus).

29.5.2.2 Andrological Relevance

Psoriasis is significantly associated with the occurrence of erectile dysfunction. Men with psoriasis are twice as likely to have erectile dysfunction compared with men with other skin conditions (Goulding et al. 2011).

The reasons for these associations are not only due to psychogenic factors such as the clustered occurrence of anxiety and depression (Kurizky and da Mota 2012). Psoriasis is disproportionately associated with cardiovascular and endocrine disorders (coronary artery disease, myocardial infarction, atherosclerosis; metabolic syndrome), which in turn are risk factors for erectile dysfunction.

Patients with psoriasis have problems with dating (social contacts) and initiation of sexual relationships in up to more than 50% (Ramsay and O'Reagan 1988; Gupta and Gupta 1997; Cather et al. 2017).

Infestation of the genito-anal region not only affects patients' sexuality but also affects their quality of life in general (da Silva et al. 2020).

Andrological aspects of psoriasis vulgaris such as associations with reduced semen quality (Caldarola et al. 2017)



Fig. 29.16 Psoriasis vulgaris

and reduced serum total testosterone (Allam et al. 2019; Perez-Garcia et al. 2020) are still insufficiently studied.

Psoriasis therapy (e.g., with methotrexate) may also acquire andrologic relevance when counseling patients or in response to queries from medical colleagues.

29.5.3 Atopic Dermatitis

29.5.3.1 Introduction

Atopic dermatitis is an inflammatory disease that preferentially affects the flexor sides of the extremities but may also occur on the other integument and is usually associated with severe pruritus. It affects people of all ages. The etiopathogenesis is complex and includes epidermal dysfunction and inflammatory reactions in addition to genetic predisposition (Langan et al. 2020).

Atopic dermatitis manifests as scaly erythema in sebostatic skin that is prone to lichenification and scratch excoriations from frequent itching (Fig. 29.17).

In industrialized countries, approximately 10% of the adult population is affected (Tsakok et al. 2019). **More than 40% of patients (both men and women) report concurrent genital atopic dermatitis** (Woo et al. 2021).

29.5.3.2 Andrological Relevance

Atopic dermatitis is associated with limitations in quality of life (Woo et al. 2021). Due to the impairment of self-esteem as a result of skin changes, many dimensions of patients' sexuality are disturbed (Misery et al. 2019; Ludwig et al. 2020).

However, data on a possible association of atopic dermatitis with erectile dysfunction are still conflicting (Chung et al. 2012; Egeberg et al. 2017).



Fig. 29.17 Atopic scrotal dermatitis with severe pruritus

29.5.4 Acne Inversa (Hidradenitis Suppurativa)

29.5.4.1 Introduction

Acne inversa is a chronic inflammatory disease of hair follicles associated with painful nodules and abscesses and prone to fistulation and scarring. Regions affected are those with apocrine glands such as the axillae, inguinal, and genito-anal regions. **The confirmed prevalence is up to 0.5%** (Nikolakis et al. 2020).

29.5.4.2 Andrological Relevance

The sexuality of patients with acne inversa is impaired, especially when the genito-anal region is affected, due to the often chronic inflammatory changes with secretion and pus secretion as a result of fistula formation. Erectile dysfunction is described in up to more than 50% (Cuenca-Barrales et al. 2021). Limitation of quality of life was correlated with severity of acne inversa, while disease severity was not related to the extent of sexual dysfunction.

29.6 Genital Dermatoses Due to Infections

29.6.1 Viral Infections

29.6.1.1 Human Papillomaviruses

29.6.1.1.1 Introduction

Anogenital warts (synonyms: genital warts or condylomata acuminata) are caused by human papilloma viruses (HPV). These belong to the DNA viruses and infect the squamous epithelium of the skin and mucous membranes (Köhn 2016c). Transmission occurs through direct contact; although sexual intercourse plays a primary role in genital warts, smear infections are also possible after contact with contaminated surfaces due to the viruses' resistance to desiccation. The **incubation period** after infection with HPV is approximately **4 weeks to several months.**

29.6.1.1.2 HPV Types

Approximately 200 different HPV types are known, of which about 40 can infect the anogenital region. **The vast majority of anogenital warts** are caused by **HPV types 6 and 11**, which are not associated with an increased risk of developing malignant changes (**low-risk HPV).**

Some HPV types (**HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59**) are oncogenic (**high-risk HPV**). They are considered a major factor in the development of cervical, penile, and other anogenital carcinomas or their precursors (e.g., cervical intraepithelial neoplasia (CIN), vulvar intraepithelial neoplasia (VIN), and penile intraepithelial neoplasia (PIN)).

29.6.1.1.3 Epidemiology

Approximately **1% to 2% of the sexually active population between the ages** of 15 and 49 are affected by anogenital warts. In Germany, the incidence for anogenital warts is estimated to be 169.5/100,000 persons (aged 10–79 years) per year. Most infections affect individuals between 15 and 45 years of age (Robert Koch-Institut 2018).

However, human papillomaviruses can **also be detected in clinically healthy genital skin in more than 50% of men** (Sichero et al. 2014).

29.6.1.1.4 Clinic

In most cases, infection with HPV appears to proceed without clinically apparent skin lesions. Clinical manifestations of HPV infection in the male anogenital area include:

- Anogenital warts (condylomata acuminata).
- Bowenoid papulosis (severe intraepithelial neoplasia in the penile region in the form of flat, reddish-brownish maculo-papular skin lesions, usually with evidence of HPV 16)
- Condylomata gigantea (Buschke–Löwenstein tumors, large, verrucous-appearing tumors in the genital area)
- Perianal intraepithelial neoplasia (PAIN)
- Anal intraepithelial neoplasia (AIN)
- Penile intraepithelial neoplasia (PIN)

The typical genital warts initially begin as pinhead-sized, skin-colored or even slightly pigmented, sharply demarcated papules (Fig. 29.18), which may confluate in the course of their development or may also form cockcomb-like structures. They then present as variously sized, reddish tumors with a fissured surface. In men, genital warts preferentially occur on the **glans and foreskin (75%) or on the penile trunk (25%),** but can also be found in the anal region or on other parts of the body. Intraurethral and intraanal condylomas require special attention.



Fig. 29.18 Condylomata acuminata on the penile trunk

Condylomata acuminata may persist for months to years and in about one-third of cases may heal spontaneously.

29.6.1.1.5 Differential Diagnoses

Condylomata lata in syphilis, soft fibromas, papillae coronae glandis, verrucae seborrhoicae, papillomatous pigmented nevus cell nevi, lichen ruber planus, and mollusca contagiosa.

29.6.1.1.6 Diagnostics

The macroscopic findings are usually so typical that a diagnosis can be made without difficulty. In cases of doubt or atypical clinical presentation, histological examination is useful. Histological examinations are also recommended in cases of increase in size under therapy or resistance to therapy, anogenital warts in immunosuppressed patients, and large (> 1 cm) condylomas.

The so-called acetic acid test can be used to support the diagnosis. In this test, suspicious lesions of the skin (e.g., on the penile trunk) are wetted with (3)–5% acetic acid. A white coloration appearing after a few minutes may indicate HPV-induced skin changes. Only 3% acetic acid should generally be used on the mucosa.

Routine proctoscopy or urethroscopy is not indicated and is only performed in suspicious cases. Since more than 90% of urethral condylomas are localized in the region of the meatus urethrae, spreading of the urethral orifice and careful examination of this region is of particular relevance. Prior to this, external condylomas should be adequately treated to avoid smear infection from the examination.

HPV typing with differentiation between "high-risk" and "low-risk" types should be performed in very large condylomas and in children. It is usually not relevant for diagnosis.

If **HPV-induced changes** are diagnosed, **screening for other sexually transmitted diseases** such as HIV, lues, chlamydia, or hepatitis B and C is also recommended (Gross et al. 2018; Workowski et al. 2015).

29.6.1.1.7 HPV Screening and Prevention

Routine HPV typing in men with anogenital warts or to rule out infection without evidence of genital warts is not recommended (Gross et al. 2018; Workowski et al. 2015).

Given the high rates of HPV detection in healthy genital skin of men, appropriate HPV typing in healthy skin of men whose partners have condylomas also cannot be recommended. However, careful clinical examination of men for the presence of anogenital warts and their therapy are mandatory in this case.

Each patient must be informed about the cause, complications (development of penile carcinomas in case of infection with "high-risk" HPV types), and possibility of infection of sexual partners including risks (development of cervical carcinomas in case of infection with "high-risk" HPV types). The different transmission routes (e.g., by orogenital contacts) should be known to the patient. Psychological and sexual-medically relevant aspects, which affect the patient, should also be addressed. It is advisable to emphasize to couples and patients that both partners usually have common HPV types and that the infection of one partner does not necessarily have to be caused by sexual contacts with other persons.

All persons, with whom the affected man has had sexual contact in the past 6 months, should be informed so that they can also be tested (Gross et al. 2018).

Reducing the number of sexual partners as a preventive measure should be emphasized.

Because HPV-induced skin lesions also occur on proximal portions of the penis, scrotum, or abdominal wall, condoms do not provide complete protection. In addition, latex condoms may be damaged by prior application of imiquimod to the penile skin.

It is possible that circumcision has a protective effect on the incidence of genital HPV infections (Yuan et al. 2019).

29.6.1.1.8 Therapy

Treatment of anogenital warts involves **procedures** that patients can perform under **home conditions** and others that can only be performed by a **physician.**

Following instructions, patients can apply imiquimod cream, epigallocatechol gallate/sinecatechins (green tea extract), or podophyllotoxin. Side effects (irritation) and limitations of use (e.g., size of area to be treated) should be considered.

Physician-only methods include cryotherapy, trichloroacetic acid, surgical removal, electrocoagulation, and ablation by laser (Cave! HPV in vapors).

29.6.1.1.9 Vaccination

Vaccination with HPV vaccines is not uniformly regulated, neither worldwide nor at the European level. Basically, **bivalen**t (HPV types 16 and 18), **quadrivalent** (HPV types 6, 11, 16, and 18), and **9-valent** (HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58) **vaccines** are available.

In Germany, the Standing Commission on Vaccination currently recommends vaccination of 9- to 14-year-old girls and boys, ideally before sexual contact occurs [AG HPV der Ständigen Impfkommission (STIKO) 2018].

The current German S3 guideline on vaccine prevention of HPV-induced neoplasms includes a recommendation to vaccinate HPV vaccine-naive adults aged 18–26 years regardless of sex (Gross et al. 2021).

The U.S. Center for Disease Control and Prevention advocates universal vaccination coverage by age 26 years if vaccination has not previously occurred (at age 11–12 years). Vaccination at age > 26 years is not recommended, but could still be applied between 27 and 45 years of age after individual consideration (Meites et al. 2019).

The consensus to date is that vaccination is not recommended for the treatment of preexisting HPV-induced changes.

29.6.1.1.10 Andrological Relevance

Genital warts diagnosed during an andrological examination provide opportunities for therapy and further diagnostics in sexual partners.

HPV can also be detected in the ejaculate of asymptomatic men. An influence on sperm quality and sperm functions has been demonstrated in various studies, but cannot be conclusively assessed at present (Moreno-Sepulveda and Rajmil 2021; Wang et al. 2021).

29.6.1.2 Herpes Simplex Virus (HSV)

29.6.1.2.1 Introduction

Genital herpes infection is **usually triggered by herpes simplex virus type 2 and less commonly by herpes simplex virus type 1**. HSV are DNA viruses that persist in sensory ganglia and then re-form clinical lesions upon reactivation (Möhrenschlager et al. 2004). Reactivation can be triggered by immunosuppression, UV light exposure, endocrinological factors, emotional stress, and neurosurgical procedures on ganglia. Infection can occur even with macroscopically unremarkable skin and mucosal findings (Patel et al. 2017).

29.6.1.2.2 Clinic

Primary infection with HSV has an **incubation period of 3 to 7 days**. This is followed by the development of erythema with grouped, pruritic, painful, and serous vesicles (Fig. 29.19). Concomitant inguinal lymphadenitis, fever, headache, and general ill feeling may occur. Primary HSV infection is followed by at least one symptomatic recurrence in approximately 85% of patients. Recurrent genital lesions occur in approximately 20% of infected individuals. Overall, however, the frequency of recurrence is subject to large intra- and interindividual variations. Clinically, only about 30% of cases of genital HSV manifest.

The diagnosis can often be made clinically. However, DNA detection by PCR is also possible to confirm the diagnosis (Patel et al. 2017).

29.6.1.2.3 Differential Diagnosis

In the acute stage, acute contact dermatitis, fixed drug exanthema, bullous sting reactions, bullous lichen sclerosus et atrophicus, primary effect of syphilis, ulcus molle,



Fig. 29.19 Genital herpes with typical grouped vesicles

lymphogranuloma venereum, autoimmune and bullous diseases, Behcet's disease, and zoster, among others, must be excluded.

29.6.1.2.4 Therapy

Therapy with viral drugs (aciclovir, valaciclovir, famciclovir) is effective only if given early. In the case of frequent, recurrent reactivations of genital herpes, suppressive therapy with the above-mentioned viral drugs can be carried out over a period of months. In addition, analgesics and, in the case of superinfections, local antibiotically or antiseptically effective topical preparations can be used.

29.6.1.2.5 Andrological Relevance

Very frequent (sometimes also monthly) recurrences of genital herpes can considerably impair the sexuality of affected patients due to subjective discomfort and concern about infection (e.g., also in the context of targeted sexual intercourse at the ovulation optimum).

Furthermore, herpes simplex virus can also be detected in human ejaculate (Kurscheidt et al. 2018). However, a conclusive assessment of a relevance for male fertility is not yet possible (Teixeira et al. 2021).

29.6.1.3 Molluscum Contagiosum Virus

29.6.1.3.1 Introduction

Molluscum contagiosum virus belongs to the group of DNA poxviruses. **Two main types (types 1 and 2**) are distinguished; type 2 occurs somewhat more frequently in the genital area. Transmission occurs through skin-to-skin contact, but also in swimming pools and through the use of shared towels. **If mollusca occur in adulthood, immunosuppression** (e.g., due to HIV infection) must be considered (Edwards et al. 2021).

29.6.1.3.2 Clinic

After an incubation period of 2 to 7 weeks (occasionally up to 6 months), centrally bifurcated, skin-colored, hemispherical papules or nodules (mollusca) 1 to 10 mm in diameter develop (Fig. 29.20). Larger, sometimes solitary lesions (giant molluscs) also occur. Occasionally, itching is complained of. Mollusca may also regress spontaneously after several months.

Diagnosis is usually possible upon inspection. Histological confirmation is rarely necessary in cases of clinical doubt.

29.6.1.3.3 Differential Diagnoses

Milia, condylomas, histiocytomas, and pedunculated soft fibromas must be differentiated.

29.6.1.3.4 Therapy

Mollusca can be treated by local application of potassium hydroxide solution, cantharidin, or cryotherapy or can be removed after local anesthesia by curettage, expression, or electrocautery.

29.6.1.3.5 Andrological Relevance

Infection with Molluscum contagiosum virus has no direct effect on male fertility. However, detection during an andro-



Fig. 29.20 Mollusca

logic examination may result in early therapy and thus prevention of transmission. In addition, Mollusca contagiosa may be indicative of immunosuppression, e.g., in the context of HIV infection, especially in cases of extensive infestation.

29.6.2 Fungal Infections

29.6.2.1 Candida Albicans

29.6.2.1.1 Introduction

Candida albicans is a saprophyte on human skin and mucosa that is prevented from uncontrolled multiplication by the immune system and other germs. Clinical relevance can be gained by this yeast as a consequence of a **general** (e.g., HIV, cancer or other general diseases such as diabetes mellitus) **or local** (e.g., use of glucocorticoids or changes in germ colonization by antibiotics) **impairment of the immune system** (Haag et al. 2004).

29.6.2.1.2 Clinic

Yeast infections of the genital region manifest as erosive, macerated skin and mucosal lesions.

In addition, whitish plaques (Fig. 29.21) and marginal folliculitis ("satellite papules," e.g., on the inner thighs) are typical of soorbalanitis or balanoposthitis candidomycetica. The skin lesions may itch or burn.

Diagnosis is made clinically and by detection of Candida albicans in culture.

29.6.2.1.3 Differential Diagnoses

Gonorrhea, trichomoniasis, genital herpes, chlamydial infection, lichen ruber (mucosae), lichen sclerosus et atrophicus, psoriasis inversa, nonspecific ("irritative") balanitis, toxic or allergic contact dermatitis, bacterial intertrigo, and Hailey–Hailey disease must be excluded.

29.6.2.1.4 Therapy

Localized yeast infections can usually be treated locally with antifungal agents. In addition, disinfecting baths are recommended. Circumcision may be considered for frequently recurrent disease.

29.6.2.1.5 Andrological Relevance

Mostly incidental finding without effect on male fertility. In case of extensive findings, further internal clarification (immune status, diabetes mellitus) may be necessary.

29.6.2.2 Dermatophytes

29.6.2.2.1 Introduction

Dermatophytes (filamentous fungi) most frequently infect the inguinal and gluteal regions in the genito-anal area. Infection occurs through contact with fungal skin flakes. A predisposing factor is a concurrent foot mycosis. The most common causative agent of **tinea inguinalis** is **Trichophyton rubrum** (Haag et al. 2004).

29.6.2.2.2 Clinic

Occasionally pruritic, roundish, and red to brown-red macules with typically **marginal accentuated scaling** that spreads centrifugally are characteristic (Fig. 29.22). Multiple lesions may confluence to form polycyclic foci.

In addition to clinical findings, positive native preparations with evidence of hyphae (fungal filaments) and a positive fungal culture allow correct diagnosis. The skin scales for this diagnosis should be taken from the edge of the lesions.



Fig. 29.21 Infection due to Candida with strippable plaques



Fig. 29.22 Infection by Trichophyton mentagrophytes with marginally accentuated scaling erythema

29.6.2.2.3 Differential Diagnoses

Tinea inguinalis must be differentiated primarily from erythrasma (lack of scaling, brick-red fluorescence in Wood's light).

Other differential diagnoses are bacterial intertrigo, psoriasis inversa, lichen simplex chronicus, contact dermatitis, candidiasis, and Hailey–Hailey disease.

29.6.2.2.4 Therapy

The basis of therapy is local drying measures and the use of antimycotics. Other affected skin areas (e.g., feet, interdigital spaces, nails) should also be disinfected.

If these measures are unsuccessful, systemic therapy becomes necessary, especially in cases of extensive infestation.

29.6.2.2.5 Andrological Relevance

Mostly incidental finding without effect on male fertility.



Fig. 29.23 Primary effect of syphilis I

29.6.3 Bacterial Infections

29.6.3.1 Syphilis

29.6.3.1.1 Introduction

Syphilis (lues) is a systemic disease occurring in **different stages** caused by the spiral bacterium **Treponema pallidum** (Köhn and Spornraft-Ragaller 2021).

In recent years, it has regained importance due to an **increase in prevalence**. The number of new cases in Germany increased from 3033 (2011) to 7889 in 2019 (Jansen 2020). The proportion of women is about 6%. The highest incidence with 35–40/100,000 inhabitants is observed in 25- to 39-year-olds. Over 85% of infections are due to **homosexual** contact among men.

29.6.3.1.2 Clinic

After an **incubation period of 21 days on average** (with variability from 10 days to 2 months), a painless ulcer with regional lymph node swelling develops at the site of bacterial entry (Fig. 29.23). Depending on sexual practice and route of infection, the ulcer may be located genitally, anally, labially, pharyngeally, or at any other body site. Clinical manifestation is observed in only 50–60% of infected individuals.

In **stage I**, syphilis is highly infectious. If no sufficient therapy is given, secondary syphilis (**Lues II**) develops after 2–6 months, which is accompanied by changes in the skin (exanthema, leukoderma), lymph nodes (lymphadenopathy), mucous membranes (plaques muqueuses of the tongue, anogenital condylomata lata), and hair (alopecia). The skin alterations heal clinically without therapy. Even in stage II, affected patients are still infectious. If untreated, syphilis progresses to the third stage (**Lues III**) after 3–5 years. This manifests itself in 30–40% with granulomatous changes of the skin and vessels with sometimes serious complications (aneurysm dissecans). After several years without sufficient therapy, quaternary syphilis (**Lues IV**) with neurological symptoms (neurosyphilis) and personality changes finally follows.

In early stages diagnosis is based on direct pathogen detection (dark-field microscopy from irritant secretions, PCR), later also on serological tests.

29.6.3.1.3 Differential Diagnosis

Depending on the stage of the disease, a variety of dermatological diseases must be considered in addition to other sexually transmitted infections.

29.6.3.1.4 Therapy

To determine therapy of syphilis, it is divided into early syphilis (up to 1 year post-infection), late syphilis (all later stages of syphilis or with unknown duration), neurosyphilis, or connatal syphilis. Therapeutic agents used are primarily penicillin or doxycycline.

29.6.3.1.5 Andrological Relevance

Incidental diagnosis of syphilis during andrologic examination is certainly limited to a few isolated cases. This is consistent with results from larger studies of lues serology prior to assisted reproduction methods, which found only one patient with Lues latens seropositiva in a collective of over 4000 patients (Lattes et al. 2013). Treponema pallidum DNA and RNA have been detected in ejaculate even without concurrent genital or anal lesions in a casuistic setting

(Godornes et al. 2019). Immunocompromised patients may also develop epididymo-orchitis due to Treponema pallidum (Agrawal and Ranjan 2019).

29.6.3.2 Gonorrhea

29.6.3.2.1 Introduction

Gonorrhea is caused by the gram-negative diplococcus **Neisseria gonorrhoeae** (Köhn and Spornraft-Ragaller 2021). WHO estimates over **85 million new cases per year** in men and women in the 15- to 49-year-old age group worldwide (WHO 2021). The focus of infections is in the Western Pacific region and the African continent.

After the introduction of the Infection Protection Act (IfSG), gonorrhea was no longer reportable in Germany. Thus, there are no nationwide figures on its occurrence. Only the state of Saxony maintains a reporting system, which recorded an increase in cases with gonorrhea from 1.8/100,000 inhabitants in 2011 to 19.9/100,000 inhabitants in 2019 (Selb et al. 2020). As of March 1, 2020, Germany has a non-name-based, laboratory-based reporting requirement under Section 7 (3) of the IfSG for Neisseria gonorrhoeae with reduced susceptibility to azithromycin, cefixime, or ceftriaxone.

29.6.3.2.2 Clinic

After an **incubation period of 2–6 days**, the infection manifests itself in an uncomplicated course by urethritis with typical purulent discharge, especially before first morning urine. Depending on sexual practices performed, proctitis, pharyngitis, or conjunctivitis may also occur.

As a result of complicated gonorrhea, ascending infections lead to prostatitis and epididymitis in men and salpingitis or acute peritonitis ("pelvic inflammatory disease") in women. The rare hematogenous spread is accompanied by arthritis, perihepatitis gonorrhoica, skin changes, endocarditis, or meningitis.

While the typical course in men with infestation of the urethra is asymptomatic in less than 10%, a specific clinic is absent in more than 50% with infestation of the urethra or cervix in women. The proportion of asymptomatic courses is even higher (>80–90%) for disease of the rectum or pharynx. This is also the reason why the true prevalence is always significantly higher than the number of cases reported in epidemiological surveys.

The **diagnosis** of gonorrhea is mainly based on **direct pathogen detection** (detection of intracellularly located diplococci in smears, e.g., by methylene blue staining), culture methods, and DNA amplification methods (Unemo et al. 2021).

It should be noted that coinfections with Chlamydia trachomatis occur in 10–40% (WHO 2021). Other sexually transmitted infections must also always be excluded.

29.6.3.2.3 Differential Diagnosis

Other causative agents of urethritis.

29.6.3.2.4 Therapy

Therapy is applied using ceftriaxone and azithromycin, alternatively with cefixime. Ciprofloxacin, ofloxacin, or doxycycline is only possible if sensitivity is proven (Deutsche STI Gesellschaft 2019; Unemo et al. 2021). The current and also regionally **varying resistance situations** of Neisseria gonorrhoeae should be noted.

29.6.3.2.5 Andrological Relevance

The data situation regarding andrological relevance of infections with Neisseria gonorrhoeae is inconsistent (Fode et al. 2016). However, the occurrence of epididymitis due to Neisseria gonorrhoeae provides evidence that impairments of male fertility are possible (Chen et al. 2017).

29.6.3.3 Soft Chancre (Ulcus Molle)

29.6.3.3.1 Introduction

Globally, the prevalence of chancre is not fully documented but appears to be decreasing. In regions such as Malawi and northern India, Haemophilus Ducreyi used to be responsible for 15–24% of genital ulcers. In South Africa, the proportion was over 60%. In recent years, the proportion has decreased to less than 10% in most regions (Lautenschlager et al. 2017). In Europe, **Haemophilus Ducreyi** infections are limited to isolated cases. Due to increased migration, anyone performing external genital examinations should be aware of this disease.

29.6.3.3.2 Clinic

After an **incubation period of 3–7 days**, papulopustules develop at the site of entry of the Haemophilus Ducreyi bacterium and eventually painful ulcerations of varying size with accompanying regional lymph node swelling. Unlike the ulcer in syphilis, chancre is soft to palpation ("soft chancre").

The diagnosis can be confirmed by culture or PCR. Other concomitant sexually transmitted diseases (especially HIV and syphilis) should always be excluded.

29.6.3.3.3 Differential Diagnosis

Syphilis, genital herpes, lymphogranuloma venereum.

29.6.3.3.4 Therapy

Therapy is carried out applying ceftriaxone and azithromycin, alternatively with ciprofloxacin and erythromycin.

29.6.3.3.5 Andrological Relevance

The likelihood of incidental diagnosis of chancre in the course of an andrological examination varies widely from

region to region worldwide. In Europe, it occurs only sporadically.

29.6.3.4 Inguinal Lymphogranuloma

29.6.3.4.1 Introduction

Lymphogranuloma inguinale is a staged sexually transmitted infection caused by **Chlamydia trachomatis serovars L1–L3** (mainly L2 and L2b in Europe). In Southeast Asia, Africa, South America, and the Caribbean, these pathogens are responsible for 2–10% of all genital ulcerations. In Europe, lymphogranuloma is rarely observed and is found here mainly in direct sexual contacts of homosexual men (de Vries et al. 2019).

29.6.3.4.2 Clinic

After an **incubation period of 3–30 days**, proctitis or a small painless papule, pustule, or erosion with scarless healing occurs within 1 week (phase 1). After 2–6 weeks, painful regional lymph node swelling with melting begins (phase 2). After a latency period of 5–10 years, ulcerations, fistulas, and sometimes massive local lymphedema ("elephantiasis") are observed (phase 3). Diagnosis is based on direct pathogen detection (PCR), serology or culture. Other sexually transmitted infections must be excluded (Lautenschlager et al. 2017).

29.6.3.4.3 Differential Diagnosis

Syphilis and ulcus molle.

29.6.3.4.4 Therapy

Doxycycline, erythromycin, or azithromycin is effective agent.

29.6.3.4.5 Andrological Relevance

Regarding the probability of an accidental diagnosis of lymphogranuloma inguinale in the course of an andrological examination, the same assessment applies as for ulcus molle.

Chlamydia trachomatis of serovars L1–L3 may be of relevance for female fertility which makes its avoidance additionally important, i.e., possible infection of the partner. Thus, Chlamydia trachomatis L2 was found in the portio smear of infertile women even without other evidence of lymphogranuloma venereum (de Jesús De Haro-Cruz et al. 2011).

29.6.3.5 Acute Gangrene of the Male Genitalia (Fournier's Gangrene)

29.6.3.5.1 Introduction

This is an infectious, **acute, and gangrenous inflammation with necrotic disintegration of scrotum and penis within a very short time**. Isolated involvement of the penis is rare (Lahouar et al. 2021).

A mixed infection with various aerobic and anaerobic bacterial pathogens is almost always an underlying cause, so that a localized, necrotizing, and subcutaneous infection with involvement of the fascia and musculature is likely (Köhn 2012). Predisposing factors include injury, urologic surgery, or previous surgery, as well as diabetes mellitus, immunosuppressive therapy, tumor disease, or HIV infection. Thrombosing vascular occlusion is thought to explain the rapid progression.

29.6.3.5.2 Clinic

The disease begins apparently spontaneously or takes its origin from an often unnoticed trivial excoriation. Quite suddenly, under high fever with chills and severely impaired general condition, inflammatory edema of the penis and scrotum develops. After only 1 day, bluish-blackish or whitish plaques are seen on the penis, which disintegrates gangrenously, spreading, and destroying the penile skin, even including the corpora cavernosa, as well as the scrotal skin. The testes may be exposed. The disease may also spread to the abdominal wall and/or lower extremities. There is marked leukocytosis.

Mortality ranges from 10% to 30%. If healing occurs, mutilating scarring sometimes remains.

29.6.3.5.3 Therapy

This must begin immediately on a massive scale. Immediately after removal of material for bacterial examination, high-dose broad-spectrum antibiotic therapy should be administered. Complete surgical removal of the diseased tissue in healthy tissue is obligatory. In addition, early intravenous administration of glucocorticoids is also recommended.

29.6.3.5.4 Andrological Relevance

The disease is rare and represents a urological emergency, so it acquires andrological relevance only in isolated cases when gangrene of the testes also occurs in younger men as a result of this necrotizing fasciitis (Gupta et al. 2007).

29.7 Phimosis

Clinically, primary and secondary phimosis, complete and incomplete phimosis, and **acute and chronic phimosis** are distinguished. Consequences may include smegma congestion, smegmolith (cobblestone-like whitish buildup of smegma concretions on the glans penis), inflammatory changes (balanoposthitis), and penile carcinoma.

Acquired phimosis is caused by inflammatory or degenerative processes of various causes that make it difficult or impossible to reposition the prepuce (Köhn 2012).

In neonates, the glans penis and inner preputial leaflet still adhere to each other in over 90% of cases. Normally, this epithelial connection loosens during the first years of life due to degenerative processes in the epithelial layers connecting the glans and prepuce. **In men older than 18 years, phimosis can be detected in up to 13%** (Morris et al. 2020).

Risk factors of acquired phimosis include obesity, diabetes mellitus, and lichen sclerosus et atrophicus.

Early circumcision has protective effects with respect to the likelihood of penile carcinoma later in life (Douglawi and Masterson 2019; Thomas et al. 2021).

A special form of acute phimosis and urologic emergency is **paraphimosis**, in which the phimotic prepuce stripped back behind the glans cannot be repositioned forward and causes severe pain. The glans is usually discolored (blue-reddish) and swollen. Behind it, a collar-shaped ("Spanish collar"), single or double annular swelling of the prepuce leaves is seen. Causes may be inflammatory processes or trauma.

29.8 Malignant Changes of the External Genitalia

Malignancies of the penis may be primary (e.g., melanoma, spinocellular carcinoma, rarely basal cell carcinoma) or may manifest secondarily as part of a general skin involvement of the penis (e.g., cutaneous lymphoma).

29.8.1 Erythroplasia Queyrat

29.8.1.1 Introduction

Like bowenoid penile papules, erythroplasia Queyrat should be considered a **non-invasive carcinoma** or **penile intraepithelial neoplasia** with a predilection for the glans. It has similarities to Bowen's disease, an in situ carcinoma of the skin. Concerning pathogenesis, infection with human papillomavirus type 16 is essential (Köhn et al. 2016b).

29.8.1.2 Clinic

Clinically, older, uncircumcised men present with sharply demarcated, irregularly configured redness that appears wet-glistening or finely granular (Fig. 29.24). Progression of untreated erythroplasia Queyrat to invasive spinocellular carcinoma is possible.



Fig. 29.24 Erythroplasia Queyrat

29.8.1.3 Differential Diagnoses

Balanitis plasmacellularis Zoon, psoriasis vulgaris, seborrheic eczema, contact dermatitis, balanitis, or balanoposthitis of other etiologies.

29.8.1.4 Therapy

The skin lesions can be removed surgically or by laser therapy. Other therapeutic options include the use of imiquimod, 5-fluorouracil, or photodynamic therapy (PDT) (Kravvas et al. 2020).

29.8.1.5 Andrological Relevance

Erythroplasia Queyrat may very rarely be an incidental finding during andrologic examination. Since this change occurs mainly in older men, and the age of men at the time of desired paternity has steadily increased in recent years, it must be considered in the future in cases of prolonged circumscribed redness of the penis. Knowledge of this condition is also important when examining older hypogonadal men or in the workup of gynecomastia in older age as an incidental finding.

29.8.2 Extramammary Paget's Disease

A **rare variant of intraepidermal adenocarcinoma** is extramammary Paget's disease, which can progress to invasive carcinoma if not treated adequately and in a timely manner (Dauendorffer et al. 2021). It primarily affects **elderly patients**. In studies of patients with genital involvement, the average age was approximately 62 years (Phyo et al. 2020). In men, extramammary Paget's disease manifests preferentially in the inguinal–scrotal region as sharply demarcated, infiltrative erythema that may also scale easily and shows a tendency to grow (Fig. 29.25). It is not uncommon for it to be treated unsuccessfully for years under the diagnosis of eczema.



Fig. 29.25 Extramammary Paget's disease presenting as chronic intertriginous dermatitis

Complete surgical removal of the lesions must be carried out.

29.8.2.1 Andrological Relevance

See Sect. 29.8.1.

29.8.3 Invasive Squamous Cell Carcinoma of the Penis

Squamous cell carcinoma of the penis is rare in industrialized countries. The prevalence there is only **0.1–1.0/100,000 population**; however, in some **regions of Africa, Asia, and South America**, they account for up to **10% of all carcinomas of the male** (Thomas et al. 2021). There are often **predisposing factors** such as **human papillomavirus** infections, **chronic inflammation**, **phimosis, inadequate hygiene, or nicotine use**. In the area of the glans or foreskin, there is growth of a reddish tumor with a tendency to bleed with possible metastasis (Fig. 29.26). Treatment of the carcinoma should therefore take place at an early stage.

29.8.3.1 Andrological Relevance

The probability of diagnosing invasive penile carcinoma during an andrologic examination is very low. Therefore, the andrologic relevance is more in the timely counseling of patients for possible provoking factors (see above).



Fig. 29.26 Carcinoma of the penis

29.9 Penile Injuries

29.9.1 Penile Rupture ("Penile Fracture")

Penile rupture results from blunt trauma (usually during sexual intercourse) to the erect penis and is characterized by **tearing of the tunica albuginea of the corpora cavernosa**. After immediate relatively localized pain, a massive **hematoma** may develop **in the rupture area** or spread diffusely to the penis, scrotum, lower abdomen, and beyond. Hematuria may indicate co-injury to the urethra (10–40% of cases). Penile rupture is a urologic emergency. Surgical care should be immediate if needed (Ge et al. 2021).

29.9.1.1 Andrological Relevance

Consequences of rupture of the tunica albuginea of the corpora cavernosa may include erectile dysfunction or structures of the urethra in 10–30%.

29.9.2 Side Effects of Penile Augmentation Injections

The injection of **paraffin, mineral oils, or petroleum jelly** for penile augmentation, which was more widespread at the beginning of the twentieth century, is still practiced sporadically in Eastern European countries and Asia and leads to **granulomatous inflammation** (paraf-



Fig. 29.27 Granulomas after injection of paraffin for penile augmentation



Fig. 29.28 Scarred condition after Prince Albert piercing

finomas, Fig. 29.27). (Rosellen et al. 2020). Heroin injections into the penis can lead to extensive infections. Self-injection of powdered tablets dissolved in hydrogen peroxide (aciclovir for genital herpes recidivans) has also been reported sporadically, resulting in granulomatous inflammation of the penile skin. Keloids of the penile skin are a rarity.

The most common complications of injections are observed in the context of intracavernosal self-injection for erectile dysfunction treatment, in which vasoactive substances are injected intracavernosally. Fibrosis occurs in the area of the tunica albuginea of the corpora cavernosa (Köhn 2012].

29.9.2.1 Andrological Relevance

Due to the granulomas thus consequence of the injection of, e.g., paraffin, pain during erection and disturbances of seminal deposition may occur.

29.9.3 Self-Injury of the Penis

A relatively common trauma to the penis in children is due to strangulation by hair, as a result of which lymphedema and skin necrosis may occur. In adults, strangulation injuries are observed due to the use of ring-shaped objects (metal rings, bottle necks). Injuries from medically prescribed penile rings or vacuum pumps are very rare when contraindications are observed (use for more than 30 min; concurrent use of coumarins) (Köhn 2012).

29.9.4 Piercings

Dermatologically and andrologically relevant changes can occur with piercing due to the **sometimes very long healing phases** (up to 9 months) and scarring of the urethra in this localization (Fig. 29.28) (Lee et al. 2018). Male genital piercing can occur in the area of the frenulum, the prepuce, between the scrotum and the base of the penis (“Hafada”), between the urethral opening and the frenulum (“Prince Albert”), vertically through the penis (“Apadravya”), transversely through the glans (“Ampallang”), or through the glans rim (“Dydoes”) (Köhn 2012).

29.10 Induratio Penis Plastica (Peyronie’s Disease)

29.10.1 Introduction

Induratio penis plastica (IPP, Peyronie’s disease, penile fibromatosis) is a **circumscribed, plaque-like, fibromatous change of the tunica albuginea of the corpora cavernosa** (Köhn 2012). Local trauma, presumably during sexual activity, mediated by various cytokines, initially leads to an inflammatory reaction with subsequent fibrosis (Patel et al. 2020). A hereditary disposition is assumed. The **prevalence is estimated to be approximately 0.4–13%** (Ziegelmann et al. 2020).

IPP may be associated with palmar (Dupuytren’s disease) or plantar (Ledderhose’s disease) fibromatoses in up to 20–30% (Patel et al. 2020).

29.10.2 Clinic

It primarily affects men between the ages of 40 and 70 years. Symptoms include deviation of the penis (>80%), pain (50–70%), and, in more severe cases, erectile dysfunction (30–50%).

29.10.3 Therapy

The current drug therapy approaches are not satisfactory. In very pronounced cases with functional limitation, surgical plaque removal must be performed or erectile tissue implants must be used (Chung et al. 2020).

29.10.4 Andrological Relevance

A consequence of IPP is erectile dysfunction in up to approximately half of affected men. Pain at the onset of the disease, erectile dysfunction, and impairment of the external appearance of the penis during erection negatively affect the quality of life of affected men.

In addition to erectile dysfunction, deviations of the penis, some of which are considerable, can affect deposition of semen in men who wish to have children.

The association of IPP with testosterone deficiency has not yet been conclusively established (Aditya et al. 2019).

29.11 Congenital Malformations of the Penis

29.11.1 Introduction

Relevant congenital defects of the external male genitalia are **mainly hypospadias**, the much rarer **epispadias** or the so-called **micropenis**.

Hypospadias is one of the most common congenital malformations of the penis, with a prevalence of approximately **20/10,000 births in Europe and approximately 34/10,000 in the United States** (Springer et al. 2016). The international prevalence is 20.9/10,000 births and shows an increase in many regions especially from the 1990s (Yu et al. 2019). An association with exposure to estrogen-like environmental toxins in utero is discussed. In approximately 25%, patients report ventral penile deviation (Spinoit et al. 2021).

The prevalence of **epispadias is 1/100,000 births**. Surgical interventions aimed at achieving adequate erections and ejaculations are the main focus (Wood and Woodhouse 2011).

Micropenis can be a consequence of various diseases (primary testicular dysfunction due to Klinefelter syndrome, anorchia or testicular dysgenesis, hypogonadotropic hypogonadism in, e.g., Kallmann syndrome, testosterone insensitivity due to androgen receptor disorders or 5-alpha-reductase deficiency, and genital developmental disorders). The true "micropenis" must be distinguished from the apparently too small penis in obesity, where the penis is partly hidden in the fat apron ("buried penis").

29.11.2 Andrological Relevance

The andrological relevance of congenital malformations of the penis includes reduced quality of life and impairment of self-esteem with limitations of sexual relations, as well as the association with erectile dysfunction and disorders of seminal deposition due to ejaculatory dysfunction. Thus, in summary, all three dimensions of human sexuality are frequently affected.

Men with hypospadias are less likely to become biological fathers; when they do have children, they are more likely to use assisted reproduction methods (Skarin Nordenvall et al. 2020). Reduced fertility in men with hypospadias is also related to the association with undescended testis; reduced spermatogenesis appears to occur with proximal hypospadias (Punjani and Lamb 2020).

The above changes also have andrologic relevance when counseling patients before performing IVF and ICSI. The risk of children born after these procedures for hypospadias (OR = 1.87, 95% CI: 1.47–2.40) and malformations of the genitourinary tract in general (OR 1.61, 95% CI: 1.41–1.85) is increased (Zhang et al. 2021).

29.12 Scrotal Skin Lesions

29.12.1 Scrotal Cysts (Scrotal Calcinosis)

29.12.1.1 Introduction

Scrotal cysts are classified as variants of epidermal cysts on the scrotal skin that calcify and lose their cyst wall as they progress. Due to their tendency to calcify, they are also referred to as scrotal calcinosis, especially in multiple occurrences in English-speaking countries (Köhn 2014f; Syed et al. 2018).

29.12.1.2 Clinic

Scrotal cysts occur as **early as adolescence or by the third decade of life** and usually show growth as they progress. They present as rough palpable, hemispherical to round protruding, whitish-yellowish nodules usually 5–10 mm in diameter, but sometimes with a much larger circumference

(Fig. 29.29). Sometimes, a small pore is visible centrally in the skin, from which whitish friable material can be emptied on pressure.

Initially, scrotal cysts are usually asymptomatic; later, they may cause pain with scarring as a result of calcification, inflammation, and infection.

29.12.1.3 Therapy

Solitary scrotal cysts without growth tendency and tendency to infections do not necessarily require therapy. Especially in the case of multiple infestations, men with scrotal cysts already feel limited because of the externally visible changes.

Surgical removal is then the only reasonable option.

29.12.1.4 Andrological Relevance

Frequently, mostly younger patients use an andrological examination in order to be informed about the significance, prognosis, and therapeutic options of these changes. There is no relevance beyond this.

29.12.2 Pruritus Scroti

29.12.2.1 Introduction

The scrotum, together with the eyelids, is one of the regions with the thinnest skin. Therefore, **mechanical and other irritative influences** (e.g., moisture and heat) can lead to disturbances of cutaneous integrity associated with pruritus and scratch excoriations. **Dermatoses such as atopic dermatitis, seborrheic eczema, or psoriasis** also affect the scrotal skin and are the cause of itching. In other cases, **psychosomatic factors** must be considered or no causes are found (Köhn 2012).



Fig. 29.29 Multiple scrotal cysts

29.12.2.2 Clinic

Often, only a subtle redness is noticed, sometimes associated with minor edema and scratch excoriations. Epidermal involvement may vary in severity. With a chronic course, more pronounced lichenification and redness of the scrotal skin (red scrotum syndrome) occur, especially in older adults. If scaling occurs, material should be taken for mycologic examination or contact dermatitis should be considered. Patients are often confused and require competent medical guidance. Inspection of the entire integument is always required.

29.12.2.3 Therapy

Uncritical therapy with local corticosteroids or antifungals usually does not lead to success. The underlying disease should always be treated. Psychosomatic causes should be managed appropriately.

Key Points

- An initial andrologic examination should always include careful inspection of the external genitalia.
- This may reveal harmless findings as incidental findings or relevant findings.
- It also offers patients the opportunity to address changes that bother or unsettle them.
- The andrological examination also opens up the possibility of early preventive consultation and treatment of patients (e.g., phimosis or infections with HPV).
- Papillae coronae glandis and heterotopic sebaceous glands represent norm variants without pathological value.
- In addition, pigment spots, angiokeratomas, angiomas, cysts, and fibromas are also without clinical relevance in most cases.
- Other changes such as infectious or malignant diseases, however, have clinical relevance for the patient and his partner.
- Diseases such as phimosis or induratio penis plastica may affect the patient's sexuality by causing pain during intercourse, erectile dysfunction, or disorders of seminal position.

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Disorders of Erection, Cohabitation, and Ejaculation

30

Armin Soave and Sabine Kliesch

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Abstract

Sexual dysfunction in men can be manifold. Purely mechanical disorders of seminal deposition due to anatomy are to be differentiated from functional libido and orgasmic disorders, from ejaculation problems or the inability to achieve or maintain erections. While pure orgasmic disturbances are almost in all cases due to psychological or psychiatric causes, libido and erectile dysfunction may also have hormonal causes and thus be symptoms of androgen deficiency. Additional clinical symptoms of hypogonadism or objectifiable findings such as testicular atrophy, changes in secondary sexual characteristics, or ejaculate volume allow the organic genesis to be identified in pronounced cases, while in other cases the corresponding indications must be actively sought. Thus, while functional orgasmic and libido disorders are the

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domain of psychological psychiatric or endocrinological therapeutic approaches, this chapter deals primarily with disorders of seminal deposition and functionally or organically caused disorders of cohabitation.

30.1 Erectile Dysfunction

30.1.1 Definition, Epidemiology, and Risk Factors

Erectile dysfunction (ED) is defined as **the persistent inability to achieve and/or maintain a penile erection sufficient for satisfactory sexual activity or intercourse** (NIH 1993). ED is the most common male sexual dysfunction with an age-related incidence, averaging 20% in Germany and internationally. The longitudinal Massachusetts Male Aging Study showed an overall prevalence of 52% in a total of 1709 men between 40 and 70 years of age (Johannes et al. 2000) and reached values of over 70% in the group over 70 years of age (Feldmann et al. 1994). The Cologne study, which included validated questionnaires answered by 4489 men between 30 and 80 years of age, showed an overall prevalence of 19%, with a strong age-dependent increase: in 30- to 39-year-olds (2%) and in 70- to 80-year-olds (53%) (Braun et al. 2000). The most recent survey, which for the first time allows a projected prevalence estimate for Germany according to the International Classification of Diseases (ICD)-11, included 2322 men between 18 and 75 years of age. A lifetime prevalence of erectile problems that may indicate ED was found to be 22%, with a strong age-dependent 12-month prevalence: 6.5% of 18- to 25-year-olds and 34% of 66- to 75-year-olds (Briken et al. 2020). ED can severely affect the **psychosocial health** and **quality of life** of patients and their partners; in particular, patients' sexual satisfaction and self-confidence may decrease, and depressive symptoms may increase (McCabe and Althof 2014). In view of this, the new 11th International Classification of Disease (ICD 11), which will come into force in 2022, is to be welcomed as it will abandon the separation into organic and non-organic sexual dysfunctions and group them together in the chapter on "conditions related to sexual health" and divide them into four main groups. A sexual problem becomes a sexual dysfunction only through additional qualifiers (duration, severity of a symptom, and suffering as morbidity criteria). These qualifying characteristics and current prevalence estimates have not been available for the general population in Germany. The study by Briken et al. (2020) fills this knowledge gap with a prevalence estimate for Germany according to the new ICD-11 criteria for sexual dysfunction, taking into account morbidity criteria and age (Kliesch 2020).

This rethinking and further de-tabooization of erectile dysfunction are urgently required. Evert since the "Report on the Health Situation of Men in Germany" by the Robert Koch Institute in 2014, it has been demonstrably known that sexual dysfunction, in particular erectile dysfunction, represents so-called **harbingers of ischemic heart disease** manifesting at a later time. The men's health report postulated that patients with erectile dysfunction should be examined thoroughly (RKI 2014).

Sexual dysfunction can be associated with cardiovascular disease, diabetes mellitus, and neurological disorders, but only one in five men actually seeks medical help (Braun et al. 2000). Conversely, physical and mental illnesses can, in part, significantly influence sexual activity and satisfaction, with sexuality experienced as satisfying having a substantial positive impact on quality of life.

In recent decades, new insights into the pathogenesis and role of **endothelial dysfunction** have placed ED in a completely new context. Now accepted as established risk factors of ED are, in addition to age, especially hypertension, smoking, obesity, metabolic syndrome, dyslipidemia, diabetes mellitus, and cardiovascular disease (Salonia et al. 2020). Up to 70% of patients with dyslipidemia, diabetes mellitus, or hypertension develop ED. As early as 2004, Rosen et al. (2004) demonstrated in a large epidemiological study of approximately 28,000 respondents and over 4000 patients that the incidence of these comorbidities was twice as high in patients with manifest ED compared with an age-matched comparison population, and approximately 65% of all ED patients had at least one of these risk factors. Up to 25% of all patients with ED have manifest **diabetes mellitus**, and up to 17% show previously unknown impaired glucose tolerance with elevated fasting blood glucose levels as an early stage and initial manifestation of the disease (Sairam et al. 2001). ED represents an early symptom and clinical predictor of **cardiovascular disease** and events. On average, ED occurs 25 months before cardiac symptoms in patients with coronary artery disease and myocardial infarction (Vlachopoulos et al. 2013). In addition, ED patients have a 33% increased all-cause mortality compared with non-ED patients (Zhao et al. 2019). Cardiology exclusion diagnosis of cardiovascular disease is therefore reasonable in ED patients (Kostis et al. 2005).

Elevated prolactin concentrations may have inhibitory effects on erectile function (Xu et al. 2019), and therapy for **hyperprolactinemia** results in improved erectile function in patients with macro- and microprolactinomas (Shimon et al. 2019). Similarly, a large number of studies have demonstrated that **thyroid dysfunction** can contribute to ED (Bates et al. 2020). Other conditions associated with ED include sleep disorders, obstructive sleep apnea, psoriasis, gout, non-alcoholic fatty liver, chronic liver disease, inflammatory bowel disease, chronic fatigue syndrome, and allergic rhinitis (Salonia et al. 2020).

Urological disorders are also associated with ED, including complaints of micturition with "**lower urinary tract symptoms" (LUTS)** due to **benign prostatic enlargement** and **M. Peyronie** (see Chap. 29). The severity of LUTS correlates with ED independently of age and previous diseases of the patients (Rosen et al. 2003).

30.1.2 Anatomy

Arterial inflow to the penis occurs via the internal pudendal artery. Considerable variation up to unilateral supply without any functional impairment has been described. After delivery of a pelvic floor branch, division into the dorsal penile artery, profunda penis artery, and bulbar artery occurs (Fig. 30.1).

The dorsal artery runs superficially with the dorsalis penis profunda vein and the dorsalis penis nerve between the fascia penis profunda (Buck) and the tunica albuginea, supplying the skin and glans penis. In many patients, there are anastomoses between these superficial vessels and the intracavernosal vascular circulation and to the C. spongiosum.

Numerous helical arteries arise from the **profunda penis artery**, which supplies the corpora cavernosa, and are contracted and **corkscrew-like** in the flaccid state of the penis. The direct transition of these arteries into wide, communicating sinusoids is characteristic of the vascular architecture of the human corpus cavernosum. Between helical arteries and cavernous veins, only short arterial terminal stretches are interposed; the absence of an actual capillary bed thus acts in the sense of an arteriovenous fistula and, at rest, with the penis not erect and the afferent vessels contracted, causes merely nutritive minimal perfusion of the corpora cavernosa (CC).

The trabecular meshwork of the **cavernous sinusoids** consists of smooth muscle cells lined with endothelium toward the cavities. Within the trabeculae, the intracavernous helicine arteries and draining veins are located. Unlike the arteries, the **veins** (Fig. 30.1) do not have their own musculature and thus depend for their width on the state of tension of the trabeculae. These venules and intermediate venules, which drain the sinusoids, collect to form a subtunical plexus from which the emissary veins arise. These emerge dorsally or laterally and join the dorsalis profunda vein directly or via the circumflex veins. Proximally, they join the cavernous and crural veins, which give rise to the internal pudendal vein. The superficial dorsal vein drains the superficial skin layers and not infrequently shows drainage into the great saphenous vein.

Stabilization of the corpora cavernosa is provided by the tunica albuginea, which gives off multiple septa and guarantees the three-dimensional structure. As a result, changes in the tone of the cavernous musculature affect the entire system unit tunica albuginea—trabeculae—draining veins and not only individual components. The septum penis is incomplete in humans, so that the corpora cavernosa clinically forms a functional unit (Fig. 30.1).

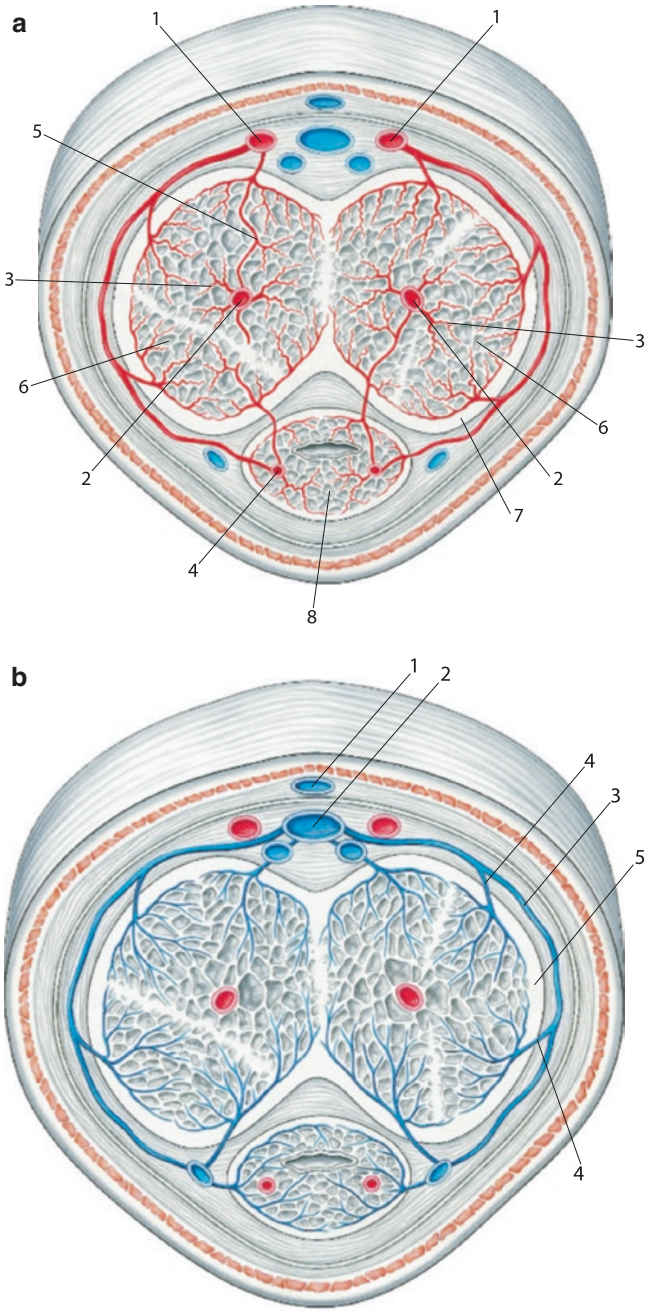


Fig. 30.1 Cross section of the penis. (**a**) Arterial vasculature. (*1*) Dorsalis penis artery; (*2*) profunda penis artery; (*3*) helical arteries; (*4*) urethral artery; (*5*) anastomoses between dorsal and profunda penis arteries; (*6*) corpora cavernosa; (*7*) tunica albuginea; (*8*) corpus spongiosum. (**b**) Venous vasculature. (*1*) V. dorsalis superficialis; (*2*) V. dorsalis profunda; (*3*) V. circumflexa; (*4*) V. emissaria

Innervation occurs via both the autonomic and somatosensory nervous systems. Parasympathetic fibers originate in the sacral erectile center (S2–S4). Sympathetic fibers originating in the thoracolumbar junction (Th12–L2) pass through

the preaortic plexus and border cord into the hypogastric plexus. Preganglionic parasympathetic fibers from the sacral erectile center unite in the pelvic plexus with sympathetic fibers from the hypogastric plexus to form the cavernous nerves, which thus represent the autonomic innervation of the corpora cavernosa. These cavernosal nn. follow the internal pudendal artery and extend laterocaudally to the apex prostate before entering the corpora cavernosa. This exposed location explains the damage to the nerves and vessels during urethral injuries and pelvic procedures such as radical surgery of the prostate and rectal extirpation.

Somatosensory innervation occurs via the dorsalis penis nerve, an end branch of the pudendal nerve. Sensory afferents reach spinal cord segments S2–S4 via the posterior roots and from there anterolateral spinothalamic pathways and the integrative medial preoptic area (MPOA). However, the pudendal nerve also gives off efferent motor fibers to the pelvic floor muscles, innervating the bulbocavernosus and ischiocavernosus muscles. These compress the CC against the bony pelvis during erection and increase intracavernosal pressure.

30.1.3 Physiology of Erection

30.1.3.1 Hemodynamics

Erection requires **three hemodynamic factors** (Fig. 30.2):

- Intracavernosal resistance decrease due to the relaxation of the smooth muscles of the corpus cavernosum.
- Increase in arterial inflow due to dilation of the smooth muscle vessel walls.
- Restriction of venous outflow by compression of the intracavernosal and subtunical venous plexuses.

Due to the reduction in tone of all smooth muscle in the CC, increased arterial inflow occurs in addition to relaxation of the sinusoids. Clinically, this phase of erection correlates with increasing tumescence and elongation of the penis (Fig. 30.2). In the venous limb, there is a reduction in the unobstructed outflow in the flaccid state due to a reduction in venous cross section. Due to the maximal expansion of the cavernous cavities, increasing compression of the venules in the trabeculae and of the vv. emissariae at the tunica albuginea occurs. A complete erection results (Fig. 30.2). Hemodynamically, five phases of erection can be distinguished (Table 30.1).

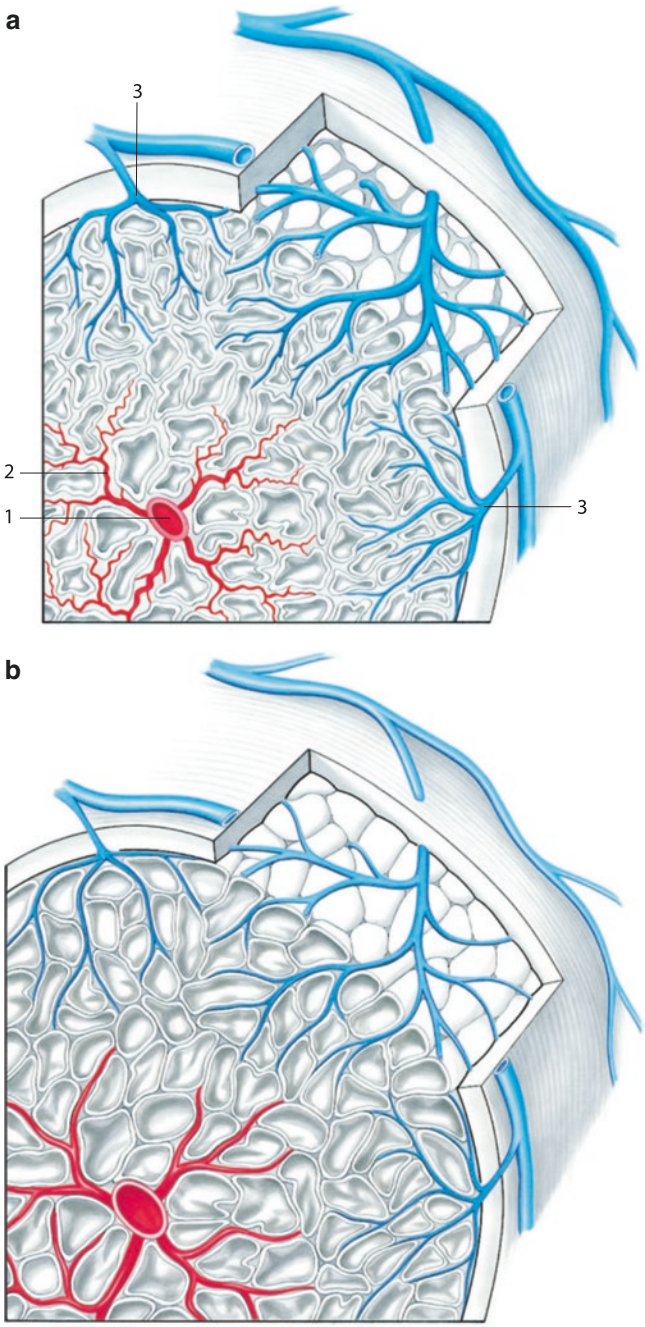


Fig. 30.2 Physiology of erection. (**a**) In the flaccid state, contracted profunda penis artery (*1*)/helical arteries (*2*), collapsed sinusoids, undisturbed venous outflow of cavernous blood via emissary veins (*3*). (**b**) In the tumescent state, marked dilation of the profunda penis artery and sinusoids, stretching of the helical arteries, compression of the intersinoidal venules, and mechanical occlusion of the subtunical emissary veins

In the latency phase, animal experiments have shown that immediately after stimulation of the cavernous sinuses, pressure in the corpus cavernosum drops while arterial perfusion simultaneously increases. As a result of the elastic–fibromuscular architecture of the CC, this leads to elongation without

Table 30.1 Five phases of erection

I. Latency phase	Penile shaft elongation, constant intracavernosal pressure due to a decrease in the cavernosal resistance
II. Tumescence phase	Increase in tumescence and rigidity with increase in arterial inflow, cavernosal volume, and intracorporal pressure
III. Erection phase	Completely elongated, pulsating penile shaft, stabilization of intracavernosal pressure at a plateau, 10–20 mmHg below systolic blood pressure; constant penile volume due to a reduction in the arterial inflow to baseline values
IV. Rigidity phase	Full tumescence and rigidity with intracorporal pressure that can clearly exceed systolic blood pressure due to the bulbocavernosus reflex; only minimal arterial inflow but complete venous occlusion
V. Detumescence phase	Loss of rigidity, decrease in penile volume and intracavernosal pressure with in- and outflow from the corpora cavernosal reaching baseline values

a change in pressure. In the tumescent phase, increasing compression of the venous outflow tract develops. At an intracavernosal pressure just below systolic blood pressure, a plateau is established, which is hemodynamically characterized by an equilibrium of arterial inflow that has dropped to baseline values and only minimal venous outflow (**erection phase**). Maximum rigidity is brought about by contraction of the pelvic floor muscles, and the pressures in the corpus cavernosum may rise to well above systolic pressure, reaching peak values of more than 500 mmHg (**rigidity phase**). Finally, as the nervous impulses subside, rigidity and tumescence decrease (**detumescence phase**).

30.1.3.2 Neurophysiology

Clinically, **three types of erections** are distinguished—reflexogenic, psychogenic, and nocturnal. **Reflexogenic erections** are triggered by direct stimulation of the genital area and are mediated by the dorsalis penis nerve. Intraspinally, switching to efferent parasympathetic fibers occurs in the sacral erectile center (S2–S4) (nn. erigentes), which, after switching in the pelvic plexus, leads to erection induction via the cavernosal nerves. The somatic portion of the pudendal nerve causes adequate rigidity via contraction of the pelvic floor muscles (especially the bulbospongiosus and ischiocavernosus muscles). Pathophysiologic correlations explain the occurrence of cohabitating reflexogenic erections in supranuclear spinal cord lesions, in which central perception may be preserved or abolished to varying degrees for the patient.

In **psychogenic erections**, erection-promoting neurotransmitters (especially dopamine and nitric oxide) (NO) are released by erotic stimuli in the central sexual centers. Via activation of the parasympathetic nervous system and switching in the sacral erectile center, signals are transmitted to the erectile tissue, where nitric oxide and vasoactive intestinal polypeptide (VIP) act as the main transmitters. Predominantly erection-inhibiting influences originate from the thoracolumbarly located erectile center (TH 11/12–L 2/3). After switching in the hypogastric plexus, the sympathetic fibers reach the penis together with the parasympathetic fibers in the cavernous nerves. Here, the sympathetic fibers terminate predominantly at α-receptors of the corpus cavernosum muscle cells or at α2-receptors of the penile arteries. Via these, there is then an erection-inhibiting effect on the smooth muscles of the corpus cavernosum. Clinical experience shows that in the case of a high sympathetic tone, an erection can fail to occur even under stimulation.

Physiologically, the corpus cavernosum is subject to more erection-inhibiting factors than erection-promoting factors. This explains why the penis remains in a contracted, or flaccid, state at rest.

Nocturnal erections are a consequence of the reversal of the day/night pattern with parasympathetic tone predominating at night, leading to the occurrence of intermittent autonomic erections. These nocturnal erections occur primarily during rapid eye movement (REM) sleep episodes. During sleep, typically 4–5 REM sleep phases occur, accompanied by autonomic nocturnal erections. With increasing age, the frequency of occurrence decreases: in 13- to 15-year-olds, nocturnal erections occur during 30% of sleep, and in 60- to 69-year-olds, nocturnal erections occur during 20% of sleep (van Driel 2014).

30.1.3.3 Cellular Control of Erection

The parasympathetic erection-promoting nerve terminals are predominantly non-adrenergic and non-cholinergic in nature. The most essential neurotransmitter in penile erection is **nitric oxide (NO)**. On the one hand, NO is released from the NANC nerve endings of the cavernous nerve and, on the other hand, from the endothelial cells lining the entire sinusoids and penile vessels. Local synthesis occurs under the influence of testosterone-dependent endothelial and neuronal nitric oxide synthase (eNOS and nNOS, respectively), with L-arginine serving as substrate. While eNOS is mainly downregulated in hypoxia, nNOS is predominantly influenced by various metabolic factors (diabetes mellitus, elevated LDL levels, hypertension, and metabolic syndrome) (Musicki et al. 2015; Traish and Kim 2005).

Under sexual stimulation, the primary neuronal nitric oxide is released. This in turn activates NO, the membrane-bound guanylate cyclase, which results in the release of 3′5′-cyclic -guanosine monophosphate (cGMP) from GTP. This second messenger is the major transmitter leading to the activation of cGMP-dependent protein kinase G (PKG). Under the influence of PKG, there is an uptake of free intracellular calcium into the endoplasmic reticulum, a

throttling of Ca efflux, and an increased efflux of potassium from the cell, ultimately resulting in smooth muscle cell depolarization and relaxation with consecutively increased blood influx (Burnett 2004). Supported by the mechanical changes and stretching of the muscle, there is then further activation of endothelial NOS with corresponding endothelial NO release, which clinically contributes to the maintenance of smooth muscle relaxation and thus erection. Analogous to the formation of cGMP, activation of membrane-bound adenylate cyclase by VIP generates cAMP. Both second messengers are subject to physiological degradation by various phosphodiesterases, of which type 3 and type 5 are of particular clinical importance. These lead to the degradation of cGMP to 5′GMP and of cAMP to 5′AMP, respectively, which are biologically inactive.

Sympathetic innervation occurs predominantly via adrenergic 1- and 2-receptors, which lead to an increase in intracellular calcium levels and thus to contraction of the muscle cell.

In addition, various local substances are synthesized in the corpus cavernosum that have a predominantly erectile inhibitory effect. In particular, prostanoids, endothelin, and angiotensin should be mentioned here. Overall, local control of erection is more subject to the influence of **testosterone** than previously thought. Thus, long-term **testosterone deficiency** leads to a reduction of NO-synthetase-containing nerve fibers, to a downregulation of nNOS and eNOS, and to a continuous reduction in the number of smooth muscle cells through apoptosis (Traish and Kim 2005).

30.1.4 Pathophysiology of Erection

Psychogenic, vascular, neurogenic, hormonal, or myogenic disorders can independently be the **cause of erectile dysfunction (ED)**, but can also act in combination. Due to modern methods of diagnostics allowing examination of the functions crucial for induction and maintenance of an erection, there is often a strong focus on organic causes. However, psychological effects and psychogenic factors are almost always observed, especially in long-term erectile dysfunction. From the perspective of sexual medicine, the separation into organic versus non-organic ED has been critically discussed for quite some time against the background that ED, as a sexual dysfunction exercises psychosocial effects on the affected patient and partners (Jannini et al. 2010). From 2022, the diagnostic classification (ICD-11) will no longer make this distinction (Kliesch 2020).

30.1.4.1 Psychogenic Influences on Erection

Psychogenic stimuli, such as sensory or mental stimuli, can provide a strong erection impulse. On the other hand, opposite stimuli, especially fear or previous traumatic events, may significantly reduce or completely eliminate a man's erectile capacity. In primary disorders, the causes often lie in upbringing and family environment. Secondary disorders usually occur acutely and partly depending on the situation or partner. They are often accompanied by other characteristic sexual disorders. Of prognostic–therapeutic importance is whether a disorder is predominantly characterized by loss of libido, or whether the initial arousal cannot be maintained due to fear of failure or other disorders. **Libido disorders** may be the result of organic causes, but in the case of psychogenic etiology they are not infrequently partner-dependent and prognostically unfavorable, since they are usually symptoms of a profound **partnership crisis**. Depression, anxiety disorders, and problems in the couple relationship can condition psychogenic erectile dysfunction (Nguyen et al. 2017). Anxiety related to sexual performance may occur in 9% to 25% of men and contribute to the development of psychogenic ED (Pyke 2020).

30.1.4.2 Vascular Erectile Dysfunction

To induce an erection, a sufficient cavernous occlusion mechanism is required in addition to an adequate perfusion reserve of the arterial pathway. Therefore, **changes in arterial inflow and venous outflow** can basically be differentiated.

About 50% to 80% of all organically caused erectile dysfunctions are mainly due to arterial circulatory disturbances of the penile vessels.

As discussed in the epidemiology section, a majority of patients have single or multiple risk factors. In addition to arteriosclerotic changes, perineal trauma with injury to the pudendal vessels or iatrogenic causes (vascular surgery and radical pelvic surgery) may also be considered. Clinically, purely arterial disorders are characterized by difficult or delayed erection onset. Symptomatology develops slowly and is typically not partner or situation-dependent.

Venous outflow disorders can have a variety of causes:

- Congenital outflow obstruction (ectopic veins).
- Morphological changes in the smooth muscles of the corpus cavernosum (reduced relaxation capacity due to different causes).
- Functional changes in the smooth muscles of the corpus cavernosum (transmitter and receptor disorders and hormonal changes).
- Morphological changes in the tunica albuginea (age, Peyronie's disease, and penile fracture).
- Pathological shunt connections between C. cavernosum and Glans/C. spongiosum.

Ectopic veins are a very rare cause of primary ED that can be surgically corrected, at least temporarily. Secondary venous erectile dysfunction is due to changes in the erectile tissue itself or in the tunica albuginea. In most cases, degenerative changes in the corpus cavernosum smooth muscle with connective tissue replacement of the muscle cells by fibroblasts and consecutive loss of elasticity are responsible for the incompetence of the cavernous closure mechanism and the associated venous outflow. Such changes did not infrequently occur in the wake of arterial perfusion defects, thus demonstrating the same pathophysiologic mechanisms known from other organ systems. Extensive fibrosis is also observed after priapism, trauma, or Peyronie's disease.

Circulatory disturbances of the small intracavernous vessels (small vessel disease) can lead to insufficient activation of the cavernous closure mechanism and thus to relative venous insufficiency in the presence of concomitant morphological changes in the tunica albuginea via insufficient filling of the corpora, so that venous insufficiency almost never occurs in isolation but reflects a combined problem of vascular corpus cavernosum function and is better summarized under the term **cavernous insufficiency**.

Furthermore, there is the possibility of functional impairment of the corpus cavernosum muscles due to deficient neurotransmitter release or depletion, competitive receptor inhibition, or quantitative receptor dysfunction. For example, in patients with diabetes mellitus, a marked quantitative transmitter reduction has been demonstrated in addition to impaired endothelium-dependent and electrically stimulable relaxation capacity (Maiorino et al. 2018). Competitive receptor inhibition may be drug-related, but may also be present in psychogenic ED when adrenergic tone prevents adequate relaxation of cavernous smooth muscle. Morphologic changes in the tunica albuginea (e.g., Peyronie's disease) are much more common than functional or quantitative changes in intracavernosal receptors.

Clinically, the erection is markedly weakened or shortened in time with rapid loss of primary rigidity and, in advanced cases, complete ED. It is not uncommon in these cases to find a combination of reduced arterial perfusion and cavernous insufficiency.

30.1.4.3 Neurogenic Erectile Dysfunction

Neurological disorders can lead to erectile problems at different levels of stimulus generation or conduction. Among the most common causes are spinal disorders. About 95% of patients with supranuclear lesions have preserved reflexogenic erections, whereas only about 25% of patients with sacral disorders have normal, psychogenic erections. This indicates a dominant role of the sacral erectile center over the thoracolumbar center.

Central processes such as **Parkinson's disease, encephalitis disseminata, and inflammatory or tumorous changes** show highly variable findings. Erectile dysfunction is to be interpreted as an expression of an imbalance of facilitating and inhibiting influences and is very heterogeneous in its clinical presentation. **Peripheral neuropathie**s usually occur in the wake of metabolic diseases such as diabetes mellitus and alcohol abuse. In diabetes, erectile dysfunction has been described as the initial manifestation in 12% to 30% of patients (Kamenov 2015). However, neuropathy, like vascular changes, usually occurs with increasing duration of the disease and is not only dependent on the quality of the drug regimen. Reactive psychogenic disorders due to drastic changes in lifestyle should certainly not be overlooked.

30.1.4.4 Endocrine-Related Erectile Dysfunction

The role of **androgens** in the regulation of sexual function is complex and their significance in erection is not clearly defined. While complete erections can be observed in infants and castrates (Greenstein et al. 1995), low serum testosterone levels lead to sexual dysfunction, such as ED, loss of libido, decreased semen production, and reduction in nocturnal erections (see Chap. 5). In patients with hypogonadism, **testosterone replacement** leads to significant improvement in erectile function, nocturnal erections, and libido. In the presence of normal testosterone serum levels, however, substitution is not superior to placebo (see Chap. 36). A marked testosterone deficiency is associated with androgen deficiency symptoms and can be detected in up to 19% of ED patients (Bodie et al. 2003).

Pathophysiologically, **androgen deficiency** leads to a reduction in NO-containing nerve fibers and apoptosis of cavernous smooth muscle, as well as a consequent impairment of signal transduction at the cellular level. For this reason, a review of testosterone serum levels is indicated, especially in the absence of response to therapy with **PDE5 inhibitors** (Salonia et al. 2020). Whether dihydrotestosterone (DHT) or dehydroepiandrosterone (DHEAS) levels also have an impact on erectile function is not conclusively established (El-Sakka 2018; Hsu et al. 2015).

As described above, hyperprolactinemia and hypo- and hyperthyreosis can negatively impact erectile function.

30.1.4.5 Drug-Induced Erectile Dysfunction

Various drugs can play a major role in the development of erectile dysfunction (see also Chap. 34). Most of them are preparations with central nervous effects, with targets at the hypothalamic–pituitary–gonadal axis (e.g., GnRH agonists and antagonists), manipulation of the androgen balance by enzyme blockade or receptor modulation (e.g., finasteride and antiandrogens), or at the autonomic nervous system. Of particular importance are antihypertensives, and here, particularly the group of **non-cardioselective beta-blockers,** as

well as **thiazide diuretics,** is clearly associated with an increased risk of ED. Beta-blockers in particular not infrequently also lead to libido disturbances. In antihypertensive therapy, **angiotensin-2 antagonists** and **ACE inhibitors** are considered to be potentially erection-neutral or, in some studies, erection-protective. Alpha-blockers and sartans may even be beneficial for the erection. Most cardiac medications, such as **digitalis drugs** or **antiarrhythmics,** can also worsen erectile function (Nicolai et al. 2014).

Psychotropic medications represent another group of medications that can lead to erectile dysfunction. In the case of psychotropic drugs, especially **tranquilizers and antidepressants**, sedative effects, but also anticholinergic or antidopaminergic effects, are predominant. In this context, both the tricyclic antidepressants and the serotonin reuptake inhibitors, which have dominated in recent years in particular, should be mentioned (Reichenpfader et al. 2014). These lead to an inhibition of the erectile and ejaculatory centers, especially at the cerebral level. With the antidepressants paroxetine, citalopram, and venlafaxine, ED develops in 30% to 40% of patients at the commonly used dosage. Here, switching to agomelatine, nonserotoninergic medications, and desvenlafaxine or vortioxetine may be useful (Montejo et al. 2019). Bupropion, a norepinephrine and dopamine reuptake inhibitor, appears to have little to no negative effects on sexual function in patients with depression or in healthy subjects (Montejo et al. 2015).

Other pharmaceuticals such as **clofibrates, antihistamines, and antifungals** are also associated with the manifestation of erectile dysfunction. In many cases, a patient is dependent on taking several preparations because of different diseases, so that, on the one hand, an accurate assessment of the role of individual preparations is difficult, and on the other hand, therapeutic alternatives are not always available. Particularly, in the case of antihypertensive and antidepressant therapy, it is advisable to check whether it is possible to switch to more erection-friendly drugs, although this should of course only be done in consultation with the treating internists or psychiatrists.

30.1.5 Diagnostic Assessment of Erectile Dysfunction

The complexity of the physiological erectile process and the often multifactorial genesis of erectile dysfunction make subtle diagnostics, as they were regularly performed in the past, seem reasonable, and positive. In practice, however, a **diagnostic nihilism** has unfortunately become established in the age of limited budgets and inadequate reimbursement and an oral pharmacotherapy that is effective for most patients. However, even in the age of oral therapeutics, adequate diagnostics should not be completely abandoned for fundamental reasons. This creates security not least for the patient himself and satisfies his fundamental need for a rational justification for his clinical symptoms. Especially in view of the possible underlying concomitant diseases, the documentation of pathological vascular findings is of particular importance. For these fundamental reasons, the diagnostic workup is given a broader scope than the current practice in many places.

History and clinical examination provide the first important clues regarding the etiology of ED. It is necessary to select from the range of diagnostic procedures those which, taking into account availability, objectivity, reasonableness, and time and cost expenditure, lead to a reliable diagnosis and thus ultimately to a therapeutic decision, since the selection of subsequent forms of treatment is based not only on personal preferences of the patient and partner but also on the most precise knowledge possible of the individual etiology. A **differential diagnostic step-by-step plan** helps to exclude or document and quantify organic factors in particular (Fig. 30.3) (Salonia et al. 2020). If a psychogenic etiology is mainly or additionally suspected, partnership conflicts aggravate, or the partner is not in agreement with ED treatment, further sexual medicine presentation and treatment are useful.

A prerequisite for diagnosing erectile dysfunction is that the affected person seek and find medical advice. Questions about sexual health are not usually included in the medical history of patients who visit their primary care physician or internist. Here, it is important to free this subject area from its taboo, to raise awareness of the topic during medical training and continuing education, and to impart competence in addressing these very personal areas of life in order to lower the inhibition threshold among physicians and patients (Kliesch 2020).

30.1.5.1 Anamnesis and Sexual Anamnesis

For most patients (and some physicians), the first conversation about sexual disorders is a shameful and difficult topic and forms the crucial foundation for a trusting patient–doctor relationship. Not infrequently, it has a guiding character in the classification of a sexual disorder and can be of great practical importance for further diagnostic and therapeutic progress. Therefore, this first patient–doctor discussion should take place undisturbed by external influences, with the necessary intimacy and within an adequate time frame.

The general anamnesis must primarily cover **predisposing diseases and risk factors** that may lead to atherosclerosis, especially diabetes mellitus, arterial hypertension, dyslipidemia, and cardiovascular disease. The recommendations of the second and third Princeton Consensus Conferences call for increased diagnostic efforts in patients presenting with the primary symptom of erectile dysfunction (Nehra et al. 2012). Also, prognostically significant is **nicotine abuse**, which, in addition to acute circulatory disturbances, can lead to impaired erectile smooth muscle function and thus may be a significant modifiable risk factor for ED. The history also includes alco-

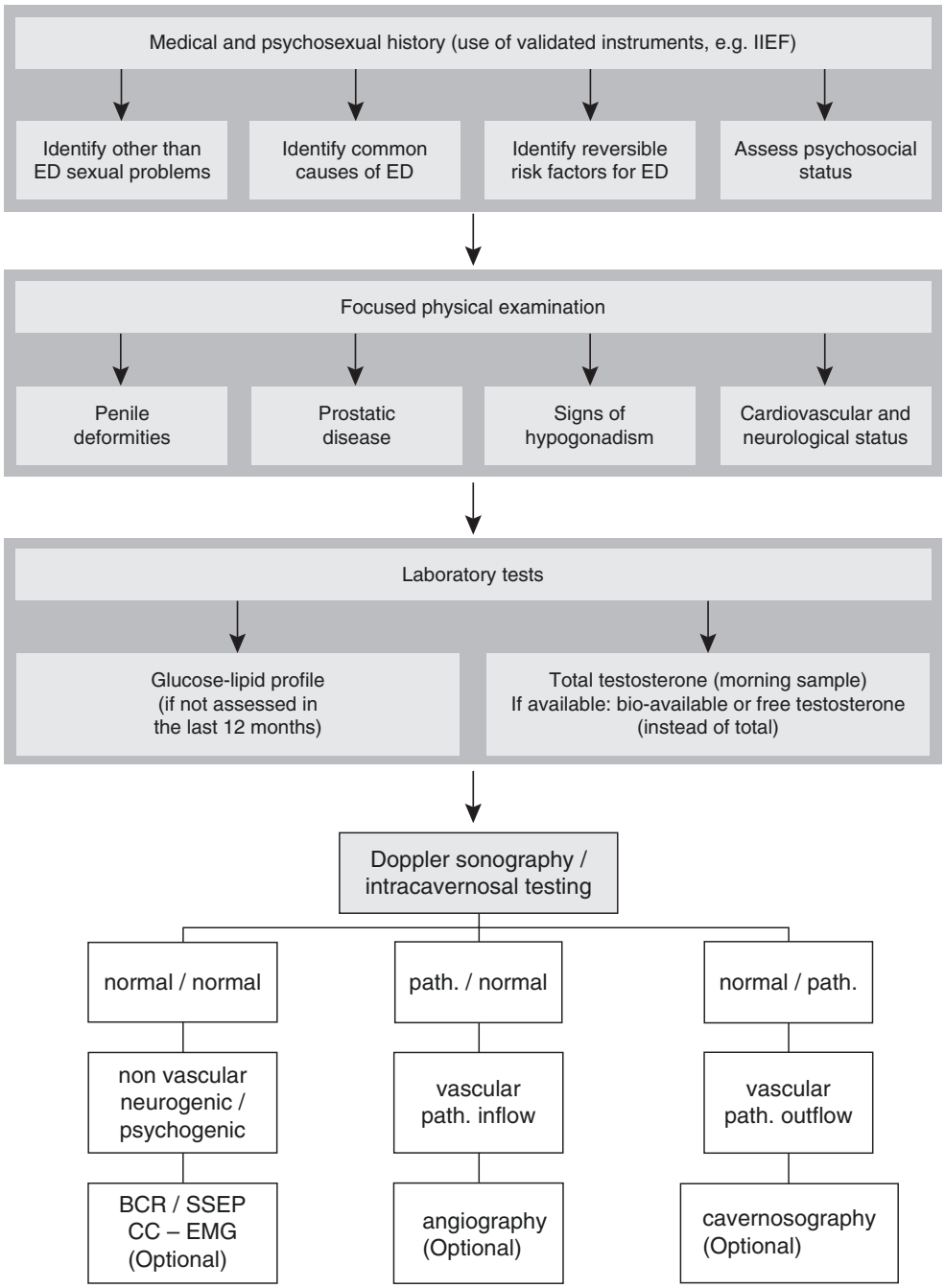


Fig. 30.3 Flowchart for diagnosis of erectile dysfunction. BCR, bulbus cavernosus reflex; SSEP, somatosensory evoked potentials; CC-EMG, corpus cavernosum EMG

hol and recreational drug use (e.g., amphetamines, cocaine, marijuana, and anabolic steroids). In addition to any cardiovascular disease, the self-history should include neurologic systemic diseases, such as epilepsy or multiple sclerosis, and in paraplegic patients, the type and extent of concomitant deficits. Surgical procedures in the pelvic region can have a negative impact on both penile perfusion and innervation. Not only urologic tumor surgery should be inquired about here; also of importance are sigmoid and especially rectal resections, as well as reconstructive vascular procedures of the aorta and iliac vessels (Towe et al. 2019). Reconstructive urologic procedures of the urethra can also negatively impact erectile function. Pelvic injuries can also lead to disruption of arterial perfusion and/or penile innervation, depending on the pattern of injury. In contrast to prostate surgery or open urethral surgery, perineal traumas are usually remembered only when specifically questioned.

A detailed **medication history** is indispensable today, since numerous widely used medications, especially antihypertensives and diuretics or lipid metabolism preparations, can lead to erectile dysfunction. On the other hand, cardiological or other concomitant medication with nitrates and other drugs can limit the possibilities of oral therapy (see Sect. 3.1.4.5).

The general medical history is completed by recording the patient's **social and psychological situation**, since latent depressive moods can be a major cause of functional and temporary erectile dysfunction. This leads to a detailed **sexual anamnesis**, which first determines the type of sexual disorder. In principle, libido disorders, erectile dysfunction, and ejaculatory disorders are to be differentiated. Evaluation and interpretation should always include all these aspects of sexuality (see Chap. 44).

Whereas **primary ED** exists from puberty and can be attributed to congenital vascular malformations with otherwise normal pubertal development, **secondary ED** is characterized by its onset after at least temporarily normal erectile function. In the assessment of the etiology of ED, the precise description of the sexual disorder is often already of decisive importance. Erectile dysfunction as a main subjective symptom is clarified by asking about erection quality, masturbation, and coitus frequency. An age-related decrease in coitus frequency is confirmed, but is often not accepted by the patient. It is therefore important to clarify how the patient assesses his situation before the onset of erectile dysfunction. Discontinuously occurring, situation- or partner-related disorders of erection indicate a predominantly psychogenic etiology. Similarly, act-related disorders, i.e., problems occurring only during sexual intercourse but not in the morning or at night or during masturbation, are to be interpreted as predominantly functional and not organogenic in nature. Concomitant premature ejaculation may indicate psychogenic factors. All these patients should be referred to experienced specialists in sexual medicine, psychologists, and psychotherapists for further clarification and treatment at an early stage (Schmidt et al. 2014).

Psychosexual developmental disorders and partnership-related conflict situations should be a reason to **include the partner in the psychosexual evaluation**. Especially in the case of sexual deviations, the involvement of a psychiatrist is essential. The diagnosis of a psychogenic and especially an "idiopathic" ED should be a diagnosis of exclusion that is made only after organ-related diagnostic possibilities have been exhausted (see Chap. 44).

30.1.5.2 Clinical Examination

During the clinical examination, attention is directed primarily to the **secondary sexual characteristics** (see Chap. 5) and the **external genitals** (see Chap. 29). Any abnormalities in the intensity of beard growth, hair pattern, body fat distribution, and general constitution as well as accompanying clinical symptoms such as fatigue, sleep disturbances, increased sweating, or loss of libido raise suspicion of the presence of hypogonadism. Since a severe androgen deficiency is often associated with testicular atrophy, the assessment of testicular size and consistency is of particular importance. However, other forms of hypogonadism, such as Klinefelter syndrome (see Chap. 21), are not infrequently discovered during the evaluation of erectile dysfunction.

During the clinical examination, blood pressure is also measured (if this has not been done in the last 3 to 6 months), the patient's body weight is measured, and the BMI is determined. Measurement of abdominal circumference is helpful, as elevated values here may indicate metabolic syndrome (Salonia et al. 2020).

30.1.5.3 Validated Questionnaires

Various **psychometric questionnaires** are available for the diagnosis of ED and for objectifiable assessment of progression under therapy. These include the International Index of Erectile Function (IIEF), which measures the domains of sexual desire, erectile function, orgasm, sexual intercourse, and satisfaction, and the abbreviated versions IIEF erectile function domain (IIEF-EF) and Sexual Health Inventory for Men (SHIM). The Erectile Hardness Score (EHS) captures penile rigidity (Salonia et al. 2020). The questionnaires are internationally accepted, although not all are validated for different countries in different languages.

30.1.5.4 Laboratory Medical Tests

To exclude endocrine causes of erectile dysfunction, first and foremost **serum testosterone** levels must be determined. The blood sample should be taken in the early morning hours between 7:00 and 11:00 a.m. due to circadian rhythms (Dohle et al. 2020) (see Chap. 7). In case of low values, LH should be additionally calculated to assess the pituitary–gonadal axis, prolactin to exclude an inapparent prolactinoma, and, if necessary, free testosterone using SHBG, and testosterone metabolites (DHT and estradiol) should be determined.

Further laboratory examinations should exclude latent diabetes mellitus by determining **fasting blood glucose** and **HbA1c** as well as lipid metabolism disorders by means of an appropriate **lipid profile**. In addition, thyroid dysfunction can be excluded or detected by determining **TSH** and fT3 and fT4. A blood count, liver values, and the PSA value are also of additional importance.

30.1.5.5 Oral Pharmaceutical Test with PDE-5 Inhibitors

Even though the **oral pharmacontest** with a PDE-5 inhibitor is not explicitly recommended in the guidelines of the urological societies, it is established in clinical practice for the diagnosis of ED. In the absence of contraindications, the patient takes a PDE-5-I at a medium or high dosage after receiving medical instruction on the intake modalities and mode of action. If an erection is subsequently formed that the patient subjectively perceives as sufficient for the performance of sexual intercourse, it can be assumed with a high degree of probability that the cavernous occlusion mechanism is intact.

30.1.5.6 Cavernous Body Pharmacoinjection Test

In the diagnostic workup of erectile dysfunction, the corpus cavernosum **pharmacoinjection test is still of central importance today to detect vascular ED** and is combined with **color-coded duplex sonography of the profundal penile arteries** (Salonia et al. 2020). In this procedure, injection of vasoactive substances into the right or left corpus cavernosum medically induces an erection that is largely physiological in its course (Fig. 30.4). Thus, bypassing nervous stimulation, the blood flow reserve and the functional integrity of the smooth muscles of the corpus cavernosum and the cavernous closure mechanism can be examined globally and, in particular, vascular disorders can be assessed. However, the occurrence of a complete erection after the application of small doses of pharmaceuticals also allows conclusions to be drawn indirectly and in combination with results of other examination procedures about neurogenic or functional or psychogenic disorders.

The injection of the vasoactive substance in an initially low dosage (e.g., 10 µg prostaglandin E1) into one of the two corpora cavernosa (i.e., intracavernous) is always performed from strictly lateral with a 27G insulin needle after careful disinfection to avoid injury to the dorsal nerve and vascular bundle and the urethra. Due to the anatomical connections between the CC, an even distribution is ensured. Additional compression at the base of the penis is not required (Fig. 30.4).

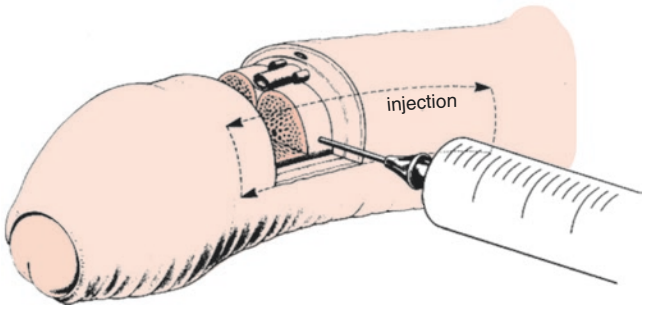


Fig. 30.4 Intracavernosal injection technique

15 min after the intracavernosal injection, the test result is best assessed on the standing patient with regard to the achieved tumescence and rigidity and classified as follows:

- E 0 = no visible reaction
- E 1 = low tumescence, no rigidity
- E 2 = medium tumescence, low rigidity
- E 3 = full tumescence, low rigidity
- E 4 = full tumescence, medium rigidity
- E 5 = full tumescence, full rigidity

Reactions of levels E 0 to E 3 are classified as **negative tests**, and patients are referred to as "non-responders." A negative test correlates with venous or cavernous insufficiency in >90%. If a **cohabitating erection (E 4 to E 5)** occurs with the injection of the vasoactive substance, this is classified as a **positive test** (responder). If multiple testing (with dose escalation) is required, the patient can be instructed in the basics and technique of self-injection therapy (cavernous body autoinjection therapy) already in this phase in individual cases. Both the intracavernous injection for testing and therapy should be preceded by a written explanation to the patient about the risks and recommendations for action in the event of the occurrence of a prolonged erection (>3 h) or priapism (>4 h).

In addition to the quality of the erection, the time of onset and the duration of the erection are also taken into account in the assessment of the test. The course and result of the test allow important conclusions to be drawn about the etiology of erectile dysfunction:

- If testing shows a rapid onset and sustained full erection even at low doses in the presence of unremarkable Doppler sonography, the presence of an arterial perfusion disorder or venous/cavernosal insufficiency and thus **a vascular etiology of ED is largely excluded**. Neurological diagnostics (BCR/SSEP) (see below) may be helpful for differential diagnosis between neurogenic and psychogenic etiology.
- If the response increases only slowly and an adequate erection develops only after 15–30 min, a hemodynamically relevant **arterial perfusion disorder** is usually present. This can be objectified by a pathologic finding on Doppler sonography of the penile vessels.
- A **positive test result** largely excludes relevant venous or cavernous insufficiency.
- A **negative test result** correlates with venous/cavernous insufficiency in more than 90% of cases.

In routine diagnostics, **prostaglandin E1 (PGE1)** has replaced **papaverine** as a vasoactive substance, either as a monosubstance or in combination with an α-adrenoreceptor blocker. Papaverine is an opium alkaloid in which inhibition of phosphodiesterase leads to an increase in intracellular cAMP and calcium efflux from the smooth muscle cell, resulting in a relaxing effect. α-Adrenoreceptor blockers

(e.g., **phentolamine**) are used because sympathetic nervous system activity is responsible for the flaccid state of the penis at rest. Clinically, they show only a weak effect as a monosubstance, but when combined with papaverine, they lead to a favoring of erection induction by reducing sympathetic tone and thus to an increase in effect.

Prostanoids have quite different effects on human erectile tissue in vitro. In particular, under PGE1, there is a pronounced relaxation of human erectile tissue through an increase in intracellular cAMP via specific **PGE1** receptors. Moreover, in vitro, PGE1 leads to presynaptic receptor-mediated inhibition of electrically stimulated norepinephrine release from sympathetic nerve terminals, thereby modulating endogenous sympathetic tone. In vivo, PGE1 leads to potent vasodilation. The substance is metabolized very rapidly, and degradation occurs predominantly in the lungs.

In principle, intracavernous injection testing is possible with all the substances mentioned above, although papaverine and phentolamine as monosubstances are generally no longer used nowadays. In addition to the combination of **papaverine and phentolamine** in a fixed dose ratio (so-called bimix; Androskat®) and **prostaglandin E1**, (Viridal®, Caverject®), the combination of all three preparations mentioned (so-called Trimix) is also used. In addition, a combination of vasoactive intestinal peptide (VIP) and phentolamine is available (Invicorp™) (mainly in Scandinavia) (Salonia et al. 2020). In the usual therapeutic range, **PGE1 is the most potent monosubstance.**

The efficacy of the various vasoactive substances can vary widely intra- and interindividually in terms of erectile strength and erection duration and is often difficult to predict based on anamnestic data alone. Since the effect of the preparations is in principle proportional to the applied dose and an erection duration of 2–3 h should not be exceeded even in primary testing, a low initial dose is always primarily recommended. If a complete erection does not occur after the first injection, further testing with increased doses is necessary, in which the dose can be increased gradually. The time interval between two injections should be at least 24 h during the testing phase.

The initial doses and increments of the various vasoactive agents are as follows:

- PGE1–5; 10; 20; (40) µg.
- Papaverine/phentolamine (so-called bimix) (30 mg + 1 mg/mL)—0.5; 1; 2 mL.
- Papaverine/phentolamine/alprostadil (so-called Trimix) (30 mg + 1 mg + 10 µg)—0.05; 0.1; 0.15 to max. 0.5 mL.
- VIP/phentolamine (Invicorp™) (25 µg + 1 mg)—0.35 mL.

Escalation of the dose of PGE1 beyond 20 µg clinically improves efficacy only in a maximum of 20% of patients, as in the majority of cases receptor saturation is already present at this dose.

Contraindications to intracavernous injection testing are as follows:

- Severe, decompensated cardiovascular insufficiency
- Unstable angina pectoris
- Pronounced liver dysfunction (papaverine)
- Glaucoma (Papaverine)
- Prostatic hyperplasia with high residual urine volumes (papaverine)

Current anticoagulant therapy with Marcumar, low-molecular-weight heparins, or platelet aggregation inhibitors is at most a relative contraindication; increased complication rates with the use of vasoactive substances in this patient group do not occur.

Adverse side effects may include the following:

- Hematomas
- Pain
- Prolonged erections (>3 h)
- Priapism (>4 h)
- Erectile tissue infections

Pain is reported with papaverine/phentolamine by 10–80% of patients and is usually of very short duration, but may last for a few minutes. With PGE1, uncomfortable tightness and pressing pain are observed in 10–40% of cases, but only in a few cases are they pronounced enough to interfere with intercourse. The cause of these complaints is unclear. A dependence on the solvent used in the various PGE1 preparations is discussed. A slight drop in blood pressure with dizziness is occasionally observed with papaverine.

As a **complication, minor hematomas** are not infrequently observed. These are harmless and do not require therapy. **Septic cavernous body infections** are feared, but extremely rare. The most significant complication of intracavernus injection therapy is an undesirable long-lasting effect of the substances. The transitions between **prolonged erection and priapism** are fluid and not clearly defined. A full erection lasting more than 3 h is called prolonged and requires no or only limited therapeutic measures, depending

on the triggering cause or substance. Priapism is characterized by an erection duration of >4 h. Depending on the duration of the event, low-flow priapism, the associated metabolic changes, and acidosis lead to severe damage and even complete destruction of the smooth muscles of the corpus cavernosum with consecutive fibrosis.

Therapeutically, puncture of the corpus cavernosum with drainage of 50–200 mL of stasis blood is usually necessary until a clear arterialization of the corpus cavernosum blood occurs. Only then should sympathomimetic preparations be applied (e.g., Effortil® 10–30 mg). These substances should always be administered under hospital conditions and under strict circulatory control because of possible, vitally threatening circulatory complications with hypertensive blood pressure crises. In the case of incipient increases in blood pressure, the simultaneous application of nitro preparations (e.g., Adalat® 10–20 mg) has proven effective.

In addition to the poorer efficacy, the significantly more frequent occurrence of prolonged erections and priapisms under papaverine and the combination of papaverine and phentolamine during the test and therapy adjustment phase (7%) compared with PGE1 (1%) has led to PGE1 becoming established as the drug of first choice in recent decades. The reason for the significantly reduced priapism incidence under PGE1 may be due to the local intracavernosal metabolism of prostaglandin.

In the choice of vasoactive agent, papaverine theoretically offers the advantage of **lower cost** with a comparatively high risk of side effects. This is offset by limited efficacy and, in some circumstances, a significantly higher number of diagnostic injections due to the slow dose escalation required. PGE1 is much more expensive, but requires a smaller number of injections due to better efficacy and greater therapeutic margin.

30.1.5.7 Doppler Sonography

Doppler sonography of the penis is still an integral part of organically oriented diagnostics and, as a **noninvasive, inexpensive method, detects disturbances of the arterial penile circulation**, which etiologically plays the most essential role in the pathogenesis of erectile dysfunction. The introduction of corpus cavernosum stimulation with vasoactive substances has made possible a systematic study of the arteria penis profunda perfusing the corpora cavernosa. This is difficult to visualize at rest with predominantly intracavernosal perfusion because of its small diameter of only about 0.5 mm, even with high-resolution transducers of 8–10 MHz. Therefore, Doppler sonography should always be performed in combination with the application of vasoactive substances as part of an intracavernous injection test.

Whereas in the past, baseline findings were always obtained on the dorsalis penis artery and, as far as possible, the deep penis artery before application of the vasoactive substance, today the majority of examiners perform the examination only 5–10 min after injection of the vasoactive substance with still increasing tumescence. Only the examination before the onset of a rigid erection allows a reliable assessment of the dilation capacity and thus the perfusion reserve of the examined vessel, since physiologically arterial perfusion decreases sharply after the onset of phase III of the erection (see Table 30.1) and practically falls back to the initial level.

A purely acoustic assessment of the Doppler signal must be rejected as insufficient. The minimum requirement is a registration of the waveform with a continuous-wave Doppler (cw Doppler). Since cw Doppler registers all vessels located in the sound cone, special care must be taken to avoid confusion of the cavernous arteries with superficial branches of the dorsal penis artery. This is usually possible by dosed compression of the penile skin and based on the different sound characters.

The assessment of the Doppler examination is based on the absolute height of the pulse curve and thus the maximum flow velocity and, if necessary, on its relative increase compared with the initial findings immediately before the application of the vasoactive substance. A broadened pulse curve complex with a reduced rate of rise and low amplitude is a sign of arterial reduced perfusion.

30.1.5.8 Duplex Sonography

Duplex sonography, which like conventional cw Doppler examination is always combined with intracavernous injection testing, examines the penile arterial vasculature with **a combination of ultrasound cross-sectional (B-scan) and Doppler**. This allows the examiner to position the cursor, and thus, the measurement point of the pulsed Doppler, precisely in the lumen of the vessel, is examined. In addition to precise imaging of an isolated vessel, this ensures targeted pulse curve recording and perfusion measurement. In this way, changes in the diameter of individual vessels can be recorded and flow velocities are determined with computer support (Fig. 30.5).

A normal value according to intracvernous injection test is considered to be a vessel diameter of the profunda penis of 1 mm and vigorous pulsations of the vessel and a pharmacon-induced dilation of >75% compared to baseline findings. **Maximal flow velocities of > 30 cm/s** are considered normal and largely exclude a serious arterial perfusion defect (Sikka et al. 2013). Because increased flow velocities can be measured, especially in the area of vascular stenoses, due to physical factors, both flow velocity and diameter increase should be included in the assessment of vascular status. Maximum flow velocity as the sole assessment criterion must be viewed critically. The determination of blood flow measured as volume/time (mL/min) has not been accepted because of too large fluctuation ranges. An improvement of

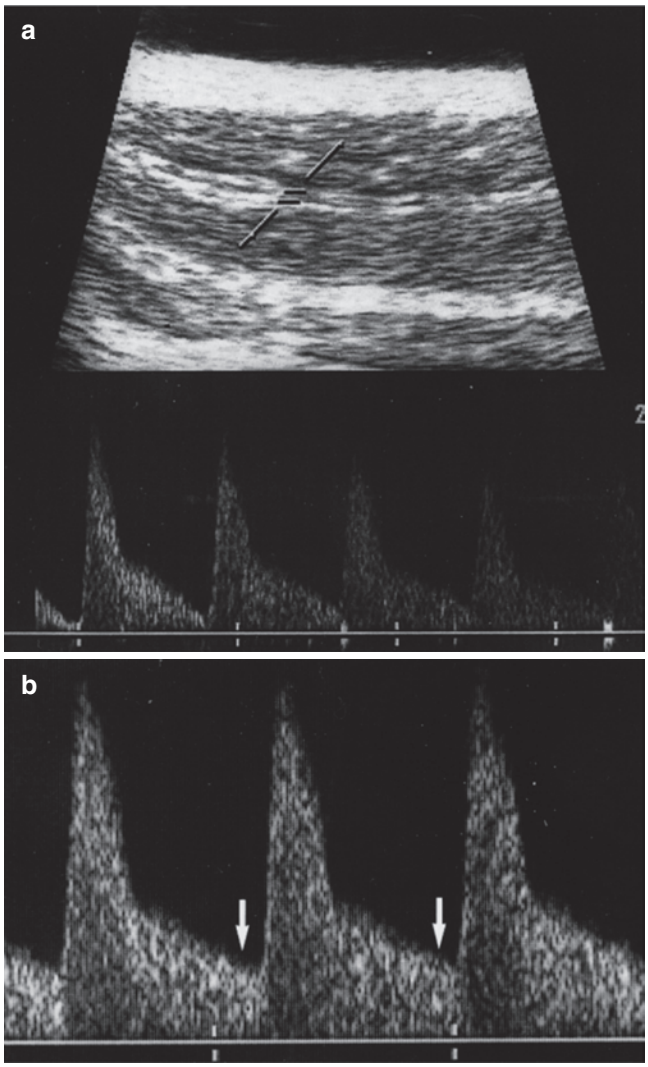


Fig. 30.5 Duplex sonography of the profunda penis artery after intracavernosal injection of 10 µg PGE1. (**a**) In the upper part of the image, representation of the corpus cavernosum and the A. profunda penis in the B-scan. The measurement point of the Doppler probe is positioned in the vessel lumen. In the lower part of the image, simultaneous display of the pulse curve (normal findings). (**b**) Representation of a pulse curve of the profunda penis with clearly increased diastolic flow (arrow). Flow (arrow) as an indication of a cavernous occlusion disorder

the significance is given by the additional assessment of the curve shape and the determination of the systolic rate of rise. Short times below 110 ms indicate an intact dilation capacity of the examined vessel and prove the integrity of arterial perfusion. Increased end-diastolic flow velocities above 3 cm/s may be indicative of insufficient relaxation of the corpus cavernosum muscles or the presence of cavernous insufficiency with increased venous drainage (Sikka et al. 2013) (Fig. 30.5). Furthermore, the determination of the **resistance index (RI)** is an accepted parameter for assessing venous integrity of the system and is dependent on end-diastolic flow. An **RI < 0.8** in the presence of normal systolic flow velocity and incomplete erectile response is indicative of venoocclusive insufficiency (Sikka et al. 2013; Salonia et al. 2020).

Pulsed Doppler sonography is now virtually ubiquitously available, and is considered a **near-ideal screening test**, and has largely supplanted conventional cw Doppler sonography. It is easy to learn and can be performed with virtually no additional time expenditure. The combination of morphological and functional observation allows a much better assessment of the functional blood flow reserve than would be possible by conventional Doppler sonography.

Color-coded duplex sonography represents a further technical improvement. The ultrasound signals reflected by moving erythrocytes are color-coded as a function of flow direction and flow velocity, so that arterial and venous vessels can also be visually separated. In addition to the quantitative measurement of flow velocity, the technique enables the visual representation of flow conditions over the entire penile vessel course with simultaneous imaging of several vessels or vessel sections up into the helical vessels.

Normal values of the systolic and end-diastolic flow velocities of the penile profunda artery and the **resistance index** according to intracavernous injection testing are summarized here. If these values are present, vascular ED is excluded:

- Maximum systolic flow velocity > 30 cm/s
- End-diastolic flow velocity < 3 cm/s
- Resistance index >0.8

In view of the fact that erectile dysfunction is often an early warning sign of cardiovascular disease in men, it makes sense to perform duplex ultrasonography of the carotid arteries with the determination of the intima–media thickness and—if there are signs of peripheral arterial occlusive disease—also to examine the femoral vessels. Carotid intima–media thickness appears to be associated with response to PDE-5-I therapy in patients with vascular ED (Pelit et al. 2019). These examinations can be performed, for example, by a cardiologist or internist or endocrinologist/andrologist, or by appropriately trained urology–andrologists to provide initial guidance for further diagnosis.

30.1.5.9 Penile Angiography

In the past, angiography of the penile vessels was long considered the gold standard in the evaluation of vascular disorders. However, it is a complex and considerably invasive diagnostic procedure with relevant risks. Penile angiography mainly allows conclusions about the anatomy and not the

functionality of penile perfusion (Sikka et al. 2013). Conventional cw Doppler sonography under correlates in more than 90% with the results of selective angiography. With the technical advancement of noninvasive duplex sonography, which is much less expensive in terms of time, personnel, and cost, angiography is now indicated only in forensic or expert opinion problems or in patients with primary erectile dysfunction and suspected congenital vascular dysplasia or malformation before any vascular surgical intervention (e.g., preoperative visualization of the internal pudendal artery and inferior epigastric artery before vascular reconstructive surgery).

MRI-guided angiography of the penis allows visualization of the internal iliac artery and vein; however, this imaging modality does not provide adequate resolution of the penile vessels, which is why it has not gained acceptance over conventional penile angiography (Kirkham 2012).

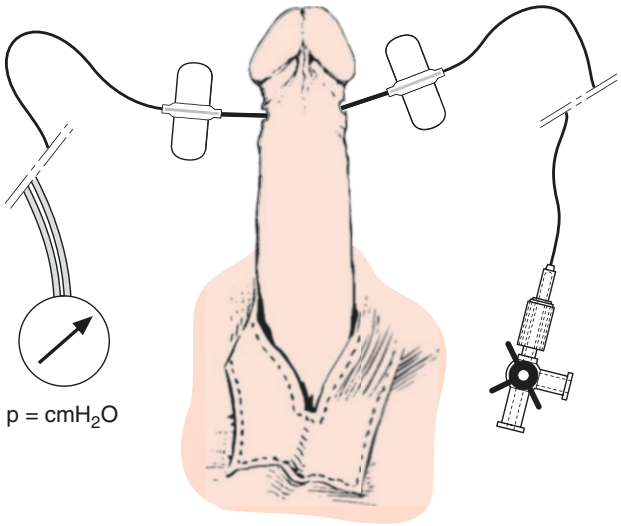


Fig. 30.6 Principle of cavernosometry and cavernosography

30.1.5.10 Venous Outflow Diagnostics

In about 10% of all patients with erectile dysfunction, no adequate erection can be induced with the usual vasoactive substances; even with the highest dosage and appropriate combinations, the intracavernous injection test is negative, and the patient is called a non-responder. Such symptomatology may indicate incompetence of the cavernous closure mechanism with the increased outflow of venous blood from the corpus cavernosum, a phenomenon often referred to as "venous leakage." However, this is very rare and may also be visualized less invasively by a good contrast-enhanced MRI examination.

Therapeutic consequences of cavernosometry for the diagnosis of venous outflow conditions would be the implantation of a penile prosthesis in the majority of cases nowadays after disappointing long-term results of venous surgery and reconstructive vascular surgery measures. This has led to the fact that the indication for pharmacocavernosography is nowadays drastically limited and the examination is only indicated in exceptional cases, e.g., for special expert opinion questions. Nowadays before implantation of a penile prosthesis, the examination is no longer required (Salonia et al. 2020).

The diagnosis of cavernous outflow conditions basically consists of two parts. It includes **bloody intracavernosal pressure measurement under conditions of artificial erection during infusion with controlled flow rates.** Saline is infused into the CC via a puncture cannula in the corpus cavernosum using a roller pump, and at the same time, the intracavernosal pressure is continuously recorded via another cannula and recorded using a recorder. This part of the examination is called **cavernosometry** (Fig. 30.6).

Since adequate compression of the draining veins by the relaxed cavernous sinusoids occurs only with increasing rigidity, ultimately only examination under drug-induced erection, so-called pharmacocavernosography, is meaningful. A high dosage of the vasoactive substances is necessary, because the indication arises only in the case of negative intracavernous injection test.

In cavernosometry, once a suprasystolic pressure plateau is reached, the infusion volume is reduced and the flow rate/minute required to maintain a defined intracavernosal pressure is determined. This is referred to as maintenance flow and is one of the most essential criteria in the assessment of cavernous compliance. A linear correlation of maintenance flow rates and intracavernosal pressures is indicative of complete smooth muscle relaxation. Maintenance flow rates of less than 5 mL/min are now considered normal. Another parameter that is considered a relevant criterion of cavernous function is the pressure decay rate. After reaching a defined pressure plateau (usually 150 mmHg), the pressure drop is measured over 30 s at the infusion stop; normally, there is a slow, linear drop in intracavernosal pressure. Exponentially decreasing pressure values with half-lives of less than 1 min or a pressure reduction of >50 mmHg within 30 s demonstrate cavernous/venous dysfunction.

If flow rates are elevated, radiologic imaging of the draining veins and thus localization of a venous leak under fluoroscopic control can be obtained by contrast application (cavernosography). Venous outflow shows great anatomic variability without prior drug stimulation. In general, the proximal CC is drained via cavernous veins, whereas the drainage of the middle and distal portions of the CC is via vv. emissariae and vv. circumflex into the superficial and profunda dorsal veins. Ectopic veins draining the cavernous blood via a direct shunt connection, e.g., into the femoral artery, are rare (Fig. 30.7). In most cases, cavernous insufficiency involves increased drainage via physiologic veins. In this regard, insufficiency types with increased drainage via

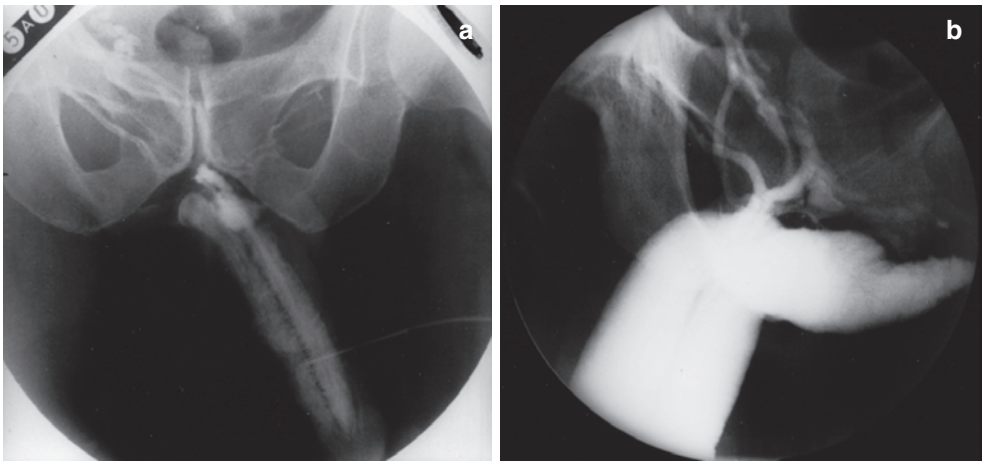


Fig. 30.7 Pathologic cavernosographic findings. (**a**) Isolated dorsal venous insufficiency with inadequate filling of the corpora cavernosa with the rapid outflow of applied contrast via the dorsalis profunda vein. (**b**) Increased outflow via crural veins leaving the corpora cavernosa proximally dorsomedial

crural and cavernous veins or via the glans penis and the C. spongiosum especially evade sufficient surgical therapy (Fig. 30.7).

Complications of cavernosometry and cavernosography are especially local hematomas, which are resorbed within a few days without consequences. Cavernitis is extremely rare. The instrumentation for cavernosometry is not readily available nowadays due to low demand.

30.1.5.11 Neurophysiological Assessment

The patient's medical history must always include accompanying symptoms such as motor or sensory lesions, which may be indicative of a neurological disorder. Attention is directed in particular to neurological systemic diseases, which are usually clinically known or easily detectable, a primary manifestation via the symptom erectile dysfunction is extraordinarily rare.

The bulbus cavernosus reflex (BCR), which records the conduction pathways between the penis and the sacral erectile center, is clinically and electrophysiologically measurable. A defined electrical impulse is applied to the penile shaft via ring electrodes and the reflex response is derived laterally separated via two needle electrodes in the bulbocavernosus muscles. Simultaneous registration of cortical evoked potentials is performed via needle electrodes attached to corresponding reference points on the scalp (somatosensory evoked potentials; SSEP). The absolute latencies of the BCR, as well as its differences in side-to-side comparison with repeated stimulus application, provide information about the integrity of the short conduction pathways between the penis and the sacral erectile center. The evoked potentials allow conclusions to be drawn about the function of the long cerebrospinal tracts in the case of prolongations exceeding 50 ms as a function of the patient's body size. Only the standardization of these examination procedures made it possible to make at least the somatosensory aspect of penile innervation accessible to analysis and thus to objectively detect a large proportion of neurogenic erectile dysfunction. However, nowadays the diagnostic value of measuring BCR and SSEP for the evaluation of ED is controversial (Giuliano and Rowland 2013).

Another part of the efferents is attributable to the parasympathetic nervous system and, after switching in the inferior hypogastric plexus, runs as the cavernous nerve to the corpus cavernosum, where the tonic activity of the smooth muscles of the corpus cavernosum is inhibited. Direct detection of autonomic lesions below the pelvic floor level has not been possible. Indirect signs of autonomic innervation dysfunction include weakening of the urinary stream or bladder voiding dysfunction detectable in urodynamics without a tangible morphologic correlate. Direct recording of the electrical activity of individual smooth muscle cells of the corpus cavernosum (corpus cavernosum EMG, formerly also called single potential analysis of cavernous electrical activity; SPACE) is technically possible today, but the interpretation of the acquired data is difficult, regardless of the type of qualitative, semiquantitative, or computer-assisted evaluation. Despite defensible reproducibility, the value of this method must still be considered experimental (Giuliano and Rowland 2013). Signs of sympathetic innervation dysfunction may also result from the absence of the sympathetic skin reflex. In combined disorders, but also in preserved somatosensory innervation and normal BCR, the sympathetic skin reflex may provide evidence of an isolated autonomic neuropathy and thus a neurogenic etiology in some patients, so it can be performed as an additional examination if the patient has an appropriate history.

The partially regionally separated anatomical course of somatic and autonomic nerve fibers may be the cause of selective damage, especially iatrogenic or traumatic. Given the uncertainties of the existing examination methods for the parasympathetic innervation part, this often causes considerable diagnostic difficulties. In addition to the electrophysiological findings, anamnestic and clinical data must therefore be taken into account, especially in the case of expert opinions. Clinically, the lack of therapeutic relevance at a high

examination technical effort has relegated all procedures to the background (Giuliano and Rowland 2013; Salonia et al. 2020).

30.1.5.12 Nocturnal Rigidity and Tumescence Measurements

In healthy males, four to five erectile phases of approximately 20- to 30-min duration occur physiologically at night during sleep REM phases. Assuming that psychological factors do not affect this form of erectile function, the measurement of nocturnal erections has long been considered the reference method for differentiating organogenic from psychogenic erectile dysfunction (Qin et al. 2018a). While studies in the sleep laboratory are very personnel- and time-intensive, various forms of nocturnal erection registration are now available. In this context, simple methods such as the postage stamp test or the snap gauge tape only provide information about the achieved tumescence increase and only allow a rough qualitative assessment. When **registering nocturnal erections** with the RigiScan®, tumescence changes at the base and tip of the penis can be continuously recorded and correlated with the respective rigidity, the most relevant parameter for cohabitation ability. However, the sensitivity and specificity of the method are much lower than previously thought, and interpretation of the findings is only possible in conjunction with the findings of other organ-oriented examination methods (Zou et al. 2019). Because tumescence and rigidity measurements record the quality of nocturnal erections but not sleep quality, the results are not usable in sleep disorders. Various medications or mental disorders such as severe depression may interfere with nocturnal erections. Also, with increasing age or hypogonadism, the number and rigidity of nocturnal erections decrease significantly. It is known from various neurological diseases such as multiple sclerosis that despite preserved nocturnal erections, psychogenic and reflexogenic erections may be severely impaired, making sexual intercourse impossible.

Due to the rather low sensitivity and specificity at high acquisition costs, the method is not suitable for routine diagnostics. For clinical questions, the method is only applicable if the possibilities of a sleep laboratory are available at the same time. It has its place rather in forensic questions and in the assessment of postoperative or post-traumatic ED, especially if isolated lesions of the autonomic innervation (cavernous nerve) are under discussion, as well as in scientific questions, such as investigations of penile rehabilitation.

Criticism of all diagnostic procedures listed here is justified as even in the case of pathological organ findings, often an unambiguous, cause–effect relationship cannot be assured, so that supposedly scientifically exact diagnoses can always only be used as working diagnoses with restrictions. It is not uncommon for patients without clinically manifest erectile dysfunction to have comparable organic findings as patients with pronounced symptoms. Also, in very few cases it is a monocausal problem, usually several factors are involved in the development of erectile dysfunction, so that not only a multidisciplinary evaluation but also a corresponding multimodal therapeutic strategy should be the goal of all efforts.

Despite the revolutionary developments in the therapeutic sector in recent years and the repeatedly cited limited therapeutic consequences of diagnostic procedures, a basic diagnostic workup should not be dispensed today in the interest of many patients, especially in view of the growing awareness that erectile dysfunction is the first early warning symptom of (cardio) vascular disease.

30.1.6 Therapy of Erectile Dysfunction

In principle, the treatment of erectile dysfunction can be divided into conservative, noninvasive or semi-invasive, and surgical forms of treatment. ED therapy follows a staged approach: First, conservative methods are attempted, which include lifestyle and risk factor modifications, as well as the various psychological-behavioral therapies, oral drug therapy, topical application of medications, and the use of external erectile aids and low-intensity shock wave therapy (li-ESWT). The next stage includes semi-invasive treatments such as autoinjection therapy. The final stage includes surgical options such as penile prosthesis implantation and, in very rare and special cases, vascular surgery, or angiographic interventions.

30.1.6.1 Lifestyle Modification and Risk Factor Reduction

Modifiable risk factors of ED include smoking, inadequate physical activity and exercise, obesity, excessive alcohol consumption, and drug abuse. Moderate and vigorous physical activity may reduce the risk of ED, according to the results of a meta-analysis. Prospective studies showed that physical activity can also improve erectile function in ED patients. Weight reduction and a switch to a Mediterranean diet may also lead to improvement in erectile function in ED patients. Stopping smoking also leads to an improvement in ED after about 1 year. Moderate alcohol consumption (1–20 "standard drinks" per week (1 "standard drink" = 375 mL of beer with 3–4% alcohol) is negatively associated with ED (Maiorino et al. 2015).

30.1.6.2 Psychological/Psychotherapeutic Interventions

Strictly speaking, psychological care of the patient begins with the first conversation about ED. In many cases of only short term, not yet fixed ED without relevant organic deficit, a few conversations are already therapeutically effective and sufficient. Not infrequently there is a completely unrealistic and exaggerated expectation of one's own sexual performance, not least induced by the media. The patient's individual ideas about his or her sexuality should be asked, and fears of expectation and failure should be revealed and reduced through targeted education about physiological processes. For example, it is already extremely helpful for many patients to recognize and understand the biochemically almost compelling connection between fear of failure and increased sympathetic tone on the one hand and functional erectile dysfunction on the other. Such counseling sessions should also be an occasion for the patients and their partners to analyze their relationship more intensively. The goal of psychological/psychotherapeutic care and therapy is to address erectile dysfunction; notwithstanding, treatment should attempt to improve communication within the partnership.

Over the past few years, classical psychodynamic therapy methods have been largely replaced by sex therapy approaches (see Chap. 44). Sex therapy, in turn, is based on insights from other forms of treatment, such as couple therapy, family therapy, or behavioral therapy. In this context, overcoming fears of failure is one of the prominent concerns of sex therapy, which substantially goes back to the work of Masters and Johnson (Masters and Johnson 1970). For this purpose, the couple is prohibited from coitus for a limited period of time, which in itself helps to reduce fears of failure. The purpose of therapy is to create a tension- and anxiety-free atmosphere between the partners with the encouragement and development of non-coital sexual practices. This alone usually leads to a significant improvement in communication skills and thus in the partner relationship. Central to the therapy is "Sensate Focus": The couple is given behavioral instructions and performs "exercises" between therapy sessions. During the therapy sessions, the couple's experiences with these "exercises" are discussed. These "exercises" range from stroking the whole body, leaving out the genital region, through up to eight intermediate stages, to unrestricted sexual activity (for a detailed overview, see Hauch et al. 2007). If possible, the involvement of both partners in the therapeutic process should always be sought. Long-lasting disorders, concomitant partnership problems, or loss of libido is prognostically unfavorable. Psychological/psychotherapeutic treatments are time-consuming and usually require numerous sessions. In addition, appropriate motivation of patients and partners is an important prerequisite. However, problems of feasibility arise not only as a result of the high time expenditure of such psychological/psychotherapeutic treatment, but also due to the limited availability of adequately qualified sex therapists.

Overall, psychological/psychotherapeutic interventions currently available include sex therapy, couples therapy, group therapy, psychoeducation, and behavioral therapy. Meta-analyses have shown that the effectiveness of psychological/psychotherapeutic interventions is mixed and the overall evidence is limited. Most meta-analyses on the effectiveness of psychological/psychotherapeutic interventions in ED therapy have limited patient numbers (16 to 235) (Ciocanel et al. 2019). A Cochrane systematic review found that focused group sex therapy can improve erectile function in selected ED patients. In addition, it was shown that there was no difference in effectiveness between psychological/psychotherapeutic interventions and the use of a vacuum pump or autoinjection therapy (Melnik et al. 2007).

Psychological/psychotherapeutic treatments should not be exclusive to psychogenic ED, but should also be considered and offered when there are proven organic causes. Rarely are erectile dysfunctions due to one cause alone, and even in severe, prolonged organic findings, concomitant psychogenic components are rarely absent. On the other hand, even if the cause of erectile dysfunction is predominantly psychogenic and symptoms persist for a long time, organically oriented treatment may be useful in addition to psychological/psychotherapeutic intervention. Thus, studies have been able to impressively demonstrate the special value of a combined therapeutic approach—e.g., the combination of behavioral therapy and PDE-5 inhibitor therapy (Ciocanel et al. 2019; Khan et al. 2019). It is quite a positive experience for the patient when the PDE5 inhibitor or intracavernous injection therapy allows him to experience a full erection again.

30.1.6.3 Hormonal Treatment

Hormonal treatment should generally be reserved for patients with proven endocrine etiology of their erectile dysfunction. A serious androgen deficiency, relevant even in the absence of other measurable organic factors, is present only with testosterone values <12 nmol/L. Values in the lower normal range or slightly below should first be checked and further clarified in terms of the diagnosis of hypogonadism (Dohle et al. 2020; Salonia et al. 2020). As the efficacy of androgen substitution is not superior to placebo when serum testosterone levels are normal, probationary hormone therapy is not indicated without a proven deficiency. However, in cases of repeated subnormal serum testosterone levels, it must be considered that testosterone is a crucial conditioning factor for central, but also peripheral–intracavernosal signal transduction, on which, for example, NO synthetase is dependent. In these cases, raising the decreased testosterone levels to a

physiological range can lead to an improvement in the effectiveness of PDE-5 inhibitor therapy and, especially in patients with mild ED, to a normalization of erectile function (for the implementation of testosterone substitution, see Chap. 36), which has been demonstrated by a large number of studies and meta-analyses (Ciocanel et al. 2019; Corona et al. 2017; Snyder et al. 2016).

In the case of **hyperprolactinemia**, it should first be verified whether it is merely minor stress-related elevations in serum levels or whether drug-related causes should be considered. Hyperprolactinemia can be accompanied by a testosterone deficiency and can correspondingly aggravate symptoms of ED. In the presence of prolactinoma, therapy is given with dopamine agonists, which can have a positive effect on erectile function (Shimon et al. 2019) (see Chap. 15).

For treatment of thyroid dysfunction, refer to internal medicine and endocrinology textbooks.

30.1.6.4 External Erectile Aids

The **principle of venous restriction by compression of the base of the penis** has always been known. Various **constriction rings** (called penile rings) on the market are intended to induce an adequate erection by reducing venous outflow at the base of the penis. These rings are only considered in patients with intact arterial perfusion. However, because cavernous insufficiency is usually associated with marked penile underperfusion and secondary degeneration of erectile smooth muscle, the applicability of penile rings as monotherapy is severely limited.

External erectile aids have become established primarily in the form of **vacuum pumps**. The various systems available differ essentially only in terms of the pumping devices used and the tourniquets. The devices consist of a plastic cylinder that is slipped over the flaccid penis. A vacuum is created in this cylinder by a manual or battery-powered pump. The vacuum causes blood to fill the entire penis, resulting in an erection-like state. Finally, to prevent premature drainage of this blood and to allow sexual intercourse, a tension ring or special rubber band is slipped from the base of the cylinder to the base of the penis (Fig. 30.8).

While adequate arterial blood flow is a prerequisite for the successful use of erection rings, vacuum pumps can be used for all causes of erectile dysfunction and are suitable for inducing adequate erections. This also applies to erectile dysfunction of severe arterial-vascular origin. The effectiveness of vacuum systems is limited in cases of pronounced intracavernosal scarring, as observed after long-lasting priapisms or after the explantation of infected prostheses. In the case of Peyronie disease, the application may be difficult or impossible due to penile deviation.

The main advantage of vacuum systems is their freedom from complications to the greatest possible extent, even with long-term use. However, the erection induced by vacuum systems differs in essential points from a physiological erection. Overall, the quality of the erection in terms of the rigidity achieved is not always completely satisfactory. While in normal cases only the corpora cavernosa are filled, the use of the pump results in blood stasis in the entire penile tissue distal to the tourniquet. This can cause **petechial bleeding and ecchymosis** of the penile skin with a subjective feeling of **coldness and numbness** on the one hand and **stasis edema** on the other. For this reason, manufacturers recommend not leaving the tourniquets in place for **more than 30 min** and then removing them. Another typical problem can result from insufficient rigidity of the erectile tissue proximal to the tourniquet, as this can lead to significant instability at the base of the penis. Ejaculatory disorders are common. These may be mechanical, but may also have functional causes in the form of inadequate precoital stimulation.

The therapeutic efficiency of vacuum pumps is high: 90% of patients have erections sufficient for sexual intercourse. Patient satisfaction is highly variable, ranging from 27% to 94%. After 2 years, 50% to 64% of patients are still using a vacuum system for ED therapy. Most commonly, therapy is discontinued 3 months after initiation (Salonia et al. 2020). After radical prostatectomy, early use of a vacuum system may improve erectile function and prevent penile length loss, which has been supported by a systematic review and meta-analysis that included 273 patients from six randomized trials (Qin et al. 2018b). It is not uncommon for younger patients to reject the pump because of the considerable technical effort required and a subjective perception of poor integration options in the context of their sexuality. In older patients with a long-standing partnership, slowly developing or postoperative erectile dysfunction, experience has shown that acceptance is often better. Reasons for discontinuation of therapy are usually lack of "cosmetic" acceptance—especially on the part of the patient himself, insufficient effectiveness of the system with regard to the rigidity achieved, pain, or declining sexual appetence. Therapy with vacuum systems is contraindicated in patients with blood coagulation disorders, priapism, and therapy with anticoagulants such as Marcumar (Salonia et al. 2020; Trost et al. 2016).

30.1.6.5 Oral Therapy

Since the introduction of **PDE-5 inhibitors (PDE-5-I)** in the late 1990s, oral pharmacotherapy of ED has been revolutionized. Basically, preparations with a central mechanism of action (yohimbine, apomorphine) can be distinguished from those with a peripheral mechanism of action (yohimbine, phentolamine, and PDE-5 inhibitors). Phentolamine and apomorphine are no longer used for ED therapy due to limited efficacy and less favorable side effect profile compared to PDE-5-I.

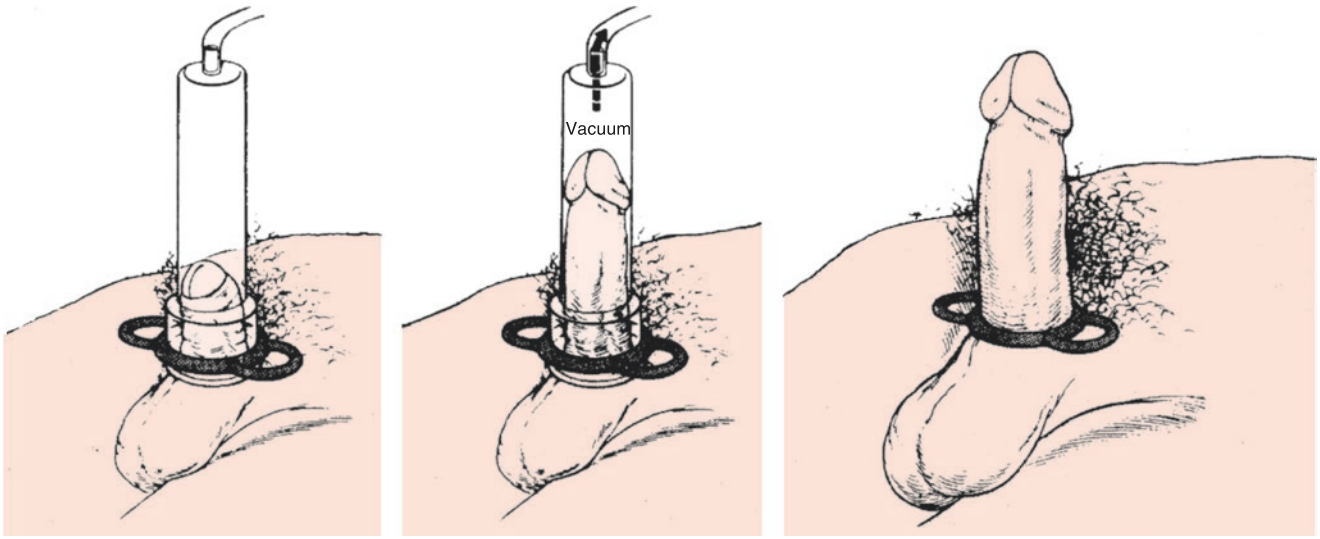


Fig. 30.8 Principle of vacuum systems (courtesy of the Osbon Company)

One of the oldest substances is **yohimbine**. Several prospective, randomized, double-blind studies, with response rates of about 30%, demonstrate the efficacy of the substance in patients with psychogenic erectile dysfunction and without severe reduction in arterial perfusion. The main cause of efficacy is 2-adrenolytic action centrally and peripherally. Full efficacy usually occurs after a therapy duration of 4–8 weeks. The required dose is 3 × 5 to 3 × 10 mg per day. Alternatively, "on-demand" therapy can be given approximately 1 h prior to intercourse. Yohimbine is primarily suitable for patients who have no or only minor organic disorders and are averse to a more invasive form of therapy. Systematic reviews and meta-analyses have shown that yohimbine is well tolerated with minor and reversible side effects (rash, hypertension, dizziness, headache, gastrointestinal, anxiety, and others). Overall, yohimbine has positive therapeutic effects; however, the evidence is considered limited because efficacy has been investigated in a limited number of studies, each of which, in turn, included rather small numbers of patients (Ciocanel et al. 2019). Clinically, the use of yohimbine has virtually ceased to play a major role.

A revolutionary change in therapeutic options for erectile dysfunction occurred with the introduction of the first oral **phosphodiesterase inhibitor, sildenafil**, in 1998. Not least, the media hype associated with this introduction has led to an unprecedented change in the therapeutic landscape for erectile dysfunction. Overnight, physicians of all specialties became designated ED therapists, and healthy men became patients, unfortunately not without fatal consequences in individual cases. In the meantime, PDE-5-I has established themselves as reputable and safe drugs whose scope of application increasingly goes beyond the treatment of erectile dysfunction and which is also approved for the treatment of LUTS in prostatic hyperplasia and pulmonary hypertension. Last but not least, the realization that erectile dysfunction is not infrequently the marker or at least accompanying symptom of serious cardiovascular disease, preceding serious cardiac events in many cases by 2–3 years, has permanently changed the way patients and physicians look at it. The current EAU guidelines recommend PDE-5-I as first-line therapy for ED (Salonia et al. 2020). It makes sense to test them out at an early stage in the diagnostic workup in order to avoid unnecessarily invasive diagnostics.

The **effect of the substance group** is based on a biochemically induced modulation of smooth muscle cell relaxation. A parasympathetic erection-inducing nerve impulse leads to the release of NO at the endothelial cell of the corpus cavernosum. Activation of guanylate cyclase results in the degradation of guanosine triphosphate to the "second messenger" cGMP. As described above, cGMP leads to smooth muscle cell relaxation through intracellular calcium depletion. PDE-5-I now leads to a reduced metabolism of cGMP by inhibiting local phosphodiesterase, which accumulates in the corpus cavernosum and thus leads to an improvement in the relaxation of the smooth corpus cavernosum muscles of the sinusoids as the accumulator of the penile vessels and thus clinically also to an improvement in erection through improved blood flow (Fig. 30.9). Thus, the effect of PDE-5-I is dependent on the transmission of an appropriate nerve impulse via an intact peripheral reflex arc, not least by means of adequate sexual stimulation, and on the presence of sufficient amounts of NO in the corpus cavernosum.

Phosphodiesterases are enzymes that are ubiquitously detectable in the human body with at least 11 subtypes. Types 3 to 5 predominate in the corpus cavernosum, with phosphodiesterases of types 3 and 4 catalyzing in particular the degradation of cyklo-AMP in the smooth muscle cell, and phosphodiesterases of type 5, which are particularly

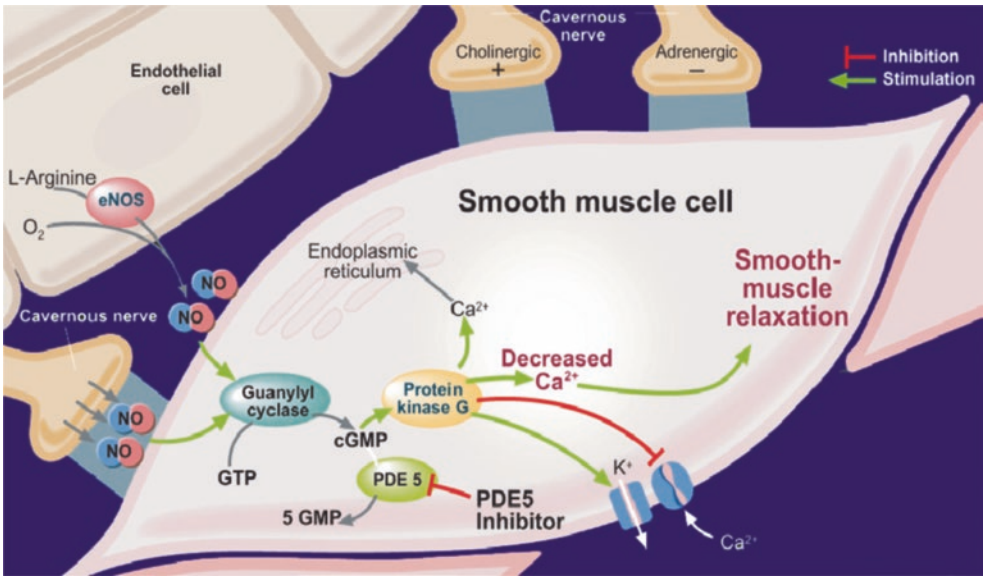


Fig. 30.9 Scheme of action of phosphodiesterase inhibitors on the smooth muscle of the corpus cavernosum. After stimulation, the released nitric oxide (NO) activates guanylate cyclase, which converts GTP (guanosine triphosphate) to cyclic guanosine monophosphate (cGMP) and activates protein kinase G (PKG), resulting in a decrease in free intracellular calcium. The decrease in intracellular Ca2+ levels results in smooth muscle relaxation. Through the use of phosphodiesterase inhibitors, the function of 5′-phosphodiesterase type 5 (5′-PDE-5) is inhibited, so that cGMP is not metabolized to 5′-GMP. Accumulation of cGMP results in increased protein kinase activation, further decreasing intracellular calcium concentration and mediating enhanced vascular smooth muscle relaxation. →: stimulation, --|: inhibition

abundant in the penile vessels and intracavernous endothelium, that of cGMP. PDE-6 is found in the retina and is responsible for the visual disturbances (mild blurred vision and blue vision) that occasionally occur under higher doses of sildenafil as a cross reaction. PDE-11 is detectable in higher concentrations in the testis and prostate, although the exact function of the enzyme in these organs has not been conclusively clarified.

Four substances are currently approved and available in Europe: **Sildenafil (Viagra®)**, the first oral substance approved worldwide in the treatment of ED, as well as **Vardenafil (Levitra®)** and **Tadalafil (Cialis®)** since 2003, and **Avanafil (Spedra®)** since 2013. The substances differ in terms of pharmacological parameters and, above all, have different pharmacokinetic properties.

Clinically, the registration studies and clinical experience have not revealed any relevant differences in the pure efficacy of the substances at the respective maximum therapeutic doses. In many cases, clinical comparative studies show a considerable bias and are often open to challenge with regard to study design and interpretation of results and are therefore problematic. Currently, there are no double-blind or triple-blind multicenter studies that have compared the efficacy of the available PDE-5-I. One meta-analysis was able to find that patients who want the highest possible efficacy can be treated with sildenafil 50 mg, while patients who place the greatest value on good tolerability should take tadalafil 10 mg initially (Chen et al. 2015). In addition, a randomized, placebo-controlled, double-blind trial in 623 patients who had previously taken PDE-5-I as needed and had partial treatment response among them demonstrated that daily use of 5 mg tadalafil improved erection (Burns et al. 2015).

Relevant clinical differences in efficacy may be most likely to exist at submaximal doses of the compounds. Overall, selectivity for PDE-5 over other phosphodiesterases is greatest with Avanafil, which is why it is postulated that the side effect spectrum should theoretically be lowest here. However, as mentioned above, there are currently no direct comparative studies to support this (Salonia et al. 2020). The most important difference between the four substances is their pharmacokinetics. In addition to the speed of drug accumulation, the half-life in particular is very different (Fig. 30.10). Whereas the time of onset (Tmax) is about 40 min for Vardenafil and about 70 min for Sildenafil, the mean time of onset is 120 min for Tadalafil and 30 to 45 min for Avanafil. Clinically, in many cases a good erection can be induced with all substances earlier than at Tmax, which is due to the inhibition of PDE-5 that has already occurred by this time. Thus, with Sildenafil and Vardenafil, erections can be induced in some patients after 10 to 15 min, and with Vardenafil, more than 50% of responders achieve a sufficient erection after 25 min. With Tadalafil, an erection can also be induced in a proportion of patients after 30 min, although the pharmacokinetics mean that the effect can occur much later in individual cases. With Avanafil, 15 min after taking 50 mg, 64% experience erections that enable sexual intercourse. When taking 200 mg of Avanafil, this is the case in 71% of patients after 15 min (Salonia et al. 2020). Only in

Parameter	Vardenafil 20 mg*	Sildenafil 100 mg**	Tadalafil 20 mg***	Avanafil 200 mg****
T_{max} (min)	40 (15–180)	70 ± 59	120 (30–720)	31 (19.8–49.8)
$T_{1/2}$ (h)	3.94 ± 1,31	3.82 ± 0.84	17.5	10.7

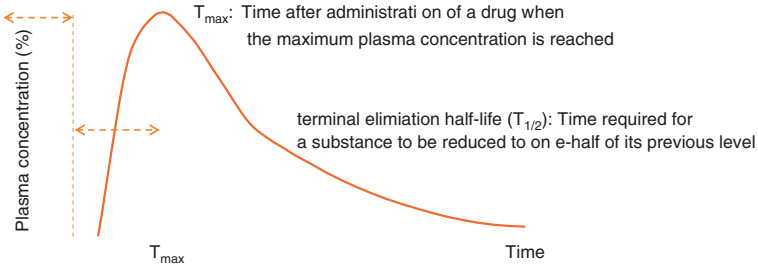


Fig. 30.10 Pharmacokinetics of available phosphodiesterase inhibitors (*Klotz et al. 2002; **Viagra product monograph; ***Patterson et al. 2002; **** Jung et al. 2010)

individual cases, however, are these pharmacological differences clinically relevant for the patient.

Prior food and alcohol intake play some role in the rate of absorption and maximum plasma levels for the shorter-acting agents; this is not the case for Tadalafil. For Sildenafil and Vardenafil, the half-life is in virtually identical range at just under 4 h, whereas it is 17.5 h for Tadalafil and 6 to 17 h for Avanafil. The clinical efficacy for all substances is 2–3 times the half-life, i.e., 8–12 or 36 or more hours. During this period, an erection can be induced under adequate stimulation. It has proven to be particularly important to specifically point out the need for adequate stimulation to the patient during the patient education process, since the occurrence of an adequate erection is not an automated, purely pharmacologically induced process as is the case with intracavernous injection therapy. This fact is often of particular importance to female partners as well, since the compelling need for stimulation highlights the role of the partner in the treatment, thus helping to address concerns and worries about a person-independent increase in libido.

At this point, therapists have a special opportunity to point out to the patient and partner the importance of communication in the partnership burdened by ED and thus to lay perhaps the most decisive foundation for a successful therapy (see also Chap. 44). Studies have convincingly shown that male ED has a lasting negative impact on the sexuality of both partners and that successful treatment of ED has a significantly positive effect on sexual and partnership satisfaction for both partners. In this context, not only the individual quality of life of the patient and partner but also that of the couple is significantly improved, and in many cases, a level of joint sexual satisfaction is achieved that existed before the development of ED (Burns et al. 2015; Huang and Jiann 2013).

In principle, PDE-5-I shows excellent efficacy across a broad spectrum of etiologically different forms of erectile dysfunction. This reaches rates of up to 80% in patients with organic erectile dysfunction, and even higher in the presence of predominantly psychogenic disorders. In patients with vascular etiology of their ED due to a wide variety of pathophysiological causes such as arterial hypertension, lipid metabolism disorders, or diabetes mellitus with a predominance of angiopathy, the substances are excellently effective. The basic prerequisite is an intact neuronal erection induction via the cavernous nerve. As expected, significantly lower efficacy is observed in patients with diabetes mellitus and latent or clinically manifest polyneuropathy, in whom, on the one hand, signal transmission is disturbed by the polyneuropathy and, on the other hand, peripheral transmitter depletion results in the course of the disease. It is therefore not surprising that patients with paraplegia and, as a rule, an intact sacral reflex arc show a good response rate of more than 80%, even in larger study series.

The required **therapeutic dose** depends on the etiology of erectile dysfunction. Since doses of 25 to 50 mg (Sildenafil), 5 to 10 mg (Vardenafil, Tadalafil), or 50 to 100 mg (Avanafil) are usually quite sufficient for psychogenic erectile dysfunction, the dose can be increased up to 100 mg (Sildenafil), 20 mg (Vardenafil, Tadalafil), and 200 mg (Avanafil) for organic disorders. Under higher drug doses, the level and, consequently, the duration of the therapeutically effective plasma concentration increase in particular. This correlates clinically in part with a certain prolongation of the time in which an erection can be induced. The effectiveness of the substance, on the other hand, can only be increased by about 10% per dosage level. It is not uncommon, however, for an increase in dose to be accompanied by an increase in therapy-related side effects. Therefore, as with other therapy modalities, a gradual dose finding and adjustment are advisable to avoid unnecessary side effects.

The occurrence of such **side effects**, the changing effectiveness of the preparations during initial use, not least due to the extraordinarily high expectations of the patient and partner after mostly long-lasting erectile dysfunction and the associated partnership problems, makes a control after the first therapeutic attempt extremely important. During this

control, the effectiveness of the medication and the occurrence and intensity of side effects should be inquired about in order to be able to make an individual dose adjustment. With all substances, even depending on the initial dose, successful sexual intercourse occurs in only about 60% of patients and full effectiveness is achieved only after two to four doses have been taken. This is the reason why some therapists deliberately use high doses in the initial phase of treatment in order to ensure optimal treatment success at an early stage, but accept somewhat increased side effects in return, while others initially start with low doses in order to achieve a certain degree of familiarization with the special features of PDE-5-I and to relieve the patient's initial anxiety.

Overall, approximately 20% of patients are **non-responders** to PDE-5-I therapy, meaning they do not have an erection sufficient for intercourse despite taking PDE-5-I (Park et al. 2013). Regardless of the strategy chosen, especially in the case of insufficient effectiveness of the substances during the first attempts, a renewed, intensified counseling regarding specifics of tablet intake (food and alcohol consumption, timing of intake, and sexual activity depending on the substance used) and the required adequate sexual stimulation is mandatory ("re-counseling"). This therapy evaluation and **re-counseling** is crucial for the success of treatment in individual cases, as 24% to 49% of patients discontinue therapy (Carvalheira et al. 2012; Kim et al. 2014) and about 60% of patients discontinue therapy after only two unsuccessful attempts (Valiquette et al. 2008).

Basically, PDE-5 inhibitors in the treatment of ED are taken by most patients in the sense of an **on-demand medication**. This corresponds to the specificity of the symptoms, but also takes into account the lack of reimbursement of the drugs by payers. It is not uncommon for a **dose reduction** to be possible after successful initiation of treatment. On the other hand, a genuine, organically induced improvement in erectile quality after a 6-month therapy interval, as can occasionally be observed with corpus cavernosum injection therapy due to histologically detectable hypertrophy of the smooth corpus cavernosum musculature, is not observed with PDE-5 inhibitors. The efficacy of the substances is also persistently good when taken over years, and tachyphylaxis has not yet been observed for any of the substances (Musicki et al. 2005).

For patients who value more spontaneous sexuality, daily use of the long-acting tadalafil at a dosage of 2.5 to 5 mg daily is an alternative. The long half-life and corresponding accumulation result in therapeutically adequate levels despite the low individual doses and the associated low side effects. For the patient, this offers the advantage of continuous effectiveness and thus a more spontaneous erectile function (Salonia et al. 2020). The extent to which this brings psychological benefits for the patient and positively affects the quality of life compared with on-demand therapy has not been clarified. A combination of taking tadalafil 5 mg daily plus an additional 50 mg of sildenafil as needed is also possible: A prospective open-label study was able to show that the combination leads to a significant improvement in erectile function compared with monotherapy with 5 mg of tadalafil daily, with no differences in terms of side effects between the two groups (Cui et al. 2015). For the treatment of LUTS in prostatic hyperplasia, tadalafil 5 mg daily is approved and also has beneficial effects on erectile function in these patients (Bechara et al. 2014). In principle, daily dosing is possible and also permissible for all substances, although the shorter half-life eliminates the advantage of continuous active levels, which is likely to be of secondary importance when taken in the evening.

In the case of supposed **non-responders** in primary therapy, sufficient efficacy of PDE-5-I can still be achieved in about half of the patients by re-counseling, increasing the dose to the maximum therapeutic dose, and repeated use within a narrow time frame. Only if after at least 8–10 attempts with repeated application of the maximum therapeutic dose no effect sufficient for successful termination of sexual intercourse can be achieved, can the patient be classified as a non-responder. In these cases, **further diagnostics** should be performed. At this point, at the latest, a determination of the **serum testosterone level** is required to exclude a relevant deficiency. In the case of proven testosterone deficiency or values in the lower normal range with concomitant clinical symptoms, substitution should definitely be carried out in these cases, as this not infrequently also leads to a significant improvement in the efficacy of the PDE-5 inhibitors (Salonia et al. 2020) (see Chap. 36).

Another option in these cases is the **regular daily use of PDE-5 inhibitors** in low doses (usually ¼ of the maximum dose). In this way, it is possible in some patients to achieve efficacy of the substances with additional on-demand use in the maximum therapeutic dose by conditioning the corpus cavernosum. After exhausting these possibilities, the combination of different therapy options such as PDE-5-I/vacuum system or PDE-5-I/ intracavernous injection therapy remains in individual cases.

A special indication for the regular use of PDE-5 inhibitors is **penile rehabilitation** after radical surgery in the small pelvis, especially after radical prostatectomy. It has been increasingly promoted as a first-line treatment option in recent years, following the previous description of a significant improvement in erectile function 1 year postoperatively with regular intracavernosal use of prostaglandin E1. Thus, regular evening use of the various substances in low doses (1/4 of the maximum dose) also appeared to contribute to a more rapid and complete restitution of erectile function. The basic pathophysiological idea here is that regular nocturnal oxygenation of the smooth muscle of the corpus cavernosum could prevent fibrosis and atrophy of inactivity, thus preserv-

ing the functional capacity of the corpus cavernosum. Several experimental and clinical data seemed to support such a concept. The recommended duration of therapy is between 3 and 18 months. In this regard, the efficacy of PDE-5-I reported in the literature in up to more than 75% of patients (Salonia et al. 2020) requires an optimal surgical technique with bilateral preservation of the nerve and vascular bundle, which is not feasible in all patients due to oncological considerations. Also, postoperative erectile function depends quite significantly not only on the quality of nerve preservation, but especially on the patient's age, comorbidities, and preoperative erectile function. After bilateral nerve-sparing radical prostatectomy, demand-controlled therapy over a postoperative study period of 10 months showed in no way worse long-term results than daily use of a medium therapeutic dose (Montorsi et al. 2008). Similarly, a Cochrane systematic review including eight randomized trials, and thus, a total of 1699 patients showed that, after radical prostatectomy, scheduled PDE-5-I therapy is not superior to on-demand use in terms of recovery and restoration of erectile function. This review strongly suggests that the overall evidence according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) is low to very low (Philippou et al. 2018). These results confirm the finding that none of the available substances can restore erectile function, even with regular use and only a moderately pronounced organic correlate. Thus, irrespective of the duration of treatment, a spontaneous deterioration of symptoms to the original level occurs within a few weeks after discontinuation of therapy (Montorsi et al. 2014; Zumbé et al. 2008). A recent meta-analysis that considered results from 22 studies with a total of 2711 patients was able to show that of the 16 different rehabilitation strategies, pelvic floor muscle training (odds ratio: 5.2) and daily use of sildenafil 100 mg (odds ratio: 4.0) led to the recovery of erectile function after radical prostatectomy compared to placebo. The reliability of the results was reported to be low for the therapeutic strategy of pelvic floor muscle training and moderate for daily sildenafil use. No statement is made regarding the required duration of therapy (Sari Motlagh et al. 2021).

Thus, after radical prostatectomy, the current practice of penile rehabilitation by regular (daily, if necessary) use of any PDE-5-I represents a concept that requires further review. This needs to be emphasized, as public health insurers do not reimburse the costs of PDE-5-I therapy, and patients ultimately have to bear these costs themselves.

What makes sense at this stage is an as-needed therapy that, if clinically effective, can be continued at an individually adapted dose for as long as the patient deems it necessary. If the treatment proves ineffective in the early postoperative period, this may be a consequence of a still prevalent postoperative neuropraxia and can ultimately only be assessed prognostically after 12–18 months. In case of non-response, the patient should be consequently adjusted to intermittent autoinjection—therapy to avoid inactivity and atrophy of the smooth muscles of the corpus cavernosum. After 6 and 12 months, a new oral treatment trial with any PDE-5 inhibitor at a maximum dose should then be attempted. With the neurovascular bundle preserved, significant improvement in response is then not uncommon during the interval (Mulhall and Simmons 2007).

The spectrum of **side effects of PDE-5-I** results from its effects on phosphodiesterases of other body regions and from interactions with other phosphodiesterase isoenzymes. Because phosphodiesterase type 5 is present in relatively high concentrations in vessels of the nasopharynx and CNS, flu-like symptoms with a **mild headache, nasal congestion**, and a very common flush-like **facial flushing** predominate. Mild gastrointestinal symptoms are also not uncommon. The blood pressure effect with a drop in systolic and diastolic pressure is very mild. Interaction with retinal phosphodiesterase type 6 results in color vision disturbances with mild **blue/green shifts**, increasing sensitivity to light, and blurred vision. This side effect, which occurs exclusively with Sildenafil, is particularly dose-dependent and occurs in only about 2% of cases at a dosage of 50 mg. At 100 mg, 11% of patients already report these side effects, and when the dose is increased to 200 mg, which is not clinically recommended, the side effect rate in this regard increases to 40%. Based on clinical and experimental data, it can be assumed that this is a harmless side effect. Sildenafil and Vardenafil should not be used in the **known presence of retinitis pigmentosa, a genetic defect of phosphodiesterases in the retina. The use of Avanafil and Tadalafil is safe** (Hellstrom 2007).

A relationship between the use of PDE-5-I and **NAION (nonarteritic anterior ischemic optic neuropathy)** has not been established. NAION is the most common acute optic nerve disease, occurring particularly in patients with the risk factors that also cause ED (hypertension, diabetes, hypercholesterolemia, and nicotine abuse). However, the Summaries of Product Characteristics (SmPCs) of all PDE-5-Is list NAION as a contraindication to use.

Because sildenafil was developed primarily as a circulatory drug for the treatment of angina and as an alternative to nitrate therapy, the cardiovascular risks of PDE-5-I are generally low. The moderate reduction in blood pressure by an average of 5 mmHg in individual cases is generally not clinically significant. Experience in patients with hypertension and concomitant antihypertensive therapy demonstrates unchanged efficacy of the substance compared to other patient populations. Cardiac side effects are not expected

because type 5 phosphodiesterase is not active in the heart itself, so there is no effect on contractility or pulse rate. The side effect profile with antihypertensive monotherapy or combination therapy with up to three antihypertensive drugs is not altered (Pickering et al. 2004). The administration of PDE-5-I is **contraindicated** with the concomitant use of nitrates or NO donors. This results from the mechanism of action of the substance, since nitrates and NO donors attack the same mechanism of action of vascular muscle, cGMP. While nitrates and molsidomine significantly increase the intracellular concentration of cGMP, metabolism is inhibited by PDE-5 inhibitors, which may lead to the potentiation of the circulatory effects of nitrates and NO donors. Although studies of all preparations with simultaneous application of nitrates and PDE-5-I have not shown any serious changes in blood pressure in healthy individuals, it cannot be ruled out that in individual cases **blood pressure** may be lowered to values of 40 to 50 mmHg, thus also triggering prolonged syncope-like states.

- Therapy with PDE-5-I in patients with coronary artery disease and angina pectoris taking nitrates or NO donors is strictly contraindicated. Because more than 100 different nitrates and NO donors are currently available, a detailed drug history is mandatory in this setting before initiating therapy with PDE-5-I. Interdisciplinary consultation with the treating internist/cardiologist is necessary.
- Conversely, a patient must be prohibited from taking PDE-5-I if a nitrate preparation is prescribed. In particular, any emergency cardiovascular patient should be asked about the previous use of a PDE-5-I before administration of a nitrate preparation. Nitrates should be applied no earlier than 24 h after the last ingestion for Vardenafil and Sildenafil, 72 h for Tadalafil, and 12 h for Avanafil (Salonia et al. 2020).

Contrary to initial concerns, a retrospective analysis of 80 clinical trials impressively demonstrated that the rate of serious cardiovascular events was not more frequent with Sildenafil than with a placebo. Similar data are also available for the other PDE-5-Is, clearly documenting that, contrary to initial assumptions, the use of these substances does not lead to an increased incidence of infarction and is safe (Kloner et al. 2018).

Because the practice of intercourse, with a circulatory burden averaging three to four metabolic units (METS), is no more stressful than other daily activities such as rapid walking (1 mile in 20 min) or moderate-impact gardening, ED treatment does not pose an incalculable risk, regardless of treatment modality. The third Princeton Consensus Panel makes the following recommendations in this regard in patients with cardiovascular disease (Nehra et al. 2012):

- In patients with low cardiovascular risk (heart failure NYHA 1–2, asymptomatic controlled hypertension, mild valvular disease, coronary artery disease after successful revascularization by stent, bypass, or angioplasty), no additional diagnostic testing by the cardiologist is required.
- In patients with intermediate cardiovascular risk (mild or moderate stable angina, status post-myocardial infarction 2–8 weeks ago without intervention, heart failure NYHA 3, non-cardiac sequelae of atherosclerosis (e.g., cerebral insult and transient ischemic attack), cardiological diagnostics with stress test (e.g., Bruce protocol and adenosine with imaging), and if necessary measurement of the intima–media thickness of the carotid artery and the ankle–brachial index are necessary.
- In patients at high cardiovascular risk (unstable or refractory angina, uncontrolled hypertension, NYHA 4 heart failure, status post-myocardial infarction less than 2 weeks ago without intervention, high-risk arrhythmia (ventricular tachycardia, poorly controlled atrial fibrillation, and frequently triggering implanted cardioverter defibrillator), obstructive hypertrophic cardiomyopathy with severe symptoms, and moderate-to-severe valvular heart disease), sexual activity is discouraged and ED therapy should be withheld until the cardiac situation is stabilized. Cardiologic presentation for further diagnosis and therapy is essential for this purpose.

LUTS due to prostatic hyperplasia represents an associated disease of ED. Since alpha-blockers are frequently used for the therapy of LUTS in prostatic hyperplasia, it should be noted here that in clinical practice PDE-5-I can be taken with concomitant alpha-blocker medication under certain conditions. This is the case in patients in whom alpha-blocker therapy is without problems and stable. Initially, it is recommended to use PDE-5-I at a low dosage in these patients. However, during therapy with doxazosin, the use of PDE-5-I should be omitted (Salonia et al. 2020).

In summary, the introduction of modern orally active drugs has resulted in a dramatic change in therapeutic options for erectile dysfunction and has led to a now established stepwise treatment schedule (Fig. 30.11). The efficacy and therapeutic safety of currently approved oral agents is very high. This offers the possibility of successful oral therapy for a broad spectrum of psychogenic and organic erectile dysfunction. At the present time, PDE-5-Is represent by far the most effective oral substance class, comparable in effectiveness to intracavernous injection therapy. However, due to the specific mode of action of the treatment and contraindica-

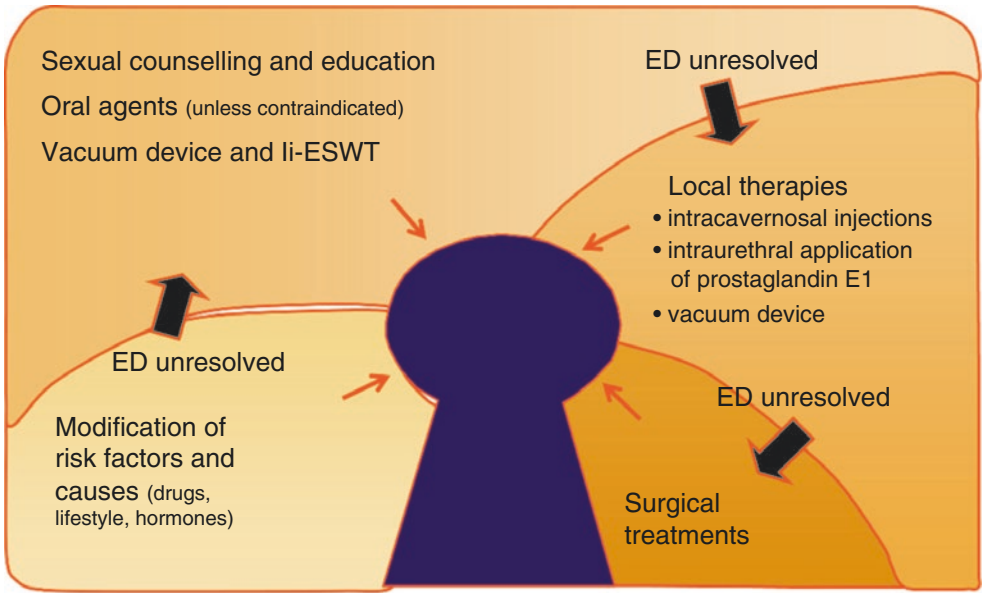


Fig. 30.11 Therapy of ED according to WHO (modified after Jardin et al. 2000)

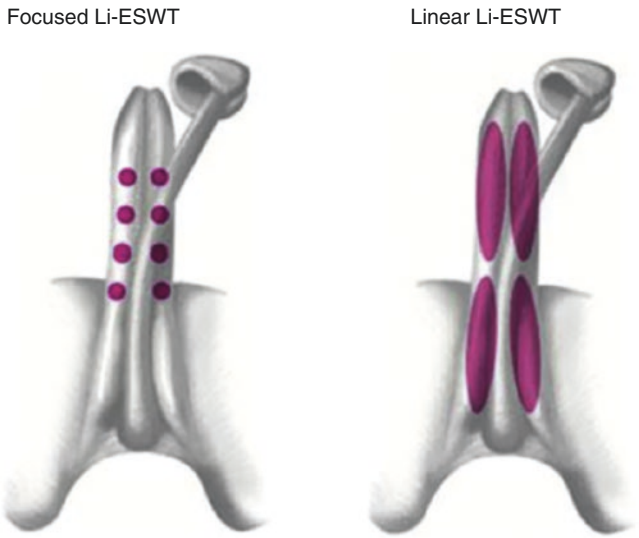


Fig. 30.12 Focused and linear li-ESWT. Figure modified according to Porst (2021)

tions, oral therapy will not be able to completely replace other treatment modalities.

30.1.6.6 Topical Therapy

For topical therapy, alprostadil is available in two formulations. Vitaros®, an alprostadil cream with dosages of 200 and 300 µg, is applied to the glans penis and meatus urethrae and results in improved erectile function in patients with mild-to-severe ED (Cai et al. 2019). Side effects may include burning pain and penile erythema, which usually resolve spontaneously within 2 h of application. MUSE® (medicated urethral system for erection) contains alprostadil in doses of 500 to 1000 µg and is applied intraurethrally via a special small applicator pen. It is less effective than intracavernous injection therapy, with 30% to 66% of patients achieving erections sufficient for sexual intercourse. Burning pain in the urethra is a typical side effect, affecting 29% to 41% of patients. A total of 2% to 14% of patients report dizziness, and 5% complain of urethral bleeding. Approximately 70% of patients discontinue therapy with MUSE® after 15 months.

Condom use is recommended when using topical therapy with Vitaros® and MUSE®, as significant resorption-related headaches may occur in the partner. In case of (potential) pregnancy of the partner, condoms must be used due to the high PGE1 dosages during topical therapy.

30.1.7 Low-Energy Extracorporeal Shock Wave Therapy ("Low-Intensity Shock Wave" Therapy)

Extracorporeal shock wave therapy (ESWT) is a noninvasive technique that uses shock waves to target tissue. In urology, ESWT was initially used to treat urolithiasis. Subsequently, the technique has also been used in the treatment of cardiovascular and musculoskeletal diseases, as well as for wound healing (Rizk et al. 2018).

The exact mechanism of action of low-intensity shock wave therapy (li-ESWT) in ED is not fully understood. The energy of the shock waves seems to activate cellular signaling pathways that enhance the expression of growth factors (e.g., VEGF and SDF-1) and thus could lead to an improvement in endothelial function and (neo-)angiogenesis and even neurogenesis. Therefore, it is postulated that li-ESWT might not be a symptomatic but a durable curative therapy for ED (Fode et al. 2017; Rizk et al. 2018).

Focused and linear li-ESWT has been available since 2010 (Fig. 30.12). In focused li-ESWT, sound waves are

focused with a probe to a defined point at a specified tissue depth. In linear li-ESWT, the sound waves reach a broader area at a specified tissue depth. There are currently several li-ESWT devices from different manufacturers that generate shock waves electrohydraulically, electromagnetically, piezoelectrically, or electropneumatically. However, the systematic reviews and meta-analyses published so far have mainly considered studies in which six different devices were used (Sokolakis and Hatzichristodoulou 2019). The applied energy is usually 0.09 mJ/mm^2, but there is currently no universally accepted standard treatment protocol. Usually, 1500 to 2000 shock waves per session are applied to multiple sites of the penis, particularly the proximal and distal portions of the corpora cavernosa and both crura. One to two outpatient sessions per week occur over a period of 5 to 10 weeks, with each session lasting approximately 20 min. The cost is not covered by health insurance and is approximately (99–)200 € per session depending on the estimated rate (Rizk et al. 2018).

Several systematic reviews and meta-analyses have shown that li-ESWT positively affects erectile function, leading to an increase in two to four points in IIEF-EF and improvement in EHS compared to placebo treatment (EHS ≥ 3: odds ratio = 4) (Dong et al. 2019; Lu et al. 2017; Sokolakis and Hatzichristodoulou 2019). Penile hemodynamics also appear to be positively affected by li-ESWT, as PSV increases by a mean of 4 cm/s compared to baseline before therapy initiation and compared to the placebo group (Sokolakis and Hatzichristodoulou 2019). All meta-analyses have decidedly highlighted that most included studies had a low risk of bias (Dong et al. 2019; Lu et al. 2017; Sokolakis and Hatzichristodoulou 2019). Side effects such as pain, bruising, and hematuria do not occur with li-ESWT. Critically, as follow-up increases, the difference in IIEF-EF between li-ESWT and placebo decreases (Sokolakis and Hatzichristodoulou 2019), which may indicate that li-ESWT is precisely not a durable curative therapy for ED. The most recent EAU guidelines recommend the use of li-ESWT in patients with mild vascular ED when they are poorly or inadequately treatable with PDE-5-I, or do not want PDE-5-I therapy and are fully informed about the opportunities and limitations of li-ESWT (Salonia et al. 2020).

30.1.7.1 Intracavernous Auto-Injection Therapy

Inracavernous autoinjection therapy is a semi-invasive treatment option for ED. In principle, autoinjection therapy can be offered to all patients. Particularly suitable are patients with normal or pathological arterial inflow, but intact cavernous occlusion mechanism, as well as patients with neurogenic disorders, since, on the one hand, a causal therapy is not available here, and, on the other hand, the PDE-5-I is only suitable to a limited extent due to their mechanism of action, and the effectiveness can be significantly reduced depending on the extent of nerve damage. In particular, these include patients with postoperative erectile dysfunction due to a surgery-related lesion of the autonomous cavernous body innervation after radical pelvic surgery or in diabetes mellitus with advanced polyneuropathy, and patients with paraplegia or multiple sclerosis. Especially with morphologically still intact erectile tissue in the early postoperative phase and perfect perfusion conditions, usually only small doses of vasoactive substances are required to induce adequate erections. With increasing perfusion disturbances and resulting morphological changes in the sense of atrophy or fibrosis of the cavernous musculature, there is then also a limited effectiveness of autoinjection therapy and the need for dose escalation.

Especially in arterial ED, oral therapy with PDE-5-I has replaced autoinjection therapy as first-line therapy due to its excellent and equivalent efficacy with significantly less invasiveness.

Also for patients with predominantly psychogenic, functional ED, autoinjection therapy, and the use of orally active substances may be indicated as adjuvant therapy in addition to appropriate psychologically oriented treatment and significantly shorten the duration of treatment. Particularly in refractory or long-standing psychogenic disorders, such as adjuvant therapy, in addition to behavioral or psychotherapy, it provides the patient with greater reassurance and helps reduce fear of failure. However, such a strategy can be problematic for psychological reasons and always requires detailed consultation between the therapists involved. Patients without a demonstrable organic deficit in particular must be fully aware of complications and potential side effects of autoinjection therapy. Not least, these limitations have led to oral PDE-5-I therapy largely replacing autoinjection therapy as an adjuvant measure here as well.

Patients with cavernous ED are generally unsuitable for cavernous injection therapy because they respond inadequately or not at all to the injection of vasoactive substances or the administration of PDE-5-I due to the underlying pathomechanism. If invasive surgical forms of treatment are not an option for the patient, the use of a vacuum system or, if the effect is insufficient, a combination of the various methods can be considered as an alternative.

Thus, from a medical point of view, autoinjection theray appears to be suitable in principle for the vast majority of patients with proven organic ED, **provided that the following conditions are met:**

- Adequate patient selection and information about potential side effects
- Cooperative, reliable patient
- Regular control examinations
- Experience in the treatment of potential side effects

In principle, it should only be offered to patients who, in addition to a proven organic cause, have sufficient manual dexterity and, last but not least, the necessary basic intellectual requirements. In the case of physical disability of the patient, limited vision, or pronounced obesity, which may make it impossible for the patient himself to use autoinjection therapy in a controlled manner, the indication must be carefully reconsidered. In these cases, consideration may be given to having the partner administer the vasoactive substance as an alternative. Regular check-ups must be ensured for all patients because of the risk of serious complications. A physician experienced in the treatment of acute complications such as priapism should always be available.

Contraindications to performing autoinjection therapy, as with the diagnostic use of vasoactive substances, are primarily:

- Decompensated cardiovascular diseases
- Poor intellect
- Lack of compliance

Taking anticoagulant preparations such as coumarins or antiplatelet drugs is not a contraindication; an increased complication rate is not to be feared in this, often well-motivated and disciplined patient collective, if sufficient compression is applied to the injection site. In patients with coagulation disorders, the most current EAU guidelines do not recommend the use of autoinjection therapy (Salonia et al. 2020).

The injection technique is similar to that used in the diagnostic application of vasoactive substances. The entire length of the corpus cavernosum is available for injection, and the patient is encouraged to vary the site of injection. This should help to minimize the risk of plaques on the tunica albuginea or localized cavernous fibrosis.

In principle, two different forms of autoinjection therapy are possible and established.

In periodic interval therapy, performed at fixed intervals by the physician or the patient himself, the vasoactive substance is applied in the previously tested dosage at 1- to 2-week intervals. Patients who still have a residual erectile function and only a slight restriction of perfusion are particularly suitable for this form of therapy. In the case of demand-controlled autoinjection therapy, the patient himself or her partner injects approximately 20 min before the desired sexual activity.

The dosage of the vasoactive substance is based on efficacy; the goal is an erection duration of about 30 to 60 min. In principle, analogous to the diagnostic use of vasoactive substances, the applied drug dose is slowly increased after initial testing with low doses in order to determine the correct therapeutic dosage with minimal risk of priapism. In most cases, there is a better response to a defined drug dose under home conditions, so that it is often possible to reduce the dose by up to 30%. This is due on the one hand to a stabilization of concomitant psychological causes through a corresponding "sense of achievement," and on the other hand, to an improved compliance of the cavernous vessels and musculature. In addition, when injected immediately before intercourse, there is an additive effect of pharmacostimulation and residual sexually inducible endogenous neurotransmitter release.

Autoinjection therapy requires great discipline from the patient regarding dose consistency and application interval. In the absence of drug effect, same-day re-injection should be avoided because of the risk of prolonged erections. Priapisms, which can pose a significant risk and were previously observed under papaverine/phentolamine in up to 10% of cases even with careful patient selection, occur in approximately 1% of patients during long-term therapy with alprostadil.

The most serious late consequence of autoinjection therapy may be the occurrence of localized or generalized cavernous fibrosis. Recurrent, needle-related microtrauma at the injection site, resorption of small local hematomas in the muscle trabeculae, or local toxic reactions, often in the context of long-lasting erections with a relative overdose of the vasoactive substances, must be discussed as causes of local fibrosis. A particular concern is generalized fibrosis of the corpus cavernosum due to prolonged use of vasoactive substances. Such fibrosis is reversible only in about half of the cases, even with immediate cessation of therapy (Fig. 30.13). The incidence is particularly high under papaverine, which has led to the fact that the substance is hardly ever used anymore. In combination with phentolamine, the substance seems to be much better tolerated, and the same applies to PGE1. Ideas that the low pH of papaverine (pH 2.7) is the cause of scarring cannot be sustained based on blood gas analyses and systematic studies using different osmolar and acidic solutions. Rather, it is probably a matter of different, substance-specific toxicity of the preparations.

This side effect is related to application frequency and duration. Therefore, patients should limit the number of injections to two to three injections per week regardless of the vasoactive agent used. All patients should be informed in detail about the possible complications, in particular infections and progressive, therapy-related damage to the corpus cavernosum with consecutive fibrosis and complete loss of erection. Regular check-ups by the attending physicians are obligatory.

Patients who present with neurogenic ED or patients in whom primary high-grade perfusion defects are already

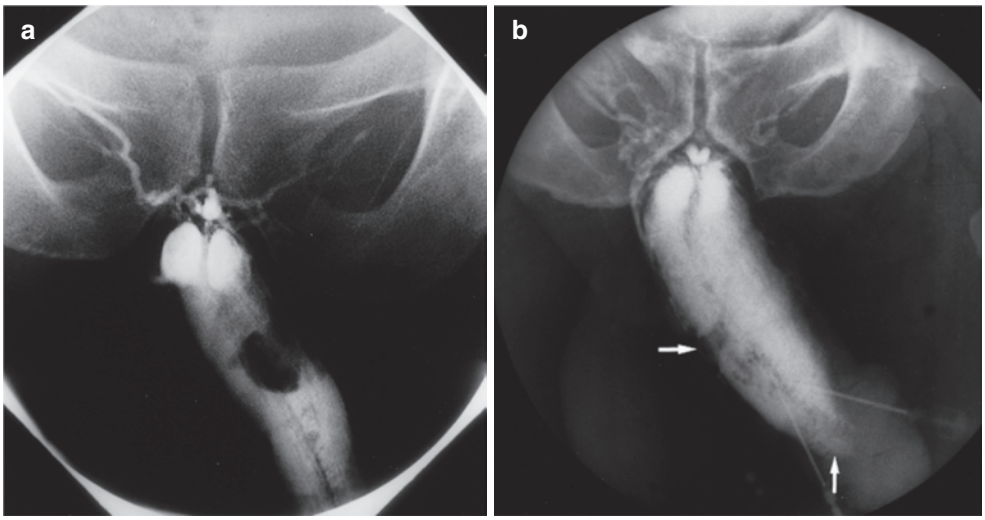


Fig. 30.13 Corpus cavernosum fibrosis after autoinjection therapy. (**a**) Extensive local fibrosis with cavernosographic filling defect with palpable induration and deviation. (**b**) Diffuse fibrosis with marginal filling defect in the middle of the corpus cavernosum (arrow) and marked narrowing of the distal tips of the corpus cavernosum (arrow) as a sign of diffuse fibrosis. Clearly pathologic contrast medium outflow with clinical findings of cavernous insufficiency

detectable are immediately referred to autoinjection therapy. Due to the limited efficacy of oral agents in special patient collectives (diabetes mellitus, postoperative ED), autoinjection therapy will undoubtedly claim a significant role here, as success can be achieved in up to 90% of patients when all options are exhausted. In severe organic ED, in principle there is also the possibility of a combination therapy of the various oral substances and the vasoactive substances.

30.1.7.2 Surgical Treatment

Even though very effective treatment options are available today with the noninvasive forms of therapy described, quite a few patients would prefer surgical correction of organic factors in order to regain undisturbed sexuality that is independent of aids. In selected cases, interventions on the penile vascular system offer the possibility to regain physiological erections without other interventions. Basically, the surgical options are divided into isolated interventions on the venous system, which are prone to recurrence in cases of increased venous outflow from the corpus cavernosum and reconstructive vascular surgery measures, which are only rarely indicated overall in cases of arterial disorders, and prosthetic treatment in cases of erectile dysfunction refractory to other therapies and special indications. Although overall the indications for surgical measures have decreased even further since the introduction of orally active substances and are practically irrelevant today except for prosthesis implantations, the basic concerns will be briefly summarized here. The current and recommended surgical procedures are presented in Chap. 40.

30.1.7.2.1 Vein Surgery

The basic principle of vein surgery is to increase venous outflow resistance. Vein surgery offers good prospects for success in patients with congenital primary erectile dysfunction due to an ectopic vein. Simple surgical resection of this vessel results in correction of the underlying disorder with (usually only temporary) resolution of clinical symptoms. Similar success can be achieved in the rare cases of penile fracture with fistula formation and secondary communication between the C. cavernosum and C. spongiosum. Here, surgical resection of the fistula can causally eliminate the disorder.

In all other patients, the surgical strategy is to resect angiographically detectable outflow tracts as radically as possible. Resection of the superficial and deep dorsal veins with ligation of all confluent vv. circumflexa at the base of the penis is uncomplicated and complications are rarely observed, but long-term results are poor, with success rates around 25%, regardless of the severity of the detectable leak. Endovascular embolization with histoacryl–lipiodol has shown success rates in small numbers of cases with no conclusions about long-term outcome (Aschenbach et al. 2013).

In the presence of concomitant severe arterial perfusion defects, as is the rule, and cavernous insufficiency, purely venous surgery is primarily not promising and, accordingly, is not recommended in the current EAU guidelines (Salonia et al. 2020).

30.1.7.2.2 Revascularization Surgery

The goal of revascularization surgery is to optimize erectile blood flow by bypassing an existing arterial obstruction. Direct corpus cavernosum revascularizations, in which the inferior epigastric artery is directly anastomosed to the tunica albuginea and the cavernosum, are plagued by an increased rate of postoperative priapism and rapid spontaneous closure of the anastomosis. Complications result from high flow rates that regularly lead to intimal hyperplasia with consecutive fibrosis and secondary shunt occlusion and from poor drainage into the CC itself. Free patency of the bifurcation of the penile artery is a prerequisite for anastomosis. This allows for increased blood flow in the deep penis artery via

retrograde perfusion of the dorsal artery. In the case of bilateral obstruction of the profunda penis, arterialization of the deep dorsal vein is an option. Retrograde perfusion of the emissary veins should thus ensure improved blood supply to the corpus cavernosum. For all these surgical procedures, success rates are low at 20–50%.

The surgical method most frequently used in the past is the modification presented by Hauri in terms of a three-vessel anastomosis between the dorsal artery, dorsal vein, and inferior epigastric artery, which combines the principles of the aforementioned procedures (Hauri 1984). The success rates of the operation have been reported in the literature to be 30% to 70%. The most significant complication is glans hyperemia, which is observed in up to 20% of patients. This glans hyperemia, resulting from increased arterial perfusion of the glans, may also occur secondarily up to 2 years after surgery and be clinically associated with stasis edema, meatus stenosis, and severe glans necrosis, necessitating reversal of the arteriovenous fistula.

Balloon angioplasty in patients with isolated stenoses of the penile arteries did result in successful dilation of 91% of the stenotic lesions in a prospective study of 22 patients (with a total of 34 stenotic lesions). However, more than 50% had re-stenosis at 8 months, and only 50% of patients had sustained clinical success (defined as IIEF-EF $\geq$ 22) 12 months after intervention (Wang et al. 2016).

The unclear pathophysiological mode of action of the various operations, the overwhelmingly disappointing postoperative results, and the not insignificant complications of the procedure have led to the fact that nowadays revascularization surgery is recommended exclusively in young patients after pelvic and perineal trauma with isolated vascular injuries (Salonia et al. 2020).

While prosthetic penile surgery is offered as a primary treatment option in the United States regardless of the pathogenesis of ED and is accepted by patients, in Germany it represents the last stage and **ultima ratio** of ED therapy.

These are patients who do not respond or no longer respond adequately to other forms of therapy or for whom other forms of therapy are contraindicated. In the majority of cases, the indication for implantation of a penile prosthesis is given in patients with refractory cavernous insufficiency, cavernous fibrosis with concomitant Peyronie disease, or postoperative ED after pelvic surgery.

Modern prosthetic surgery dates back to the fundamental developments of Scott (Scott et al. 1973) and Small and Carrion (Small et al. 1975). All currently available prosthesis types can basically be traced back to these two original types. In addition to semirigid, bendable prostheses (e.g., AMS Tactra™; Boston Scientific; Genesis™, Coloplast; ZSI 100TM, Zephyr), various hydraulic implants are available today (Fig. 30.14) (see Chap. 40).

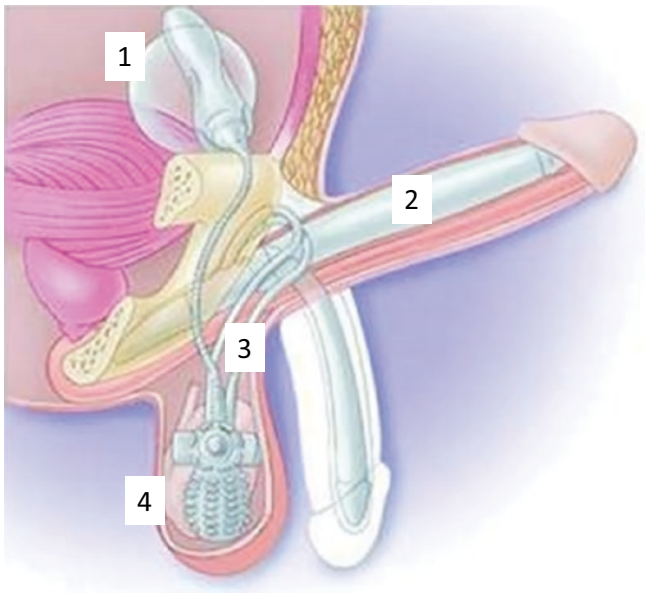


Fig. 30.14 Functioning of a three-piece inflatable penile prosthesis. The three-piece inflatable penile prosthesis consists of a reservoir (*1*), two cylinders (*2*), connection tubes (*3*), and a pump (*4*). Figure modified according to Boston Scientific, American Medical Systems

The main advantage of the hydraulic, multi-part implants is the functional principle, which is based on the physiological process of a natural erection. By means of the pump located in the scrotum, physiological saline solution is transferred from the reservoir placed intraperitoneally or extraperitoneally paravesically into the cylinders implanted in the corpora cavernosa, thus imitating, as required, a stiffening of the penis largely mimicking the natural erection with an increase not only in rigidity but also in penile girth and length (Fig. 30.14). Of particular importance for acceptance, in addition to optimal longitudinal stiffening, is adequate flaccidity under resting conditions. This also reduces the permanent pressure on the tissue, so that the risk of local pressure lesions with perforations of the prosthesis proximally or distally is considerably reduced. This risk is particularly high in paraplegic patients and diabetics with reduced sensitivity and trophic disturbances due to the underlying disease.

The most serious complication of prosthesis surgery overall is an infection, the rate of which is 2% to 3% in primary procedures in high-volume centers and low-risk patients. Antibiotic impregnation of modern three-piece implants has further reduced infection rates to 1% to 2% (Mahon et al. 2020). The most recent prospective and largest multicenter study to date (902 implantations) demonstrated that diabetics with poorly controlled blood glucose are at higher risk for prosthetic infections than diabetics with well-controlled blood glucose when prostheses not coated with antibiotics are used (Habous et al. 2018). Due to the colonization of the implant with a penetration of the germs into the silicone sub-

stance, antibiotic therapy is generally unsuccessful, so that when a prosthesis infection occurs, explantation of the entire implant is practically unavoidable. Other possible complications include perforation of the corpora cavernosa into the urethra and/or glans in 1% to 6% of patients (Scherzer et al. 2019) or glans ischemia or glans necrosis in up to 2% of patients (Hebert and Kohler 2019).

High-risk patients should preferably be treated in centers with appropriate surgical experience to achieve the lowest possible complication rates (see also Chap. 40).

The two surgical access routes—penoscrotal or infrapubic—showed no differences in complication and satisfaction rates in a systematic review (Palmisano et al. 2018).

The acceptance and patient and partner satisfaction of penile prostheses are very high if the indication is correct and adequate preoperative education (also of the partner) is provided. **Such information must include that**

- A "normal" erection cannot be regained by implanting a penile prosthesis.
- The penis will be relatively thinner and also shorter than before, even with the new, bidirectionally expandable implants.
- Only the corpora cavernosa is prosthetically supplied, but not the glans, and this, in contrast to the physiological erection, remains small and soft and can occasionally be perceived as very disturbing.
- The implantation of a corpus cavernosum prosthesis irreversibly destroys the corpora cavernosa.
- At least one of the two partners must have sufficient manual dexterity to activate and deactivate the prosthesis (caution: finger arthrosis).
- That the prosthetic fitting of the corpora cavernosa is not capable of improving or even saving an inadequate partnership relationship.

Subjective satisfaction is 92% to 100% for the patient and 91% to 95% for the partner even with a longer observation period (Salonia et al. 2020; Chierigo et al. 2019).

30.2 Ejaculation Disorders

Ejaculation is a complex process in which, in the first phase, contraction of the smooth muscles of the epididymal tail, vas deferens, seminal vesicles, and prostate leads to emission of seminal fluid into the prostatic urethra and simultaneous bladder neck closure, and in the second phase, contractions of the urethra and the M. bulbospongiosus and ischiocavernosus cause the expulsion of the seminal fluid from the urethra and the meatus urethrae. Emission and bladder neck closure are subject to predominantly sympathetic control from the thoracolumbar segments Th 9–L 3, whereas expulsion is a reflex via the parasympathetic nervous system (S2–4).

30.2.1 Anejaculation and Retrograde Ejaculation

Disorders of emission of seminal fluid, **anejaculation or aspermia**, must be distinguished from disorders of transport of seminal fluid within the urethra, **retrograde ejaculation**. Functional disorders such as **ejaculation praecox** are also of considerable clinical importance. A prerequisite for normal ejaculation is, in addition to an intact innervation with sequential contraction, a quantitatively adequate production of seminal fluid. Orgasm may be present in both anejaculation and retrograde ejaculation. However, in retrograde ejaculation in particular, reduced intensity of orgasm is also possible. The primary absence of ejaculation with unchanged orgasms indicates a disorder of emission, insufficient production of seminal fluid, or insufficient bladder neck closure with retrograde ejaculation.

In cases of secondary ejaculatory loss, it is necessary to determine whether possible causes of the problem can be elicited anamnestically. Surgery on the prostate or bladder neck are typical causes of retrograde ejaculation. Retroperitoneal lymphadenectomy for testicular tumor, pelvic surgery, aortofemoral bypass, or sympathectomy can lead to loss of ejaculation due to damage to the autonomic innervation of the bladder neck and ductus deferens. Side effects of drugs (e.g., adrenoreceptor blockers) and neurologic systemic diseases must be excluded. Spontaneous loss of ejaculation is also observed as a symptom of diabetogenic polyneuropathy or in secondary post-inflammatory obstruction of the ductus ejaculatorii. Thus, any process that interferes with the innervation of the bladder neck, vas deferens, or seminal vesicles may be the cause of loss of emission and ejaculatory dysfunction. Differential diagnosis should be made for androgen deficiency, which can lead to silencing of seminal fluid production. The absence of virilization due to a disturbance of pubertal development is accompanied by underdevelopment of the accessory sex glands and absence of seminal fluid production (see Chaps. 5 and 14).

In all patients with absent ejaculation or conspicuously low ejaculate volume, it must first be clarified whether it is a **loss of emission due to anejaculation** or **retrograde ejaculation.**

30.2.1.1 Diagnostics

The **microscopic examination of postcoital or postmasturbatory urine** and **transrectal sonography** are important diagnostics. Transrectal sonography reveals seminal vesicle

aplasia or ectasia and congestion of the ductus deferens. Obstructions are possible at all levels between the vas deferens and the ductus ejaculatorius. The most common cause is **congenital, cystic, or secondary inflammatory obstruction** of the ductus ejaculatorius, typically leading to dilation of the seminal vesicle and ductus deferens. Sonographically detectable cysts of a ductus ejaculatorius can be opened transurethrally. Only in the case of unclear findings may a patency test be indicated in individual cases. Today, this should be performed with inert dyes, since radiological imaging with contrast media, which was frequently practiced in the past, is associated with the risk of secondary occlusions. Detection of more than 15 spermatozoa/facial field in postcoital or postmasturbatory urine sediment is considered evidence of retrograde ejaculation.

30.2.1.2 Therapy

Therapeutic treatment may be attempted initially with medication. This applies both to cases with **retrograde ejaculation** and in individual cases to those with **anejaculation**. Directly or indirectly sympathomimetic substances are used, such as **Gutron®** 3–4 × 25–50 mg/day or the tricyclic antidepressant **imipramine** (**Tofranil®**; 2 × 25 mg/day) (Kamischke and Nieschlag 2002). The goal of treatment is to achieve improved contraction of vas deferens and internal sphincter by increasing adrenergic tone. A systematic review found that the antegradation of ejaculation occurs in 39% of cases with the combination of sympathomimetics plus anticholinergics, compared with 28% with monotherapy with sympathomimetics and 22% with monotherapy with anticholinergics (Jefferys et al. 2012). The effect can be increased by taking medication for several days. If antegradation is successful, the retrieved sperm can either be cryopreserved for later-assisted fertilization or, if intercourse is not possible, self-insemination can be performed with the retrieved ejaculate. In the case of retrograde ejaculation, it is occasionally possible to preserve sperm from postmasturbatory urine after sufficient alkalinization and, after appropriate preparation, to use it for an **assisted fertilization** procedure (see Chap. 42).

In case of **anejaculation**, it is possible to use electrovibrator stimulation. The patient can learn how to use electrovibrator stimulation and then apply the procedure independently. The electrovibrator stimulation on the glans penis in the area of the frenulum triggers the ejaculation reflex. Thus, a prerequisite for the effectiveness of electrovibrator stimulation is intact lumbosacral pathways. If electrovibrator stimulation is not successful, the more invasive **transrectal electrostimulation under anesthesia** is available therapeutically. **Electrovibrator stimulation** and **transrectal electrostimulation** can induce antegrade or retrograde ejaculation in up to 97% of cases (Sinha et al. 2017). Alternatively, there is also the option of surgical sperm retrieval by TESE. All procedures are used with cryopreservation of sperm for later use in artificial insemination (see Chap. 43).

30.2.2 Premature Ejaculation

Premature ejaculation is characterized by the inability of the man to voluntarily control the timing of ejaculation. Some widely varying definitions of premature ejaculation exist, including those based on the International Classification of Diseases (ICD)-10, the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV, the EAU, and the American Urological Association (AUA) guidelines. The International Society of Sexual Medicine (ISSM) suggests that premature ejaculation is present when:

- Ejaculation always or almost always occurs in ≤1 min of vaginal penetration.
- Ejaculation can never or almost never be delayed.
- This is associated with negative consequences (stress, frustration, and avoidance of sexual activity) for the patient (Serefoglu et al. 2014).

30.2.2.1 Epidemiology

Due to the varying definitions, highly variable prevalences of premature ejaculation have been published. These range from 3% to 84% depending on the study and whether acquired or lifelong ejaculatio praecox is thought to be present. In the general population, both acquired and lifelong ejaculatio praecox appear to have a prevalence of 5% (Salonia et al. 2020). This is also consistent with the most recent prevalence estimate from a representative population study of health and sexuality in Germany, which showed a prevalence of 4.5% (Briken et al. 2020).

A unified concept of pathogenesis does not exist. In many cases, functional causes underlie the disorder; factors such as a decreased threshold of the ejaculatory reflex, decreased sensory genital feedback, and, as potential organic causes, a seminal tract infection, especially prostatitis, or, alternatively, thyroid dysfunction are also discussed. In addition, ED may be a risk factor for but also relevant comorbidity of premature ejaculation. Chronic pelvic pain syndrome may also be associated with premature ejaculation. Finally, illicit drug use and certain medications may also play an important etiologic role in premature ejaculation. Psychological causes of premature ejaculation include anxiety disorders, fearfulness, sexual inexperience, and reduced sexual activity. Conditioning to rapid ejaculation due to taboos in early sexual development is also discussed as a possible psychological etiology (Althof et al. 2014). Genetic factors are also suspected, particularly in lifelong premature ejaculation.

30.2.2.2 Diagnostics

First and foremost is the sexual history, which aims, among other things, to be able to differentiate between lifelong and acquired and between consistently versus situationally present ejaculatio praecox. In addition, other sexual dysfunctions, especially the presence of ED, must be clarified. Of course, the history also serves to disclose risk factors (e.g., use of illicit drugs, medication use, and concomitant diseases).

The intravaginal ejaculatory latency time (IELT) is difficult to objectify, but is nevertheless used for the diagnosis of premature ejaculation. In daily clinical practice, patient-estimated IELT is sufficient because measured and estimated IELT correlate adequately. Several questionnaires are available for the evaluation of premature ejaculation, for example, the Premature Ejaculation Profile and the Index of Premature Ejaculation and Premature Ejaculation Diagnostic Tool.

During the physical examination, the urological, neurological, and endocrinological status is determined in order to identify the underlying causes of premature ejaculation. Diagnostics to detect or exclude seminal tract infection and prostatitis have a special role.

30.2.2.3 Therapy

First and foremost is the medical consultation with information for the patient about the physiological processes involved in ejaculation and realistic classification of the duration of penetrative sexual intercourse: In the general population, the median IELT is 5.4 min, as demonstrated in a study of 491 men from Europe and the USA (Waldinger et al. 2005).

In patients with proven prostatitis, the antibiotic treatment appropriate to the test makes sense if germs are detected (El-Nashaar & Shamloul 2007). Behavioral therapy methods and cognitive psychosexual interventions are available, especially for acquired premature ejaculation. The involvement of the partner is useful. It should be noted that there is little evidence for the use of psychotherapeutic procedures alone for the treatment of premature ejaculation (Salonia et al. 2020). **The stop-start and squeeze** techniques of Masters and Johnson are based on the patient learning to perceive the moment that inevitably leads to ejaculation and thereby being able to influence ejaculation. In the stop–start technique, the patient masturbates until the time that inevitably leads to ejaculation and then stops. Only after reaching a lower level of sexual arousal is masturbation continued. Through this, the patient learns to better perceive arousal and becomes more sensitive to the moment that inevitably leads to ejaculation. In the **squeeze technique**, a strong pressure of 3–4 s duration is applied in the region of the frenulum immediately before the onset of ejaculation, which weakens the erection and reduces the urge to ejaculate. With increasing practice, the man thus succeeds in better controlling arousal and the time of ejaculation. The success rate of the method is reported to be about 60% to 90% (Althof et al. 2014). In patients with lifelong premature ejaculation, psychotherapeutic interventions and behavioral therapy measures are not very promising.

Drug therapy options include **local anesthetics**, which are applied to the glans in **gel form** or as an **aerosol**. In Europe, the combination aerosol lidocaine (150 mg/mL) plus prilocaine (50 mg/mL) is approved for this purpose. Lidocaine/prilocaine gel combinations can also be used, with randomized studies showing similar results. In order not to trigger any disturbing hypesthesia in the partner, condoms should be used or, alternatively, the gel should be washed off before sexual intercourse after a sufficiently long exposure time. It should also be taken into account that the use of local anesthetics can have an unfavorable effect on the quality of the ejaculate (Salonia et al. 2020).

Alternatively, there is the possibility of **oral pharmacotherapy. Dapoxetine**, **a selective serotonin reuptake inhibitor (SSRI**), is approved for the treatment of acquired and lifelong premature ejaculation in Europe and is taken orally at a dosage of 30 to 60 mg 1 to 2 h before sexual intercourse. Adverse side effects include nausea, diarrhea, dizziness, and headache. Vasovagal syncope is rare and has been described in 0.1% of patients. Nevertheless, blood pressure and heart rate should be measured before the initial use of the drug. The tricyclic antidepressant **clomipramine** can also be used on demand 2 to 6 h before sexual intercourse. Off-label—because not approved for this indication—other **SSRIs (e.g., citalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline**) can also be used for the treatment of premature ejaculation; these substances are taken daily for this purpose. Adverse side effects include loss of libido, ED, anorgasmia, and anejaculation. SSRIs should be used very cautiously in patients with concomitant depression and in patients ≤18 years of age because of the risk of increased suicidality. **Tramadol**, in addition to analgesic effects, also has an ejaculation-delaying effect via reuptake inhibition of serotonin and norepinephrine and can increase IELT 2.5-fold depending on dosage. Therapy with **PDE-5-I** alone does not prolong IELT but increases self-confidence and sexual satisfaction in patients. Several non-randomized studies showed that the combination of **PDE-5-I with SSRI** leads to a greater prolongation of IELT than SSRI monotherapy (Salonia et al. 2020).

30.3 Penile Abnormalities

Congenital or acquired anatomical norm variations can make cohabitation or proper seminal position impossible. Congenital anatomical abnormalities include, in particular, urethral malformations (hypospadias and epispadias), but also penile deviations. Among the acquired disorders, penile deviations due to Peyronie's disease play a major role.

30.3.1 Hypospadias and Epispadias

In **hypospadias**, the urethra does not open at the tip of the glans but on **the** ventral aspect **of the penis**. The meatus may be located at varying distances from its regular position between the glans and perineum, depending on the severity of the malformation. The majority of cases are **glandular, coronary, or distal penile hypospadias**, while the severe **penoscrotal or perineal forms** represent only a small percentage.

Surgical correction within the first years of life with a distal displacement of the meatus ensures an unaffected seminal position in the majority of cases. Thus, with an erect penis, distal position, and normal width of the meatus, the vast majority of patients are not likely to have impaired potentia coeundi or generandi, and **distal hypospadias are more of a cosmetic** than a functional problem. Mechanical problems may result if the connective tissue replacement of the missing urethral portion results in ventral penile deviation under erection. Therefore, in addition to optimization of the meatus position, complete resection of an existing chorda as part of the primary operation is the basis of any surgical correction of hypospadias. If fertility problems occur due to the altered position of the semen in the vaginal tract, **intrauterine insemination** is the method of choice.

Epispadias are also a congenital malposition of the urethra. In contrast to hypospadias, the urethra opens dorsally on the **dorsum of the penis** and often forms a **wide, open groove**. Often epispadias is part of an extensive genital malformation and is practically regularly observed in bladder extrophy. On the penis, it is almost always accompanied by significant dorsal deviation and fixation, which can make intercourse considerably difficult or completely impossible. Cosmetically and functionally satisfactory surgical correction of this deformity can be extraordinarily technically difficult and demanding, so that postoperative results do not always meet expectations. As a result, **cohabitation problems due to penile deviation and disorders of the sperm position due to the proximal position of the meatus are frequently to be expected in epispadias**. In case of desire for a child, **insemination** may lead to success.

30.3.2 Phimosis

Phimosis is defined as a narrowing of the foreskin (prepuce) of the penis, which is usually congenital. Secondary acquired forms are usually post-inflammatory due to diabetes mellitus, after pronounced balanitis or in lichen sclerosus et atrophicus. In **complete phimosis**, the foreskin cannot be retracted over the glans when the penis is flaccid; in **relative phimosis**, there are difficulties in retracting the foreskin only during erection and **paraphimosis** may occur (entrapment of the phimotic foreskin behind the glans corona with edematous swelling and circulatory disturbance). In individual cases, phimosis that has not been eliminated can impair the position of the semen and thus cause infertility.

The therapy of choice is usually plastic or radical **circumcision**. It should be noted that phimosis from birth to the completion of puberty is not a pathology but actually a physiological condition (impossibility of atraumatic retraction of the prepuce). In infancy, circumcision is indicated only in the presence of other urological pathologies. In principle, this also applies in infancy until the completion of puberty. Especially if recurrent urinary tract infections and other congenital changes of the urinary tract are present, circumcision is also useful as a prophylactic measure. Initially, conservative therapy should be attempted. Here, corticoid-containing ointment or cream is applied locally twice a day for 4–8 weeks. If conservative therapy is not successful, circumcision is indicated (AWMF guideline 2017). Circumcision is recommended after the completion of the second year of life (Radmayr et al. 2020). Circumcision motivated by religion, which is often summarized under the term "ritual circumcision," must be distinguished from the medically justified indication of circumcision.

30.3.3 Penile Deviation

Penile deviations can basically be divided into **congenital and acquired forms** according to their etiology. Since minor deviations of the penis from the longitudinal axis are clinically insignificant and are at most perceived as cosmetically disturbing, the **basis of a need for treatment is always the mechanical impairment of cohabitation** or pain during erection or **sexual intercourse, irrespective of the cause of the penile deviation. Secondary penile deviations** can occur after blunt trauma with hemorrhage into the corpus cavernosum, after penile fractures, urethrotomy, or due to fibrosis in the course of intracavernous autoinjection therapy for erectile dysfunction. By far, the most common cause, however, is **Peyronie's disease** named after its first describer **François Gigot de la Peyronie, 1743**.

30.3.3.1 Congenital Penile Deviation

Congenital forms are a distinct clinical picture based on **asymmetry of the tunica albuginea of the corpora cavernosa** or its bony fixation, resulting clinically in a **bending of the penis**. The prevalence is 0.5% to 10%, with patients usually presenting to a physician for the first time during adolescence or young adulthood. The deviation itself may be large-arched or have a sharp kink and may cause the penis to deviate ventrally or laterally, and rarely dorsally. In ventral deviation, relative shortening of the urethra ("short urethra") or hypospadias may be the cause of the bending. The history

and clinical examination should therefore address the presence of micturition symptoms and the localization of the meatus urethrae. Depending on the extent of the angle of deviation, ventral bends may mechanically result in significant **impairment of cohabitation ability**. Erectile function is typically not impaired in these patients and the penis tends to be long (Sokolakis and Hatzichristodoulou 2020).

The therapeutic gold standard for pronounced findings is **surgical correction** (Salonia et al. 2020). The basic principle here is a plication of the tunica albuginea on the convexity of the curvature. This leads to the elimination of the deviation via relative shortening of the ipsilateral corpus cavernosum. The most commonly used method of treating congenital penile deviation is **Nesbit's corporoplasty**, in which one or more segments of the tunica albuginea are excised, depending on the severity of the findings, and the edges of the resulting defect are subsequently sutured. Careful placement of the resected rhomboids and corrective sutures thus allows correction not only for lateral or ventral deviations but also for torsional components (Salonia et al. 2020). Alternatively, plication sutures have been described in which parallel longitudinal sutures are placed with nonabsorbable suture material without prior fenestration of the tunica (e.g., according to Schroeder-Esset) or also with incision of the tunica (e.g., according to Yachia). The advantage of these procedures is the lower preparatory effort with shortened operation times. However, a relevant disadvantage is the increased recurrence rate and necessary re-interventions of 10% (range: 0% to 50%). In contrast, an advantage of the original method with the excision of tunica albuginea in combination with plication compared to pure plication techniques is the formation of a fixed scar in the tunica, which is not dependent on the permanent stability of the suture material used. If the penile length is very limited and the length of the urethra allows it, grafting procedures can also be used in cases of high-grade ventral penile deviations.

Postoperative results are good, especially for congenital deviations, and serious complications are not to be feared if the structures of the dorsal vascular nerve bundle are carefully spared. Overall, success rates of 80% to 100% have been published for the techniques with excision and incision of the tunica albuginea and highly variable success rates of 35% to 100% for the techniques without incision of the tunica albuginea. For the grafting techniques, published success rates range from 93% to 100% (Sokolakis and Hatzichristodoulou 2020).

30.3.3.2 Peyronie's Disease

30.3.3.2.1 Definition and Pathophysiology

Peyronie's disease (PD) is an acquired, chronic disease of the tunica albuginea of the corpora cavernosa with a sudden onset of disease (acute disease phase) followed by a chronic and more stable disease phase. The disease is characterized by localized and often progressive fibrosis at the interface between cavernous tissue and tunica albuginea (Fig. 30.15). Plaque formation is histologically preceded by perivascular inflammatory infiltration, the etiology of which remains largely unexplained to date. There appears to be a rupture of elastic fibers and fibrin deposition in the tunica albuginea due to inflammatory reactions. Subsequently, cytokine production is increased and macrophages, mast cells, and lymphocytes migrate into the tissue (Al-Thakafi and Al-Hathal 2016). These processes eventually lead to an excessive increase in collagen fiber production and the destruction of elastic fibers (Al-Thakafi and Al-Hathal 2016; Mulhall 2003). The fibrotic changes in the tunica albuginea lead to deviations and deformities of the penis, which are particularly visible in the erect state.

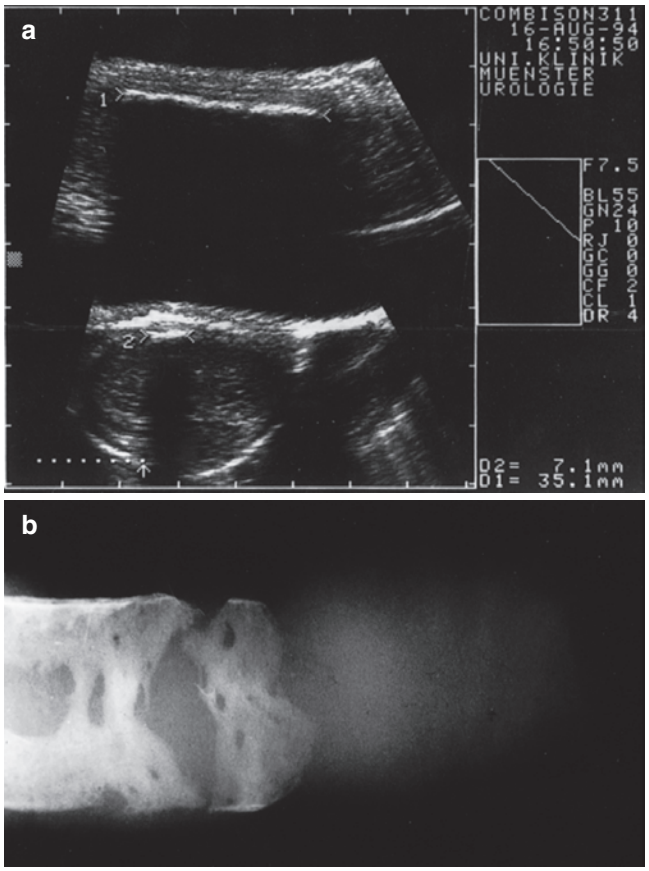


Fig. 30.15 Typical findings in Peyronie's disease. (**a**) Sonography of the penis with extensive dorsal plaque (><) (upper half of image in longitudinal section, lower half of image in cross section). (**b**) Soft tissue image of penis in mammography technique; extensive ectopic calcifications

30.3.3.2.2 Epidemiology and Risk Factors

The prevalence of PD is 0.3% in the general population and 20% in patients with diabetes mellitus and concomitant ED (Al-Thakafi and Al-Hathal 2016). The predilection age is

55 to 60 years; however, the first onset of symptoms occurs before the age of 40 years in 10% of cases (Mulhall et al. 2006). PD is associated with medical conditions and potential risk factors that include diabetes mellitus, arterial hypertension, hyperlipidemia, and excessive alcohol and nicotine use (Bjekic et al. 2006; Hussein et al. 2015; Rhoden et al. 2010). Furthermore, there is a coincidence with Dupuytren's disease: Up to 40% of PD patients suffer from Dupuytren's disease, and a total of 4% of patients with Dupuytren's disease have PD (Deveci et al. 2007). Confirmed data on the coincidence with Ledderhose disease are not available.

30.3.3.2.3 Clinical Findings

PD typically occurs in two distinct phases of disease, which is also clinically relevant with regard to therapeutic options. In the first (active) phase of the disease, inflammatory reactions occur in the tunica albuginea. Here, especially during erection but also in the flaccid state, pain and paresthesia occur in the penile region and usually subside within 3 (to 6) months in the further course. In the second (stable or chronic) phase, there is progressive fibrosis of the tunica albuginea with (nodular) deposits of collagenous connective tissue (plaques). In up to 30% of cases, there is ectopic **calcification** of the plaques with true bone formation on the penis (Fig. 30.15).

The vast majority of plaques are localized dorsally on the dorsum of the penis or dorsolaterally on the shaft of the penis (Pryor and Ralph 2002), but they may also (16%) occur ventrally (Di Maida et al. 2021). Depending on the extent and localization, clinically, due to insufficient elasticity of the tunica albuginea in the area of the plaques due to a scarring shrinkage process, there is therefore a mostly **dorsally directed penile deviation, but also dorsolateral or ventral deviations**. The deviation can take on different dimensions and lead to such grotesque deformities that sexual intercourse becomes impossible. If the process spreads to the corpora cavernosa, in addition to band-like constrictions of the penile shaft, there may be the inadequate filling of the distal portions of the cavernous body with inadequate stiffening or pseudoarticulation and consequent inability to cohabitate ("goose gurgle phenomenon"). These complexes, e.g., hourglass-like deformities, occur in approximately 23% of patients (Margolin et al. 2018). Fibrosis results in loss of penile length in 70% of patients (Farrell et al. 2013). The **spontaneous course** of the disease is **highly variable and hardly predictable**. During the course of the disease, spontaneous recovery may occur in 13% of patients, whereas progression occurs in approximately 40% of patients (Yafi et al. 2015). It usually takes 12 to 18 months for final consolidation with the achievement of a clinical end state. In most cases, the final extent of deviation and the associated subjective impairment of the patient determine the further therapeutic approach. Concomittant ED is present in 71% of PD patients. The penile deviation and penile deformity can lead to detrimental psychological sequelae. As a consequence of PD, 54% of patients have relationship problems, and 48% have a clinically relevant depression. These psychological problems illustrate why the treatment of PD remains challenging (Soave et al. 2021).

30.3.3.2.4 Diagnostics

In addition to the medical history, a clinical examination of a palpable plaque is essential. These plaques or an increasing penile deviation is usually what leads the patient to the physician. Measuring the length of the penis under standardized conditions is essential, especially for forensic reasons, to assess the progression before and after therapeutic interventions.

It is important to document the deviation and deformity of the penis in the erect state. This is done by self-photography by the patient, whereby the penis is photographed from the sides, from above, and from the front (in three planes).

In terms of technical examinations, sonography of the erectile tissue is of particular importance. This allows the plaque to be localized and measured to its extent. However, the accuracy of plaque measurement is prone to error depending on the examiner and the localization (Salonia et al. 2020). Imaging techniques, especially X-ray imaging of plaques or MRI, play no role in the diagnosis of PD and are obsolete in routine clinical practice.

To assess psychological and physical symptoms and pain in PD, a questionnaire developed by a pharmaceutical company (Auxilium Pharmaceuticals Inc., Pennsylvania, USA) in collaboration with the FDA and validated in 2013 is available, the Peyronie's disease questionnaire (PDQ). This asks about psychological and physical discomfort (six questions), burden of PD (three questions), and penile pain (three questions) via three subscales (Hellstrom et al. 2013).

30.3.3.2.5 Therapy

Conservative Therapy

Conservative therapy options include oral pharmacotherapy, intralesional injection therapy, low-intensity extracorporeal shock wave therapy, and mechanical stretching. Oral pharmacotherapy is used exclusively in the active disease phase. The therapeutic effectiveness of conservative treatment options—particularly with respect to improving penile deviation and deformity—is limited. The current guideline of the European Association of Urology (EAU) no longer recommends oral pharmacotherapy except for analgesic use when needed (Salonia et al. 2020).

Oral Pharmacotherapy

Potassium paraaminobenzoate (POTABA™) is thought to have an antifibrotic effect via an increase in tissue oxygen. In a prospective randomized study of 41 PD patients, potaba showed no reduction in plaque size and no decrease in deviation. There was a decrease in penile pain when taking 12 g of potaba daily for 12 months. Another randomized trial (51 patients potaba vs. 52 patients placebo) failed to demonstrate a decrease in penile pain and no improvement in deviation. However, the extent of deviation remained stable under potaba, whereas progression occurred under placebo (Weidner et al. 2005). Gastrointestinal side effects (including nausea, vomiting, and loss of appetite), rash, muscle and joint discomfort, and elevation of transaminases occur with potaba use (Cephlapharm 2015). Potaba is contraindicated in concomitant sulfonamide medication, hyperkalemia, elevated transaminases, and renal insufficiency. In the latest version of the EAU guidelines, in contrast to all previously published versions, the use of potaba for the treatment of PD is not recommended, which cannot be justified based on the data available (Salonia et al. 2020). However, in Germany, potaba is currently the only oral drug specifically approved for the treatment of PD by the German Federal Institute for Drugs and Medical Devices.

Vitamin E (tocopherol) is an antioxidant that can bind oxygen free radicals and is thought to prevent progression. It is usually taken once or twice daily at a dose of 400 IU. Placebo-controlled studies have failed to show therapeutic efficacy for vitamin E. At similar doses, vitamin E may even have negative cardiovascular effects (Abner et al. 2011). Its use in the therapy of PD should therefore be viewed critically, and the most current EAU guidelines advise against it (Salonia et al. 2020).

In animal studies, the **PDE-5 inhibitor** vardenafil had shown antiproliferative effects on PD-like plaques (Ferrini et al. 2006). Taking 2.5 mg of tadalafil daily for 6 months resulted in a decrease in scars in 69% of cases versus 10% in the control group in a non-randomized, retrospective study of 65 men who had penile septal scars due to PD (Chung et al. 2011). Currently, the overall evidence for therapy of PD with PDE-5-I remains limited and their use represents an off-label use as they are not approved for therapy of PD (Hatzimouratidis et al. 2018).

A variety of other substance groups have been studied for efficacy in treating PD, including tamoxifen, pentoxifylline, and colchicine. However, they were found to have limited efficacy, and none of the compounds studied resulted in a reduction in deviation (Kadioglu et al. 2000; Paulis et al. 2015; Ralph et al. 2010; Teloken et al. 1999).

Intralesional Injection

Intralesional injection involves injecting agents directly into plaques. **Collagenase clostridium histolyticum** (CHH) is a bacterial enzyme that disrupts the collagen fibers of PD plaques (Jordan 2008). It is or has been marketed under the trade names Xiaflex® (USA) and Xiapex® (EU) and is currently the only intralesional agent approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of PD (Hatzimouratidis et al. 2018). Injections are given twice at 24–72 h intervals. Treatment cycles are repeated after 6 weeks up to four times (Gelbard et al. 2013). Two double-blind, placebo-controlled trials (IMPRESS I and II) demonstrated benefits for CCH in 551 patients at 52 weeks in terms of the extent of deviation in 34% versus 18% in the placebo group (with 281 patients) and psychological impairment from the disease. Patients with an extent of dorsal- or lateral-directed deviation between 30° and 60° benefited the most, with a mean deviation reduction of 17° (±14°). In order to perform treatments with CHH, physicians must previously participate in a certified "Risk Management Plan" (www.ema.europa.eu). Intralesional injection must be very precise to avoid damage to the healthy erectile tissue. In IMPRESS I and II, a rupture of the corporal bodies was described in 0.3%. In contrast, a survey among PD treating physicians of the Sexual Medicine Society of North America revealed that 34% of the treating physicians found ruptures of the corpora cavernosa (Yafi 2016). Health insurance companies often do not reimburse the costs of treatment with Xiapex®. Xiapex® has since been withdrawn from the market, which is why it is no longer available in Germany and Europe and must be obtained from a pharmacy abroad (as of December 2020). For these reasons, treatment with Xiapex® is not widely available in Germany.

Low-Intensity Extracorporeal Shock Wave Therapy (Li-ESWT)

In older studies, ESWT was used to direct shock waves to fibrotic plaques with the aim to eliminate them. Thereby, the direct impact of the shock waves should induce remodeling of the penile plaques (Husain et al. 2000). Through a systematic review, ESWT was shown not to improve penile deformity and deviation (Fojecki et al. 2017). ESWT has been shown to improve penile pain (Hauck et al. 2004; Palmieri et al. 2009). However, it must be critically noted that almost 90% of PD patients are pain-free after 18 months of disease onset without any therapy (Young Academic Urologists Men's Health 2017). For li-ESWT, the data for the application in PD patients are currently insufficient, so that it is currently only recommended within clinical trials (Chung and Wang 2017; Salonia et al. 2020). ESWT is currently not recommended for the treatment of penile deviation, but only for pain management (Salonia et al. 2020).

Penile Traction Therapy

This therapy uses special devices to stretch the penis daily for 2 to 8 h for a period of up to 6 months. The goal of the therapy is to reduce the extent of the deviation and prevent or mitigate penile length loss due to PD. There are currently four different specific stretching applicators available (Fast Size®, Andropenis®, PeniMaster®PRO, and Restorex®), but a vacuum pump can also be used for stretching. Use over several hours can only be considered for highly motivated patients. The use of vacuum erection devices is particularly useful for patients with concomitant erectile dysfunction.

Surgical Therapy

Surgical therapy represents the gold standard for the treatment of severe PD. The prerequisites for surgical therapy are as follows:

- The presence of the stable phase of the disease for at least 6 months, i.e., stable extent of deviation or deformity and stable extent of plaques to keep the risk of recurrent deviation low.
- Restricted sexual activity, i.e., sexual intercourse and/or masturbation are no longer possible or only possible to a very limited extent (deviation >30°) due to pain caused by the deviation/deformity or due to the deformity and/or the angle of the deviation.

The goal of surgical therapy is to straighten the penis while preserving erectile function so that the patient can be sexually active again. There are generally two surgical approaches available, and they distinguish

- Tunica albuginea shortening procedures
- Tunica albuginea non-shortening procedures

An overview of shortening and non-shortening corporoplasty procedures for surgical therapy of PD is provided in Chap. 40 (Surgical Therapy).

In cases where the erectile function is already impaired preoperatively, the abovementioned surgical procedures lead to a straightening of the penis, but erectile dysfunction usually increases. In addition to psychological factors and arterial circulatory disorders, disorders of the cavernous closure mechanism or fibrosis of the erectile tissue can be involved in pre-existing erectile dysfunction. The pronounced deviation may complicate the correct assessment of erectile function.

Penile Prosthesis Implantation

In PD, the organic findings and associated erectile dysfunction may be so severe that simultaneous penile prosthesis implantation is indicated, combined with corporoplasty if necessary. In principle, two-stage, i.e., delayed, implantation of a penile prosthesis is also possible (Osmonov et al. 2020) (see also Chap. 40).

After surgical therapy for PD, patient satisfaction is highly variable (after Nesbit corporoplasty 76% to 88% (Salonia et al. 2020), after "small intestinal submucosa" (SIS) corporoplasty 35% to 86% (Hatzichristodoulou et al. 2017), after penile prosthesis 79% (Khera et al. 2018). Unfavorable effects on patient satisfaction after surgical therapy using SIS corporoplasty include subjective penile length loss as experienced by the patient, negative physical as well as sexual self-image, and declining erectile function (Soave et al. 2019). Therefore, patient selection and preoperative patient information and education are crucial to avoid creating unrealistic expectations about the opportunities and limitations of surgical therapy (Hatzichristodoulou et al. 2017).

30.4 Priapism

30.4.1 Definition and Epidemiology

An erection lasting longer than 4 h is referred to as priapism, whether this occurs in the context of or without prior sexual stimulation. The term priapism can be traced to Priapus, the god of fertility in Greek mythology. An erection that lasts up to 4 h is called a prolonged erection. In clinical practice, the transitions between prolonged erection and priapism are often variable. Priapism is a rare disorder, with an incidence of 1.5 per 100,000 person-years in the general U.S. population (Mishra et al. 2020) (see also Chap. 29).

30.4.2 Classification and Clinical Findings

A distinction is made between ischemic and nonischemic priapism. Recurrent (so-called "stuttering") priapism represents a special form of ischemic priapism. In all forms of priapism, there may be a complete or only partial erection, although in ischemic priapism, in contrast to nonischemic priapism, there is often complete rigidity of the cavernous sinus. Pain is most common in ischemic priapism (Mishra et al. 2020).

30.4.3 Ischemic Priapism

Ischemic priapism is the most common form of priapism, accounting for 95%. Typically, there is severely reduced or completely absent intracavernosal arterial perfusion, which is why it is also referred to as "low-flow" priapism. The lack of arterial perfusion results in hypoxia, hypercapnia, and acidosis of the cc. cavernosa, analogous to compartment syndrome (Vreugdenhil et al. 2019). Histologically, the cc. cavernosa show interstitial edema, destruction of the endothelium in the sinusoids, thrombosis in the sinusoids, smooth

muscle necrosis, and ultimately fibrotic remodeling of the cavernous tissue. Ischemic priapism is a medical emergency.

30.4.4 Etiology and Risk Factors

In most cases, ischemic priapism occurs idiopathically, with **sickle cell disease** being the most common cause in childhood. Other causes of ischemic priapism include other hematologic disorders (e.g., thalassemia, leukemia, and multiple myeloma), metabolic disorders (e.g., gout), neurogenic disorders (e.g., cauda equina syndrome, neuropathy, spinal stenosis, and brain tumors), metastatic or infiltrative malignancies (e.g., prostate and urinary bladder), infections, and toxins (e.g., rabies, stings, and bites of certain scorpions/spiders). Certain medications (e.g., certain antihypertensives, antidepressants, anticoagulants, and alpha-receptor blockers) may also cause ischemic priapism. The risk of ischemic priapism after intracavernosal injection of prostaglandins is <1% and up to 35% after injection of papaverine. Ingestion of PDE-5-I is not a risk factor for the development of ischemic priapism. Dysregulation of the NO/cGMP signal transduction pathway appears to represent the molecular mechanism in ischemic priapism (Anele et al. 2015).

30.4.5 Recurrent Priapism

Recurrent or intermittent priapism (often referred to as "stuttering" priapism) is characterized by the repeated development of episodes of painful ischemic priapism. In most cases, these priapisms, which may occur mainly at night and often following ischemic priapism, resolve spontaneously. The duration and frequency of priapism occurrence in patients with recurrent priapism are highly variable. Recurrent priapism can sometimes progress to persistent ischemic priapism. Etiologically, sickle cell anemia and neurologic diseases may be considered. At the molecular level, alterations in the NO/cGMP signal transduction pathway appear to be causative for the presence of recurrent priapism. Excessive NO secretion due to hemolysis (e.g., in sickle cell disease) may also lead to dysregulation of the NO/cGMP/PDE-5 signal transduction pathway and thus to recurrent priapism (Joice et al. 2021).

30.4.6 Nonischemic Priapism

In nonischemic priapism, uncontrolled and persistent arterial perfusion of the cc. cavernosa is present. Nonischemic priapism is often not painful, and the erection is not fully formed. Patients are typically still able to obtain full erections with sexual stimulation. Most commonly, nonischemic priapisms occur as a result of perineal or penile trauma. It is not uncommon for the time interval of the trauma to be 2 to 3 weeks. Trauma results in fistula formation between penile arteries and sinusoids. Less commonly, etiologic infiltrating metastases or iatrogenic as a result after urethrotomy and corporoplasty play a role. Nonischemic priapism is not a medical emergency.

30.4.7 Diagnostics

The patient history serves to assess previous diseases, duration of erection, use of medications and drugs, and any trauma to the genital and perineal region that may have taken place. Physical examination is used to palpate the cc. cavernosa and the C. spongiosum.

30.4.8 Laboratory Tests

Blood sampling is necessary for a differential blood count and a comprehensive coagulation status workup. Drug screening may be performed if needed and clinically suspected.

A puncture of the cc. cavernosa is performed for further examination of cavernous blood. The punctured blood is immediately submitted to blood gas analysis. Based on the results, ischemic and nonischemic priapism can be differentiated.

	Nonischemic priapism	Ischemic priapism
pO_2 (mmHg)	>90	<30
pCO_2 (mmHg)	<40	>60
pH	7.40	<7.25

30.4.9 Sonography

Dynamic sonography of the cc. cavernosa (and especially the A. penis profunda) in cw Doppler mode, in addition to blood gas analysis from the punctate of the corpora cavernosa, allows differentiation between ischemic and nonischemic priapism (Halls et al. 2012). It is important that sonography is obtained before puncturing the corpora cavernosa.

30.4.10 MRI

The value of an MRI of the penis is currently unclear. MRI may be useful in ischemic priapism to visualize and quantify infarcted cavernous tissue (Halls et al. 2012).

30.4.11 Therapy

30.4.11.1 Therapy of Ischemic Priapism

30.4.11.1.1 Nonsurgical Therapy

Although regional anesthesia using penile block does not reduce ischemic pain, it may be useful to increase the tolerability of the nonsurgical therapies mentioned below. In children, general anesthesia is a better option.

In the first step, the cavernous bodies are punctured with a 16 or 18 G butterfly cannula. Subsequently, cavernous blood is both aspirated and the cc. cavernosa is flushed with physiological saline. Puncture with two cannulas makes it possible to aspirate and irrigate simultaneously. These measures can eliminate priapism in 30% of cases (Burnett and Sharlip 2013).

If the above measures fail to treat priapism, different sympathomimetic substances can be injected intracavernously. Intracavernosal injection can sufficiently treat approximately 80% of priapisms. Drugs available for intracavernosal injection include phenylephrine, methylene blue, and epinephrine. Orally, terbutaline, a beta-2 agonist, may be tried. Because sympathomimetic drugs can have cardiovascular side effects (e.g., hypertensive crisis, reflex bradycardia, tachycardia), circulatory monitoring must be used during intracavernous injection. The use of sympathomimetic agents is contraindicated in poorly controlled hypertension and during ongoing therapy with monoamine oxidase inhibitors.

30.4.11.1.2 Surgical Therapy

If nonsurgical therapy does not result in the resolution of priapism, surgical therapy is indicated. Surgical therapy is based on the principle of shunt surgery and decompression of the cc. cavernosa by diverting blood flow from them via a connection to the C. spongiosum or a vein. A distinction is made between distal and proximal shunt surgery. A variety of different techniques have been described, and they diverge primarily in their invasiveness, which is greatest for proximal shunt operations (Burnett and Sharlip 2013). After distal shunt surgery, approximately 25% of patients suffer from ED, whereas after proximal shunt surgery, 50% do. The Winter, Ebbehoj, T, the Al-Ghorab, and Burnett's shunt are among the distal shunt procedures (Fig. 30.16). In these procedures, a connection is made between the glans penis and the cc. cavernosa. Winter's shunt results in cessation of priapism in 66% of patients and Ebbehoj's shunt in 73%. Proximal shunts include the Quackles and Grayhack techniques. A connection between the cc. cavernosa and C. spongiosum is established via a scrotal or perineal approach in the Quackles technique. In the Grayhack technique, an end-to-side anastomosis is created between the saphenous vein and a C. cavernosum. Success rates are 77% for the Quackles technique and 76% for the Grayhack technique. If ischemic priapism persists for more than 36 to 78 h, it results in marked cavernous fibrosis and ED. Therefore, in these cases, a penile prosthesis implantation may be appropriate, as it may prevent major penile length loss at best (Mishra et al. 2020).

30.4.11.2 Therapy of Nonischemic Priapism

30.4.11.2.1 Conservative Therapy Methods

Conservative treatment options include local compression of the perineum and cooling, which can close the arteriovenous fistula. Nonischemic priapism spontaneously resolves in 62% of patients over the course of up to 6 months without therapy (Mishra et al. 2020). In contrast to the treatment of ischemic priapism, aspiration of blood and irrigation of the cc. cavernosa are not effective, and injection of sympathomimetic substances is even contraindicated, as this can lead to severe systemic side effects.

30.4.11.2.2 Surgical Therapeutic Procedures

Arterial angiography can selectively close the fistula with autologous clots or absorbable gel, resulting in successful therapy of nonischemic priapism in nearly 80% of cases. Nonabsorbable materials such as ethanol or coils lead to ED in nearly 40% of patients so treated, which is an eightfold increased risk compared with absorbable materials (Mishra et al. 2020). In terms of other complications, gluteal ischemia, perineal abscesses, and also gangrene of the penis may occur. Recurrences after selective embolization can be observed in up to 27% of cases. Technically demanding duplex sonographically guided open surgical ligation of the arteriovenous fistula is the ultima ratio of therapy for nonischemic priapism (Mishra et al. 2020).

30.4.11.2.3 Therapy of Recurrent Priapism

For the acute episode, the same therapeutic procedures are used for ischemic priapism. To prevent the recurrence of recurrent priapism, therapy trials may include α-receptor agonists (e.g., phenylephrine 50–100 mg daily). Success rates of up to 72% have been published on this (Olujohungbe et al. 2011). In addition, anti-androgenic therapies can also be used (e.g., GnRH agonists, antiandrogens, or 5α-reductase inhibitors). However, it must be considered here that there are undesirable side effects on male sexual function. Other substances that can be used on a trial basis include, for example, digoxin, baclofen, gabapentin, or terbutaline, although there are limited data regarding the efficacy. PDE-5-I can also be used if the penis is completely flaccid (Mishra et al. 2020; Joice et al. 2021).

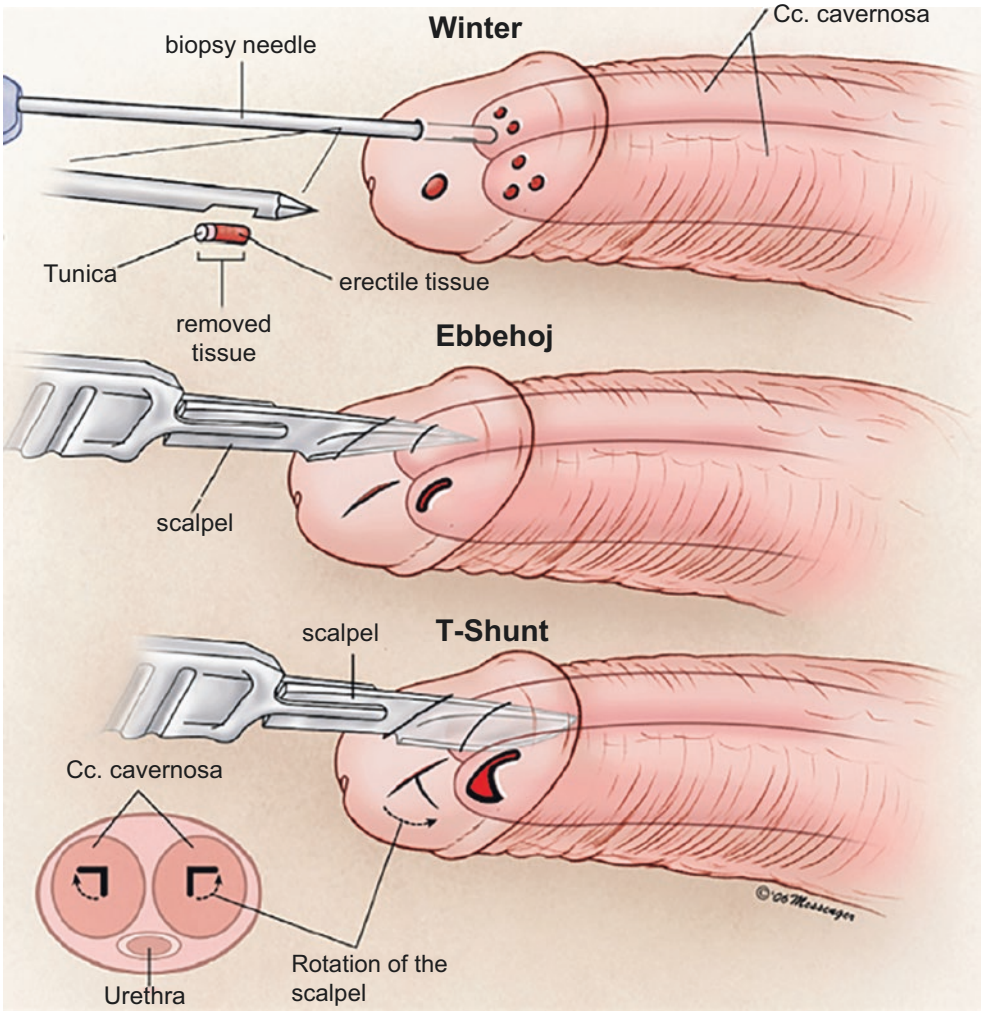


Fig. 30.16 Percutaneous corporoglanular shunts. Percutaneous corporoglanular shunts result in a direct connection of the corpora cavernosa with the glans penis (corpus spongiosum). Thus, the arterial blood flow into the corpora cavernosa is reestablished. The figure displays the Winter, the Ebbehoj, and the T-Shunt technique. Figure modified according to Burnett and Sharlip (2013)

Key Points

- Sexual dysfunction in men can be multifaceted. Purely mechanical disorders of seminal deposition due to anatomy should be differentiated from functional libido and orgasmic disorders, ejaculation problems, or inability to achieve or maintain an adequate erection.
- While pure orgasmic disturbances are almost in all cases due to psychological or psychiatric causes, libido and erectile dysfunction may also have hormonal causes and thus be symptoms of androgen deficiency.
- Additional clinical symptoms of hypogonadism or objectifiable findings such as testicular atrophy, changes in secondary sexual characteristics, or ejaculate volume allow the organic etiology to be identified in pronounced cases, while in other cases the corresponding indications must be actively sought.
- Thus, whereas functional orgasmic and libido disorders are the domain of psychological psychiatric or endocrinological therapeutic approaches, this chapter deals primarily with disorders of seminal deposition and functionally or organically determined disorders of cohabitation.

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Part VI

Clinics in Andrology: Disorders of Sexual Differentiation and Androgen Target Organs

Variants of Sex Development

31

Isabel Viola Wagner and Olaf Hiort

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Abstract

Variations (formerly "disorders") of sex development (DSD) describe congenital conditions with atypical constellations between the chromosomal, the gonadal, and the phenotypic sex. This definition encompasses a variety of clinical entities and is not strictly defined regarding its inclusive- and exclusiveness, because the classification allows for both a clinical and a (molecular-) genetic nomenclature and classification. In addition, the DSD complex is associated with a concept of clinical care and medical management, as affected persons may have a very special gender self-perception, which may impose a sociocultural challenge. In the following chapter, we therefore introduce the present classification and the current discussion about medical management before we explain defined conditions.

31.1 Introduction

The definition of variants (originally "disorders") of sex development (**DSD**) was established in 2005 at a consensus conference of major pediatric endocrine societies held in Chicago in order to deal with a mismatch between chromosomal, gonadal, and phenotypic sex. The intention behind this was to replace the previously used terms such as **intersexuality** or **(pseudo) hermaphroditism,** which were thought to be pejorative**.** However, it is precisely this that has sparked a social dispute, as the definition is imprecise and is also unrelated to a sociocultural context of the self-perception of those affected. Therefore, in many countries, especially in Western countries, a broader view has been established, which on the one hand is based on the biological definition, but on the other hand also associates DSD with a special, medically oriented care concept within the social context of gender diversity.[1]

31.2 Nomenclature and Classification

The definition of DSD allows for the inclusion of a broad spectrum of possible biological variants of sex development. According to current knowledge, DSD is characterized by a dynamic in the developmental biological processes of sex development. These in turn allow a range of expression of sex and gender differentiation. What is known is the initial omnipotence of the embryo, which does not prescribe any differentiation or categorization of sex. Only with the **differentiation and development of the gonadal system** under the control of a complex genetic regulation into a testicular or ovarian orientation are the main directions "male" or "female" predetermined. With further development, a **dominance of testicular hormone synthesis is** assumed, which causes the formation of the internal and external genital structures via transcriptional regulation. Ultimately, these regulatory events allow a high degree of variability, which can also be seen within the reproductively relevant categories of "male" and "female." DSD as variants of sex development therefore has diverse characteristics, which will certainly also have to be defined more accurately in the future.

31.2.1 The Classification of DSD

Consideration of the biological **classification of DSD** arose from the understanding of the genetic and hormonal cascades of sex development in their spatiotemporal context and should allow a diagnostic classification. It does not serve the gender categorization of affected persons, which must follow individually, considering a variety of other biological-medical but also sociocultural aspects.

The classification follows the developmental biological understanding that the usual direction is given with the determination of chromosomal sex. Thus, a classification is first made into the categories of numerical chromosomal inconsistencies and **46,XX or 46,XY DSD**. According to this classification, Klinefelter syndrome and Ullrich-Turner syndrome also belong to the group DSD forms even though Klinefelter syndrome is a particularly frequent variant (see Chap. 21) and certainly both Klinefelter and Ullrich-Turner syndromes require different approaches to medical care than is assumed for many other forms of DSD. Other numerical abnormalities include mixed gonadal dysgenesis with **45,X/46,XY karyotype** and the rare cases of **46,XX/46,XY chimerism**.

Within the numerically inconspicuous categories 46,XX and 46,XY DSD, a distinction is then made between congenital abnormalities of gonad development and tangible

[1]For reasons of better readability, the masculine form is used for personal names and personal nouns in this chapter. Corresponding terms apply in principle to all genders for the purpose of equal treatment.

disorders of androgen biosynthesis (with increased or decreased androgen formation) or androgen action. This subdivision allows future inclusion of new findings of molecular genetic or biochemical causes in the main categories. In addition, however, clinical descriptions of genital abnormalities not yet defined at the molecular genetic level, such as pronounced **hypospadias** or complex malformations such as **cloacal exstrophy,** are also permitted in each main category. Table 31.1 summarizes the classification according to consensus.

This categorization emphasizes that it has not yet been clearly defined whether every genital abnormality should be classified as DSD. For example, isolated unilateral undescended testis is certainly a genital abnormality, but is not counted as DSD according to current interpretation. Similarly, it is not clear to what degree hypospadias should be classified as DSD. On the other hand, a clear separation should be made from **gender dysphoria/transgender identity** entities. An update of the DSD consensus from 2016 draws the line at pronounced **hypospadias in combination with cryptorchidism** and gives the **frequency for DSD as** about 1:4000–5000 (Lee et al. 2016). However, this certainly does not provide satisfactory and exact clarification. Further consensus work is needed on this topic.

Table 31.1 Classification of DSD (modified according to Hughes et al. 2006)

DSD due to numerical aberrations of the sex chromosomes	46,XY-DSD	46,XX-DSD
A: 47,XXY	**A: Disorders of gonadal/testicular development**	**A: Disorders of gonadal/ovarian development**
Klinefelter syndrome and variants	• Ovotesticular DSD	• Gonadal dysgenesis
B: 45,X	• Complete or partial gonadal dysgenesis (e.g., SRY, SOX9, SF1, GATA4, WT1, DHH, WNT4 duplication, DAX1 duplication, etc.)	• Ovotesticular DSD
Ullrich-turner syndrome and variants	• Gonadal regression	• Testicular DSD (e.g., SRY+, SOX9 duplication)
C: 45,X/46XY mosaic	**B: Disorders of androgen biosynthesis or androgen action**	**B: Androgen excess**
Mixed gonadal dysgenesis	• Disorders of androgen biosynthesis	• Fetal androgen excess
D: 46,XX/46XY	– LH receptor mutations	– 3 β-Hydroxy-steroid dehydrogenase type-2
Chimerism	– Smith-Lemli-Opitz syndrome	– 21-hydroxylase
	– Steroidogenic acute regulatory protein (STAR)	– P450 oxidoreductase
	– P450 side chain cleavage (SCC)	– 11 β-hydroxylase
	– 3 β-Hydroxysteroid dehydrogenase type-2	– Glucocorticoid resistance
	– 17 α-hydroxylase/17,20-lyase	• Fetoplacental androgen excess
	– P450 oxidoreductase	– Aromatase deficiency
	– 17 β-Hydroxysteroid dehydrogenase type-3	– P450 oxidoreductase
	– 5-Reductase type-2α	• Maternal androgen excess
	• Disorders of androgen action	– Virilizing tumor (luteoma)
	– Complete and partial androgen resistance	– Application of androgenic substances
	– Endocrine disruptors	**C: Other**
	C: Other	• Syndromal forms
	• Syndromal forms	– Cloacal malformations
	– Cloacal malformations	– Et al.
	– Aarskog syndrome	• Agenesis/ hypoplasia of the Müller structures (MURCS)
	– Hand foot genital syndrome (HOXA13)	• Vaginal atresia (McKusick-Kaufmann syndrome)
	– Robinow syndrome	• Labial synechiae
	– Et al.	• Etc.
	• Persistent Müllerian duct syndrome (AMH and AMH receptor disorders)	
	• Vanishing testis syndrome	
	• Isolated hypospadias	
	• Cryptorchidism	
	• Etc.	

31.3 Clinical Examination and Medical Classification

Any suspicion of a congenital abnormality of sex development requires a systematic patient history, especially with regard to family characteristics, previous surgeries, and the onset and course of pubertal development. According to the classification, DSD covers a very **broad phenotypic spectrum of genital findings**, only some of which become conspicuous during childhood. These include the obvious findings of ambiguous genitalia at birth, or a deviation of the phallic structures from the usual reference, an unusual localization or absence of gonadal structures, an only partial fusion of the labioscrotal structures, and a deviating localization of the meatus urethrae. In the course of time, various scoring systems have been used for the complete recording of a genital finding in childhood, which, however, had the disadvantage that they could not be used for the range of gender expression, but were oriented toward a "male" or "female" gender. Thus, the **Prader score** describes genital abnormalities in 46,XX adrenogenital syndrome (Prader 1945) and the **Quigley score** describes the range of 46,XY androgen resistance (Quigley et al. 1995). Recently, this shortcoming was overcome by the European definition of an **External Genitalia Score (EGS),** which attempts to establish a common description (van der Straaten et al. 2020) (Table 31.2).

Another possibility for the clinical evaluation of a genital abnormality in childhood is the determination of the **anogenital distance**. It is considered a sensitive measure of androgen action during embryonic development and is sexually dimorphic. For this purpose, the distance of the anus from various genital structures is measured (Fig. 31.1).

Some affected persons do not come to medical attention until adolescence or even adulthood. In this case, special attention should focus on the sex-related physiognomy and on the (secondary sex characteristic) hair structure and its distribution pattern, breast development, and fat distribution. In general, a **complete physical examination is** always part of a clinical diagnosis when a variant of sex development is suspected, which should describe any atypical findings, since some forms of DSD are associated with other malformations of the skeletal system, the internal organs, especially the heart and kidneys, or the nervous system.

Adults with DSD have been paid little attention in terms of their healthcare needs. However, the European study DSDLife, as well as those nationwide studies of the German network "Intersexuality"(from 2003 to 2008), found a high dissatisfaction with the medical care of 46.XY DSD people (Thyen et al. 2014). This may be due to the special issues and needs of this patient group. Among other things, this concerns **hormone substitution** during puberty and adulthood. There is little reliable data on this. Recently, we were able to show that in 46,XY women with complete androgen resistance, therapy with both estrogens and androgens can be considered safe, but testosterone therapy given at the usual male substitution dose leads to a significant improvement in libido (Birnbaum et al. 2018). Whether and what conditions need to be considered for the initiation of puberty as well as hormone replacement in adults living in the male gender is currently unresolved. Similarly, questions about **fertility options have** not yet been addressed or have only been addressed hypothetically. In 2015, the German Medical Association issued a detailed statement on this issue (Bundesärztekammer 2015). Currently, guideline-based care for individuals with DSD in Germany is being addressed by the Federal Ministry of Health and the quality indicators for the provision of specialized centers are to be developed and reviewed in a "DSD-care" research project (Jürgensen et al. 2021).

In addition, challenging disease entities sometimes occur, e.g., prostatitis is conceivable in a 46,XY woman with an androgen biosynthesis defect. How should both medical and holistic care be provided in such cases? A frequently discussed topic is the possible tumor formation and its status in 46,XY DSD. Only sparse epidemiological data are available

Table 31.2 The "External Genitalia Score" describes the clinical findings at five anatomical sites of the genitals: the extent of the labioscrotal fusion, the length of the genital tubercle (GT), the position of the urethral opening, and the localization of the right and left gonads

External genitalia score	Labioscrotal fusion	Length of the genital tubercle (mm)	Urethral opening	Right gonad	Left gonad
3	Merged	>31	At the top of the GT		
2.5		26–30	Coronary		
2			On the shaft		
1.5	Partial fusion posterior	21–25	At the base of the GT	Labioscrotal	Labioscrotal
1		10–20	Labioscrotal	Inguino-labioscrotal	Inguino-labioscrotal
0.5				Inguinal	Inguinal
0	Not merged	<10	Perineal	Not palpable	Not palpable

The final score is calculated from the sum of the points from all five findings
EGS, external genitalia score; *G,* genital tubercle
According to van der Straaten et al. (2020)

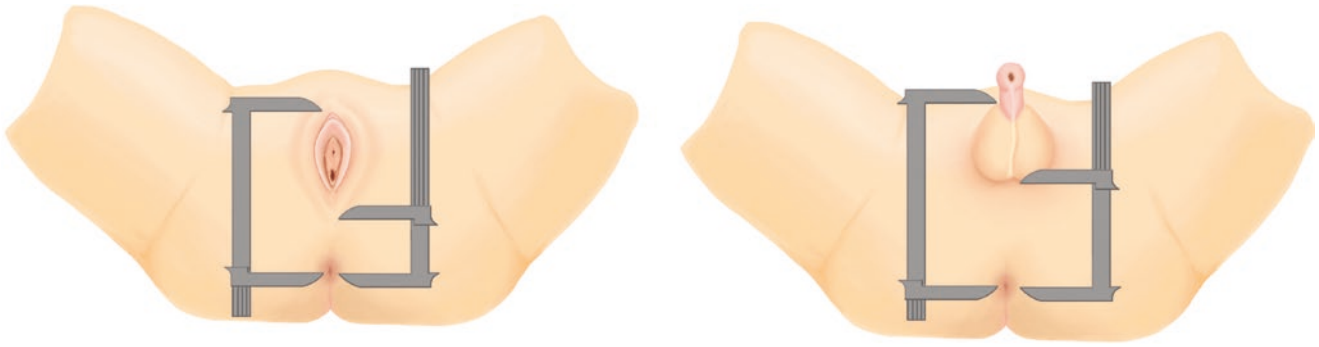


Fig. 31.1 Measurement of the anogenital distance (Thankamony et al. 2016). For this purpose, the distance from the anus to the posterior commissure of the labioscrotal folds is measured, as well as the distance from the posterior commissure to the root of the phallus. The greater the ratio of the lower to the upper measurement, the more androgen effect can be assumed. (drawn by Cand. Med. Mirkka Hiort)

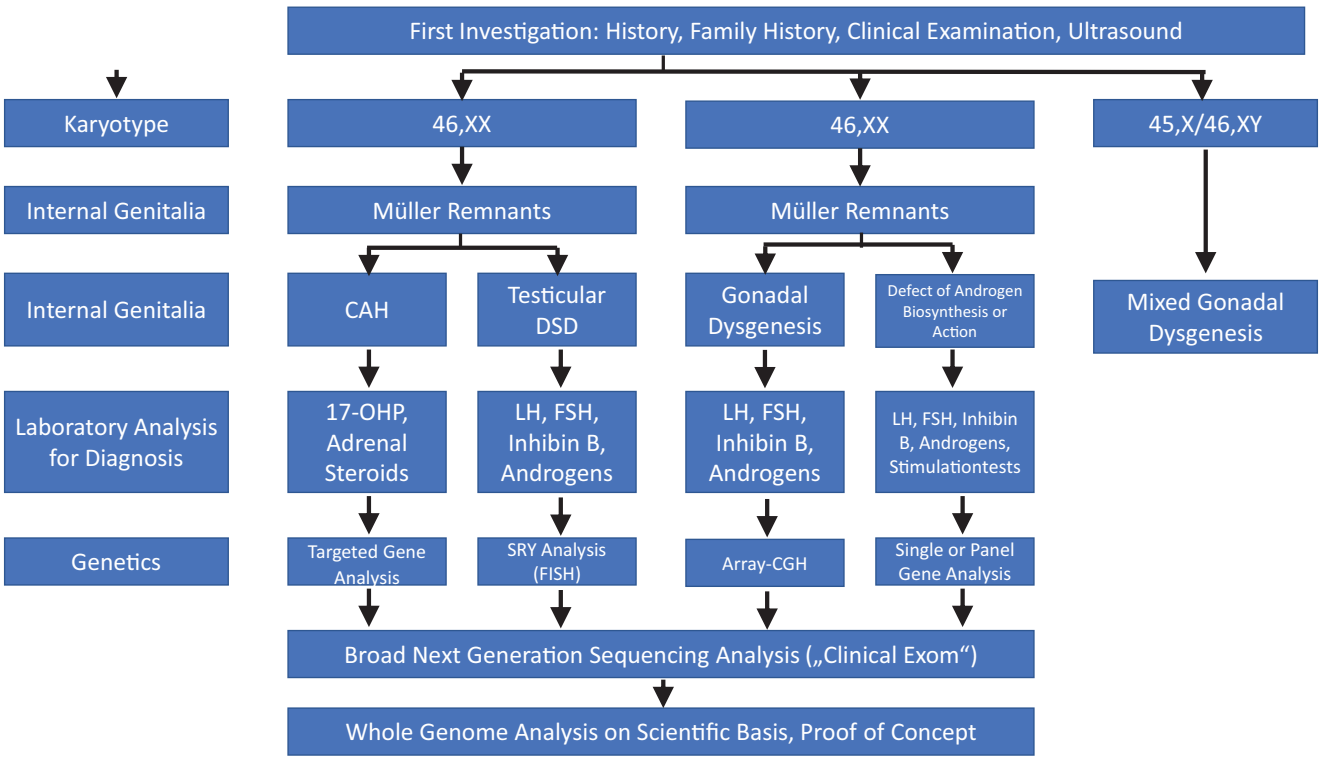


Fig. 31.2 Linking clinical, biochemical, and (molecular) genetic diagnostics in DSD (from Hiort et al. 2019)

on this. Even if tumors are described, their status is usually unclear and not considered life-threatening. Very recently the German Federal Cabinet banned **any gender reassignment surgery** up to the age of consent.

In the following, we will discuss the individual entities of DSD in the context of this book. The linkage of clinical, biochemical, and (molecular) genetic diagnostics is shown in Fig. 31.2.

31.4 Structural Chromosomal Abnormalities

Klinefelter's syndrome is dealt with in Chap. 21. Similarly, the Ullrich-Turner syndrome itself does not play a significant role here in a textbook of andrology. Affected individuals have a female habitus and are cared for by gynecologists and endocrinologists because of ovarian insufficiency.

31.4.1 Definition and Etiology

In this chapter, we would like to focus on the special features of numerical chromosomal abnormalities with a **45,X/46,XY mosaic karyotype,** which are important for andrology. The frequency of 45,X/46,XY mosaics is given as about 1:15,000 live births (Nielsen and Wohlert 1991). The 45,X cell line probably results from a loss of an inconspicuous or structurally altered Y chromosome during early meiosis, leading to the mosaic finding.

31.4.2 Diagnosis

Diagnosis is made by chromosomal analysis. In many cases, this may be an incidental finding in the differential diagnosis of Turner syndrome. The clinical picture can be highly variable and includes genital abnormalities, short stature, varying degrees of gonadal dysgenesis and function, and impaired fertility. Phenotypic variability ranges from a completely female phenotype with typical findings of the Ullrich-Turner syndrome to only mildly underandrogenized males. It is even likely that the majority of affected children will be born as inconspicuously male (Lindhardt Johansen et al. 2012). A relevant number of children have comorbidities such as kidney or heart malformations.

31.4.3 Therapy

From an andrological perspective, the risk of gonadal tumor formation, sex hormone substitution for puberty induction, and treatment of hypergonadotropic hypogonadism, as well as the assessment and treatment of fertility restriction are important. In a recently published study, patients with 45,X/46,XY mixed gonadal dysgenesis and male sex assignment were followed up (Ljubicic et al. 2019). This showed that about half of the children had a genital abnormality with hypospadias and undescended testes at birth, while the other half were initially classified as phenotypically normal males. While the majority entered puberty spontaneously, the children with genital abnormalities usually had relevant hypergonadotropic hypogonadism, which was classified as requiring treatment. Thus, in addition to possible surgical correction of the genitals, therapy usually also includes puberty induction and permanent substitution with testosterone when the individual lives as a male.

The **gonadal histology** shows a variable proportion of undifferentiated stroma, while a Sertoli cell-only syndrome may be present in the testicular portion. About a quarter of patients show spermatids; therefore, infertility or reproductive dysfunction is a common finding and problem. **Germ cell neoplasias** are seen in about 10% of the cases, mostly as germ cell neoplasia in situ, possibly also as a gonadoblastoma (Lindhardt Johansen et al. 2012). Therefore, regular ultrasound controls of the gonads should be carried out.

31.5 46,XX Men (with 21-Hydroxylase Deficiency)

31.5.1 Definition and Etiology

The **46,XX testicular form of DSD** is dealt with in another chapter (see Chap. 22). However, the 46,XX highly androgenized forms of other causes must also be considered here for differential diagnosis. These can be children with increased testosterone production due to an **aromatase deficiency or testosterone production during pregnancy** in the mother due to a luteoma. In these cases, a detailed history of the pregnancy should be taken to determine whether the mother also had hyperandrogenemia.

Another rare cause of a 46,XX DSD with marked androgenization and male-appearing external genitalia can occur in **21 hydroxylase deficiency.** The children have an inconspicuously male phallus, but the scrotum is empty and gonads cannot be detected. Abdominal ultrasound reveals typical female internal genitalia with uterus and ovaries.

31.5.2 Diagnosis

In many Western countries, the children are usually detected through newborn screening, but we ourselves were able to identify several **46,XX children with 21 hydroxylase deficiency** and male gender assignment very late, because either the screening findings were not understood by the parents and no clarification took place, or the parents had consciously decided for male gender assignment due to the clinical appearance of the external genitalia. In addition, we were able to identify children with a migration background in whose home countries no newborn screening takes place. Overall, however, male gender assignment in 46,XX children with adrenogenital syndrome is very rare and epidemiological studies do not exist.

31.5.3 Therapy

46,XX males with congenital adrenal hyperplasia require **lifelong substitution with hydrocortisone and fludrocortisone**. This treatment is important to treat the adrenal insufficiency, but at the same time the adrenal hyperandrogenemia is reduced. Therefore, for these individuals, if the male sex assignment is maintained, adequate testosterone therapy must be given in adolescence. This simultaneously leads to

the absence of menstruation and suppression of ovarian function. In this respect, surgical removal of the internal genital structures is not absolutely necessary. The procedure is usually analogous to the treatment of transmen.

31.6 46XY-DSD

31.6.1 Gonadal Dysgenesis

31.6.1.1 Definition and Etiology

The term **gonadal dysgenesis** defines a group of genetically caused conditions affecting gonadal sex differentiation. Mutations, deletions or duplications of transcription factors and genes of gonadal differentiation can lead to complete or partial gonadal dysgenesis. In case of complete gonadal dysgenesis, there are no functional gonads; instead, there are connective tissue **streak gonads (string gonads)** usually without residual function. A streak gonad is histologically characterized by stromal tissue, i.e., the absence of both germ cells and Sertoli/granulosa cells derived from the germ strands. In partial gonadal dysgenesis, hormone-active tissue is still partially present, which can lead to virilization in children with a 46,XY karyotype.

Mutations in genes responsible for early gonadal differentiation, such as *WT1* and *NR5A1*, and genes responsible for testicular differentiation, such as *SRY, DMRT, SOX9, DHH, ATRX,* and *ARX*, have been identified as a possible cause for DSD (Hughes 2008; Hughes et al. 2006). Mutations in some of these genes lead to syndromal forms of gonadal dysgenesis. For example, mutations in the *WT1* gene are also associated with nephropathy and in the *NR5A1* gene with adrenal insufficiency. Mutations in the *SOX9* gene lead to campomelic dysplasia, in the *DHH* gene to neuropathy, in the *ATRX* gene, and in the *ARX* gene to mental retardation.

31.6.1.2 Diagnosis

In gonadal dysgenesis, apart from patients with confirmed 45,X/46,XY karyotype, the diagnosis can only be confirmed definitely by **laparoscopy with gonadal biopsy.** In gonadal dysgenesis with a 46,XX karyotype, LH and FSH are elevated, and estrogen and progesterone levels are decreased. Sometimes it is a familial disorder. The endocrine situation in gonadal dysgenesis with 46,XY karyotype is identical to the 46,XX variant. Diagnostically gonadotropins are markedly elevated, and Sertoli cell markers like Inhibin B or AMH are low. The testosterone concentration is often too low for males and too high for the female reference range. After hCG administration, there is often a small but significant increase in testosterone levels. Laparoscopy mostly shows at least rudimentary remnants of the Müllerian ducts. Biopsies from the intra-abdominal streak gonads consist histologically almost exclusively of connective tissue, ovarian stroma-like tissue without evidence of germinal cells. The testicular biopsy shows isolated precursors of germinal cells with absent or barely present Sertoli cells and often Leydig cell hyperplasia. A chromosomal analysis must be carried out in any case. Mutations in the sex-determining genes must be specifically searched for Hughes (2008). Furthermore, anorchia or bilateral cryptorchidism may be considered as differential diagnoses; however, in these children the external genitalia are most often unequivocally male.

31.6.1.3 Therapy

Adequate **hormone replacement therapy** must be started at the time of expected puberty. In the case of ambiguous genitalia, surgical corrections should consider the patient's gender identity (and are currently forbidden in minors in several countries). There is a **risk of** gonadal **tumors** (gonadoblastomas, seminomas, dysgerminomas, carcinoma in situ) in all patients who have a cell line with a Y chromosome (Savage and Lowe 1990). For this reason, the gonads of these patients should be monitored and, in any case, if regular checks of the gonads by ultrasound are not possible, surgical biopsy or removal must be discussed. Infertility is not causally treatable. In countries with corresponding legal regulations, successful pregnancies have been achieved in patients with pure gonadal dysgenesis after in vitro fertilization with donor eggs (Kalra et al. 2019).

31.6.2 Gonadal Dysgenesis Due to SRY Mutation (Yp11.3)

In complete gonadal dysgenesis, a mutation in the SRY gene is present in approximately 10% of the cases. Complete gonadal dysgenesis with a karyotype of 46,XY was previously called **Swyer syndrome.** No androgens are produced during the embryonic period, so that affected individuals have **external female genitalia at** birth. Some cases present with slight clitoral hypertrophy. Since AMH production is absent, the Müllerian structures do not regress, so that the uterus and fallopian tubes persist. Later on, due to lack of sex steroids, there is increased height and at puberty the thelarche is absent and the menarche does not occur.

The **diagnosis** is usually not made until puberty, when adolescents present due to the lack of pubertal development. The XY karyotype and the proof of an existing uterus and fallopian tubes without ovaries on sonography indicate complete gonadal dysgenesis and warrant search for a mutation in the *SRY* or other developmental genes. The hormone status shows hypergonadotropic hypogonadism plus decreased AMH and inhibin B concentrations. There is a risk of degeneration of the gonads with increased risk of tumor formation such as gonadoblastoma or dysgerminoma.

31.6.3 Gonadal Dysgenesis Due to SF1/NR5A1 Mutations (9q33)

During embryonic development, the *SF1/NR5A1* gene is expressed in the gonads as well as in the adrenal glands, hypothalamus, and pituitary gland. Affected 46,XY individuals with a *NR5A1* **mutation** may rarely present with **adrenal hypoplasia**, which in the course of time usually leads to adrenal insufficiency (Achermann et al. 1999). The phenotype is usually externally female with persistent Müllerian structures and streak gonads. However, the phenotype is highly variable, and some or even the same mutations lead only to partial underandrogenization and partial or complete regression of the Müllerian structures.

Inhibin B and AMH are usually reduced. Some patients of neonatal age may already present with adrenal insufficiency. **Diagnosis is confirmed by** sequencing the *SF-1/NR5A1* gene.

Adrenal insufficiency must be treated with **glucocorticoids and mineralocorticoids.** There is a residual risk of gonadal tumor development. During puberty, sex hormone replacement therapy is almost certainly necessary.

31.6.4 Gonadal Dysgenesis Due to *WT-1* Mutations (11p13)

A mutation in the ***WT-1* gene (Wilms tumor suppressor 1 gene**) affects the development of gonads and kidneys with the risk of developing Wilms tumors and gonadoblastomas (in 46,XY patients). This form of gonadal dysgenesis can occur in 46,XX and in 46,XY individuals, as the gene exerts its effect at the level of the bipotent gonads. The androgen deficiency in 46,XY individuals leads to genital malformations, such as hypospadias. During puberty, secondary sexual characteristics are absent or diminished. Different gene alterations of the *WT-1* gene lead to different entities: such as Denys-Drash syndrome (with partial gonadal dysgenesis, Wilms tumors), Frasier syndrome (with complete gonadal dysgenesis and glomerulosclerosis leading to renal failure) or WAGR syndrome (with Wilms tumors, aniridia, atypical genitalia due to gonadal dysgenesis, and mental retardation). The diagnosis can only be confirmed by sequencing the *WT-1* gene.

31.6.5 Gonadal Dysgenesis Due to Deletion of the DMRT1 Gene Locus (9p-)

Deletion of sections of chromosome 9 (9p-) leads to a combination of 46,XY-DSD with androgenization deficiency of the external genitalia due to partial gonadal dysgenesis and is often associated with mental retardation. This can be accompanied by craniofacial abnormalities, heart defects, and kidney malformations, depending on the size of the rearrangement at this gene locus leading to a contiguous gene syndrome.

In suspicious cases, a chromosomal analysis or a FISH analysis should be performed. This can also be supported by an Array Comparative Hybridization Analysis (aCGH). A *DMRT-1* deletion is also associated with an increased risk of gonadoblastoma development.

31.6.6 *SOX9* (17q24), *DAX1* (Xp21.3), *DHH* (12q13.1), *WNT4* (1p35)

The SOX9 protein exerts its function immediately downstream of SRY and is thus of central importance for testis development. *SOX9* is expressed in embryonic Sertoli cells and in cartilage precursor cells. Thus, heterozygous mutations of *SOX9* lead to campomelic dysplasia and, due to gonadal dysgenesis, to 46,XY-DSD. Duplications of the X-linked *DAX-1* gene lead to suppression of normal testicular development and thus to impaired sex development due to gonadal dysgenesis. Rare mutations such as in the desert hedgehog (*DHH*) gene lead to gonadal dysgenesis with polyneuropathy. A duplication of *WNT4* can also lead to gonadal dysgenesis.

31.7 46,XY-DSD Caused by Defects in Androgen Biosynthesis

In a 46,XY variant of sex development, in contrast to gonadal dysgenesis, a failure of androgen biosynthesis is accompanied by normal Sertoli cell function. Therefore, persons with 46,XY DSD with a deficiency in androgen biosynthesis have absent Mullerian structures due to normal AMH secretion, but a diminished androgenization of the testosterone-dependent organ structures (external genitalia, seminal vesicles, prostate). The first steps of steroid hormone synthesis involve adrenals and gonads and can thus lead to a combination of DSD and adrenal insufficiency.

Disruption of testosterone biosynthesis from cholesterol is possible at any level. All these enzyme disorders are **inherited in an autosomal recessive manner**. In addition to obligate hypogonadism, adrenal insufficiency or mineralocorticoid excess may occur clinically, since 20,22-desmolase, 3ß-hydroxysteroid dehydrogenase, and 17α-hydroxylase are also involved in the synthesis of mineralocorticoids and glucocorticoids, respectively. Enzyme defects that selectively affect testosterone biosynthesis are the 17,20-desmolase defect and the 17β-hydroxysteroid dehydrogenase defect. The deficiency of 5a-reductase type 2 is special, because this enzyme is expressed in the peripheral target cell to convert

testosterone to dihydrotestosterone (DHT) prior to binding to the androgen receptor to facilitate androgen action.

At the time of expected puberty, testosterone and estrogen serum levels should be closely monitored in order to initiate hormone replacement therapy at an early stage if necessary. Depending on the phenotypic expression, estrogen substitution therapy or testosterone substitution is carried out for the rest of the patient's life. In order to preserve gonadal functionality in these patients, undescended testicles should be transferred to the scrotum at an early stage and closely monitored by palpation and sonography.

31.7.1 17ß-Hydroxysteroid Dehydrogenase Type 3 Defect (9q22)

The reduction of androstenedione to testosterone is catalyzed by the **17ß-hydroxysteroid dehydrogenase type 3 defect.** In both homozygous and compound heterozygous inactivating mutations, testosterone cannot be produced in sufficient quantities. Therefore, clinically individuals often present with only slightly androgenized genitalia at birth (Hiort et al. 2017). The testes are usually located inguinally, the Wolff derivatives are partially developed and the vagina is usually shortened and ends blind. During puberty, however, pronounced virilization may occur with phallic growth, vocal change and male body proportions develop. These changes can be so pronounced that in some cases a change to the male gender role takes place.

The **hCG test** may demonstrate an increased androstenedione-testosterone quotient (A:T > 1). To **confirm the diagnosis, the** HSD17B3 gene must be sequenced. Most of the time at least initially, the children grow up in a female gender role**.** The diagnosis is often not made until pubertal onset with **increasing virilization.** To gain time for diagnosis and decision-making, **puberty** can be **stopped with GnRH analogs**. If retention of the female gender role is desired, a gonadectomy can be performed after reaching consent age. If a change to the male sex role is preferred, hypospadias correction and penile reconstruction must be surgically performed. The risk of gonadoblastoma is classified as intermediate.

31.7.2 5α-Reductase Type 2 Defect (2p23) = Perineoscrotal Hypospadias with Pseudovagina

The so-called **perineoscrotal hypospadias with pseudovagina (PHP**) is a disorder of androgen action, however, not at the receptor level but at the metabolic level. All clinical manifestations of PHP are explained by a **deficiency of dihydrotestosterone** in the target cells of the genital tract. The basic defect lies in an impaired conversion of testosterone to DHT by the enzyme **(steroid) 5α-reductase** (Griffin et al. 1995; Imperato-McGinley 2002; Imperato-McGinley et al. 1982). Since DHT is responsible for virilization of the external genitalia, 46,XY newborns often have female external genitalia or—depending on the residual activity of 5α-reductase—signs of **virilization with clitoromegaly, hypospadias, and labioscrotal fusion.** Müllerian structures are absent due to the AMH effect of the testis, so that the urogenital sinus remains or a blind-ending pseudovagina results. In contrast, the Wolff ducts are formed due to testosterone action. The prostate, whose development is dependent on DHT, is localized as a rudiment dorsal to the urethra and remains small. The gonads are often located extra-abdominally, mostly in the inguinal canal or in the area of the labia majora. During puberty, the testosterone-dependent processes come into effect. Vocal change and the typical male muscle habitus occur, while DHT-dependent processes such as beard growth and acne are diminished. The hair on the head remains dense, the frontal hairline straight. At puberty, menarche and breast development do not occur, and there is a significant virilization. In many cases, a reorientation toward the male gender role has been observed (Hughes et al. 2006; Imperato-McGinley 2002; Imperato-McGinley and Zhu 2002).

There is a **positive family history in** about 40%, depending on the ethnic background. The condition is inherited in an autosomal recessive manner. In endocrinological diagnostics, an h**CG test** can be performed, whereby testosterone shows the expected increase, while DHT remains low. However, the specificity of this test is low**.** Blood estrogen levels, LH, and FSH concentrations are in the typical male reference interval or slightly elevated. A urine steroid profiling with gas chromatography mass spectrometry can also diagnose 5α-reductase type 2 deficiency. Genetic diagnosis is made by mutation detection in the corresponding gene, SRD5A2 (Audi et al. 2018; Kumar et al. 2019; Sinnecker et al. 1996).

Treatment concepts should be applied as for the 17ß-hydroxysteroid dehydrogenase type 3 defect, as DHT is currently not available for therapy. Surgical measures should only be carried out with great restraint and at the earliest, at the age of puberty.

31.7.3 Gonadotropin Receptor Mutations

Specific receptors mediate the effect of the gonadotropins LH and FSH on the Leydig and Sertoli cells, respectively. Such receptor mutations lead to functional changes in these cells with multiple clinical consequences. Basically, a distinction must be made between **inactivating and activating mutations.** While the former lead to a loss of function,

activating mutations cause a constitutive, i.e., autonomous activity of the target cells without the need for stimulation by LH or FSH. Overall, however, these clinical pictures are rare.

31.7.3.1 LH Receptor Defect (2p21) or Inactivating LH Receptor Mutations

Leydig cell hypoplasia or Leydig cell agenesis is a very rare autosomal recessive disorder with an approximate incidence of 1:1,000,000. The name of the syndrome as Leydig cell agenesis is misleading in that Leydig cells are present but cannot develop due to **inactivating mutations of the LH receptor** and therefore lack of stimulation (Huhtaniemi and Alevizaki 2006). The expression of the phenotype depends crucially on the extent of intrauterine testosterone secretion. Depending on this, either: (1) a **male phenotype** with weak virilization and microphallus or (2) **hypogonadism** with delayed puberty or (3) a **46,XY variant of sex development** develops. Inactivating mutations of the LH receptor lead to an isolated disorder of gonadal testosterone production and are inherited in an autosomal recessive manner. LH and hCG cannot act on the Leydig cells, which negatively affect Leydig cell development, in the sense of hypoplasia. This results in reduced virilization at the 46, XY chromosome set. The phenotype ranges from almost male with micropenis to an external female phenotype. Wolff structures are usually rudimentary, Müllerian derivatives are absent due to normal AMH production in the Sertoli cells. The testes are located in the inguinal canal or scrotum.

One unique case of a patient with Leydig cell hypoplasia (LCH) type II caused by a genomic deletion resulting in the complete absence of exon 10 of the LH receptor (LHR) was published by Gromoll et al. Subsequently, hCG treatment resulted in an increase in testicular volume and the appearance of spermatozoa in the ejaculate after 16 weeks of treatment. The response to hCG indicates a possible dual mechanism of hormone binding and signal transduction for hCG and LH on a LHR that lacks exon 10 (Gromoll et al. 2000).

There is no adequate testosterone response in the **hCG test** (see Chap. 7). During puberty, the gonadotropins increase and show an increased response in the LHRH test. **Diagnosis is confirmed by a** biopsy, in which hypoplastic testicles can be detected, and by **sequencing the LH receptor gene**.

Clinical management should depend on the extent of virilization and age at diagnosis. Estrogen substitution will induce the development of female body shape and sexual characteristics. In partial forms with marked virilization, male sex assignment may also occur, which would involve therapy with testosterone and surgical corrective measures. The risk of gonadoblastoma is classified as very low.

31.7.3.2 Activating LH Receptor Mutations

Activating mutations of the LH receptors cause LH-independent activity of the Leydig cells. This activation leads clinically to an **isosexual peripheral precocious puberty**, which usually manifests before the age of 4. There is usually a familial accumulation. The result is a noncentrally controlled continuous production of testosterone, also known as **testotoxicosis.** If left untreated, it leads to very early pubertal development and **short stature.** Activating LH receptor mutations have also been described in Leydig cell tumors.

The **treatment goal** is to temporarily **inhibit testosterone biosynthesis or block androgen receptors** so that normal pubertal development can occur after discontinuation of therapy and fertility is not affected. There are several treatment options: treatment with **ketoconazole** or a combination of **spironolactone and testolactone**. Also, treatments with cyproterone acetate and anastrozole have been described.

31.7.3.3 Inactivating and Activating FSH Receptor Mutations

Inactivating mutations of the FSH receptor can lead to subfertility and infertility (Simoni et al. 1997) However, these are extremely rare and occur mainly in the Finnish population. Usually, the testicular volume is smaller and the fertility status variable.

Men with activating mutations of the FSH receptor are difficult to identify clinically because they do not show any particular phenotypic features. One case was discovered by showing intact spermatogenesis only with testosterone substitution despite hypophysectomy due to an adenoma (Simoni et al. 1997).

31.8 Disorders of Androgen Action

31.8.1 Definition and Etiology

Sex differentiation is the result of a cascade involving the interaction of regulatory genes, cellular and hormonal signals (see also Chap. 2). In the first phase of sex development, the developing gonads are initially omnipotent and both Wolffian and Müllerian ducts can be detected. In the typical male pathway, starting due to the effect of the SRY gene, the indifferent gonads differentiate into testes. The Sertoli cells start to produce the anti-Müllerian hormone, which causes regression of the Müllerian ducts which would otherwise develop into the uterus, tubes, and upper part of the vagina. The Leydig cells then begin to secrete testosterone, which stimulates the differentiation of the Wolffian ducts into ductus epididymis, vasa deferentia, seminal vesicles, and part of the prostate. Testosterone is then converted peripherally to dihydrotestosterone, which causes differentiation of the external genitalia

and stimulates the development of the prostate. Since androgens can only exert their effect when they bind to a functional receptor, **mutations of the androgen receptor gene cause** different degrees of **androgen insensitivity**. Androgen action is mediated by an intracellular steroid hormone receptor that functions as a transcription factor of androgen-regulated genes. Testosterone and DHT can bind to the androgen receptor via a ligand-binding domain. This is followed by translocation from the cytoplasm to the nucleus. Then a receptor dimerization occurs and subsequently binding to the promoter region of androgen-regulated target genes in conjunction with hitherto mostly unknown cofactors. This results in an increased or decreased transcription of the target genes, which unfold their biological effects after translation into their corresponding proteins.

The spectrum of androgen insensitivities ranges from a:

- Female phenotype with **complete androgen insensitivity (CAIS)** over
- ambiguous expression of the genitalia in **partial androgen insensitivity (PAIS)** up
- to a predominantly male phenotype with infertility in **minimal androgen insensitivity (MAIS).**

According to Quigley's classification, **seven different grades** are distinguished (Quigley et al. 1995). Grade 1 is characterized by a typical male appearance of the external genitalia and grades 6 and 7 by a typical female phenotype of the external genitalia, with grade 7 having a complete absence and grade 6 having sparse axillary and pubic hair in adolescence and adulthood. Grades 2–5 describe different degrees of virilization of the external genitalia.

31.8.2 Diagnosis

The diagnosis of androgen insensitivity is now typically confirmed by mutation detection in the androgen receptor gene in the context of a genetic test (Gottlieb et al. 2004; Hornig and Holterhus 2021). Nowadays, an AR-mutation-negative form of androgen insensitivity called AIS type II has been described, where the genetic origin is speculated to be in unknown cofactors of the androgen action cascade (Hornig et al. 2018).

These mutations can be divided into four different groups (Wieacker et al. 1998):

- Complete and larger partial deletions
- Deletions and insertions of a few nucleotides
- Point mutations that act as missense, nonsense or splice mutations
- Expansion of the CAG repeat in exon 1 in X-linked spinobulbar muscular atrophy

Almost all forms of inactivating mutations can be found in the androgen receptor, including point mutations, missense and nonsense mutations, splice-site mutations as well as deletions and insertions. They diminish the transcriptional regulation, DNA binding, and ligand binding. As a result, **the clinical picture is** also **very variable**. In about 1/3 of cases, new mutations are present in androgen resistance, and in 1/3 of cases the new mutations only occur postzygotically (Holterhus et al. 2001). Complete androgen resistance is almost always associated with a mutation in the androgen receptor, while only in 1/3 of cases a mutation is found in presumed partial androgen resistance. The majority of mutations are family-specific and only a few recurrent mutations have been described so far, so that complete gene sequencing is indicated for diagnosis.

Androgen insensitivity is inherited in an X-linked recessive manner. A carrier of an AR mutation passes on the mutated hereditary trait to half of the children with an XY karyotype. Androgen-binding studies, which have to be performed on genital fibroblasts after a skin biopsy, no longer play a significant role in confirming the diagnosis. The form of androgen resistance that was historically considered receptor-positive can now be explained by mutations in the DNA-binding domain, in which steroid binding is undisturbed, as expected. Additionally, androgen insensitivity can be identified by the APOD assay, which measures the expression pattern of an AR-regulated gene in genital skin fibroblast of an affected individual. Its combination with next-generation sequencing of the AR locus uncovered an AR mutation-negative, new class of androgen resistance, whose etiopathogenesis is currently unknown (Hornig et al. 2016).

31.8.3 Therapy

Therapy varies from individual to individual and depends on age and gonadal function. Usually, no therapy is necessary before puberty and until gonadectomy. Afterwards, hormone therapy with estrogens or testosterone can be indicated.

31.8.4 Complete Androgen Insensitivity Syndrome (CAIS)

The prevalence of **CAIS** is about 1:20,000 among individuals with an XY karyotype. Due to normal testis differentiation, the Sertoli cells produce AMH, so that **oviducts and uterus are absent,** resulting in a **blind-ending vagina.** The Leydig cells produce androgens with concentrations in the typical male range or even above. Due to the AR dysfunction, however, these androgens cannot act, so that Wolffian structures regress and a **maldescensus testis** results. In the

case of complete androgen resistance, no androgens can act prenatally either, so that an **external female phenotype** is present at birth. The testes may be located intra-abdominally, in the inguinal canal or in the labia majora. The probability of androgen insensitivity in phenotypically female children with inguinal hernia is 1–2% (Grumbach and Conte 1998). Surgical inguinal hernia repair not infrequently leads to recognition of the diagnosis during childhood.

During puberty, testosterone is synthesized by the Leydig cells. Clinically primary **amenorrhea, absent (grade 7) or sparse (grade 6) axillary and pubic hair ("hairless women")** with normal **female breast development are** noticeable during puberty. The breast development might be explained by the sufficient estrogen concentrations due to the aromatization of testosterone by aromatase and the lack of androgen effect. Body size is increased compared to female siblings, probably as a result of delayed closure of the epiphyseal fossae (Han et al. 2008). The lack of androgen action causes a regulatory increase in LH, which stimulates Leydig cells. The resulting hyperplasia of Leydig cells may become visible as Pick's adenomas. Individuals with CAIS show female play behavior and a large proportion of the women who are diagnosed with CAIS show a **definite female gender identity**.

In some cases, CAIS can be **diagnosed in the newborn period** if testicles are palpable or an inguinal hernia occurs. If the diagnosis is not made at that period, it is usually made in **young adulthood when** menarche does not occur. Prepubertal patients usually have normally low LH and testosterone levels. In postpubertal patients, testosterone levels are in the typical male range and estradiol levels are higher than in men but lower than in females. LH can be markedly elevated, while FSH may be only slightly elevated or within the normal range (Doehnert et al. 2015). Inhibin B levels are in the pubertal male range but there is no suppression of AMH levels, which remain in the prepubertal male range, demonstrating the role of testosterone binding to its receptor for the negative regulation of AMH production (Johannsen et al. 2020). In CAIS, **no uterus** is detectable **sonographically.** The diagnosis is confirmed by **DNA sequencing of the androgen receptor gene**. However, the same mutations can be associated with very different degrees of reduced androgen effect, so that it is often not possible to make a precise prognosis about the course of the condition. In CAIS, there is a higher risk for malignant germ cell tumors (Hughes et al. 2006; Looijenga et al. 2007; Looijenga et al. 2019).

Among the tumors that do not originate from germ cells, Sertoli cell adenomas are more frequent than Leydig cell adenomas. It is proposed that morphological and histological evaluations of gonadal tissue, in combination with OCT3/4 and TSPY double immunohistochemistry and clinical parameters, are most informative in estimating the risk for germ cell tumor development in the individual patient, and might in future be used to develop a decision tree for optimal management of patients with DSD (Looijenga et al. 2007; Looijenga et al. 2019). Prophylactic gonadectomy has been recommended in complete androgen insensitivity syndrome (CAIS) because of an increased risk for the development of malignant germ cell tumors in the intra-abdominal gonads for many years (Doehnert et al. 2017). Because the tumor risk before puberty is very low, the timing of gonadectomy has been postponed to allow spontaneous puberty and involvement of the patients in important decisions affecting their body and health. Even after puberty, gonadectomy is still discussed controversially. Currently, the absolute malignancy risk for individuals with CAIS cannot be determined and endogenous hormone profiles show very specific features that influence bone health, psychosocial well-being, and many other aspects. For women with CAIS who wish to keep their gonads, we propose a biannual screening program which has to be evaluated in a prospective multicenter trial (Doehnert et al. 2017).

In CAIS, no **therapy** needs to be carried out until the onset of puberty, unless there is an inguinal hernia. The risk of degeneration of the testicles is considered to be rather low, so that the testicles should be left in situ for as long as possible. During puberty, this can lead to spontaneous pubertal development. The testicles then produce testosterone, which is partially aromatized into estrogens. In most cases, hormone replacement therapy is not necessary as long as the testicles remain in situ. However, an **imaging of the gonads** should be done every 6–12 months (Doehnert et al. 2017).

After gonadectomy, **hormone replacement therapy with** estrogens or testosterone (Birnbaum et al. 2018) is necessary. Corrective genital surgery, such as **vaginoplasty,** should ideally be performed after puberty and should be based on the individual wishes and needs of those affected. Patients mostly identify with the **female gender role** but there are some individuals that identify with the male gender identity (T'Sjoen et al. 2011).

31.8.5 Partial Androgen Insensitivity Syndrome (PAIS)

The **phenotype in partial androgen resistance** covers a broad spectrum corresponding to grades 2 to 5 according to the Quigley classification. This ranges from predominantly female phenotypes with clitoral hypertrophy, **ambivalent genital findings** with partial labioscrotal fusion to an almost male phenotype with hypospadias and micropenis. During puberty, there is then also increased aromatization, resulting in female body shapes and often a distressing and pronounced **gynecomastia**. **Axillary and pubic hairs** are present to a variable extent.

Diagnostically, PAIS usually shows ambiguous genitalia at birth. The hormone parameters in PAIS are comparable to those in CAIS. The hormone status shows **increased LH and testosterone,** as the regulatory circuit no longer functions due to the receptor defect. In PAIS, a molecular genetic diagnosis is only possible in 1/3 of the cases. In PAIS, the testicular tumor risk is obviously dependent on the localization of the testes. According to a consensus paper (Hughes et al. 2006), a **malignancy risk of** about 50% is cited for PAIS with intra-abdominal gonads, although this risk figure is based on a small number of patients. Gonadectomy at the time of diagnosis is recommended. In PAIS with scrotal gonads, a lower risk is assumed, although reliable quantification does not appear possible at present. In PAIS, the **risk of breast cancer is** also increased. In three patients with PAIS and breast cancer, a missense mutation in the DNA-binding domain was detected (Lobaccaro et al. 1993; Wooster et al. 1992).

Treatment of PAIS should be interdisciplinary, especially for grades 3 and 4. It should be based on the needs of the patient and consider, among other things, the risk of degeneration of the gonads and the surgical options in adulthood.

In PAIS, the measures depend on the degree of clinical virilization and the gender in which the child will be raised. Depending on the residual function of the androgen receptor, the response of the genital tissue to androgens during puberty may vary greatly. If gynecomastia is pronounced, plastic surgery should be offered if necessary. If patients have a male psychosexual orientation, therapy aims to influence the phenotype in a male direction. Surgical measures will therefore aim to correct hypospadias, cryptorchidism, and gynecomastia. After orchidectomy, exogenous sex hormone substitution must be carried out. In individual cases, an attempt can be made to achieve a certain intensification of androgenization through high-dose testosterone therapy. The risk of degeneration of the gonads is significantly higher than in CAIS. Therefore, regular checkups are necessary and possibly a gonadectomy during adolescence or adulthood.

31.8.6 Minimal Androgen Insensitivity Syndrome (MAIS)

Minimal androgen insensitivity (MAIS) corresponds to grade 1 according to Quigley. Except for a possible **micropenis,** the external genitalia are inconspicuously male. **Gynecomastia** typically occurs at puberty. There is infertility due to **azoospermia or severe oligozoospermia**. MAIS also includes male infertility due to a mutation in the AR gene. However, only a few AR mutations have been found in infertile males so far. Also, elongation of the variable polymorphic CAG trinucleotide region in the N-terminal end of the AR gene has been associated with MAIS and male infertility (Zitzmann et al. 2005). Endocrinological indications of MAIS are normal or increased testosterone concentrations with increased LH.

From a therapeutic point of view, fertility could be restored by androgen therapy if necessary.

31.9 Persistent Müllerian Duct Syndrome

The syndrome of **persistent Müllerian ducts** results from a **lack of AMH secretion or also action** (via the AMH type 2 receptor). Affected 46,XY individuals have unremarkable virilized genitals with normally developed Wolff ducts (ductus deferens, epididymis) and normally developed testes, which are usually located in the abdomen. However, despite the male phenotype, the uterus and fallopian tubes are present (Imbeaud et al. 1996).

Diagnostically, AMH concentrations that cannot be measured are found in AMH gene defects and normal or even increased AMH concentrations in AMH receptor defects.

The testicles should be orchidopexied. Laparoscopically, the Müllerian derivatives can be surgically removed, however, as the ductus deferens is usually adherent to the uterus, the surgical approach to testicular descent and uterine removal needs to be performed by an experienced surgeon (Josso et al. 2005).

31.10 Vanishing Testis Syndrome

In the **Vanishing testis syndrome,** no gonads can be detected with a chromosome set of 46,XY. The external genitalia usually correspond to a male phenotype with anarchy. This means that testicles must have been developed prenatally at least until the 12th week of pregnancy (see also Chap. 17), and their dysfunction is secondary.

Hormone measurements reveal **elevated gonadotropins** with very low to undetectable AMH and inhibin B levels. **Hypergonadotropic hypogonadism** is latest seen at puberty. There is no increase in testosterone in the **hCG test** (see Chap. 7). Laparoscopies sometimes show so-called "nubbins," "granules," and blind-ending blood vessels and ductus deferens. These have no risk of degeneration but are usually removed in the course of testicular prostheses. Puberty induction and hormone replacement therapy with **testosterone** must be given **lifelong.**

31.11 Ovotesticular DSD

31.11.1 Definition and Etiology

An **ovotesticular feature of** sex development (Hughes et al. 2006) is present when **ovarian and testicular tissues are** present **simultaneously.** The gonads can consist of ovotestes on one or both sides with both tissue parts. However, ovaries may also be present on one side and testes on the other**.**

The exact prevalence of the disease is not known, but no more than a few hundred patients have been described so far. Approximately **2/3 of all patients have a 46,XX karyotype, 10% have a 46,XY karyotype, and the** remaining patients have a **chromosomal mosaic** with at least one Y cell line. In a proportion of patients with an XX karyotype, SRY is detectable, in the presence of which the primarily undetermined gonad differentiates into a testis (McElreavey and Fellous 1997). Overall, this is a very rare DSD diagnosis.

The genital phenotype is highly variable and can range from predominantly male to ambivalent genital findings to female external genitalia. At birth, 90% of patients with ovotesticular disorders of sex development present with ambiguous **genitalia.** The remaining 10% of cases have unequivocally female or male external genitalia. Due to the asymmetry of the gonads, asymmetrical findings for the labioscrotal folds are also found, and the position of the gonads is also variable.

Depending on the proportion of ovarian or testicular tissue and thus their local AMH and testosterone secretion, different findings are found for Wolff and Müllerian structures. The end of the vagina and a rudimentary uterus are often present. In up to 50% of those affected, inguinal hernias develop, which is sometimes the first reason for the diagnosis. Hormone production during puberty often leads to virilization and gynecomastia (caused by androgens and estrogens). In a study by Melardi et al., puberty was evaluated in 20 patients, being spontaneous in 12 of them. Four patients with partial gonadectomy in infancy were able to enter female puberty spontaneously. It was observed that patients who preserved gonadal tissues were able to enter puberty spontaneously more often (Kilberg et al. 2019; Melardi et al. 2020). About half of the phenotypically female patients have a menstrual cycle, and pregnancies have also been described in individual patients with a 46.XX or 46.XX/46.XY karyotype (Narita et al. 1975). In the ovotestes, normal spermatogenesis does not develop, whereas in the testes, spermatogenesis can be observed in every tenth patient.

31.11.2 Diagnosis

Gonadotropins may be normal or elevated. The extent of estrogen and progesterone concentration depends on a possible ovarian cycle. The functional detection of testicular tissue is achieved by the hCG test and of ovarian tissue by the hMG test. To assess testicular function, the determination of inhibin B and AMH is also useful. The diagnosis can ultimately only be made by biopsy of seminiferous tubules, follicles, and ovarian stroma in the gonads. Karyotyping should be performed in all cases. For differential diagnose, the different forms of 46,XY- and 46,XX-DSD can be considered.

31.11.3 Therapy

Gender assignment in ovotesticular disorders of sex development must consider a variety of aspects. For ethical and legal reasons, interventions and irreversible measures should be postponed as far as possible so that those affected can decide for themselves. The malignant degeneration tendency of the gonads in ovotesticular disorders of sex development is 10% in cases with a Y chromosome and 4% in patients without a Y chromosome, i.e., significantly lower than in gonadal dysgenesis. The decision to extirpate the gonads must therefore not only take the karyotype of the patient into consideration but also, in particular, the fertility status and hormonal status of the patient. If the gonads are left in place, close ultrasound monitoring of the gonads is recommended. If the gonads are removed, lifelong estrogen or androgen replacement therapy must be carried out according to the phenotype.

Key Points

- DSD includes both a biological definition of variants of sex development and a concept of care for affected people, some of whom describe themselves as intersexual.
- The classification follows a biological ordering principle, but does not specify a gender assignment.
- Individuals with a 45,X/46,XY karyotype can have a very variable clinical picture, from completely male to completely female.
- 21-Hydroxylase deficiency with 46,XX DSD and marked androgenization may present with male-appearing external genitalia and inconspicuous female internal genitalia with uterus and ovaries. Diagnosis is made by newborn screening. 46,XX males with congenital adrenal hyperplasia require lifelong hormone replacement therapy with hydrocortisone and fludrocortisone.

- Gonadal dysgenesis is the term used to describe the incomplete development of the gonads. A distinction is made between "mixed gonadal dysgenesis" with a 45,X/46,XY karyotype and "partial gonadal dysgenesis" with a 46,XY karyotype with androgenization (Andrade et al. 2019). The cause has been identified as mutations in genes responsible for early gonadal differentiation such as *WT-1* and *NR5A1*, and those such as *SRY*, *DMRT, SOX9,* and *DHH,* which are instrumental in testicular differentiation. The diagnosis is made by laparoscopy, gonadal biopsy, and endocrine and genetic analysis.
- In case of a 46,XY disorder of androgen biosynthesis, female or ambiguous external genitalia are formed due to the disturbance of androgen production. The cause is either defects in steroid biosynthesis, which can then also lead to adrenal insufficiency requiring treatment, or mutations in the gonadotropin receptors. In the course of the disease, a gonadectomy and hormone replacement therapy must usually be carried out.
- Mutations in the androgen receptor lead to a disturbance of the androgen effect. A distinction is made between complete androgen insensitivity (CAIS) and ambiguous genital manifestations in partial androgen insensitivity (PAIS) and a predominantly male phenotype with infertility in minimal androgen insensitivity (MAIS). The gonads should be examined regularly by ultrasound due to the risk of degeneration, but should be left in situ as long as possible to benefit from their own hormone production.
- An ovotesticular feature of sex development is present when ovarian and testicular tissue are present simultaneously. The clinical phenotype, hormone concentration, and gonadal function are highly variable, so that individual treatment concepts must be developed for each patient.

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Gynecomastia

32

Eberhard Nieschlag

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Abstract

Gynecomastia is a benign enlargement of mammary gland tissue in males and must be distinguished from pure lipomastia and carcinoma. By itself, gynecomastia is not a disease entity, but a symptom of a variety of causes leading to a dysbalance between androgens and estrogens. Gynecomastia can cause considerable psychological and psychosocial problems (body dysphoria, reduced self-esteem, etc.). Gynecomastia of the newborn, at puberty and in old age, are considered almost "physiological." It is important to recognize whether gynecomastia is caused by an estrogen or hCG-producing testicular tumor requiring prompt treatment. Common causes of gynecomastia are primary (e.g., Klinefelter syndrome) and secondary hypogonadism. Numerous medications, drugs, and products of daily use (cosmetics and food) as well as occupational exposure can cause gynecomastia. Treatment consists first of all in the removal of causative agents. In addition, there is no evidence-based pharmacological therapy, only treatment trials with drugs not approved for this indication. Empathic guidance of the patient is the task of the treating physician. Ultimately, only gynecomastectomy remains, which should be performed by a plastic surgeon experienced in senology.

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32.1 Definition of a Multicausal Symptom

Gynecomastia is a benign enlargement of the mammary gland tissue in the male sex. The causes are manifold, so that gynecomastia can be considered more as a symptom than as an independent clinical picture. Gynecomastia can occur at any age from newborn to senile with an accumulation at puberty. The various causes of gynecomastia are discussed in this chapter.

Gynecomastia must be distinguished from **lipomastia (pseudogynecomastia)**, which is a pure accumulation of fat in the breast area and has no further significance as a symptom of obesity. In addition, **accessory mammilla (hyperthelia)** can occur along the embryonic milk duct and may include scattered breast tissue, which is rarely affected by gynecomastia (**hypermastia**).

32.2 Prevalence of Gynecomastia

Data on the frequency of gynecomastia vary considerably. This is partly due to the fact that diagnosis remains subjective and standardization is lacking. For example, data on gynecomastia in newborns fluctuate between 65 and 90%, which can thus be described as "physiological." Pubertal gynecomastia can also affect 50% of pubescent boys, but in 90% of those affected it regresses spontaneously within 2 years. For adult males, the prevalence rates indicated diverge between 36 and 57%, with a marked increase with age (Kanakis et al. 2019).

Men have a lifelong risk of **breast cancer** of 0.1% (Yu et al. 2015), for which gynecomastia can only exceptionally be considered a predisposing factor (e.g., Klinefelter syndrome, radiation of the thorax, family history of breast cancer).

32.3 Pathophysiology

In a Danish analysis of 786 adult men with gynecomastia, no clear cause could be identified in 57% (Mieritz et al. 2017). These cases are often referred to as "idiopathic" (Fig. 32.1). The high percentage of unexplained cases illustrates the incomplete knowledge about the pathophysiology of this common disorder.

It is generally assumed that the cause of gynecomastia is a dysbalance of androgens and estrogens. This can be caused by:

- Reduced androgen and increased estrogen production,
- Decreased testosterone activity in the target organs,
- Exogenous intake of estrogens, drugs, food, or cosmetics, and
- Multifactorial causes.



Fig. 32.1 "Idiopathic" gynecomastia. Apart from obesity, no other causal factors could be identified. In addition to the mammary gland tissue, fat pads were palpable

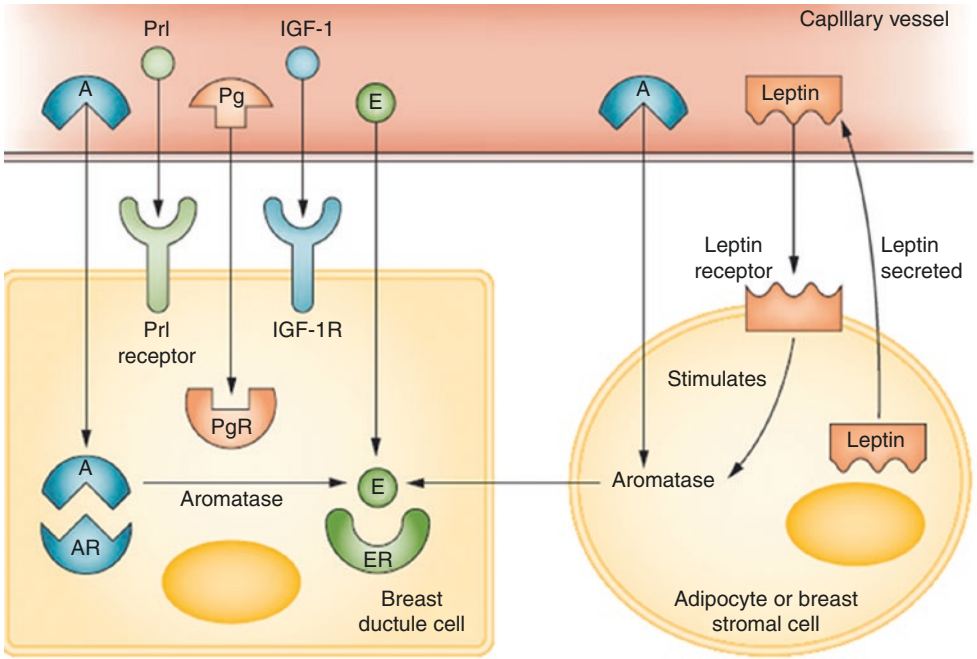


Fig. 32.2 Influence of different hormones on breast and fatty tissue in the development of gynecomastia. A, androgen; AR, androgen receptor; E, estrogen; ER, estrogen receptor; IGF-1, insulin-like growth factor 1; IGF-1R, IGF1 receptor; LR, leptin receptor; Pg, progesterone; PgR, progesterone receptor; Prl, prolactin (Narula and Carlson 2014)

Breast tissue has both estrogen and androgen receptors; while estrogen receptors α and β mediate growth of the breast tissue, androgen receptors have an inhibitory effect. Decreased androgen effect may be the result of primary, secondary, or functional hypogonadism and androgen receptor insensitivity. In general, adipose tissue, and particularly the adipose tissue of the mamma, is rich in **aromatase**, which converts androgens into estrogens. Aromatase is stimulated by other hormones such as leptin, prolactin, and IGF1 (insulin-like growth factor-1), whose receptors are found in breast tissue (Narula and Carlson 2014) (Fig. 32.2). In boys with pubertal gynecomastia not only elevated levels of free testosterone and estradiol but also of IGF1 were found; these boys underwent the growth spurt earlier than controls (Mieritz et al. 2015). Furthermore, the concentration of sex hormone binding globulin (SHBG) has an influence on the balance between androgens and estrogens, so that SHBG must also be taken into account in diagnosis.

32.4 Psychosocial Aspects

The development of gynecomastia is a disturbing event for every affected boy or his parents. Especially in adolescents, who have to deal with the transition from boy to man and thus with a new understanding of their role, **pubertal gynecomastia** can cause a number of problems.

First of all, there are concerns about possible pathological aspects of breast formation and doubts about masculinity. The affected boy experiences impairment of his self-esteem, is ashamed to expose his body publicly, e.g., during sports and bathing, and fears being teased and bullied. Then the boy withdraws and isolates himself. Especially in obese boys, the thought that gynecomastia may have alimentary causes can lead to bulimia and anorexia. In addition, there are concerns by parents and relatives, who also doubt that the phenomenon is harmless (Rew et al. 2015).

Table 32.1 Psychosocial and psychological aspects of gynecomastia

- Anxiety and fear
- Self-esteem as a man
- Body dysphoria
- Sense of shame
- Social integration (attire, sports, swimming)
- Bullying because of phenotype
- Emotional stress
- Depressive moods
- Libido loss
- Erectile dysfunction
- Bulimia >>> anorexia
- (Concern by parents and relatives)

To a lesser extent, these problems also concern the **adult male** affected by gynecomastia, who may also be affected by psychosocial and psychological problems and thus experience a reduced quality of life. These problems include depression, anxiety, reduced self-esteem, and eating disorders (Ordaz and Thompson 2015). In addition, **loss of libido** and **erectile dysfunction** can occur (Nieschlag 2018) (Table 32.1).

Therefore, after careful diagnosis and exclusion of a serious cause, the first priority of therapeutic measures is to clarify findings and to provide understanding medical guidance, which does not consist in mere trivialization.

32.5 Diagnostics

32.5.1 Clinical Examination

A gynecomastia can be more pronounced on one side, symmetrical on both sides, or unequal on both sides. A tumor must be excluded.

First, the presence of **real gynecomastia** or **pseudogynecomastia** (in the sense of lipomastia) must be clarified. Through careful palpation, a distinction can usually be made between breast gland tissue and diffuse fatty tissue. Lipomasty is mainly expected in obese men. If the palpation findings are uncertain, an ultrasound examination can provide clarity.

The extent of gynecomastia can be determined semi-quantitatively using the Tanner stages (see Chap. 5) (Fig. 5.3 as well as Fig. 32.3). Gynecomastia is present if a skin fold formed by light pressure with thumb and index finger above the nipple is more than 2 cm in diameter and/or if the areola is more than 3 cm in diameter (Braunstein 2007; Schanz et al. 2017).

The extent of virilization should also be determined. Secondary hair and testicular size should be recorded, as well as any indications of a suspected tumor in the testis. Sonography of the testes for exclusion of a **testicular tumor** is an essential part of every clarification of a gynecomastia. The patient should also be asked about symptoms indicating an androgen deficiency, such as a decrease in libido, potency problems, and changes in beard growth. Finally, indications of systemic diseases such as liver or kidney disease should be sought. A detailed **medication anamnesis** is required. Information on use of lifestyle substances, suspicious diets (e.g., soy products, essential oils), and cosmetics (e.g., lavender products, hair lotion) must be requested.

The S1-Guideline on Gynecomastia (Schanz et al. 2017) recommends a **step-by-step diagnostic procedure** (basic or advanced diagnosis) depending on whether the case is typical or atypical [Table 32.2]). Typical cases have a longer anamnesis and rather benign findings, while atypical cases have a short anamnesis, suspected hypogonadism, testicular tumor or carcinoma, and only a unilateral finding.

Table 32.2 Step-by-step diagnostics gynecomastia

Adolescence	Adult
• Clinical examination	• Clinical examination
• Palpation mammae and testicles	• Palpation mammae and testicles
• T, E2, LH, hCG	• T. E2, LH, hCG, PRL?
• Testicular sonography	• Testicular sonography
• Follow-up	• Follow up
Extended diagnostics	
• Clinical examination	
• Palpation of mammae and testicles	
• Testicular sonography	
• Mammary sonography	
• T, E2, LH, hCG, SHBG, PRL, FSH, TSH, fT4, DHEA, AFP, liver and kidney values, chromosome analysis, karyotype	
• Further imaging and laboratory diagnostics, X-ray thorax, MRT skull, CT abdomen	

According to Schanz et al. (2017)

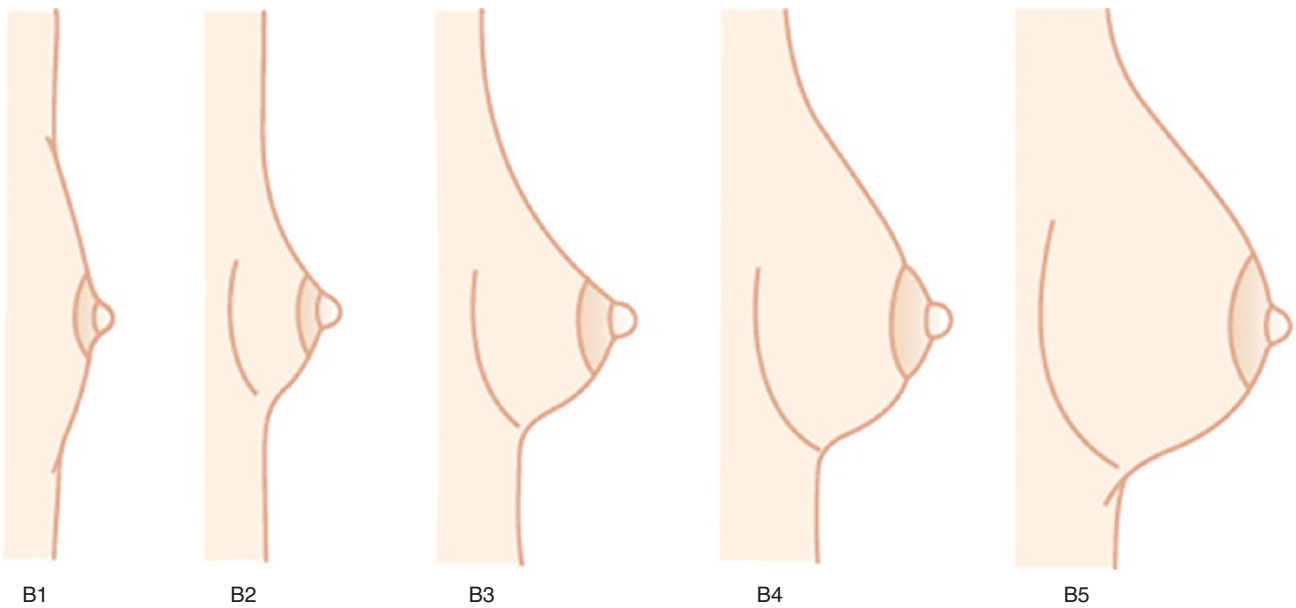


Fig. 32.3 Developmental stages of gynecomastia

32.5.2 Laboratory Diagnostics

Laboratory diagnostics should be adapted to the clinical situation. In case of non-progressive gynecomastia at puberty, extensive hormone diagnostics are not necessary. In most cases, determination of **LH**, **FSH**, **prolactin**, **testosterone**, and **estradiol** is sufficient. In addition to sonography of the testes, determination of **hCG** is part of the clarification of gynecomastia of the adult male, in pubertal boys it is only required if there is a clear suspicion of a testicular tumor. In ambiguous cases, extended diagnostics with additional determination of **SHBG**, **FSH**, **TSH**, **fT4**, **DHEA**, **prolactin**, **AFP** as well as kidney and liver function tests are useful. Depending on the case, genetic diagnostics with karyotyping and specific genetic tests may be indicated.

32.5.3 Imaging Diagnosis

To detect testicular tumors, the clinical examination must be supplemented by **sonography of the testes**. This allows testicular tumors to be detected at an early stage before they become palpable due to hardening of the testis or before being directly palpable. Sonography of the mammary gland serves to determine size. In the case of very large gynecomastia and suspicious palpation findings, a **mammography** should be arranged in order to rule out breast carcinoma. An **X-ray, computed tomography (CT),** or an **MRI** of the thorax should also be performed if a tumor is suspected (see Chap. 6).

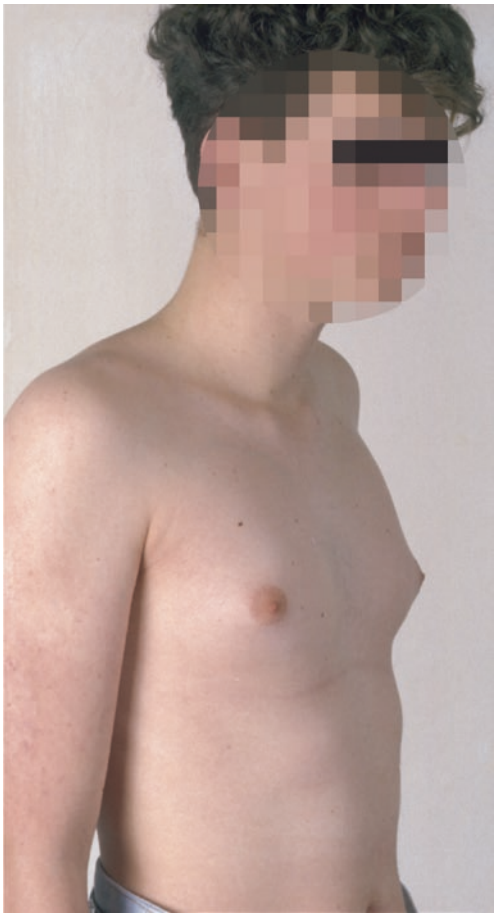


Fig. 32.4 Pubertal gynecomastia in a 16-year-old boy

32.6 Clinical Pictures

32.6.1 "Physiological" Gynecomastia

In differential diagnosis, "physiological" gynecomastia must be distinguished from pathological gynecomastia. Physiologically, transitory gynecomastia can occur in newborns (**"witch's breast"**), occasionally with low-milk secretion (**"witch's milk"**). This develops under the influence of maternal estrogens during pregnancy and usually recedes spontaneously within a few days to weeks after birth.

Pubertal gynecomastia (Fig. 32.4) usually requires no therapy. In about 40% of pubescent boys, breast tissue can be detected, which in itself is not a pathological finding. In about 4% of the boys, a gynecomastia with tissue ≥1 cm in diameter occurs (Kumanov et al. 2007). Starting from this size, cosmetic and psychological problems may occur that require intervention. In order not to overlook any endocrine or other systemic disease, basic diagnostics should be carried out if the findings are of a certain severity. Since pubertal gynecomastia develops after the onset of puberty, the degree of virilization should be assessed. If no signs of puberty are visible, the presence of an endocrine cause, e.g., a hormone-producing tumor, must be considered. Coincidentally, pubertal gynecomastia occurs at the same time as varicocele (Kumanov et al. 2007) without the connection being clear. In most cases, pubertal gynecomastia resolves spontaneously. In less than 5% of cases, gynecomastia persists.

Persistent pubertal gynecomastia is one of the most common causes of **gynecomastia in adult** men.

Men of advanced age often display gynecomastia, usually of a milder form (**age-related gynecomastia**). This can be explained by a shift in the ratio of androgens to estrogens, due to a decrease in androgen production or an increase in estrogens, since aromatase activity increases with age and the amount of fatty tissue. This mechanism is reinforced by the fact that estradiol and estrone are less strongly bound to SHBG than testosterone, thus increasing the bioavailability of estrogens. However, side effects of drugs and systemic diseases may also be possible.

32.6.2 Gynecomastia due to Reduced Androgen Production

Gynecomastia can occur in almost all diseases associated with reduced androgen production. It is a typical but not obligatory symptom of **Klinefelter's syndrome**, which is observed in 38% of patients (see Chap. 21). Gynecomastia also occurs in the **XX-Male** syndrome. In contrast, pronounced gynecomastia is rather unusual in Kallmann syndrome or other forms of congenital hypogonadotropic hypogonadism (CHH). Hyperprolactinemia caused by a micro- or macro-**prolactinoma** can lead to secondary hypogonadism and thus cause gynecomastia, the development of which is promoted by the prolactin receptors in breast tissue. This can also lead to milk secretion. Since **hypothyroidism** is associated with hyperprolactinemia, gynecomastia may also occur in this clinical picture (Sansone et al. 2017).

Enzyme defects in steroid biosynthesis associated with decreased androgen production are typically associated with gynecomastia. This is, for example, the case with 17ß-hydroxysteroid dehydrogenase deficiency, 3ß-dehydrogenase deficiency, or 17,20-lyase deficiency.

Systemic diseases such as **myotonic dystrophy** or **hemochromatosis**, in which testicular androgen production is increasingly disturbed during the course of the disease, may be associated with gynecomastia.

32.6.3 Gynecomastia due to Androgen Insensitivity

In the various forms of androgen insensitivity, gynecomastia can be explained by the lack of androgen influence (despite normal androgen production) and by the predominant influence of estrogens (see Chap. 31).

In 46,XY disorders of sexual differentiation (46,XY DSD) with complete or partial androgen insensitivity (CAIS, PAIS), inactivating mutations of the androgen receptors exist in the target organs; as a result, gynecomastia develops, which can reach female dimensions in CAIS (Fig. 32.5). The karyotype of these patients is male, but the testosterone produced in the testes cannot act due to the receptor defect. However, the estrogens produced from the testosterone by aromatization can act, resulting in an overall female phenotype (hence the former name: testicular feminization) (see Chap. 31).

X-linked recessive **spinobulbar muscular atrophy** or **Kennedy's disease** is a late manifesting motor neuron disease affecting men in whom the transactivation ability of target genes for androgens is impaired due to the expansion of the CAG repeat in the first exon of the androgen receptor gene. The typical age of manifestation is the third or fourth decade of life. Characteristic symptoms are muscle weakness (especially in the shoulder girdle and legs), muscle cramps, dysarthria, and dysphagia. There are also signs of androgen resistance such as gynecomastia and azoospermia or oligozoospermia.



Fig. 32.5 Gynecomastia in a patient with complete androgen insensitivity (CAIS) (46,XY DSD). Because of the completely female phenotype, CAIS was formerly called as "testicular feminization"

32.6.4 Gynecomastia due to Increased Estrogen Production

A predominance of estrogens can be caused by increased aromatase activity and by increased gonadal or adrenal estrogen production.

If swelling of the mammary glands occurs after puberty, a **testicular tumor** must be considered first and then be excluded or confirmed. The symptoms triad gynecomastia, libido loss, and testicular tumor are characteristics for a Leydig cell tumor and the rare **Sertoli cell tumor** (Fig. 32.6). Also **hCG-secreting tumors** typically lead to mammary gland swelling; such as embryonic carcinoma, teratocarcinoma, chorionic carcinoma, or gonadal mixed tumors. Therefore, sonographic **imaging of the testicles is an obligatory part of diagnosis in adults.** An **adrenal carcinoma** must also be considered, especially in cases of sudden and initially unilateral occurrence of gynecomastia.

In addition to hCG secretion, **paraneoplastic estrogen production** by the Leydig cells of the testicular tumor may be the cause. Also other carcinoma diseases with paraneoplastic hormone production (e.g., **small-cell lung carcinoma** or an **adrenal cortex carcinoma**) can become symptomatic due to the sudden development of a gynecomastia (Braunstein 2007).

Since the binding affinity of estradiol and estrone to SHBG is lower than that of testosterone, an increase in SHBG tends to cause a decrease in free testosterone and an increase in free estrogens. **Hyperthyroidism** is associated



Fig. 32.6 Three patients with rapidly developing gynecomastia, libido loss, and Leydig cell tumors at the ages of 22, 29, and 35 years, respectively. After surgical removal of the tumors, the gynecomastia regressed completely

with increased SHBG production, which causes a decrease in free testosterone and may explain the occurrence of gynecomastia in this disease (Sansone et al. 2017).

Chronic systemic diseases can lead to the development of gynecomastia. This is particularly common due to increased estrogen secretion in liver cirrhosis. Furthermore, a mammary gland hypertrophy can be observed in patients with **end-stage renal failure** under hemodialysis treatment. **Hunger dystrophies** in the recovery phase may also cause swelling of the mammary glands.

The **aromatase gene (CYP19A1)** in 15q21.2 is under the control of different promoters that are activated by specific hormones. So far, only a few families have been detected with autosomal dominant inheritance of gynecomastia and premature puberty, including different rearrangements such as inversions in the region of the CYP19A1 gene, which are associated with increased aromatase activity (Shozu et al. 2003).

32.6.5 Gynecomastia Caused by Drugs

A large number of drugs can cause gynecomastia via a wide variety of mechanisms of action (Deepinder and Braunstein 2012; Kanakis et al. 2019) (Table 32.3).

Direct **estrogen intake** or accidental transdermal or inhalation exposure may lead to mammary gland hypertrophy in men. Figure 32.7 shows a pharmacist active in the production of estrogen preparations who had developed gynecomastia within a short time. Even under high-dose of **testosterone substitution**, gynecomastia can develop due to the aromatization of testosterone to estrogens in fatty tissue. The same applies to intake of extremely high doses of **anabolic androgenic steroids (AAS)** by bodybuilders or athletes. Even under therapy with hCG (as part of the treatment of secondary or tertiary hypogonadism), gynecomastia can develop due to overstimulation of the Leydig cells and aromatization of testosterone.

Spironolactone displaces estrogens, more than androgens, from SHBG, causing a shift in the quotient of free testosterone to free estrogens in favor of the estrogens, and thus may lead to gynecomastia in addition to the antagonistic effect at the androgen receptor.

Table 32.3 Gynecomastia-causing drugs

Hormones	Chemotherapeutics
Estrogenic	Methotrexate
Testosterone	Imatinib
hCG	**Cardiovascular drugs**
Anabolic steroids	ACE inhibitors
GnRH agonists	Calcium channel blockers
Growth hormone	Amiodarone
Antiandrogens	Digitalis
Flutamide	Verapramil
Finasteride	Nifedipine
Bicalmutide	Methyldopa
Ketocanazole	Reserpine
Spironolactone	**Drugs**
Psychotropic drugs	Methadone
Diazepam	Heroin
Phenotiazine	Marijuana
Haloperidol	Amphetamine
Antacids	**Other medicines**
Cimetidine	Metoclopramide
Rantidine	Penicillamine
Omeprazole	
Antibiotics	
Isomiazide	
Metronidazole	

Drugs such as **ketoconazole** can cause gynecomastia by inhibiting testosterone biosynthesis. The same applies to damage to the Leydig cells by cytostatic drugs. Digitalis, marijuana, and heroin often cause gynecomastia by direct stimulation of the estrogen receptors. Cimetidine (an H2-receptor antagonist) develops an anti-androgenic effect and is therefore a possible cause of gynecomastia. 70% of patients undergoing androgen deprivation therapy with **antiandrogens** (cyproterone acetate, flutamide, finasteride, bicalutamide, etc.) or GnRH agonists because of prostate cancer develop gynecomastia (Fagerlund et al. 2015) (Fig. 32.8).

By increasing prolactin and disrupting gonadotropin secretion, drugs such as **metoclopramide**, **methyldopa**, **reserpine**, **isoniacid**, **phenotiazine**, **tricyclic antidepreesiva**, **amphetamines**, and **antiepileptic drugs** (e.g., carbamazine; Fig. 32.9) lead to gynecomastia. The mechanism of action is unknown in calcium antagonists, phenytoin, amiodarone, metronidazole, ACE inhibitors, penicillamine, and diazepam.

32.6.6 Gynecomastia Due to Food and Cosmetics

Even **everyday products** can cause gynecomastia. **Cosmetics** (creams, hair lotions, perfumes, etc.) containing estrogen-like extracts of the so-called **essential oils** or other natural products can cause gynecomastia. These are mainly cosmetics containing estrogen-like substances such as **lav-**



Fig. 32.7 A 37-year-old patient with gynecomastia, libido loss, erectile dysfunction, and oligozoospermia who was a pharmacologist working in the production of oral contraceptives. Changing the workplace was followed by complete regression of gynecomastia



Fig. 32.8 Gynecomastia in a 13-year-old boy taking carbamazepine

ender, **tea tree oil**, **eucalyptus,** or **pine oil** which can cause gynecomastia (Henley et al. 2007; Patel 2017; Ramsey et al. 2019). A thanatopractor (embalmer) who used a phytoestrogen-containing balm to prepare corpses without using gloves had not only developed stage B5 gynecomastia but also secondary hypogonadism with loss of libido, erectile dysfunction, and reduced beard growth (Finkelstein et al. 1988).

Spices such as rosemary and juniper also contain estrogen-like substances. Finally, the excessive consumption of **phytoestrogens** (flavonoids) as contained in soy products should also be mentioned here (formulas in Fig. 32.10). For example, regular consumption of large quantities of soy milk can lead to gynecomastia; these patients are referred to in the USA as "soy boys," a term that is now generally used for men who appear to be feminine.



Fig. 32.9 Pronounced gynecomastia in a 68-year-old patient who underwent androgen deprivation therapy with finasteride because of prostate cancer

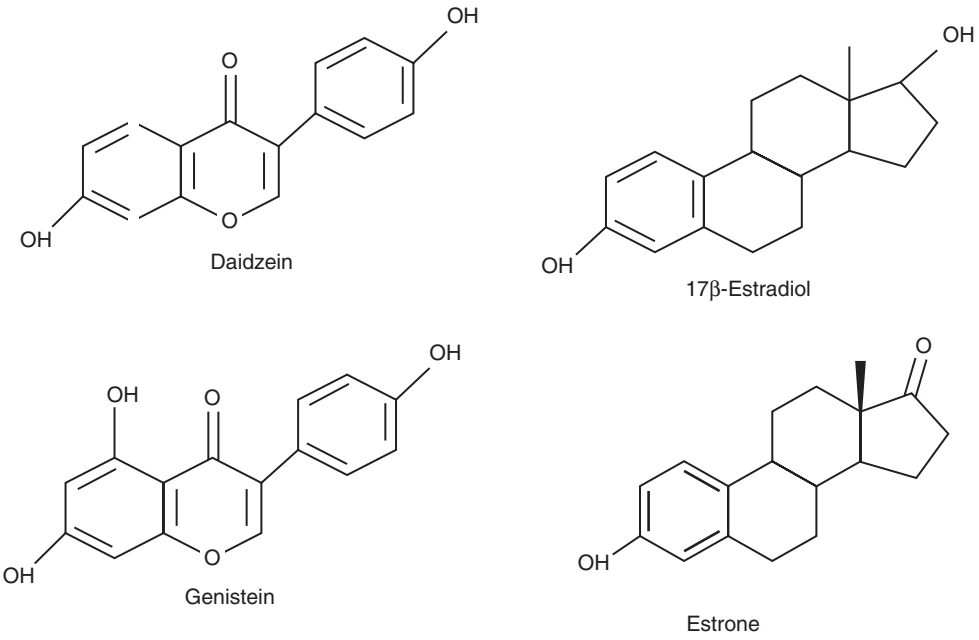


Fig. 32.10 Phytoestrogens (isoflavones), genistein and daidzein, structurally similar to 17 β-estradiol and istron, which are, e.g., present in soybean products

32.7 Male Breast Cancer

The clinically most significant differential diagnosis of gynecomastia is male breast cancer with an incidence of 0.5–1/100,000 per year (Fentiman et al. 2006; Ruddy and Winer 2013). The incidence increases with age (Abdelwahab Yousef 2017). In contrast to breast cancer in women, almost all breast cancers in men are receptor-positive; this is especially true for the androgen receptor (Gucalp et al. 2019).

Above all, a one-sided finding is suspected of being tumor-positive. A breast cancer is usually hard and located outside the areola. Bleeding from the nipple occurs in about 10% of breast cancer cases. In unclear cases, advanced diagnostics including mammography is indicated (Table 32.4).

Breast cancer can occur in the context of partial androgen insensitivity (PAI). In Klinefelter's syndrome, the malignant degeneration of gynecomastia is slightly increased. In the case of BRCA2 mutations, the risk of breast cancer in the male sex is about 7%, so that in the case of male breast cancer—especially if female breast cancer cases occur in the family history—mutation analysis of the BRCA2 gene is indicated. In the case of BRCA1 mutation carriers, an increased, but currently not exactly quantifiable risk can also be assumed. Particular attention must be paid to breast carcinomas developing under use of drugs more frequently leading to gynecomastia. The database of the Drug Commission of the German Medical Board (2008), for example, reports 32 cases of gynecomastia and 6 cases of breast cancer under finasteride use.

Table 32.4 Clinical differential diagnosis

	Gynecomastia	Breast cancer
Symmetry	Mostly double-sided	Mostly one-sided
Consistency	Mostly soft, elastic to firm	Mostly firm or hard
Location	Mostly concentric to the areola	Mostly decentralized to the areola
Symptoms	Painful	Painless, may bleed, discharge, retraction of skin or nipple

32.8 Therapy

In the treatment of gynecomastia, various factors must be taken into account: the underlying cause, the degree of gynecomastia, the pressure felt by the patient, and the presumed course without treatment. In cases of discrete manifestation, therapy is often not necessary, especially if there is no distress or if spontaneous remission is expected. This applies, for example, to many cases of pubertal gynecomastia. However, subjective suffering can be great, even if, from a purely medical point of view, there is no need for action. If the male phenotype is perceived as disturbed, this can lead to impaired

self-esteem and isolation, e.g., because certain activities such as sports and swimming or sexual contacts are avoided.

Therapeutic Principle

A warning should be given against overhasty inducement to undergo a gynecomasectomy. The gynecomastia must have been fully diagnosed and its spontaneous development best observed for some time. This ensures that surgical therapy does not prematurely remove an important indicator symptom of an underlying disease.

If the cause of gynecomastia is known, therapy should be directed to alleviation of those possible causes. Thus, treatment of hyperestrogenism or androgen deficiency can cause regression of gynecomastia. However, this therapeutic approach is not always successful, especially after a certain degree of severity and after a longer period of existence (more than 1 year), fibrosis may develop and not regress. Various hormone preparations and endocrine active drugs (e.g., testosterone, dihydrotestosterone, danazol, clomiphene, tamoxifen) have been tested in patients without proven hormonal disorders (Braunstein 2007).

There is no **evidence-based therapy**, but there are various **empirical approaches** with **anti-estrogens**, **selective estrogen receptor modulators (SERMs)**, or **aromatase inhibitors**. In dealing with these empirical therapies, different guidelines take different positions. While the most recent reference work of the German Society of Endocrinology (Diedrich et al. 2020) presents treatment with **tamoxifen** as the appropriate standard therapy, the S1 Guideline (Schanz et al. 2017) does not specify this, and the EAA guideline (Kanakis et al. 2019) generally advises against treatment with SERMs, aromatase inhibitors, and non-aromatizable androgens (Table 32.5). Evaluation of the present studies is complicated by the fact that the case and control groups (if the latter were included at all) are small and the results are difficult to classify because of the frequent spontaneous remissions.

Nevertheless, **tamoxifen** is most frequently used off-label (Lapid et al. 2013). A trial with 10–20 mg/day should be discontinued after 3 months, depending on the extent of the gynecomastia, the expected spontaneous course and the patient's level of suffering if no significant improvement has occurred. Tamoxifen may have adverse gastrointestinal and cardiovascular side effects (Wibowo et al. 2016).

Table 32.5 Pharmacological trials for the treatment of gynecomastia

Tamoxifen, off-label
Estrogen receptor inhibitor
No controlled studies
(Lapid et al. 2013)
10–20 mg/day
Trial over 3 months
Clomiphene, off-label
Estrogen receptor inhibitor
No controlled studies
Anastrozole, off-label
Aromatase inhibitor
A controlled study
(Plourde et al. 2004)
Testolactone, off-label
Aromatase inhibitor
No controlled study
Dihydrotestosterone cream, off-label
No controlled study

Correction of excess estrogen or testosterone deficiency only leads to a decrease in mammary protrusion in cases of slight mammary gland swelling without accompanying lipomastia. In particular, fibrous remodeling takes place in gynecomastia that has already persisted for a long time and prevents reversibility of the mammary gland swelling. In the early stages, however, drug therapy can lead to a reduction of swelling; pressure pain of the gynecomastia also usually improves. Tamoxifen therapy is not licensed for men.

In the event of therapeutic failure or in pronounced cases with a high degree of suffering, surgical measures can be considered at the end of puberty. This should be carried out by an experienced plastic surgeon, as otherwise the result is often cosmetically worse than the initial condition. If the plastic **mastectomy** is successful, i.e., preserving the nipple and with only discrete scars remaining as residuals, success may also be measured in psychosocial terms. As already mentioned, education and medical guidance (depending on age and also including parents or relatives) are particularly important elements of all therapeutic measures.

In a retrospective study of patients with idiopathic gynecomastia treated with the anti-estrogen tamoxifen (20 mg daily for 3 months), complete remission was observed in 78% of cases. In patients treated with danazol, however, the remission rate was 40%. If after 3 months there is no success, **gynecomasectomy** may be considered under the same restrictions mentioned above. It goes without saying that prior to such therapy side effects or a hor-

mone-producing tumor (e.g., Leydig cell tumor) must be excluded.

For the therapy of male breast carcinoma, identical oncological measures apply as for women (Zehr 2019).

Key Points

- Gynecomastia is a benign enlargement of mammary gland tissue in males and must be distinguished from pure lipomastia and carcinoma.
- Gynecomastia is not a clinical picture in itself, but a symptom of a variety of causes that lead to a dysbalance between androgens and estrogens.
- Gynecomastia can cause considerable psychological and psychosocial problems (body dysphoria, reduced self-esteem, etc.).
- Gynecomastia of the newborn, at puberty, and in old age is considered almost "physiological."
- It is important to recognize whether gynecomastia is caused by an estrogen or hCG-producing testicular tumor requiring prompt treatment.
- Common causes of gynecomastia are primary (e.g., Klinefelter syndrome) and secondary hypogonadism.
- Numerous medications, drugs, and products of daily use (cosmetics and food) as well as occupational exposure can cause gynecomastia.
- Treatment consists first of all in the removal of causative agents.
- In addition, there is no evidence-based pharmacological therapy, only treatment trials with drugs not approved for this indication.
- Empathic guidance of the patient is the task of the treating physician.
- Ultimately, gynecomastectomy remains which should be performed by a plastic surgeon experienced in senology.

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Male Androgenetic Alopecia

33

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Abstract

A first receding of the forehead-hairline border is eyed suspiciously by the majority of affected persons. Androgenetic alopecia is still interpreted as a sign of aging and those afflicted are often burdened by psychosocial stigmatization. Following anamnesis directed towards differentiated diagnosis, the treating physician must present therapeutic options with their respective pros and cons; these must be coordinated with the patient's wishes and goals. New pathophysiological approaches and a better understanding of hair cycles, genetics, sex hormones, and signal cascades form the basis for possible therapeutic developments.

33.1 Epidemiology

Androgenetic alopecia (AGA) is the most common form of alopecia. Approximately every second male of Caucasian origin is affected by alopecia during his lifetime. The condition is age-related. After the age of 30, the proportion increases by 10% in each of 10 annual decades, i.e., approximately 70% of 70-year-olds are affected by AGA, albeit to varying degrees (Rinaldi et al. 2016; Kelly et al. 2016). Prevalence is much lower in populations of other ethnicities, such as Asians and Africans.

The biological signaling functions of beauty, youthfulness, and vitality have gained importance in a society which is increasingly aesthetically oriented. With regard to AGA, **quality of life** may be limited, such as loss of self-esteem, depression, including introversion to neuroticism (Gupta et al. 2019).

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33.2 Pathophysiology

Normally, humans have between **80.000 and 120.000** scalp hairs. Hair is subject to multifactorial and polygenetic influences in its structure (shape and diameter) and growth, in addition to ethnic influences and body location.

An **active growth phase (anagen phase**) is followed by a short **regression phase** (**catagen phase**) and then a **resting phase (telogen phase)** before the hair falls out. The steps of the **hair cycle** are controlled by epithelium and mesenchyme. A hair follicle develops from mesenchymal condensation, followed by invagination of epithelium into the dermis. There, regrowth occurs around the dermal papilla, which is composed of connective tissue, blood vessels, and nerve fibers. In the piston-shaped hair bulb, new cells are constantly produced above the dermal papilla and pushed upwards. Catagenic regression is characterized by decreased cell production, throttling of nutrient supply, apoptosis, proteolysis, and remodeling of the extracellular matrix.

Daily hair loss is approximately 100 hairs (80+/−20). Normally, the anagen phase lasts about 3 years (2–6 years). In the catagen phase, the hair follicle regresses over 2–3 weeks, followed by the telogen phase of 100 days (1–3 months). The time ratio of anagen to telogen phase is about 9:1. About 90% of hair is normally in the anagen phase, 2–3% in the catagen phase, and up to 18% in the telogen phase.

In AGA, the average duration of the anagen phase shortens progressively. A premature catagen onset occurs. The control and supply center of the hair, consisting of the dermal **papilla and hair bulb**, commonly referred to as the **hair follicle**, slowly becomes smaller. The hair becomes thinner and shorter. In affected areas of the head, pigmented terminal hairs become unpigmented vellus hairs. The kenogenic interval (between telogen and renewed anagen phase) is extended.

The hair cycle represents an independently regulated epidermal-dermal regeneration and is not subject to immunological control. Nevertheless, inflammatory mechanisms influence the processes. **Oxidative stress** as well as **inflammatory mediators** (IL-1α, IL-15, TNF-α, TGF-β) cause micro-inflammation and dermal fibrosis. Senescence and apoptosis of the functional unit of hair bulb and dermal papilla are accelerated (Lolli et al. 2017). Antioxidant mechanisms as well as their regulation via transcription factors such as Nuclear factor erythroid 2-like 2 (Nrf2) remain to be investigated (Vomund et al. 2017; Prie et al. 2016).

33.3 Genetics

Polygenic modalities with high-familial prevalence and strict concordance in monozygotic twins underlie AGA (Lolli et al. 2017). Genome-wide association studies (GWAS) have identified a setting of pathophysiologically relevant genes in approximately 300 genomic regions with >600 independent genetic risk factors for AGA (Heilmann-Heimbach et al. 2020; Hagenaars et al. 2017). Based on 63 loci**, approximately 39% of AGA cases** can be explained **genetically** (Heilmann-Heimbach et al. 2020). At present, several gene loci are under discussion.

First and foremost is the **androgen receptor (AR)** (xq11–12) (Nieschlag et al. 2003). By and large the characteristic remodeling of hair follicles from fronto-parietal to vertex can be explained by the binding of dihydrotestosterone (DHT) to the AR of hair follicles. In this context, certain variants (SNP rs6152) of the androgen receptor represent a disposing factor for AGA (Ellis et al. 2007). The importance of **the androgen receptor polymorphism** in the development of alopecia is also emphasized by the fact that men with Kennedy syndrome, i.e., a high number of **CAG repeats**, are protected from alopecia (Sinclair et al. 2007). The roles of histone deacetylase 4 and 9 (HDAC4, −9), which are expressed in the hair follicle, have been implicated in the post-translational modification of AR protein (Dey-Rao and Sinha 2017).

A second crucial gene polymorphism is present in the neighboring genomic region of the AR. A missense mutation (Arg57Lys) has been detected for the **ectodysplasia A2 receptor (EDA2R).** In addition to AGA, the x-linked EDA2R/AR locus also confers an increased risk of prostate carcinoma. Such synergisms of AGA and organic disease are discussed in recent research (Hagenaars et al. 2017).

Other autosomal genes with variable expressivity predispose to AGA. The **Hedgehog signaling pathway** is involved in embryonic hair follicle morphogenesis and subsequent regulation of follicular hair growth (Paladini et al. 2005). Hedgehog agonists stimulate the transition from telogen to anagen phases in mouse skin (Sun et al. 2020). Hedgehog inhibitors, approved for the treatment of advanced and metastatic basal cell carcinoma, induce hair loss as a side-effect.

Missense mutations in the **steroid 5-alpha reductase 2 (SRD5A2) gene (2p23.1**) affect the synthesis of testosterone to DHT. Increased SRD5A2 activity is detectable in the hairy scalp of patients with AGA. Patients with 46,XY DSD have no AGA, no or little beard hair, and normal body hair due to 5αR2 deficiency (Inui and Itami 2013). Successful therapy with finasteride, a SRD5A2 inhibitor (see Sect. 33.5.1) confirms this mechanism.

Wnt and EGF signal transduction are important for hair follicle regeneration and hair growth (Choi 2020). They are involved in essential signaling cascades in hair papillae including cross-talk with the AR.

Runt-related transcription factor 1 (RUNX1) and **Kruppel-like factor 8 (KLF8)** are integrated into the Wnt/β-catenin signaling pathways. RUNX1 is responsible for hair

structure (Raveh et al. 2006). As a co-activator, KLF8 is involved in stem cell regulation and interaction with mesenchyme and adipogenesis in the hair follicle. Supplemented by other elements yet to be explored, factors can be assigned to individual cycle phases.

Hedgehog signals and Wnt signals activate the entry into anagen hair growth. Numerous growth factors, in particular vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF1), and fibroblast growth factor 5 (FGF5), play a role in follicular proliferation, differentiation, and tissue remodeling. Signal transducer and activator of transcription (STAT) 3, as well as parathyroid hormone (PTH) and parathyroid hormone-like hormone (PTHLH), are involved in maintaining the anagen phase (Kelly et al. 2016). Crosstalk between keratinocytes and surrounding mesenchyme in the hair papilla is influenced by the PTH1 receptor.

Estrogens support the anagen phase. They are antagonists of anagen shortening. In particular, the estrogen receptor α mediates 17β-estradiol-dependent effects on the hair follicle. Retinoid-related orphan receptor α (RORα) is active with respect to estrogen receptor expression and thus contributes to the maintenance of the anagen phase. Melatonin may act as an antagonist. Among other factors, melatonin is synthesized in the hair follicle of the scalp. Melatonin may act on the estrogen receptor, and androgen sensitivity is described for melatonin (Slominski et al. 2017). The therapeutic use of melatonin (see Sect. 33.5) shows that these results do not stand up to stringent evaluation.

Prolactin and corticotropin releasing hormones are relevant for the induction of the catagen phase (Foitzik 2005; Foitzik et al. 2006). Inhibitory effects on stem cells in the hair follicle are described for prolactin, and further paracrine and autocrine effects influencing the hair cycle are suspected. Via corticotropin releasing hormone receptor 1 (CRHR1), keratinocyte proliferation is inhibited in hair follicles. Transforming growth factor β (TGF-β) and Dickkopf (DKK) genes, the latter as Wnt signaling inhibitors, are active in the regulation of the catagen phase (Heilmann-Heimbach et al. 2020; Foitzik et al. 2006; Burg et al. 2017).

In addition to these switching mechanisms, hair miniaturization and conversion to vellus hair remain to be investigated. The gene interferon regulatory factor 4 (IRF4) is significant for hair pigmentation. Pigment loss is seen in association with the transformation from terminal to vellus hairs.

Genetics provide foundations and approaches to therapy influencing transcription and target genes such as using 5-alpha reductase inhibitors, AR antagonists, and growth factors (Dey-Rao and Sinha 2017).

33.4 Diagnostics

The diagnosis of AGA is based on self-report, family history, physical examination, additional tests, laboratory tests, and possibly the collection of a tissue sample and its histologic examination.

In the family history, paternal AGA and any occurrence in the mother's father in particular should be inquired about. With regard to possible differential diagnoses and systemic diseases, in addition to specific anamnestic questions, such as onset, duration and course, complete physical examination including eyebrows, eyelashes, mucous membranes, nails, and secondary body hair is necessary. Conditions that may be associated with hair loss such as hyperthyroidism/hypothyroidism, malignancies, hepatopathies, iron deficiency, anemia, and diabetes mellitus, among others, should be considered (see Table 33.1) (Phillips et al. 2017).

Table 33.1 Relevant differential diagnoses in hair loss

Differentiation of hair loss	Diagnosis	Symptoms and associated factors
Non-scarring telogen effluvium	AGA	Gradually progressive, bitemporal, frontal, vertex
	Telogen effluvium	Diffuse hair loss, up to 6 months after anesthesia, surgery, post-partum, stress-related, weight loss
	Alopecia areata	Circular hair loss, exclamation mark hairs, hairs at edge of alopecia clearly epilated during the episode
	Trichotillomania	Generally girls and young women, often unconscious pulling of hair, one-sided, different hair lengths
	Inflammatory dermatoses	More common in psoriasis capitis, greasy scales, plaques, loss of hair with keratosis plaques
	Diffuse effluvium in organ diseases	Thyroid dysfunction, stage II syphilis, acute cutaneous lupus erythematosus
	Diets/nutrition	Protein, iron, zinc, vitamin C deficiency, sudden weight loss, bulimia, anorexia nervosa
	Medications	E.g., lithium, antidepressants, anticoagulants, beta antagonists, retinoids, isoniazid, cholesterol-lowering drugs, hedgehog inhibitors
Anagen effluvium	Toxins	Thallium, mercury
	Chemotherapy	2–3 months after alkylants, vinca alkaloids, regrowth after cessation of noxious agents in 4–8 weeks, synchronous growth of hair

(continued)

Table 33.1 (continued)

Differentiation of hair loss	Diagnosis	Symptoms and associated factors
Scarring alopecia	Discoid lupus erythematosus	Follicular hyperkeratosis, photosensitive, red keratotic plaques ('cut nail phenomenon')
	Lichen ruber capillitii	Perifollicular keratosis, Wickham's striae, dystrophic nails, polygonal papules on wrists
	Frontal fibrosing alopecia	Hairband-shaped progressive atrichia, usually frontal and post-menopausal
	Pseudopelade Brocq	Idiopathic, discussed as a separate entity
	Traction alopecia	Permanent hair loss due to constant traction, e.g., hairstyle-related, mostly fronto-temporal
	Folliculitis decalvans	Pustules, crusts, tufts and brush hairs
	Folliculitis and perifolliculitis capitis abscedens et suffodiens	Males, Afro-Americans, vertex and occipital, may affect entire head, fluctuating nodules, fistulas, exudate

The use of anabolic steroids, especially in the context of increased muscle training, is to be inquired. In the context of athletic activities, DHT increases. The intake of anabolic steroids, of estrogen and androgen receptor modulators, of dietary supplements such as muscle protein concentrates (so-called whey proteins) or of amino acids such as arginine and ornithine, increase anabolic testosterone-controlled metabolic processes.

Concerning AGA, the physical examination focuses on the scalp hair: pattern and distribution of hair thinning, frontal hairline, hair length, hair diameter, scarring or non-scarring, pigment shifts, scales, crusts, papules, and pustules are to be considered. Abnormal sensations (trichodynia) in loco—such as tingling—may be felt by the patient (Mubki et al. 2014).

AGA typically starts with bitemporal hair loss. Its further course is variable in terms of expression and extent, and can be classified according to the **nomenclature by Hamilton and Norwood** (Norwood 1975) (see Table 5.1).

In the **pull test**, gentle traction on approximately 40–60 hairs is used to identify and localize loosely seated hairs. Frontal, occipital, and bilateral temporal traction are recommended. The indication for effluvium exists if 4–6 hairs are epilated during such traction.

In a **trichogram**, about 50 hairs are epilated with the help of a clamp and the hair root forms are examined microscopically. Standard values are 75–95% anagen hairs, up to 20% telogen hairs, 4% dystrophic hairs, 1–2% catagen hairs. In addition to history and clinic, a result exceeding 20% telogen hairs may be helpful in making a diagnosis.

Trichoscopy can be performed using digital video dermatoscopy (folliscope). Hair thickness is measured, peripapillary brown or white patches, yellowish color and honeycomb pigmentation are recorded along with focal atrichia. The ratio of head hair to vellus hair is calculated. Perifollicular pigmentary changes may provide initial evidence of AGA. A honeycomb-like pattern is associated with advanced AGA (Kibar et al. 2014; Ummiti et al. 2019).

Trichoscan combines digital microscopy with automated computerized image analysis. In situ hair density (number per cm^2), hair diameter (μm), hair growth rate (mm/day), and the ratio of anagen to telogen hairs are measured. The procedure is performed in an area with short hair and takes about 20 min (Hoffmann 2003). This method is particularly suitable for measuring therapeutic success in studies.

Laboratory tests are recommended in cases of unclear diagnosis and differential diagnoses that need to be considered (see Table 33.1). Hormone tests are of little use specifically for AGA. However, since it is not uncommon for patients to take a wide variety of measures on their own initiative, these may need to be checked by laboratory chemistry. For example, excessive supplementation with vitamin A may induce hair loss (Almohanna et al. 2019).

If diagnostic uncertainty persists, scalp biopsy should be considered. A sufficiently large tissue sample should be obtained from the periphery of the affected scalp area. The number of hair follicles, proportion of telogen hairs, vellus hairs, and variations in hair shaft diameters are assessed. A ratio of terminal to vellus hairs of <3:1 confirms the diagnosis of AGA (Stefanato 2010).

Table 33.1 shows major differential diagnoses of AGA. Two main groups are distinguished: non-scarring and scarring alopecia. AGA belongs to the non-scarring alopecias and to the subgroup of telogen effluvium. The pattern of hair loss, the nature of possible regrowth, and the course of effluvium provide important clues.

The presentation of infantile syndromes associated with hair loss has been omitted, as has a review of diseases of the hair structure (Itin and Fistarol 2005).

33.5 Therapy

Therapeutically possible goals are to be discussed with the patient. Priority is given to preventing further progressive and permanent hair loss and thus hair thinning.

Standard treatments for AGA in men are based on oral administration of finasteride 1 mg and dutasteride as well as topical application of a 5% minoxidil solution or a 5% minoxidil foam.

33.5.1 5-Alpha-Reductase Inhibitors: Finasteride and Dutasteride

Finasteride was first approved for the treatment of benign prostatic hyperplasia (1992). In 1994, European approval was granted for the treatment of early stages of AGA in men in age groups 18–41 years. According to the guideline, 1 mg of finasteride per day is recommended for mild to moderate male AGA (Fig. 5.1 Hamilton-Norwood-IIv-V).

Finasteride inhibits the conversion of T to DHT **via 5-alpha-reductase type II.** DHT is responsible for miniaturization of the hair follicle. After regular finasteride use, serum concentrations of DHT are reduced by 65%.

Systematic reviews and meta-analysis speak of a significant increase in the number of hairs. The average increased number of hairs after 12 months ranges from 7.2/cm^2 to 36.1/cm^2 (Blumeyer et al. 2011; Adil and Godwin 2017). Expert consensus in the guideline puts the success of finasteride at 1 year at 37–54%. Individual studies report a 65% success rate, with a 37% effect in the placebo arm (Darwin et al. 2018). Studies over 5 to 10 years point to stable efficacy with continuation of therapy (Yoshitake et al. 2015). Better success is achieved in younger patients. Generally, 40–60% of men are satisfied with treatment outcome.

Finasteride 1 mg is available by prescription, but, as a lifestyle medication, is not reimbursable. Among the side-effects, the patient should be advised of possible **loss of libido, erectile dysfunction, decreased ejaculate volume, and increased body hair**. The development of gynecomastia is possible (Lee et al. 2019). Sexual dysfunction affecting 2–3.8% of patients (placebo 1–2.1%) is increased at older ages. Although evidence remains very limited, the possibility of permanent sexual dysfunction, such as years of erectile dysfunction, should be discussed with the patient (Gupta et al. 2017). The term "**post finasteride syndrome"** was coined after licensing and summarizes depressive, neurological, and sexual symptoms (Andy et al. 2019; Goren and Naccarato 2018). A 48-week administration of finasteride 1 mg in 181 healthy men resulted in **no change in semen parameters**. Finasteride concentrations measured in the ejaculate are very low; however, treatment cessation should be considered during periods of conception.

Use in hepatic insufficiency is not recommended. Dose adjustment should be considered in renal insufficiency. Finasteride is metabolized via cytochrome P450-3A4. Interactions with drugs that are also metabolized via cytochrome P450 cannot be ruled out.

Dose comparison studies of dutasteride 0.02 versus 0.1 mg and 0.5 mg per day demonstrated significant improvement in hair diameter and hair number at a dose of 0.5 mg compared to placebo (Gubelin Harcha et al. 2014). In 31 men, dutasteride 0.5 mg was used after unsuccessful treatment with finasteride 1 mg. In 23% (7/31) no success was seen, in 77% (24/31) little to markedly better clinical outcomes were observed (Jung et al. 2014).

Based on few studies, meta-analyses have been published on the use of **finasteride** and **dutasteride** (0.5 and 2.5 mg) in AGA (Gupta and Charrette 2014; Zhou et al. 2019). **Treatment outcomes** are considered comparable. The side-effect profile of dutasteride is also consistent with the use of 1 mg finasteride and includes the risk of sexual dysfunction, which is rated as 1.55-fold for both 5-alpha-reductase inhibitors (Lee et al. 2019).

Studies of 0.2, 1, or 5 mg oral finasteride have produced comparable results. The concentration of 0.01 mg is considered too low. Work is underway on topical treatment options with finasteride 0.25% and 1% gel (Lee et al. 2018). In the guideline, topical treatment was still judged to be insufficiently evaluated. With potentially similar success with finasteride 1 mg and comparable serum levels, high-local levels in the skin and lower finasteride serum levels should be achieved by improving the vehicle. Contamination of women of childbearing age should be avoided in local treatment (teratogenic).

Increasing treatment success is attempted in combination of finasteride with minoxidil, but Mysore et al. advise against this (Mysore and Shashikumar 2016). Supplemental administration of ketoconazole 2%, as a modulator of steroid synthesis, shows little effectiveness to date.

33.5.2 Minoxidil

Minoxidil, a piperidinopyrimidine derivative, was licensed in the 1970s for the treatment of hypertension. As an unexpected side-effect, many treated patients showed increased hair growth. In comparative studies with 1%, 2%, and 5% minoxidil solutions, better response was shown with the higher concentration (Manabe et al. 2018). In 2005, 5% minoxidil was approved by the EMA as a solution and in 2006 as a foam for the treatment of AGA in the age group of 18 to 65 years.

Minoxidil is converted by the hair follicle enzyme sulfotransferase to the active form minoxidil sulfate, which promotes hair growth via the hair bulb. The success of minoxidil treatment correlates with local sulfotransferase activity. Serum levels do not correlate with response to minoxidil (Suchonwanit et al. 2019; Goren and Naccarato 2018).

Causal mechanisms of action of minoxidil include improved blood flow and VEGF priming, which has been demonstrated by laser Doppler velocimetry and electron microscopy as well as mRNA studies (Suchonwanit et al. 2019).

Minoxidil is available over the counter as 5% solution and 5% foam. 1 mL of 5% minoxidil is to be applied and massaged into the affected scalp twice daily. Treatment

should be continued for at least 6 months. An initial response becomes visible after approximately 12 weeks and can only be reliably assessed after 6 months. Continuation of therapy should be decided only then, if it is successful.

Pathological hair loss can be stopped in about 80–90% of those affected. Better results are achieved in younger candidates and at the top of the head than in the "secret hair corners" (Wolff et al. 2016). Meta-analysis and reviews report denser hair growth in 30–40% of candidates (Adil and Godwin 2017; Suchonwanit et al. 2019). The guideline speaks of an average increase in hair number of 15.5/cm^2 up to 83.3/cm^2 after 12 months. Treatment should be given on a long-term basis, as interruption is associated with gradual hair loss. After one therapy-free year, the pre-therapeutic status will be regained.

Side-effects of minoxidil include **redness, itching, scaling, skin irritation and** "**shedding"**4–9 weeks after initiation of therapy. "Shedding" describes increased loss of telogen hairs that are pushed out by re-growing anagen hairs. Less commonly, headaches and contact dermatitis are reported.

The addition of **micro-needling** in preparation for minoxidil application offers the possibility of improved drug penetration. Additional stimuli such as PDGF release and VEGF expression and thus follicular stem cell activation are discussed (Sadick 2018).

Combinations of 5% minoxidil solution with a zinc-pyrithione shampoo or in combined solution with tretinoin 0.01% have not produced significantly better results compared with standard application of 5% minoxidil solution (Shin et al. 2019; Blumeyer et al. 2011).

Smaller case studies report **oral minoxidil** in off-label use. At doses of 1–1.25 mg daily orally, increased hair growth was recorded in 28% (7/18) of patients, but this also affected body hair (Beach 2019). Due to possible cardiovascular complications, its use in AGA is not recommended.

33.5.3 Laser Therapies

With the onset of initial laser therapies in the 1960s, the experimental use of red light lasers and diode lasers to stimulate hair growth soon followed. The field of **photobiomodulation** may present opportunities for stimulating keratinocytes and fibroblasts, increasing blood flow and metabolism, influencing anti-oxidation, and inflammation.

An optimal wavelength has not been determined, 500 to 1100 nm with a power of 3.3–90 mW/cm^2 and 1–4 J/cm^2 are used. A spectrum of 650–900 nm is preferred. Possible frequency indications include 1 × per week for 30 min for 8 weeks, then every second week for 8 more weeks, and 2–4 × per year in succession (Sadick 2018).

In controlled studies, significantly increased hair density is noted after 26 weeks (Adil and Godwin 2017: 17.66/cm^2). In short-term comparison, the response is similar to 5% minoxidil solution and 1 mg finasteride. Long-term results are not as good. A 2018 review summarized 13 studies. Of 11 studies, 10 show proportional success in hair thickness and/or hair count. Small case numbers, varying diagnostic procedures, divergent efficacy measurements, as well as divergent therapy combinations, make it **difficult to assess the success of laser therapies**.

Often advertised as **low-level laser therapy (LLLT)** or **soft laser therapy**, corresponding devices are freely available, e.g., as a laser comb, laser brush, laser in cap, or helmet form. As a comb, the application is described as 20 min per day 3 times per week. Regarding side-effects, dry skin (5.1%), itching (2.5%), tension of the scalp (1.3%), irritation (1.3%), and feeling of warmth (1.3%) are mentioned. There were no treatment discontinuations or interruptions (Jimenez et al. 2014). For laser diodes in a helmet (650 nm) with an application of 18 min daily for 24 weeks or every second day for 16 weeks, a 35% hair regrowth has been described (Kelly et al. 2016).

33.5.4 Hair and Stem Cell Transplantation

Hair transplantation should be considered in patients older than 25 years with chronic androgenetic hair loss. Preoperatively, other skin and hair diseases such as lichen ruber capillitii should be excluded (see Table 33.1).

Follicular unit transplantation (FUT) is a therapy in which small areas from the hair ring are transplanted into androgen-dependent scalp. Hair in the hair ring is considered to be body hair. Hair follicles are not androgen-sensitive and thus continue to grow after transplantation. Transplantation of small follicular units should be performed in specialized centers, since the success of treatment depends, among other things, on professional assessment of the hair and on the preparation and number of transplantable, undamaged hair follicles. One option is to treat the follicular units with platelet plasma growth factor before implantation (Blumeyer et al. 2011).

Surgery-associated side-effects, including pain, scarring, infection, and treatment failure should be considered. Postoperative telogen effluvium is to be expected. Topical minoxidil treatment may promote regrowth of transplanted follicles and also - oder - Or the additional administration of 1 mg finasterid may also improve clinical outcomes (Blumeyer et al. 2011). After 6 months, the outcome should be stable. Success can only be definitively assessed after 9–12 months. A general graft survival is reported to be approximately 80%. In the majority of cases, multiple surgical grafts must be scheduled. Despite decades of use of

autologous hair transplants, randomized controlled trials are lacking (Blumeyer et al. 2011; Manabe et al. 2018).

Biotechnologically **stem cells** can be grown into a hair follicle. Developments on stem cell therapy and optimization in the procedure are summarized in a review (Gentile and Garcovich 2020). Injections of autologous dermal cells from a hair follicle into hairless scalp were studied in a phase II trial. Among study participants, 19.2% had increased hair at 6 months and 15.4% had thicker hair.

33.5.5 Other Therapeutic Approaches

Injections with platelet-enriched plasma have been used for AGA since 2014. The plasma fraction is obtained from autologous blood. Prolonged anagen phase and inhibition of apoptosis should be achievable via perifollicular vascularization and activation of growth factors (Sadick 2018). Two to four injections are administered at intervals of 2 weeks to 3 months. Although response rates of 40–54% are reported, evaluations, counts, and long-term outcomes are poorly evidence-based (Kelly et al. 2016; Giordano et al. 2018).

The substance group of prostaglandins is coming into focus due to the expression of prostanoid receptors in hair follicles (Xu and Chen 2018). **Bimatoprost**, a prostamide analog, is approved for glaucoma treatment. It has been shown to cause eyelash growth as a side-effect (Barrón-Hernández and Tosti 2017). The prostaglandin analog latanoprost 0.1% also produces visible growth of eyelashes (El-Ashmawy et al. 2018). Use in 16 men with AGA (Hamilton II-III) yielded visible hair growth (Blume-Peytavi et al. 2012).

Based on limited data and to be considered as possible developments are the topical applications of **cortexolone**, a mineral corticoid derived from the adrenal cortex, or the topical application of 8.3% **valproic acid** or **melatonin** (Jo et al. 2014). Melatonin application showed a short-term improvement of the pull test in about 50% of patients (Fischer et al. 2011).

The effects of mesotherapy, botulinum toxin, zinc pyrithione, or ketoconazole 2% in shampoo or solution or substances such as carpronium chloride, flavonoids, cytopurine/pentadecane (alkane), cepharantine (alkaloid) are not confirmed (Blumeyer et al. 2011; Reguero-del Cura et al. 2020; Zhou et al. 2020).

Based on advertising in the media and based on the guideline with additional updated research, numerous **dietary supplement products** should be identified as **non-significant and non-evidence based** in their effects: iron, copper, zinc, vitamins (vitamin B3 = niacin, vitamin B7 = biotin, vitamin D, vitamin E), caffeine, amino acids, pumpkin seed oil, selenium, green tea, probiotics, including ginkgo products (Kanti et al. 2018; Hosking et al. 2019; Almohanna et al. 2019).

Key Points

- History, clinical examination with consideration of possible differential diagnoses represent cornerstones in the diagnosis of AGA.
- Understanding signaling cascades and the genetic basis of AGA will become increasingly important, also with regard to therapeutic approaches.
- In addition to standard therapies with finasteride 1 mg and 5% minoxidil solution or foam or even dutasteride, there are numerous optional therapeutic variations and adjuncts such as needling and laser therapy.
- Hair and stem cell transplants should only be applied by specialized colleagues.

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Part VII

Clinics in Andrology: Disorders of Reproductive Health Caused by Environmental and Systemic Diseases

Testicular Dysfunction in Systemic Diseases

34

Gideon A. Sartorius and David J. Handelsman

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Abstract

General health and reproductive health are in a close, bidirectional relationship. Reproductive health, expressed through effects mediated by sex steroid production, gametogenesis and fertility, and sexuality, may, on the one hand, modify the influence of systemic non-reproductive disorders on general health. Conversely, systemic non-reproductive disorders also have a major impact on reproductive health. Therefore, the optimal clinical management of medical disorders should include knowledgeable consideration of the effects of illness and its treatment on male reproductive health (including fertility, androgenic status, and sexual function). Although not life-threatening, the diagnosis of impaired reproductive health is important as it might decrease the quality of life and worsen the course of disease. However, reproductive dysfunction including subfertility and/or androgen deficiency occurring in association with chronic disease is readily overlooked. Such underrecognition is particularly likely when the relatively subtle and nonspecific effects of reproductive dysfunction are overshadowed by a major systemic illness with more dramatic but similar nonspecific clinical features.

This chapter highlights the impact of systemic illness on testicular function by elucidating common pathophysiological mechanisms and established the effects of specific diseases on reproductive health.

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34.1 Background

Systemic non-testicular illness may have a major impact on the testis although these have not always been sufficiently recognized. Reproductive ill-health (such as androgen deficiency, infertility, and sexual dysfunction) might therefore be either the cause or consequence of adverse effects on general health. The clinical management of any medical disorder should include careful consideration of the effects of illness and its treatment on male reproductive health (including fertility, androgenic status, and sexual function). Although not life-threatening, the recognition of adverse impacts on male reproductive health may contribute to a decreased quality of life and a worse course of disease although in some instances these impacts might be rectifiable. In particular, the nonspecific symptoms of androgen deficiency coinciding with similar features of chronic disease are readily overlooked when overshadowed by a major systemic illness with more dramatic clinical features.

General health and reproductive health are in a close, bidirectional relationship (Fig. 34.1). Systemic non-testicular disease can have major effects on reproductive health. Preserving and improving **reproductive health** is an essential part of medical care of men to avoid dissatisfaction, should their management, focusing solely on the presenting disease, neglect his often unstated, but nevertheless deeply felt, expectations relating to preserving his reproductive functions, something men value highly as part of a full life.

On the other hand, reproductive ill-health might also reflect impaired general health leading to later systemic morbidity (e.g., erectile dysfunction as a sentinel sign of cardiovascular morbidity and mortality) (Nehra et al. 2012). This knowledge allows the clinician in the field of andrology not only to improve reproductive health outcomes but also to ameliorate general health.

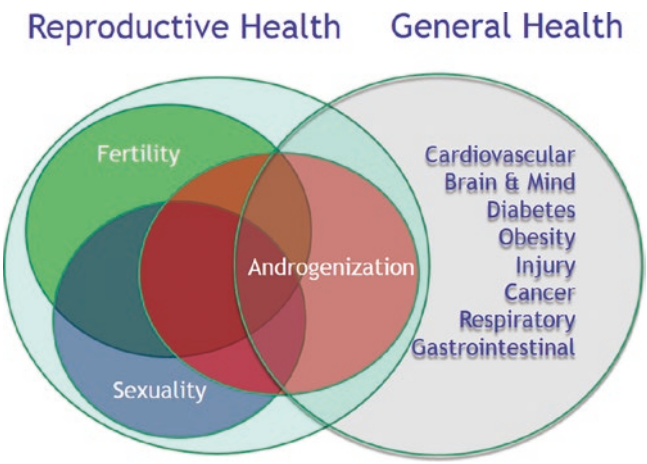


Fig. 34.1 Bidirectional relationship between reproductive health and general health

This chapter will highlight the impact of systemic, non-testicular disease on testicular function. In the first part, pathophysiological mechanisms for testicular dysfunction are considered, while the second part highlights specific diseases and their treatment by organ system. Sexual dysfunction and testosterone deficiency with its treatment are covered in other chapters (see Chaps. 5, 12, 16, 21, 25, 30, and 36) while drug effects on testicular function are covered only in connection with specific diseases for which they are prescribed.

34.2 Mechanisms of Reproductive Disruption by Systemic Diseases

The clinical reproductive effects of non-testicular illnesses depend on the epoch of life and the severity and chronicity of the underlying disease. Male reproductive dysfunction can result from the selective impact on any specific level of the hypothalamic–pituitary–gonadal axis, and chronic non-testicular disease often involves multiple levels concurrently. These impacts can occur at any epoch of life but are especially important when there are major changes in reproductive axis activity such as at birth, during puberty, and aging. The duration and reversibility of testicular axis disruption depend on the severity and the pathophysiological mechanisms involved in the underlying disease and its treatment. Disruptions of spermatogenesis can be transient, lasting from less than one spermatogenic cycle, typically associated with nonspecific features such as fever, weight loss, or cytokine effects of non-testicular illness, or may persist for prolonged periods or even being irreversible following cytotoxic drug treatment for cancer or other serious diseases.

34.2.1 Onset of Hypogonadism, Sexual Dysfunction, and Spermatogenic Failure

The expression of **androgen deficiency** is distinctive at different epochs of life. Prenatal androgen deficiency produces various degrees of incomplete development of male internal and external genitalia, varying from ambiguous genitalia arising from the partial or complete absence of masculine sexual development to various degrees of hypospadias, gynecomastia, and/or cryptorchidism (see Chap. 31) Pubertal androgen deficiency manifests as delayed puberty, eunuchoidal skeletal proportions, and impaired virilization, whereas

postpubertal androgen deficiency may present with the well-known classical features of androgen deficiency in adults (see Chap. 25).

Most classical features of androgen deficiency, apart from hot flushes in castrate men, are nonspecific manifestations of prolonged, moderate-to-severe androgen deficiency.

Sexual dysfunction becomes relevant only after puberty following the onset of sexual activity and incidence increases with the occurrence of non-reproductive health problems and with age (see Chaps. 44 and 45).

Defects in spermatogenesis are only manifest after puberty and, having no symptoms other than **infertility**, are only detected during the diagnostic evaluation for infertility or incidentally (e.g., cryostorage prior to gonadotoxic treatments) (see Chap. 43).

34.2.2 Level of Disruption in the Male Reproductive Axis

Non-testicular diseases can disrupt different levels of the hypothalamic–pituitary–testicular axis. GnRH, and consequently gonadotropin, secretion can be suppressed by acute or chronic diseases via disruption of hypothalamic regulatory mechanisms, while additionally or alternatively underlying chronic diseases may impact directly on Leydig cell steroidogenesis or spermatogenesis. The exact mechanisms linking systemic ill-health and reproductive function remain to be elucidated, but several factors have been hypothesized to play a role, such as developmental factors, lifestyle, hormonal factors, different cytokines, and genetic and epigenetic factors. However, some common modulating mechanisms seem to be shared by different pathophysiologic processes, such as cancers, infections, immune disorders, and other specific disorders highlight below. Different cytokines and the kisspeptin system, which lies upstream of the hypothalamic GnRH-secreting neurons, might play a pivotal role in this complex interplay (Loveland et al. 2017; Plata-Salaman 2000; Wahab et al. 2015).

Severe or chronic systemic illnesses often produce characteristically defective hypothalamic-–pituitary axis regulation, which is similar in mechanism to the physiological mechanism operative before and during male puberty and in the annual gonadal cycles of seasonal breeding animals. The mechanism characteristically involves a triad of features, inhibition of GnRH secretion, hypersensitivity to negative testicular feedback, and resistance to naloxone, which has been termed **ontogenic regression** (Handelsman and Dong 1992, 1993). Ontogenic regression involves a reversible variation in sensitivity to negative testicular feedback, which causes coordinate changes in gonadotropin and testosterone secretion during periods of stress unfavorable to reproductive activity. By facilitating an orderly withdrawal of reproductive function, ontogenic regression facilitates its orderly recrudescence when more favorable environmental circumstances once again prevail. Negative energy balance, for example, caused by short-term and long-term starvation, is known to lead to reproductive quiescence in rodents and nonhuman primates (Bergendahl et al. 1991; Cameron 1991, 1996). Besides other neuropeptides, the peptide hormones kisspeptin (KP) and the RF amide-related peptide 3 (RFRP3, gonadotropin inhibitory hormone (GnIH)) might not only be the main central regulators of the reproductive axis, but also be sensors relaying information on energy balance to the hypothalamic centers regulating food intake and reproductive behavior (reviewed in [Wahab et al. 2015]).

Ontogenic regression is a common underlying mechanism in a variety of systemic diseases, including chronic renal, respiratory, liver, inflammatory, nutritional diseases, and acute critical illness and severe burns. It is presumably an archaic adaptive physiological mechanism invoked by adverse environmental circumstances ensuring that no overall reproductive fitness is lost in times unfavorable for successful reproduction, while retaining the capacity for successful recovery when more positive circumstances return. In the context of chronic disease, the impact of chronic organ failure and transplantation is a particularly clear exemplar of ontogenic regression, both in its negative and positive human applications. In addition, disease-induced hypogonadism might also have favorable and protective effects as testosterone might cause the progression of certain diseases by pro-inflammatory, profibrotic, and pro-apoptotic signaling (Metcalfe et al. 2008).

Ontogenic regression refers to the orderly regression of reproductive function during adverse environmental conditions in a fashion that permits facile recrudescence when a more favorable environment once again prevails.

Most severe systemic non-gonadal illness or trauma such as **burns**, **myocardial infarction**, **traumatic or surgical injury**, and **acute critical illness** (Woolf et al. 1985; Dong et al. 1992; Wang et al. 1978) depresses testicular function as evidenced by low blood testosterone levels, unchanged immunoreactive inhibin levels, decreased or mildly increased immunoreactive gonadotropin levels with low bioactive LH levels, and abolition of pulsatile LH secretion (Dong et al. 1992; Woolf et al. 1985; Semple et al. 1987). Such **transient biochemical lowering of circulating testosterone** is a common, regular accompaniment of severe acute or chronic illness. Such lowering of serum testosterone is even more frequent than the analogous thyroid functional status known as the **sick euthyroid** or non-thyroid illness syndrome as dif-

ferent facets of reversible, functional hypopituitarism due to systemic non-gonadal illness. In theory, severe and sustained androgen deficiency caused by a chronic disease may contribute to the morbidity of the underlying disease. However, to determine whether lowering of circulating testosterone is deleterious, incidental, or even protective requires well-designed, placebo-controlled RCTs evaluating short- and long-term effects of physiological testosterone replacement. However, the primary focus of this chapter is a review of observational studies outlining the impact of systemic non-gonadal disease on the testicular axis. Therapeutic studies of physiological androgen replacement with testosterone or pharmacological androgen therapy using synthetic androgens in systemic non-gonadal diseases are reviewed in detail elsewhere (Handelsman 2011).

Testicular function is depressed by common features of systemic illness such as different **cytokines**, **fever** (Kandeel and Swerdloff 1988; Carlsen et al. 2003), **weight loss,** and **chronic illness or catabolism** (Handelsman and Staraj 1985) and distinguishing between these effects is difficult. Extremes of nutrition influence testicular function, but **male reproductive function (notably spermatogenesis) is more robust than the female reproductive system** during catabolic states such as undernutrition, trauma, and extreme physical exertion. **Strenuous physical exercise** has minimal effects on testicular function and spermatogenesis among elite athletes (Bagatell and Bremner 1990), but extreme physical exertion causes profound inhibition of testicular androgen secretion (e.g., Schürmeyer et al. 1984; Stroud et al. 1997), which may be modified by long-term anaerobic training (Slowinska-Lisowska and Majda 2002).

While androgen deficiency and sexual dysfunction are known to reflect general health, the bidirectional relationship between systemic health and male fertility has come into focus recently (Choy and Eisenberg 2018; Kasman et al. 2020). However, due to the complex nature of human reproduction it will remain always a challenge to differentiate between different causative factors, such as developmental, hormonal, genetic, epigenetic, and lifestyle factors.

Disruption of spermatogenesis can lead to infertility primarily through a reduction in the number of ejaculated sperm and possibly also to sperm function. The intense cellular and DNA replication of the germinal epithelium makes it singularly susceptible to cytotoxins such as ionizing irradiation, cytotoxic drugs (notably alkylating agents), other therapeutic agents (e.g., some antibiotics), and occupational or environmental exposures (see Chap. 35). Such agents cause all degrees of hypospermatogenesis from reversible depression to permanent ablation from germinal stem cell depletion. As spermiogenesis forms the morphological basis for sperm function, interruption at this stage of spermatogenesis can produce hypofunctional (infertile) sperm.

Age is a crucial modifier of testicular response to systemic illness (see Chap. 25). The maturing hypothalamic–pituitary testicular axis has heightened susceptibility, making adolescents particularly vulnerable to delayed puberty during chronic illness. Aging men exhibit decreased circulating testosterone levels, while SHBG and gonadotropin levels increase (see Chap. 25) changes, which might be caused by (or at least markedly accentuated) by the coexistence of chronic illness and/or its treatment (Sartorius et al. 2012). These changes reflect common alterations in hypothalamic function as features of ontogenic regression including decrease in testosterone, alterations in pulsatile LH secretion, and sensitivity to both negative steroidal feedback and opioids and the corresponding testicular manifestation such as progressive decreases in testis size, cell content, and spermatogenesis.

Drugs may impair androgen action through numerous distinct and sometimes multiple mechanisms including (i) decreasing LH secretion (e.g., opiates), (ii) inhibiting steroidogenic enzymes (e.g., aminoglutethimide, ketoconazole, finasteride, and dutasteride), (iii) increased testosterone metabolism (e.g., barbiturates, anticonvulsants, and other hepatic enzyme inducers), (iv) androgen receptor antagonists blocking testosterone action (e.g., cimetidine, spironolactone, and cyproterone acetate), or (v) acting as physiological antagonists of androgen action (estrogenic effects of digoxin and drug-induced hyperprolactinemia). Only a few therapeutic drugs, however, have had their potential effects on the human male reproductive system studied in detail, as reviewed elsewhere (Ding et al. 2017; Drobnis and Nangia 2017; Semet et al. 2017).

34.3 Specific Diseases and Disorders

34.3.1 Renal Disease

Reduced renal function may lead to androgen deficiency, sexual dysfunction, and abnormal spermatogenesis. Depending on the time of onset, gonadal dysfunction manifests as delayed puberty in adolescents and/or as testicular atrophy, impaired spermatogenesis, infertility, sexual dysfunction, or gynecomastia in adult men. The prevalence of low circulating testosterone levels in patients with a chronic kidney disease (CKD) lies between 24% and 66% (Neuzillet et al. 2016; Carrero and Stenvinkel 2012; Snyder and Shoskes 2016). Sexual dysfunction is reported in around 75% of men with CKD (Navaneethan et al. 2010).

Renal failure leads to alterations at different levels of the hypothalamic–pituitary axis with alterations in the hypothalamic regulation of pituitary–gonadal function being predominant, rather than peripheral defects intrinsic to the testis. Inhibition of both spermatogenesis and steroidogenesis

accompanied by modest-to-minimal reflex increases in gonadotropins, together with testicular histological features, is indicative of a functional hypogonadotropic state (Handelsman and Dong 1993). However, the markedly reduced gonadotropin clearance rates in uremia mask the effects of gonadotropin secretion, which is inappropriately low for the reduced testosterone production rates. Consequently, despite low blood testosterone levels, the failure of appropriate rises in blood LH levels, together with defects in pulsatile LH secretion and aberrant hypothalamic opioidergic regulation of gonadotropin secretion, reflect the predominance of aberrant hypothalamic regulation in the pathogenesis of human uremic hypogonadism, as a characteristic manifestation of ontogenic regression. GnRH pulse frequency is preserved, while the pulse strength is decreased, which leads to a decrease in LH per burst, but unchanged frequency of plasma LH pulses (Veldhuis et al. 1993). Additional secondary consequences of the underlying uremia and its treatment include relative LH insensitivity of Leydig cells (Dunkel et al. 1997), increased whole-body metabolic clearance of testosterone (Handelsman and Dong 1993), and impaired spermatogenesis (Prem et al. 1996).

Acute **renal failure** is accompanied by decreased testosterone levels with minimal changes in gonadotropin or SHBG levels and preserved responses to GnRH stimulation and is also consistent with hypothalamic (secondary) hypogonadism that is reversed following recovery of renal function. Experimental studies in rodents confirm the predominance of aberrant hypothalamic regulation of pituitary–gonadal function in the pathogenesis of uremic hypogonadism.

Reproductive dysfunction might become evident at an early stage of acute or chronic renal failure, but most men with pre-dialysis uremia have low or low-normal testosterone levels (Yilmaz et al. 2011) and the prevalence and severity of biochemical androgen deficiency increase in patients requiring dialysis (Albaaj et al. 2006; Handelsman 1985). Hypogonadism in patients undergoing dialysis shows a correlation with decreased quality of life (Bello et al. 2014), anemia (Carrero et al. 2012), reduced strength and muscle mass (Cigarran et al. 2013), and an increased overall mortality risk (Carrero et al. 2009). Low testosterone at the time of renal transplant is also linked to graft loss and survival after transplantation (Shoskes et al. 2014), but whether this is the cause or effect of underlying ill-health is unclear. Renal transplantation remains the most effective treatment to restore testicular function and reverses some of the disturbances in testosterone secretion and spermatogenesis (Reinhardt et al. 2018; Prem et al. 1996; Akbari et al. 2003; Yadav et al. 2008; Lundy and Vij 2019). Nevertheless, a minority of men with successful renal transplants have mild persistent biochemical androgen deficiency (Albaaj et al. 2006) and impaired spermatogenesis (Inci et al. 2006), probably attributable to the incomplete normalization of renal function. Sexual function improves significantly after renal transplantation, but the prevalence of erectile dysfunction remains high (Pertuz et al. 2014; Navaneethan et al. 2010; Perri et al. 2020). This might be due to sequelae of long-term uremia, persistent hypogonadism, and increased prolactin levels and to vascular causes such as iliorenal artery anastomosis or immunosuppressive drugs and psychological reasons like anxiety and depression, which are common in patients after transplantation (Fiuk and Tadros 2019; Mirone et al. 2009).

Despite the consistent findings of low circulating testosterone levels in chronic uremia, controlled studies of androgen replacement therapy are scarce but promising (Johansen et al. 1999; Ballal et al. 1991; Iglesias et al. 2012). Pharmacokinetics of transdermal testosterone in men with end-stage renal failure are comparable to healthy hypogonadal men (Johansen 2004), so that testosterone gels can be used for the treatment of men with renal failure. Even if testosterone replacement therapy is not warranted, pharmacological testosterone treatment may have benefits for men with chronic renal insufficiency. For example, pharmacological androgen therapy with testosterone or synthetic androgens as adjuvant therapy for renal anemia (Ballal et al. 1991; Teruel et al. 1996), undernutrition (Johansen et al. 1999; Eiam-Ong et al. 2007), and osteodystrophy (Doumouchtsis et al. 2008) during pre-dialysis and dialysis phases is well established but often considered reasonably effective but low-cost alternatives to modern expensive treatments such as erythropoiesis-stimulating agents (ESA) or bisphosphonates, respectively. However, the 2012 Clinical Guidelines from the Kidney Disease Improving Global Outcome KDIGO for the treatment of anemia in CKD recommends not using androgens as an adjuvant to ESA (Anon 2012) resulting from the lack of added benefit in ESA hyporesponsive patients and risks of long-term androgen use.

In summary, recommendations regarding testosterone treatment in patients with CKD are controversial. Testosterone treatment must be considered cautiously on an individual case basis until appropriately designed RCTs are available other than for men with underlying pathologic hypogonadism accompanied by low bone density and sexual dysfunction where testosterone treatment should be offered (Bao and Johansen 2015).

Claims that other treatments, including suppression of hyperprolactinemia, zinc supplementation, or erythropoietin, improve testicular function, and are not confirmed in well-controlled studies. Conventional immunosuppressive regimens involving prednisone, azathioprine, cyclosporin A, mycophenolate mofetil, or rapamycin inhibitors (sirolimus, tacrolimus, and everolimus) appear to have little or no consistent clinically significant effects on the HPG axis, but it is claimed that erectile function and sperm quality can be

impaired by rapamycin inhibitors such as sirolimus or everolimus. However, the limitation of retrospective studies makes it hard to evaluate such observational reports of potential adverse effects (Zuber et al. 2008; Tondolo et al. 2005; Huyghe et al. 2007).

34.3.2 Liver Disease

Liver function and reproductive function interact in a bidirectional manner. Sex steroids influence liver physiology and pathophysiology, while the liver function affects sex steroid metabolism and sex steroid transport via SHBG production (Grossmann et al. 2019).

Advanced liver disease correlates with low testosterone and hypogonadism (Handelsman et al. 1995). The extent of testosterone deficiency increases with disease severity in patients with cirrhosis (Zietz et al. 2003), and testosterone levels are a predictor of adverse outcome and mortality in men with end-stage liver disease independent of other predictive factors (Sinclair et al. 2016b; Grossmann et al. 2012; Paternostro et al. 2019).

Sexual dysfunction is common and occurs in up to two-thirds of patients with liver diseases—again with a positive correlation with disease severity (Paternostro et al. 2018; Maimone et al. 2019).

Scientific information about semen quality and fertility is surprisingly limited, except information about the negative impact of hepatitis viruses on sperm chromosomes and semen parameters (Safarinejad et al. 2010; Karamolahi et al. 2019).

Acute liver disease including **hepatitis** causes marked increases in circulating SHBG levels, resulting in reflex increases in gonadotropin and sex steroid secretion to maintain testicular testosterone output and tissue androgen supply. The pathophysiological significance of such transient biochemical disturbance during acute illness remains unclear.

Chronic liver failure is associated with prominent features of hypogonadism, including muscle waisting, anemia, fatigue, infertility, impaired spermatogenesis, testicular atrophy, gynecomastia, reduced body hair, and sexual dysfunction (Zifroni et al. 1991; Neong et al. 2019). Reduced muscle mass in patients with liver disease correlates with mortality, which might be linked to serum testosterone levels (Sinclair et al. 2014) without established causality.

Cirrhotic men show significantly higher levels of circulating estrogens (Sinclair et al. 2016a), while testosterone production rate is decreased, leading to lower circulating testosterone levels, although the concomitant increases in circulating SHBG levels (Luppa et al. 2006) with a consequential fall in testosterone clearance rate complicate interpretation of androgen status. Although most hepatic protein synthesis is reduced in cirrhosis, the paradoxical increased hepatic production of SHBG might be pronounced by elevated circulating estrogen levels in men with cirrhosis (Zietz et al. 2003) and observed clinical features similar to hypogonadism (Maruyama et al. 1991). Despite subnormal testosterone levels, gonadotropin levels remain in the low to normal eugonadal range with diminished pulsatile LH secretion, emphasizing the importance of hypothalamic dysregulation in the pathogenesis of hypogonadism in chronic liver disease (van Thiel et al. 1981; Zietz et al. 2003). In chronic liver disease, the link between circulating testosterone and hepatocellular carcinoma remains controversial with the strong male preponderance of that cancer remaining unexplained (Tanaka et al. 2004; Lukanova et al. 2014; Montalto et al. 1997).

Experimental data suggest that portocaval shunting, aromatase overexpression, or insulin-like growth factor I deficiency has all been attributed to possible roles in gonadal dysfunction due to cirrhosis, although none are yet confirmed in humans (Castilla-Cortazar et al. 2004; Castilla-Cortazar et al. 2000).

As **alcohol** is the most common cause of chronic liver failure in affluent societies, the usual clinical features of chronic liver disease are an amalgam of clinical manifestations due to chronic liver disease per se and chronic alcoholic toxicity. Men with alcoholic liver disease exhibit distinctly higher gonadotropin levels suggestive of additional direct testicular damage from alcohol, in addition to the predominantly hypothalamic effects of chronic liver failure; men with nonalcoholic liver failure tend to have inappropriately low gonadotropin levels, especially with severe hepatic failure and coma where gonadotropin levels are markedly reduced (Zietz et al. 2003).

Apart from direct alcohol toxicity on the testis (Villalta et al. 1997), the major pathogenic factors for reproductive effects of liver disease are the loss of hepatic parenchyma and portocaval shunting (leading to possible cerebral dopamine excess), but their relative roles remain to be clarified. In general, the amount of residual functional liver tissue correlates with the degree of biochemical androgen deficiency. Similarly, the testicular endocrine dysfunction (Handelsman et al. 1995) and consequences of partial androgen deficiency to the prostate (Jin et al. 1999) are proportional to the severity of the underlying liver function (Kaymakoglu et al. 1995; van Thiel et al. 1981) and are mostly reversed by successful **liver transplantation** (Handelsman et al. 1995; van Thiel et al. 1990; Gariani et al. 2016) despite immunosuppressive treatment (Kantarci et al. 2004).

The beneficial effect of liver transplantation on different hormones and functions was similarly independent of the underlying disease, suggesting that the restoration of liver function itself plays a more important role in reproductive function than the causative agent (Gariani et al. 2016).

Sexual function improves after transplantation, but sexual dysfunction remains common and might be multifactorial in origin (Burra 2009; Huyghe et al. 2008).

Men with chronic viral hepatitis B and C show impaired testicular function (Safarinejad et al. 2010; Hofny et al. 2011). While hepatitis C patients present with a loss of pituitary gonadotropin secretion, especially with severe liver failure (Safarinejad et al. 2010; Sinclair et al. 2015), patients with hepatitis B show increased gonadotropins, suggesting a direct testicular involvement of the infection (Serin et al. 2013). In men with hepatitis C, serum SHBG correlates with the degree of liver fibrosis (Nguyen et al. 2006).

Although low testosterone is an independent predictor of mortality in patients with cirrhosis (Montano-Loza et al. 2012; Grossmann et al. 2012), results of trials with **testosterone therapy** remain inconclusive (Sinclair et al. 2015). Testosterone administration improves the sense of well-being, increases serum proteins, and reduces edema without serious adverse effects (Puliyel et al. 1977; Kley et al. 1979; Gluud et al. 1981; Yurci et al. 2011), but meta-analysis of the best available evidence indicates no significant or sustained clinical or biochemical benefits, except reduction in gynecomastia.

Effects of chronic hepatitis C treatment with interferon-alpha 2b and ribavirin have inconsistent impact on blood testosterone levels. Some studies show a reversible reduction in total testosterone levels without consistent change in LH, FSH, or SHBG levels (Kraus et al. 2005; Sinclair et al. 2015), whereas others showed no or only minor changes in serum testosterone levels (Piazza et al. 1997). A recent prospective study showed that low circulating androgen levels persisted in approximately half of the patients despite successful HCV treatment (Chaudhury et al. 2019).

Systemic **iron overload** due to either genetic or acquired post-transfusional **hemochromatosis** often causes hypogonadotropic hypogonadism due to pituitary iron deposition, causing relatively selective damage to gonadotropes (Noetzli et al. 2012; El Osta et al. 2017). Hypogonadism is the second most common endocrinopathy in patients with hereditary hemochromatosis (HH) after diabetes (Pelusi et al. 2016). **In more advanced disease**, the additional effects of comorbidities further highlight the clinical presentation of androgen deficiency with prevalence rates of 89% in hemochromatosis patients with cirrhosis and 33% when diabetes is present (McDermott and Walsh 2005). This disorder once frequently presented with progressive androgen deficiency in middle-aged men that was readily amenable to androgen replacement therapy or, if fertility is required, gonadotropin therapy. **Testosterone replacement therapy** is often necessary and effective both symptomatically (Kley et al. 1992) and in restoring bone density loss due to androgen deficiency (Diamond et al. 1991). In patients with cardiac morbidity due to myocardial iron overload (Gulati et al. 2014), the role of testosterone treatment remains controversial considering the potential adverse cardiovascular effects of testosterone. Iron overload-induced hypogonadism is usually irreversible, but has occasionally been reversed by iron desaturation from vigorous venesection and/or iron chelation in younger (<40 years) men (Cundy et al. 1993; Hamer et al. 2001). Gonadotropin induction of spermatogenesis is particularly effective in genetic **hemochromatosis,** as the onset of gonadotropin deficiency follows normal puberty, while pulsatile GnRH therapy is ineffective since the pituitary damage means that gonadotropin secretion cannot be induced (Wang et al. 1989). The discovery of the genetic defect in hemochromatosis and development of effective pre-symptomatic family and community screening have dramatically reduced, but not eliminated, the late presentations of genetic hemochromatosis with overt clinical manifestations of hypogonadism. For post-transfusional iron overload, see Sect. 34.3.7.

34.3.3 Respiratory Diseases

Chronic sinopulmonary infections (recurrent bronchitis, bronchiectasis, chronic sinusitis, and/or otitis media) are associated with infertility due to **Young syndrome**, **cystic fibrosis** (CF), and **dyskinetic cilia syndromes** (including **immotile cilia** and **Kartagener syndrome**).

Both Young syndrome and CF have obstructive azoospermia due to the congenital absence of the vas deferens in CF. By contrast, in Young syndrome the epididymis and vas deferens are anatomically intact, but the epididymis becomes obstructed intraluminally by inspissated secretion (Handelsman et al. 1984). In contrast, the excurrent ductular system and sperm output are normal in dyskinetic cilia syndromes/motile ciliopathies, but sperm are immotile due to defects in axonemal function (Neugebauer et al. 1990). In motile ciliopathies, ciliary and flagellar beating is disturbed and can lead to abnormalities of the left–right symmetry of the body (such as situs inversus totalis or cardiac malformations), to chronic upper and lower respiratory disease, and to asthenozoospermia due to dyskinetic or immotile sperm flagella. However, rare variants of the dyskinetic cilia syndromes lacking respiratory disease, with cilial defects restricted to lungs or sperm or with ductular obstruction, have been described (Fliegauf et al. 2007; Munro et al. 1994; Dougherty et al. 2020).

Classical CF, due to mutations in the CFTR gene, is also associated with **delayed puberty** attributable to both chronic illness and suboptimal nutrition from exocrine pancreatic dysfunction. CFTR protein impacts the formation of functional spermatozoa at different levels of the male reproductive system (testis, epididymis, and vas deferens) due to different aspects of fluid and lipid metabolism, but crucially nearly all

(>95%) men with CF have a congenital bilateral absence of vas deferens (**CBAVD**), leading to a retention of spermatozoa in the epididymal compartment and therefore to azoospermia (Yefimova et al. 2019). CBAVD alone is recognized as a primarily genital variant of CF, with most affected men carrying compound heterozygotes for two different CFTR mutations. Approximately 80% of men with CBAVD show mutations in CFTR gene (Patat et al. 2016), with the most frequent mutations associated with CBAVD being the ΔF508 deletion of the CFTR gene and an intron 8 variant (IVS8-5T) (Chillon et al. 1995), but more than 2100 other mutations of this gene have been identified so far (Cystic Fibrosis Mutation Database, most of them being single nucleotide, changes often "private" to a particular family. This profusion of genotypes makes screening for sporadic cases or exclusion of a subclinical genotype in the female partner difficult as it is impossible to screen comprehensively for all mutations, many of which remain undiscovered. However, as ART can regularly achieve paternity from testicular sperm aspiration (Hubert et al. 2006; Popli and Stewart 2007) every child is an obligate CF carrier and well-informed genetic counseling is essential. What determines the clinical pattern of disease (CF vs. CABVD) remains an intriguing biological puzzle (Bienvenu et al. 2020) (see Chap. 27).

About half of the male patients with **sarcoidosis** show normo- to hypogonadotropic hypogonadism independent of glucocorticoid usage (Spruit et al. 2007) overlapping with hypothalamic infiltration causing gonadotrophin deficiency (Fraietta et al. 2013). While epididymo-testicular involvement is rare and can simultaneously occur with testicular cancer (Block and Kava 2017; Chierigo et al. 2019), neural involvement in ~5% can produce hypogonadotropic hypogonadism by pituitary infiltration (Matos 2020; Bullmann et al. 2000; Joseph and Scolding 2009). The lack of specific morphologic findings in the reproductive tract in extragenital sarcoidosis is consistent with an effect of chronic disease **ontogenic regression** mechanism. Whether the low circulating testosterone levels contribute to or are the consequence of fatigue, muscle weakness, and depressed mood remains to be established (Spruit et al. 2007).

Sleep apnea (OSA) is associated with sexual dysfunction (Liu et al. 2015) and lowered testosterone levels without a reflex rise in gonadotropin levels indicative of a central hypogonadotropic mechanism (Grunstein et al. 1989; Bercea et al. 2015; Kim and Cho 2019). The relative contributions of hypoxia and sleep fragmentation to this central hypogonadism remain to be clarified. These effects occur commonly together with obesity and symptoms of metabolic syndrome (Liu et al. 2007), with obesity being considered the main determinant of androgen deficiency (Wittert 2014). Sexual function and/or hypogonadism can possibly be reversed by uvulopalatopharyngoplasty (Santamaria et al. 1988) or mechanical maintenance of upper airway patency without weight loss (Grunstein et al. 1989). However, the majority of studies show that CPAP therapy alone does not ameliorate reduced serum testosterone and gonadotropin levels (Hoekema et al. 2007; Bratel et al. 1999; Macrea et al. 2010; Meston et al. 2003). Testosterone administration can precipitate obstructive sleep apnea in some predisposed obese men (Sandblom et al. 1983) and adverse short-term effects on sleep and breathing (Liu et al. 2003; Liu 2019) by blunting ventilatory drive and/or chemoreceptor sensitivity (Matsumoto et al. 1985; Schneider et al. 1986; Kim and Cho 2019). However, results of RCTs suggest that high-dose and replacement-dose administration of testosterone may have adverse short-term effects on sleep and breathing (Liu et al. 2003; Killick et al. 2013; Hoyos et al. 2012), but improve cardiometabolic function and sexual function (Liu 2019). Nasal CPAP can improve sexual function and quality of life in patients with OSA (Goncalves et al. 2005). Overall, data suggest that nocturnal hypoxia decreases with testosterone replacement only after prolonged exposure. Due to the potential exacerbation of OSA, especially in obese patients with untreated sleep apnea, testosterone replacement should be prescribed with caution and ideally only after rectification of OSA (Payne et al. 2021).

Chronic Obstructive Pulmonary Disease (COPD) is associated with pubertal delay due to chronic illness and systemic corticosteroid therapy, but the reported effects on postpubertal male reproductive function remain inconclusive. The prevalence of reduced serum testosterone levels in COPD lies between 22% and 69% (Kamischke et al. 1998; Atlantis et al. 2013; Van Vliet et al. 2005; Mousavi et al. 2012; Balasubramanian and Naing 2012). The suggested mechanisms include hypoxemia, systemic inflammation, and impact of corticosteroids on hypothalamic–pituitary axis regulation in an underlying **ontogenic regression** mechanism. Testosterone deficiency might exacerbate COPD either via direct effect on respiratory muscles or indirectly via reduced strength and exercise capacity (Van Vliet et al. 2005). For example, among 138 men with COPD, an exacerbation led to decreased circulating testosterone levels (positively correlated with PaO_2) and a reflex increase in gonadotropins, which were normalized in parallel with the improvement in arterial blood gases during recovery (Karadag et al. 2009; Laghi et al. 2005; Makarevich 2003). Some evidence suggests that testosterone treatment can increase muscle and bone mass, strength, and exercise capacity and improve the disease course by slowing disease progression and reducing hospitalizations (Baillargeon et al. 2019; Atlantis et al. 2013; Pan et al. 2014).

34.3.4 Malignant Disease

Cancer and its treatment with cytotoxic drugs or irradiation have dramatic effects on male reproductive function and regularly produces transient or permanent spermatogenic

damage and infertility as well as sexual dysfunction and, less often, androgen deficiency (Howell and Shalet 2005; Kim and Clavijo 2022). Spermatogenic damage particularly concerns the common malignancies of male reproductive life that are medically treated with curative intent during reproductive life comprising testicular (teratoma, seminoma) and hematological (Hodgkin and non-Hodgkin lymphoma) tumors and sarcomas. In order to preserve male fertility, sperm cryopreservation should be an integral part of a comprehensive treatment plan in men receiving gonadotoxic treatment, but it remains underutilized, potentially due to lack of availability of cryostorage facilities or failure to offer cryostorage (Shankara-Narayana et al. 2019; Schover et al. 2002b; Schover et al. 2002a). The majority of men treated in established cancer centers report to be informed about options for fertility preservation (Armuand et al. 2012; Duma and Lambertini 2020), but some men are still not well informed about infertility as a common side effect of cancer treatments (Hohmann et al. 2011).

Virtually all men presenting with cancer demonstrate moderate testicular dysfunction including particularly depression of spermatogenesis even prior to cytotoxic treatment. The pathogenetic mechanisms remain unclear, but contributions from fever, temperature fluctuations, weight loss, diagnostic procedures, or cytokines as part of **ontogenic regression** effects of chronic disease (Ostrowski and Walsh 2015; Marmor et al. 1986). Cancer patients often develop a decrease in muscle mass, anorexia, weakness, fatigue, depression, and sexual dysfunction. Such nonspecific symptoms are difficult to distinguish from symptoms of androgen deficiency. While serum FSH is consistently increased in proportion to the degree of germinal damage from cytotoxic cancer treatments, changes in the circulating levels of LH, T, and SHBG are more variable (Strasser et al. 2006; Greenfield et al. 2007; Garcia et al. 2006). It is likely that a variety of individual factors such as cytokines (IL 6, IGF-1, and others), variations in cytotoxic treatments and opiate-based pain treatment (see Sect. 34.3.13), and other factors influence the net androgen status in male cancer survivors. Among the very few well-controlled studies of testosterone treatment for subclinical androgen deficiency in various male cancers (Loprinzi et al. 1999; Darnton et al. 1999; Howell et al. 2001), the findings do not establish any consistent benefits of testosterone treatment for subclinical Leydig cell failure and further clinical trials would be required before such treatment is justified (Xu et al. 2020).

34.3.4.1 Surgery

Testicular cancer reduces fertility by local and systemic effects (Sineath and Mehta 2019; Ostrowski and Walsh 2015), but unilateral orchidectomy for testis cancer has little consistent effect on sperm output or fertility if the contralateral testis is normal. Pretreatment semen parameters show lower sperm concentrations and lower motility than in other cancers (Williams et al. 2009; Hotaling et al. 2013), which has been suggested to link to tumor histology and tumor size (Moody et al. 2018); however, this apparent difference disappears when accounting for whether sperm output is from only a single testis after tumor orchidectomy (Shankara-Narayana et al. 2019). Despite the possibility that sperm output might sometimes improve after unilateral orchiectomy, sperm cryopreservation before surgery is strongly recommended because the tumor-bearing removed testis might be the main source of sperm production. In men who are azoospermic at presentation, a salvage option for fertility preservation is sperm retrieval via microdissection testicular sperm extraction at the time of orchidectomy (onco-microTESE) (Moody et al. 2019). While testosterone levels might remain unchanged after orchidectomy due to a concomitant LH increase in some men (Petersen et al. 1999; Nord et al. 2003), other studies show that the risk of post-treatment hypogonadism is increased in patients with additional gonadotoxic therapies (Wiechno et al. 2017; Bandak et al. 2016; Bandak et al. 2018).

Radical **prostatectomy** or **rectal cancer** surgery can produce high rates of post-surgical erectile dysfunction. Improved surgical techniques, such as nerve-sparing surgery or unilateral instead of bilateral radical retroperitoneal lymph node dissection with or without robot assistance, are claimed to improve post-surgical erectile and ejaculatory functions to reduce the prevalence of dry ejaculation and erectile dysfunction as well as urinary incontinence (Capogrosso et al. 2019); however, the success of such procedures varies between reports. Different strategies exist for restoration of erectile function after prostatectomy such as the prolonged use of PDE-5-inhibitors, vacuum erection devices, prostaglandin injections into the corpora cavernosa, psychotherapeutic interventions, pelvic floor muscle training, and extracorporeal shockwave therapy (Feng et al. 2022).

34.3.4.2 Chemo-/Radiotherapy

Treatment with combination chemotherapy and/or therapeutic irradiation virtually always causes azoospermia and infertility. However, considering the steady improvement of cancer survivorship, quality of life becomes increasingly important, including the different facets of reproductive health, such as fertility, sexual health, and androgen status. The duration of azoospermia and the degree and rate of spermatogenic recovery vary according to the regimen used from full (e.g., cisplatin/vinblastine/bleomycin for teratoma), partial (e.g., combination chemotherapy for sarcoma), and dose-dependent (e.g., pelvic irradiation with testicular shielding for seminoma) reversibility over several years after treatment to essentially irreversible sterilization (e.g., MOPP for Hodgkin disease (Okada and Fujisawa 2019), but as methods to predict the loss of fertility remain imperfect, appropriate

counseling with the offer to cryopreserve sperm should be offered (Oktay et al. 2018) as part of comprehensive cancer care (Shankara-Narayana et al. 2019). In men with Hodgkin's disease the otherwise inevitable sterilization from a standard course of MVPP or MOPP may be avoided by using fewer cycles of MOPP, MOPP alternating with the ABVD regimen (doxorubicin, bleomycin, vinblastine, and dacarbazine) or ABVD alone, each of which features equivalent therapeutic efficacy with less spermatogenic toxicity. This is most likely due to the inclusion in MOPP of alkylating agents, which are particularly potent gonadotoxins (Paoli et al. 2016; Amin et al. 2021).

Chemo-therapeutic regimens used to treat non-Hodgkin lymphomas vary greatly, depending on type of lymphoma, grading, staging, and patient factors; overall, however, they are less gonadotoxic than regimens commonly used for Hodgkin disease. This is most probably due to the absence of the potent gonadotoxins, the alkylating agents procarbazine and dacarbazine. However, gonadal dysfunction remains an important treatment side effect during survivorship and fertility preservation should be offered to all patients who have not definitely completed their families (Sapkota and Shaikh 2020; Shankara-Narayana et al. 2019).

The testis is exceptionally sensitive to ionizing irradiation, with single doses of 20 rads (cGy) causing azoospermia and time-to-recovery being proportional to dose (Rowley et al. 1974). Effective testicular shielding can greatly reduce testicular dosage during pelvic irradiation, but the scatter doses (typically ~2% of dose) still well exceed the threshold for spermatogenic damage (<0.5% of dose). In contrast to other tissues, dose fractionation enhances spermatogonial killing (Meistrich and van Beek 1990; Meistrich 2013). However, Leydig cells are less sensitive to irradiation and no resulting damage is recorded in prepubertal boys given doses of up to 20 to 30 Gy doses in men (Shalet et al. 1989).

Total body irradiation (TBI) given for conditioning prior to allogeneic hematopoietic stem cell transplantation (HSCT) causes severe spermatogenic damage (Mathiesen et al. 2020). Spermatogenic recovery is more likely with younger men (<25 years of age) at HSCT, with chemo rather than radioablation of host marrow and when they remain free of chronic graft-versus-host disease (Rovo et al. 2006). A study evaluating long-term survivors after HSCT reported that 19% of male patients show hypogonadotropic hypogonadism 1.5 years after HSCT, but testosterone levels normalized after 3 years (Somali et al. 2005).

Thyroid cancer patients who receive a single or few standard dose of radioiodine ^{131}I experience usually only a transient impairment of spermatogenesis (Handelsman and Turtle 1983; Pacini et al. 1994; Rosario et al. 2006; Rosario et al. 2008).

Besides pretreatment sperm cryostorage (Kelleher et al. 2001; Kamischke et al. 2004; Shankara-Narayana et al. 2019), measures to protect reproductive health during the treatment include minimizing gonadotoxic or using non-gonadotoxic chemotherapy, maximal testicular shielding during radiotherapy, nerve-sparing operative techniques, and in the future, possibly autologous germ cell transplantation (Schlatt 1999). More speculative options for the future, especially for prepubertal boys, include ectopic xenografting or autografting of testicular tissue, in vitro maturation of testicular tissue, or the use of pluripotent embryonic stem cells. Experimental hormonal cytoprotection treatments using either steroids and/or GnRH analogs to inhibit testicular function during chemotherapy have shown only limited promise in experimental models (Crawford et al. 1998) or preliminary human or nonhuman primate studies (Meistrich and Shetty 2008).

Pretreatment testicular dysfunction is one constraint on sperm cryopreservation, although most men including adolescents as young as 14 years can cryostore sperm if they wish (Shankara-Narayana et al. 2019; Goossens et al. 2020; Picton et al. 2015). Sperm cryopreservation for men without completed families represents a cost-effective, psychologically reassuring preparation for recovery from treatment. As a form of fertility insurance, only limited use of cryostored material in artificial insemination or male factor IVF/ICSI may be expected. Nevertheless, sound medical care involves ongoing follow-up, including appropriate advice on contraception, prognosis for cure, for recovery of fertility, and potential use (or discarding) of stored sperm (see Chap. 24).

A frequent concern among cancer survivors is the risk of birth defects in their post-treatment offspring. The most comprehensive data available indicate that there is no increased teratogenic or genetic risk to such offspring (Arnon et al. 2001). In this context, elective cryopreservation of sperm prior to cancer therapy avoids exposure of sperm to potential genetic effects of cytotoxic therapy although such sperm continue to be exposed to ambient environmental irradiation during cryostorage. Nevertheless, increased aneuploidy (Robbins et al. 1997; Genesca et al. 1990) and/or in vitro DNA damage (O'Flaherty et al. 2008) in human sperm have been reported in men with cancers. The disparity between these in vitro apparently adverse findings on sperm DNA integrity with the negative clinical observations suggests that natural embryo (and possibly sperm) selection and surveillance mechanisms may protect couples naturally against teratogenic and/or genetic risks from paternally mediated cancer treatments at the cost of possibly increased miscarriage rates.

Recent years brought a major improvement in the survival and quality of life of childhood cancer survivors, but infertility remains a consequence of prepubertal gonadotoxic therapies. As sperm freezing is not possible in prepubertal boys, freezing of testicular tissue with the idea of later retransplantation of spermatogonial stem cells in the testis or in vitro spermatogenesis might restore male fertility in the future has

been suggested (David and Orwig 2020; Gul et al. 2020); however, established methods have not been developed for either option and these remain experimental options suitable for well-controlled clinical trials but not general use.

34.3.4.3 Immunotherapy

While preliminary data on the use of anti-inflammatory monoclonal antibodies (TNF-alpha blockage) appears to be safe for male reproductive function, data regarding the use of monoclonal antibodies in human cancer treatment are still scarce (Semet et al. 2017; Walter et al. 2016). Despite some optimism regarding the impact on male and female fertility (Duma and Lambertini 2020), impaired fertility has been reported experimentally with the use of the anti-EGFR (epidermal growth factor) cetuximab, which intensifies cisplatin-induced testicular toxicity in experimental mice (Levi et al. 2016). Immune checkpoint inhibitors (ICIs), which are expected to play an increasing role in the treatment of solid tumors, may affect reproductive health by causing immune reactions at several levels of the HPG axis; however, preliminary findings are reassuring. Hypophysitis is a common side effect in PD-1 and PD-L1 inhibitors (<1–3%) with a high incidence of 11% with CTLA-4 inhibitor ipilimumab treatment (Caturegli et al. 2016; Postow et al. 2018), effects which might lead to hypogonadism and impaired spermatogenesis/spermiogenesis (Rabinowitz et al. 2021). Anti-PD1-induced immune orchitis with hypergonadotropic hypogonadism was reported after treatment with nivolumab (Brunet-Possenti et al. 2017) and pembrolizumab (Quach et al. 2019).

34.3.5 Neurological Diseases

Sex steroids exert profound effects on brain development, sexual brain differentiation, reproductive behavior, and cognition. Some prenatal hormone imbalances are linked to neuro-psychological disorders such as the association between elevated testosterone levels with Tourette syndrome and obsessive–compulsive disorder (Alexander and Peterson 2004) or that between high prenatal estrogen levels and schizophrenia (Brown Jr. 2011); however, the causality of these observational findings remains unclear. The mechanism by which neurologic diseases at adult age may produce testicular dysfunction remains only partially known. In addition to the dominant effects of hypothalamic regulation of pituitary–testicular function via GnRH secretion, there is also evidence for direct neural regulation of testicular function (Selvage et al. 2006) and direct sex steroid effects on diverse brain functions including effects on cerebrovascular function, which might play an important role in neurodegenerative disorders (Abi-Ghanem et al. 2020).

The presence of androgen receptors in the central and peripheral neural system and the link between hypogonadism and certain neurodegenerative diseases suggests that androgen replacement therapy might influence disease course, as suggested in several clinical and experimental studies (Bianchi et al. 2020). However, valid data are scarce as described below.

34.3.5.1 Genetic Disorders

Myotonic dystrophy, an autosomal dominant multi-system disorder, is the most frequent inherited muscle disease of adults and is associated with reduced fertility, testicular atrophy, hypospermatogenesis, sexual dysfunction, and hypergonadotropic hypogonadism with up to two-thirds of affected men (Antonini et al. 2011; Peric et al. 2013; Orngreen et al. 2012) with an age-dependent increase in prevalence (Dahlqvist et al. 2015) and an inverse correlation of testosterone with body fat mass (Passeri et al. 2015).

Myotonic dystrophy type 1 (DM1) is the most common form and is due to a polymorphic expansion of tandem CTG triplet codon repeats (>35) in the 3′ untranslated region of the human dystrophia–myotonica–protein kinase (DMPK) gene on 19q13, showing a correlation between a longer repeat with more severe symptoms and an earlier disease onset displayed by intergenerational anticipation (Andre et al. 2018). Male fertility is reduced due to defective capacitation and acrosome reaction (Hortas et al. 2000), but the use of ART offers good reproductive outcome if involving well-informed genetic counseling with the option for preimplantation genetic diagnosis (Verpoest et al. 2008). Pharmacological testosterone treatment does not improve muscular strength despite increasing muscle mass (Griggs et al. 1989; Vazquez et al. 1990), presumably by making more dystrophic rather than normal muscle. The failure to improve disease course means that testosterone treatment has no specific role in the treatment of myotonic dystrophy patients (Schoser et al. 2019).

The genetic basis of **Kennedy disease** (late-onset, X-linked recessive bulbospinal muscular atrophy) has been identified as a variable increase in numbers of CAG triplet repeats beyond the physiological range in the first exon of the androgen receptor (AR) in a region coding for its non-binding, N-terminal domain (La Spada et al. 1991; La Spada 1993). The loss of lower motor neurons in the brain stem and the spinal cord leads to progressive neuromuscular atrophy beginning in the hips and shoulders, progressing to brainstem-innervated bulbar muscles, which results in difficulties in walking, speaking, and swallowing and eventually often in death associated with respiratory difficulties (Breza and Koutsis 2019). In addition, there is a subtle evidence of acquired mild androgen resistance including gynecomastia, androgen deficiency symptoms, testicular atrophy, and oligo-/azoospermia. The pathogenesis is still not clearly

understood, but the binding of testosterone to the poly-Q-androgen receptor with the major factors being either a toxic gain-of-function effects like other neurodegenerative diseases with or without defects in androgen receptor function (Warner et al. 1992; Simanainen et al. 2011). The number of tandem CAG triplet repeats in the mutant AR correlates with disease severity and age of onset (Atsuta et al. 2006) and alters the function of the disease protein after testosterone binding to the mutant AR. The structure and function of this toxic disease protein alter several cellular processes, such as protein folding, transcriptional regulation, and mitochondrial function. While androgen deprivation by GnRH agonists is reported to reduce disease progression (Hijikata et al. 2019; Hashizume et al. 2019), future therapeutic approaches could be a combination therapy involving anti-androgens with drugs that reduce the ligand-induced alterations of the mutant disease protein (Parodi and Pennuto 2011). However, while testosterone therapy was once considered possible (Goldenberg and Bradley 1996; Neuschmid-Kaspar et al. 1996), its use is not recommended as there is little firm evidence of benefit and some experimental evidence that it may be deleterious (Kinirons and Rouleau 2008).

Huntington's disease (HD) is an adult-onset neurodegenerative disorder, which is characterized by motor, neuropsychiatric, and cognitive abnormalities due to an expanded CAG trinucleotide repeat in the HD gene on chromosome 4p16.3, which codes for Huntington (htt) (MacDonald et al. 1993). Individuals affected by Huntington's disease have decreased levels of total testosterone and LH correlating with disease severity (Markianos et al. 2005; Saleh et al. 2009), although a recent small study of a 24-h neuroendocrine profile showed no difference between HD patients and healthy controls (Kalliolia et al. 2015). FSH levels and fertility did not differ between HD patients and controls (Mastromauro et al. 1989; Pridmore and Adams 1991; Saleh et al. 2009), while a histopathological postmortem evaluation of testes from four HD patients showed a decreased number of germ cells, and an abnormal seminiferous tubule morphology and concordant finding of late-onset testicular dysfunction in mouse models of the disease (Van Raamsdonk et al. 2007). Gene therapy using CRISPR-Cas 9n-technology remains the most promising treatment method on the horizon (Dabrowska and Olejniczak 2020), but the role of androgens and testosterone therapy still has some unexplored potential (Ransome 2012; Bianchi et al. 2020). Experimental data suggest that testosterone therapy surprisingly prevents testicular atrophy and might improve spermatogenic function (Hannan and Ransome 2012). The reason that Kennedy and Huntington's diseases caused by heritable unstable DNA replication all manifest testicular dysfunction remains unclear, but interference with the efficiency of meiotic replication is likely to impair spermatogenesis.

A variety of other rare genetic neurological disorders involving multiple congenital defects are associated with hypogonadotropic hypogonadism, presumably due to defective neural circuitry involving the hypothalamic GnRH neurons and/or their pulse generator (see Chap. 13). These eponymous syndromes include the **Prader–Labhart–Willi syndrome** of mental retardation, hypotonia, short stature, and obesity caused by deletions or uniparental disomy of chromosome 15 (Eiholzer et al. 2006; Crino et al. 2003), the **Laurence–Moon–Biedl syndrome** of retinitis pigmentosa, obesity, mental retardation, and polydactyly or other dysmorphic features, **Friedreich and other cerebellar ataxia syndromes**, **multiple lentigines syndrome**, **steroid sulfatase deficiency** (X-linked congenital ichthyosis), and other rare congenital neurological syndromes like Woodhouse–Sakati syndrome (Schneider and Bhatia 2008), **Moebius, RUD, CHARGE, Lowe, Martsolf, and Rothmund--Thomson, Borjeson–Forssman–Lehman** syndromes (Rimoin and Schimke 1971). Other non-eponymously named conditions may cause hypogonadotropic hypogonadism (Wong et al. 2017). Such patients may require androgen replacement although social factors usually dictate that fertility requiring gonadotropin induction of spermatogenesis is rarely requested.

34.3.5.2 Acquired Disorders

Several studies suggested an association between low blood testosterone levels and cognitive decline. However, most prospective studies fail to confirm a consistent association between sex steroid levels and cognitive decline or **Alzheimer's disease** in aging men (Kaufman et al. 2019). Furthermore, reverse causation, due to impaired cognitive function leading to reduced physical and other activities, is at least as likely a causative mechanism as any effect of reduced testosterone (Hsu et al. 2015). Furthermore, the largest prospective placebo-controlled study of the impact of testosterone on cognitive function in men was the Testosterone Trials Study in which 788 men were treated for 12 months with transdermal testosterone or placebo gel and found that testosterone treatment did not improve memory or cognitive function (Resnick et al. 2017; Snyder et al. 2018).

Despite gender differences in incidence and phenotype (Heller et al. 2018), the age-related decline of testosterone levels in aging men with **Parkinson's disease** (PD) was reported to be similar to control groups without dementia or neurodegenerative diseases (Okun et al. 2004). The suggested exacerbation of PD symptoms in patients by androgen deprivation for prostate cancer (Shahinian et al. 2006) was not confirmed in a larger population of PD patients with prostate cancer undergoing androgen deprivation (Chung et al. 2016). Some case reports showed the beneficial effects of testosterone treatment in patients with PD (Mitchell et al. 2006; Okun et al. 2002). However, a placebo-controlled RCT

of testosterone treatment reported no benefit on any Parkinson's clinical features in affected men although the suboptimal clinical trial reporting leaves the finding inconclusive (Okun et al. 2006). Dopaminergic medications in patients with early Parkinson's disease were reported in an observational not to reduce serum testosterone levels, but the finding was inconclusive due to the observational nature and limited testosterone measurements (Okun et al. 2014).

Although women are three times more likely to develop **multiple sclerosis (MS)** than men**,** the disease course in men is more progressive with more brain atrophy, disability, and cognitive impairment. It is speculated that this sex difference may be due to the influence of sex steroids on inflammation and neurodegeneration in MS (Gold and Voskuhl 2009). Between 25% and 39% of men with **multiple sclerosis** (MS) have decreased circulating testosterone levels with low or normal LH and FSH levels consistent with the ontogenic regression mechanism (Bove and Chitnis 2013; Safarinejad 2008a) and probably attributable to the nonspecific effects of the underlying chronic inflammatory process. There is high prevalence of sexual dysfunction between 50% and 90%, which can have multiple causes including spinal demyelinization, fatigue, spasticity, bladder, and bowel dysfunction as well as psycho-emotional reasons including depression and performance anxiety (Previnaire et al. 2014).

Sperm count, motility, and morphology are impaired in patients with MS (Safarinejad 2008a) and a recent Danish register-based study suggested that MS is linked to infertility, possibly due to autoimmune factors, shared genetic etiology, or disturbances in the hypothalamic-–pituitary–gonadal axis (Glazer et al. 2018). The correlation between infertility and autoimmune diseases was also found for other diseases, such as psoriasis, thyroiditis, and rheumatoid arthritis (Brubaker et al. 2018), but the precise pathophysiological mechanism underlying this correlation remains unclear. The preconceptional use of MS therapies such as interferon beta and glatiramer acetate was not linked to adverse neonatal outcomes (Fragoso et al. 2018).

Estrogens and androgens are reported to have potential neuroprotective and neuroregenerative effects, and the promising results of experimental and small clinical studies should be confirmed in further studies (Bianchi et al. 2020; Collongues et al. 2018). A small pilot clinical trial of testosterone treatment in male MS patients showed a stalling and even reversing effect of testosterone on the neurodegenerative processes (Kurth et al. 2014).

Temporal lobe epilepsy is associated with hypogonadism and sexual dysfunction, whereas other forms of epilepsy have little known systematic impact on testicular function (Bauer et al. 2004; Keller et al. 2012; Hamed 2016). Clinical and experimental animal data suggest that epileptiform discharges in the temporolimbic system alter the hypothalamic–pituitary regulation of GnRH and gonadotropin secretion. Temporal lobe surgery normalizes blood testosterone values (Bauer et al. 2000). Low blood testosterone levels and hyposexuality are common in anticonvulsant-treated epileptics (Fenwick et al. 1985; Isojarvi 2008; Harden and Pennell 2013; Reis et al. 2013; Svalheim et al. 2015). Anticonvulsants increase hepatic SHBG secretion, leading to decreased metabolic clearance rate for testosterone together with increases in total testosterone and gonadotropins. Semen parameters seem little affected in untreated and phenytoin-treated epileptic men, although data are scarce (Taneja et al. 1994).

Sperm output remains normal, but morphology and motility are impaired during long-term treatment with phenytoin (Schramm and Seyfeddinpur 1980). While estrogens seem mainly to be proconvulsant whereas progesterone and its metabolites or analogs have anticonvulsant effects, testosterone has mainly antiseizure effects in experimental animal models, but the effects on experimental seizures in humans are mixed (Sivaraaman and Mintzer 2011; Tauboll et al. 2015). The role of metabolization of testosterone remains to be further elucidated, but aromatization of testosterone leads to the proconvulsive estrogen, 17beta-estradiol, while the 5 alpha-reduced metabolites, DHT and androstanediol, have potent anticonvulsive effects (Frye 2010). However, larger controlled clinical studies of the effects of androgen administration on seizure control or androgenic status would be of interest.

Spinal cord damage (SCD) from trauma or neurological disease causes testicular dysfunction depending in severity on the level and extent of spinal cord interruption (Bauman et al. 2014; Sullivan et al. 2017; D'Andrea et al. 2020). Testicular function is disrupted by aberrant thermoregulation, recurrent ascending urinary tract infections from bladder catheterization, and neurogenic dysfunction and iatrogenic factors (diagnostic irradiation, drugs). Hypospermatogenesis and testicular atrophy are usually observed in men with long-term spinal injuries, but fertility may be preserved by timely sperm cryopreservation using electroejaculation coupled with assisted fertilization (Stoffel et al. 2018). Impotence is predominantly due to the interruption of neural pathways controlling erection and emission, while libido remains appropriate for age. Conservation of sexual function depends upon the level and extent of the spinal injury.

Testosterone replacement in hypogonadal men with spinal cord damage has neuroprotective effects and improves nerve regrowth (Wang et al. 2020; Gorgey et al. 2020a; Gorgey et al. 2020b), body composition parameters (Nightingale et al. 2018), and metabolic parameters (Bauman and Spungen 2008), but due to the lack of well-designed studies to assess the risk of testosterone treatment in patients with spinal cord injury, testosterone replacement has mainly been used in hypogonadal SCD patients with sexual dysfunction (Lim et al. 2020).

Head injuries may cause gonadotropin deficiency due to disruption of the pituitary portal bloodstream and/or pituitary infarction following basal skull fractures.

Sex steroids may play a role in the pathogenesis of various psychiatric disorders, which show sex differences in prevalence, symptom profiles, or disease course, and some psychiatric diseases, such as **schizophrenia**, have their first onset only after puberty. Data about the correlation between testosterone and schizophrenia are inconsistent, with some studies suggesting that low testosterone concentrations are associated with negative symptoms probably representing nonspecific consequences of reduced physical and sexual activity (Kaneda and Fujii 2000; Akhondzadeh et al. 2006; Brzezinski-Sinai and Brzezinski 2020; Misiak et al. 2018; da Silva and Ravindran 2015).

Dysthymic disorders in elderly men are associated with low blood testosterone levels when compared with men with no depression or major **depression** (Seidman et al. 2002). Androgens seem to have a protective neurobiological effect against anxiety and depression (McHenry et al. 2014), and testosterone therapy in men with low serum testosterone concentration improves mood and depressive symptoms in most studies, except in patients with major depression (Vartolomei et al. 2020).

The sex-specific preponderance of primary headaches, such as **migraine, tension-type headache,** and **cluster headache,** suggests a link to sex steroid hormones with migraine and cluster headache having been associated with low blood testosterone (Shields et al. 2019; Delaruelle et al. 2018). The modest therapeutic benefit of testosterone treatment (Stillman 2006) observed in small numbers of patients represents probably the nonspecific mood-elevating effects of testosterone treatment.

34.3.6 Gastrointestinal Diseases

Coeliac disease (CD) is associated with delayed puberty, subfertility, impaired sperm output, morphology, and motility together with elevated blood testosterone, SHBG, and gonadotropin levels, whereas dihydrotestosterone levels are decreased (Bona et al. 2002). These changes are reversible upon dietary elimination of gliadin, leading to improvement of the gluten enteropathy or decrease in small bowel atrophy. Nevertheless, some symptoms such as fatigue may persist despite optimal dietary control of CD. According to a recent case–control study, these persistent symptoms reflect rather non-responsive CD symptoms rather than signs of androgen deficiency (Kurada et al. 2016). The distinctive endocrine pattern is suggestive of acquired androgen resistance (e.g., high testosterone levels which fail to suppress LH secretion) and/or inhibition of 5-α reduction; because 5-alpha reductase type 1 is present also in the gut, it is hypothesized that the loss of enzyme activity in the small bowel contributes to low DHT levels (Ozgor and Selimoglu 2010; Matsumoto 2015). However, detailed studies of androgen receptor function or action (Farthing and Dawson 1983) are lacking. Opioidergic hypothalamic activation by gluten peptides has been suggested as a mechanism that deranges the gonadal axis.

Inflammatory bowel disease (IBD) is often associated with a delayed puberty, possibly more frequent in Crohn's disease than in ulcerative colitis. Serum gonadotropin levels are inconsistently reported to be both increased and decreased. The underlying mechanism may involve a combined effect of undernutrition and cytokines such as TNF-α, interleukin-1β, and interleukin-6. These findings are consistent with the therapeutic finding that anti-TNF-α treatment improves not only disease markers but also pubertal development and the function of the pituitary–gonadal axis (DeBoer et al. 2016).

Postpubertal testicular endocrine function is often unaffected (Farthing and Dawson 1983), but spermatogenesis is frequently impaired, leading to subfertility (Ananthakrishnan et al. 2019). Hypospermatogenesis in **Crohn's disease is** possibly related to fever, chronic illness, inflammatory mediators, and/or nutritional status (Farthing and Dawson 1983). Similarly, men with **ulcerative colitis** taking salazopyrin exhibit impaired spermatogenesis, sperm function, and male fertility (Cosentino et al. 1984). Routine use of salazopyrin for both acute and preventive maintenance therapy early in the course of **ulcerative colitis** in some countries has precluded studies of testicular function in untreated men to determine the extent of effects of ulcerative colitis per se. Sulfasalazine/salazopyrin is correlated with lower sperm count, lower sperm motility, and higher risk of oligospermia (Banerjee et al. 2019), but semen parameters improve in patients switching from salazopyrin to 5-aminosalicylic acid (Zelissen et al. 1988). Consequently, other clinical issues being equal, men who have not completed their families should be treated with the latter drug. Patients with IBD have a higher frequency of antisperm antibodies (ASA), possibly due to increased intestinal permeability and immunization against antigens of the intestinal flora (Dimitrova et al. 2005). ASA show cross-reactivity to antigens of spermatozoa and some microorganisms, as physiological and pathological genital and gastrointestinal flora. ASA are also increased after **diarrheal diseases** in shigellosis and salmonellosis (Kalaydjiev et al. 2007).

Testosterone treatment in hypogonadal patients with Crohn's disease can have a positive effect on disease course and markers of inflammation (Nasser et al. 2015), but further well-controlled studies evaluating the effect of testosterone treatment for IBD in adult males are needed. The use of short-term testosterone therapy to induce puberty is not evaluated either, but as in other chronic diseases associated with

delayed puberty, a carefully monitored testosterone therapy seems reasonable.

The differentiation between disease effects and the effect of anti-inflammatory drugs on reproductive function is difficult, and data from available studies remains inconclusive so there is a need for better-controlled studies. So far, the effect of anti-inflammatory cytokines, such as the TNF-α inhibitor adalimumab on reproductive function, seems to have no adverse effect on semen quality (Grosen et al. 2019) or outcome of offspring if used before conception (Larsen et al. 2016).

Peptic ulceration has no reported effects on testicular function, although treatment with the H_2 receptor blocker cimetidine impairs testicular function by androgen receptor antagonism unrelated to its H_2 receptor blocking activity (Knigge et al. 1983). These effects are not observed with ranitidine and other H_2 receptor blockers or other anti-acid drugs.

34.3.7 Hematological Diseases

Hemoglobinopathies are associated with delayed puberty. In regularly transfused children with t**halassemia,** transfusion-induced iron overload leads to acquired gonadotropin deficiency functionally like that of genetic hemochromatosis (see Sect. 34.3.2). Prepubertal onset of iron chelation therapy enhances pubertal maturation, presumably by preventing **pituitary siderosis** (Bronspiegel-Weintrob et al. 1990).

A large proportion of adult men **with β-thalassemia major** suffer from hypogonadotropic hypogonadism and impaired semen parameters (Safarinejad 2008b; Kidson-Gerber et al. 2008) probably due to pituitary iron accumulation (Noetzli et al. 2012)**.** Systemic and seminal iron overload may predispose sperm to oxidative injury and to increased DNA damage (Perera et al. 2002) and is linked to impaired sperm parameters (Skandhan et al. 2012; Singer et al. 2015). Patients with **sickle cell anemia** show consistently delayed growth, sexual maturation, and puberty (Martins et al. 2019). Data about the reproductive disruption are conflicting (Huang and Muneyyirci-Delale 2017) with some studies suggesting a primary testicular problem with high gonadotropin levels and a normal gonadotropin response upon GnRH stimulation (Abbasi et al. 1976) and others describing secondary hypogonadism with poor spermatogenesis (Agbaraji et al. 1988). The variation may depend on the predominant location of the ischemic micro-infarcts due to episodes of sickling. A recent study in adult men aged 18–39 years shows similar basal gonadotropin levels between patients and controls, but an increased response to GnRH, which might be due to a mechanism of peripheral androgen resistance (Martins et al. 2019). While the reproductive effect of newer, innovative therapeutic concepts for sickle cell anemia are not yet evaluated (Salinas Cisneros and Thein 2020), hydroxyurea therapy, used to treat or prevent sickling crisis, can have an adverse, but reversible effect on male fertility, even in low-dose-therapies (DeBaun 2014; Sahoo et al. 2017) with an impairment of spermatogonial DNA methylation (Portela et al. 2020). Hydroxyurea therapy in prepubertal boys leads to a significant reduction in spermatogonial stem cells in testicular biopsies (Stukenborg et al. 2018). Priapism is a complication in patients with sickle cell anemia (Arduini and Trovo de Marqui 2018), presumably related to corporal thrombosis, although the claims of an increase in priapism incidence during testosterone treatment remain controversial (Morrison et al. 2013). Boys with sickle cell anemia prepubertally treated with hematopoietic stem cell transplant using alkylator-based conditioning regimens showed normal serum testosterone 7.9 years after therapy (Elchuri et al. 2020).

Small studies show that **iron deficiency anemia** is linked to negative effects on the testicular function with low gonadotropins and impaired sperm parameters. The impairment is reversible upon normalization of hemoglobin levels after iron replenishment (Mehta et al. 2018; Soliman et al. 2014). **Folate or vitamin B_{12} deficiencies** can lead to **megaloblastic anemia** and are known to inhibit DNA replication in the marrow and might cause arrest of the germinal epithelium; although vitamin B12 deficiency was linked to abnormal semen parameters in infertile men (Pront et al. 2009), there are no reports among men with megaloblastosis available to examine this hypothesis, presumably reflecting the paucity of vitamin B_{12} deficiency in young nonalcoholic men in developed countries. Low seminal folate levels correlate with decreased sperm concentration and reduced sperm DNA integrity (Boxmeer et al. 2009), but the underlying mechanisms of this association and of the positive effects of folate and zinc treatment remain to be elucidated (Wong et al. 2002; Ebisch et al. 2006; Yuan et al. 2017).

Hemophilia can lead to hematospermia and is associated with a striking reduction in male fertility (Francis and Kasper 1983). Whether this striking subfertility is explained fully by voluntary restraint of fertility is unclear as no studies of testicular function in hemophiliacs are available.

Fanconi anemia is an autosomal recessive genetic disorder leading to various symptoms including bone marrow failure with aplastic anemia, head and neck cancers, leukemia, skin manifestations, and diverse congenital malformations. HPG axis disruption and disturbed genital development are frequent with increased prevalence of changes in timing of pubertal (mostly delay), small testes, hypospadias, and small phallic size. Hypogonadism is a frequent finding in Fanconi anemia patients with both hypergonadotropic and hypogonadotropic forms reported (Petryk et al. 2015). Discerning between disease-inherent causes, effects of chronic disease,

and effects of treatments including for cancer or hematopoietic cell transplantation remains difficult (Trivin et al. 2007). Infertility is frequent due to social aspects of the disease load, but also linked to disrupted sperm production due to mutations in the FANC genes. These genes disrupt one DNA repair mechanism in spermatogenesis, the FANCA pathway (Kee and D'Andrea 2012; Larder et al. 2004), and may also disrupt gonadotropin secretion.

34.3.8 Endocrine and Metabolic Diseases

Thyroid disease influences male reproductive function with the most striking effects manifest through changes in circulating SHBG levels (Krassas and Pontikides 2004). Thyroid hormones stimulate hepatic SHBG synthesis so **hyperthyroidism** increases circulating SHBG levels and **hypothyroidism** causes low SHBG levels, changes that are reversed by normalization of thyroid hormone levels (Kumar et al. 1990). Approximately 30% of patients with primary hypothyroidism show TRH-induced hyperprolactinemia (Staub et al. 1992). The rise in SHBG decreases the testosterone clearance rate, resulting in increased total testosterone, estradiol, and gonadotropin levels. The net effect on overall tissue androgen action remains unclear. Clinical features include gynecomastia and galactorrhea, erectile dysfunction, and ejaculatory disorders in a substantial number of cases (Carani et al. 2005). Patients with hyperthyroidism (but not with hypothyroidism) show a significant increase in erectile dysfunction in comparison with age-, BMI-, and testosterone-matched controls (Corona et al. 2012). These symptoms resolve when a euthyroid state is regained. Hypo- and hyperthyroidism are also linked to reduced fertility with sperm motility being the major fertility parameter affected in hypothyroidism (Ambigapathy et al. 2020) and hyperthyroidism (Krassas et al. 2008) (Krassas et al. 2010). Sperm parameters normalize upon establishing euthyroidism (Krassas et al. 2010). The mechanisms by which thyroid hormones influence fertility include indirect effects via pituitary gonadotropin secretion but also direct testicular genomic and non-genomic effects, as thyroid hormone receptors are expressed in the testis including Sertoli, Leydig, and germ cells and in the epididymis and the penis (Carosa et al. 2018; La Vignera and Vita 2018).

Congenital adrenal hyperplasia (CAH), most commonly caused by **21-hydroxylase deficiency,** can lead to secondary testicular failure, impaired spermatogenesis, and infertility (Jaaskelainen et al. 2000; Tiitinen and Valimaki 2002; Engels et al. 2018), although the majority of treated and untreated men are fertile (Urban et al. 1978) but show distinctive effects of late diagnosed or long-term untreated disease such as short stature and/or testicular adrenal rest nodules (Bachelot et al. 2015; Nermoen et al. 2012). The majority of men with nonclassic CAH are asymptomatic (Nandagopal et al. 2011), but even in salt wasters with appropriate follow-up reproductive implications of impaired testicular function are easily overlooked (Bouvattier et al. 2015). Gonadal dysfunction can be due to the inhibition of gonadotropin secretion by high blood concentrations of adrenal androgens, but aberrant adrenal cells in the testes, so-called testicular adrenal rest tumors (TART), can lead to azoospermia and eventually severe testicular damage by obstruction and congestion of seminiferous tubules after stimulation by the chronically elevated adrenocorticotropic hormone (ACTH) (Kim et al. 2017; Pierre et al. 2012; Chihaoui et al. 2016; Claahsen-van der Grinten et al. 2020; Bachelot et al. 2015; Nermoen et al. 2012).

Hypercortisolism from any cause can inhibit testicular function at multiple levels of the HPT axis, leading to a reduction in circulating testosterone and gonadotropin levels, which are reversed by interruption of the exposure to excessive glucocorticoids (Luton et al. 1977). The degree to which androgen deficiency contributes to the catabolic state and symptoms of sexual dysfunction and weakness during hypercortisolism is unclear. The mechanisms involve multiple levels of the HPT axis including inhibition of hypothalamic GnRH secretion, of GnRH-stimulated pituitary LH secretion, and of LH stimulation of Leydig cell testosterone biosynthesis.

The effects of **diabetes mellitus (DM)** on male reproductive function are primarily due to neuropathic, myopathic, or angiopathic complications of type 1 diabetes causing erectile and/or ejaculatory dysfunction (Dunsmuir and Holmes 1996; Gandhi et al. 2017), whereas direct effects of hyperglycemia on testicular function are not well established. Approximately 30–50% of patients with diabetes mellitus type 2 show mildly decreased testosterone levels with low- or low-normal gonadotrophin levels (Dandona and Dhindsa 2011; Grossmann 2011; Holmboe et al. 2016), which reflect obesity-related lowering of blood SHBG and other nonspecific mechanisms (Pitteloud et al. 2005). Subjects with well-controlled type 1 DM by contrast are reported to have normal TT and gonadotropin levels (Tomar et al. 2006; Holt et al. 2014), but poorly controlled type 1 DM leads to hypogonadism via a decreased amplitude and reduced frequency of LH pulses (Lopez-Alvarenga et al. 2002).

Longitudinal studies in men with type 2 DM suggest that lifestyle optimization improves insulin sensitivity and increases testosterone levels via reduction in underlying obesity (Kumagai et al. 2015) with exercise being probably more important than reduced calorie intake (Kumagai et al. 2016).

Although not conclusively established, some data suggest that testosterone treatment in subsets of men with type 2 DM improves glucose metabolism, anemia, bone density, and body composition with an increase in lean muscle mass, sexual function, and overall QoL (Hackett 2019). A

recent, large RCT showed that pharmacological testosterone treatment for 2 years in overweight and obese men with impaired glucose tolerance but no pathologic hypogonadism reduced the proportion of patients developing type 2 DM beyond the effects of a lifestyle program (Wittert et al. 2021).

The impact of diabetes on spermatogenesis and fertility remains controversial. Increased DNA damage in nucleus and mitochondria, impact on HPG axis, oxidative stress, and the modulation of different cellular pathways have been variously proposed to have an adverse impact on fertility (Agbaje et al. 2007; Maresch et al. 2018; Ding et al. 2015) and sperm parameters and reduced semen volume attributable to defective neurally mediated ejaculatory function (La Vignera et al. 2015).

Recent studies have reported a relationship between the **metabolic syndrome** and biochemical androgen deficiency. This has been defined as a positive relationship between low blood testosterone levels and insulin insensitivity manifested clinically as an overlap between hyperglycemia, hyperinsulinemia, and obesity (Corona et al. 2011). While an increasing body of evidence suggests that pharmacological testosterone treatment improves parameters of the metabolic syndrome and additional RCTs such as the T4DM trial (Wittert et al. 2021), further studies are needed in order to change clinical policies (Cunningham 2015).

Eating disorders, such as anorexia nervosa, bulimia nervosa, or binge eating disorders show an increasing incidence in males but can cause profound inhibition of testicular endocrine function (Buvat et al. 1983). While the complex interaction between the HPG axis and body weight, adipocytes, leptin, and neuropeptides such as kisspeptin is mainly studied in females (Childs et al. 2021), epidemiological and clinical data regarding its role in male reproductive health are scarce (Raevuori et al. 2014). Anorectic males show low testosterone levels with low gonadotropin and poor responses to GnRH stimulation. Weight gain is associated with increasing testosterone and LH levels, consistent with the positive correlation with leptin levels (Wabitsch et al. 2001). However, the regulation of leptin secretion by adipocytes shows sex differences, which are also dependent on sex steroids (Jenks et al. 2017). Men show a prepubertal leptin rise, in parallel with pubertal testicular development, before it declines to significantly lower leptin levels, which are approximately 50% of those in women (Childs et al. 2021). Androgens have an inhibitory effect on leptin secretion, and testosterone substitution reduces leptin levels in adult men (Luukkaa et al. 1998). The effects of moderate undernutrition or selective dietary micronutrient deficiencies (e.g., vitamins and cofactors) on human testicular function are less clear (Smits et al. 2019).

Both extremes of body weight are not optimal for reproductive health.

Obesity inhibits testicular endocrine function and has negative effects on spermatogenesis and fertility. There is a bidirectional link between obesity and hypogonadism with hypogonadism leading to increased adiposity and obesity being the cause of low serum SHBG and testosterone with normal LH and FSH (Carrageta et al. 2019). Obese boys show delayed pubertal development with decreased testosterone levels for chronological age, but ultimately normal growth and normal testicular development (Denzer et al. 2007). The exact mechanism of puberty induction remains still unclear, but adipose tissue plays an important role as secreting endocrine triggers (Manfredi-Lozano et al. 2018). The decrease in blood testosterone and SHBG levels is proportionate to the degree of overweight and reversible with weight loss. Blood estradiol levels are increased, probably due to increased aromatase-dependent peripheral conversion of testosterone to estradiol. The latter explains the observation that decreased blood testosterone levels may be partly reversed by an aromatase inhibitor (Loves et al. 2008). The principal pathogenic mechanism of obesity-induced lowering of blood testosterone levels involves reduced hepatic SHBG secretion, which may be speculated as due to functional hyposomatotropism and/or insulin resistance. These hormonal changes are reversed by weight reduction and feature normal LH and FSH (as androgen sensors indicating normal androgen status) without overt clinical features of androgen deficiency. Moderate obesity has little overt effect on male reproductive function but may contribute to the decline of hypothalamus and pituitary testicular function in aging men (Gray et al. 1991; Travison et al. 2007).

Sperm concentration and output are related to the body mass index (BMI) in a U-shaped manner with the highest sperm production in the group with a BMI between 20 and 25 kg/m^2 (Magnusdottir et al. 2005; Kort et al. 2006; Jensen et al. 2004). The pathophysiologic mechanism of reduced spermatogenesis at high or low BMI is not clear, including whether they are direct effects of body weight or indirectly due to associated nutritional and/or lifestyle metabolic factors. Nevertheless, there is little consistent evidence for obesity as a cause of male infertility.

34.3.9 Immune Diseases

Autoantibodies to spermatozoa develop in about 70% of men after vasectomy but have no apparent deleterious effects on general health (Petitti 1986) although they may inhibit sperm function and fertility after vasectomy reversal (Linnet et al. 1981). Sperm autoantibodies are observed in 5% of nonvasectomized infertile men who also have an increased prevalence of other organ-specific autoantibodies (see Chap. 28). Immune complexes of unknown significance have been

observed in seminiferous tubular basement membranes of infertile men.

Most **autoimmune diseases** have a marked (>5:1) female predominance (e.g., systemic lupus erythematosus, chronic active hepatitis, chronic biliary cirrhosis), which remains unexplained, and testicular involvement in immune disease is unusual apart from **polyarteritis nodosa** where testicular biopsy may be diagnostic. **Rheumatoid arthritis** causes prolonged depression of testosterone levels during flares of disease activity with spontaneous recovery during remission (Cutolo et al. 1991). Among men with longstanding rheumatoid arthritis with and without glucocorticoid treatment, there is a high prevalence of impaired testicular endocrine function (Silva et al. 2002; Martens et al. 1994). However, a randomized clinical trial of testosterone substitution showed no benefit on disease activity in men with rheumatoid arthritis (Hall et al. 1996). Testicular endocrine function is normal in men with **ankylosing spondylitis** (Gordon et al. 1986; Stahl and Decker 1978), **systemic lupus erythematosus** (Stahl and Decker 1978), or **osteoarthritis** (Spector et al. 1988). Treatment with TNF-α blocker in patients with ankylosing spondylitis has no negative effect on sperm quality with some studies reporting even an improvement in sperm quality (Perez-Garcia et al. 2020b). However, immunosuppressive drugs can have an impact on reproductive function by altering the HPG axis, by causing sexual dysfunction, or by causing disruption of spermatogenesis and/or motility (Sasaki et al. 2011; Finelli et al. 2021). The common advice to stop MTX 3 months prior to conception is not based on sound evidence of adverse outcomes; rather, it reflects a reliance on the timeframe of spermatogenesis, not on sound health safety reasons (Gutierrez and Hwang 2017). Treatment of immunological diseases with cytotoxic drugs such as sulfasalazine and cyclophosphamide may lead to severe, dose-dependent, and sometimes irreversible spermatogenic damage typical of alkylating agents. As long as data from well-designed studies regarding immunosuppressive treatments remain inconclusive, clinicians have to balance the disease-inherent negative reproductive effects against the concern of drug effects, but available evidence is reassuring regarding pregnancy and offspring outcomes in fathers with immunosuppressive drug exposure before conception (Perez-Garcia et al. 2020b).

Autoimmune orchitis (see Chap. 19) is a rare component of the organ-specific autoimmune cluster and can in its secondary form be associated with purpura Schonlein–Henoch, polyarteritis nodosa of Behçet's disease (Silva et al. 2008). Antibodies against gonadotropin-secreting cells of **autoimmune hypophysitis** causing isolated gonadotropin deficiency or panhypopituitarism are uncommon (Obermayer-Straub and Manns 1998; Cocco et al. 2014). Immune orchitis as a consequence of immune checkpoint inhibitor treatment in cancer patients has been reported after treatment with anti-PD1 nivolumab (Brunet-Possenti et al. 2017) and pembrolizumab (Quach et al. 2019), but conclusive clinical studies are lacking.

34.3.10 Infectious Diseases

Systemic infections often influence testicular function even without causing orchitis or having any direct effect on testes. Many mechanisms are involved, including the effects of fever, of inflammatory mediators such as tumor necrosis factor-α and various cytokines, weight loss, and chronic catabolism. The net effects depend on the severity and duration, as well as the site of infection. However, different microorganisms such as protozoa, bacteria, and viruses can infect the male reproductive tract with bacteria affecting mostly the accessory glands, whereas viruses predominantly impact the testes directly, causing orchitis and some direct impact on spermatozoa (Bhushan et al. 2009). Some viruses are well known to have a tropism for the testes, such as mumps viruses, HIV, ZIKA, and recently SARS-CoV-2 (Liu et al. 2018). As the majority of these microbes can also be sexually transmitted (Gimenes et al. 2014), clinicians have a twofold role: reduction or treatment of infections on reproductive function as well as avoidance of sexual transmission to partners or offspring.

Epididymo-orchitis is rare in prepubertal boys, but occurs in 15–30% of **mumps**-affected pubertal or postpubertal males with 15–30% of cases being bilateral (Philip et al. 2006; Hviid et al. 2008; Rubin et al. 2015), so that, despite the well-known association, overall, mumps only rarely leads to infertility, which can then be managed as other forms of male infertility (Lin et al. 1999). The pathophysiological mechanisms include direct viral infection of the tubules, pressure-induced necrosis of seminiferous tubules due to parenchymal edema within the tight testicular capsule, and strong associated inflammatory reaction. Direct testicular damage is evident in the acute phase with the prominent decrease in blood testosterone and reflex increase in blood gonadotropins prior to the testicular atrophy, which occurs in up to half of the infected men. Reports that treatment of mumps–orchitis with interferon-alpha 2B prevents testicular atrophy and might protect fertility require further verification (Ku et al. 1999), but TNF-α probably plays a relevant role in the disruption of blood–testis barrier in mumps virus infections (Wu et al. 2019). However, vaccination remains the best protection against mumps-associated infertility.

The prevalence of androgen deficiency among **HIV-positive men** treated with highly active antiretroviral therapy (HAART) lies around 20% (Rietschel et al. 2000; Rochira et al. 2011), mostly associated with weight loss and catabolism causing low- to low-normal gonadotropin levels. However, 10–25% of hypogonadal HIV-positive men are

reported to have high gonadotropin concentrations indicative of primary hypogonadism indicative of some additional unexplained direct testicular damage (Matsumoto 2015). With disease progression, LH and FSH levels rise and postmortem evaluation shows testicular atrophy (Salehian et al. 1999). How many of these effects are due to drug treatments remains unclear (Dube et al. 2007).

Androgen deficiency in AIDS is associated with severe weight loss, including loss of muscle mass, consistent with the nonspecific effects of other chronic non-testicular diseases (Grinspoon et al. 1996), but is now rare in the era of HAART treatments.

HIV-associated malignancies such as lymphomas or opportunistic infections such as toxoplasmosis can cause mass lesions disrupting the hypothalamic–pituitary–testicular axis on all levels. Additional factors contributing to hypogonadism in HIV patients include medications commonly used in this group (such as ketoconazole, megestrol acetate, ganciclovir, and spironolactone) or the prevalent use of drugs like opiates, alcohol, and marijuana.

Testosterone treatment in symptomatic HIV-positive men has beneficial effects on the body composition (Bhasin et al. 2007; Bhasin et al. 1998) including bone density (Grant et al. 2019). It reduces depression scores and improves the quality of life in androgen-deficient men with AIDS wasting syndrome (Blick et al. 2013; Grinspoon et al. 1998). As symptomatic AIDS is greatly reduced in the era of HAART treatment, these clinical features are now rarely observed or testosterone treatment is required (Bhatia et al. 2015).

Spermatogenic damage in men with AIDS is almost universal at postmortem (de Paepe and Waxman 1989), whereas spermatogenesis (Crittenden et al. 1992) is unaffected in asymptomatic, HIV-seropositive men (van Leeuwen et al. 2007) but deteriorates with clinical status and treatment (Van Leeuwen et al. 2008a, 2008b). HIV is present in semen as free virions, virions associated with spermatozoa, and virions within leucocytes (Anderson et al. 2010), the latter being considered main vector. Postmeiotic germ cells can carry virions, but lie in the immunoprivileged luminal compartment of seminiferous tubules, where the blood–testis barrier reduces immune attack and contact with antiretroviral therapy. Therefore, the testis could act as viral reservoir (Tung et al. 2017; Liu et al. 2018). Similar to other viruses, the presence of HIV can be detected in the semen of men, in whom virions are undetectable in blood plasma (Lo and Schambelan 2001), suggesting that the testes can be a sanctuary for viruses (Epelboin et al. 2017). However, the presence of viral nucleic acids does not necessarily indicate infectious viruses.

SARS-CoV-2 can lead to COVID-19 disease with a broad spectrum of manifestations ranging from mild respiratory symptoms to severe pneumonia with multi-organ failure and death (Guan et al. 2020). The virus shows a gender preference with multimorbid men being more susceptible for infection, speculatively linked to a higher expression of the ACE2 receptor or the transmembrane protease serine 2 (TMPRSS2) receptors in men, both receptors being the portal of viral entry into the host cell via binding to the spike (S) protein. Their expression in the testes may predispose to testicular involvement subject to there being a viremic phase of SARS-CoV-2 infections (He et al. 2020). The ACE2 receptors are also found on adult Leydig cells, potentially explaining the link between low testosterone levels and COVID-19 infections (Pozzilli and Lenzi 2020).

While COVID-19 patients show a significant impairment of sperm quality, it remains unclear so far whether seminal fluid can lead to a sexual transmission of SARS-CoV-2 (Holtmann et al. 2020; Vahedian-Azimi et al. 2021; Burke et al. 2021), which is known for some other viruses such as HIV, Zika, or Ebola (Foy et al. 2011; Mate et al. 2015).

Despite increasing evidence, the amount of peer-reviewed data does not allow therapeutic consequences for reproductive health in male COVID-19 patients to be concluded at the time point of publication.

Sexually transmitted diseases may influence fertility by impairing spermatogenesis (e.g., ureaplasma urealyticum can cause asthenozoospermia and disruption of DNA condensation) or by causing urethral strictures and epididymo-orchitis (e.g., gonorrhea). The negative impact of male chlamydia infection on a couple's fertility (due to transmission to the female) is well known, but direct influence on male fertility is uncertain, although increased sperm DNA damage and a direct interaction between sperm and chlamydia have been reported (Cunningham and Beagley 2008).

The genital manifestation of **lepromatous leprosy** leads in the majority of affected subjects to testicular atrophy (Gunawan et al. 2020) and hypergonadotropic hypogonadism (Martin et al. 1968), although only a minority shows clinical reproductive symptoms (Gunawan et al. 2020), whereas tuberculoid leprosy rarely leads to hypogonadism, which is mainly secondary to granulomatous disease of the hypothalamus.

Congenital **toxoplasmosis** (Massa et al. 1989) and other congenital infections with cerebral involvement are reported to present as hypogonadotropic hypogonadism. Acute toxoplasmosis in men can precipitate hypogonadotropic hypogonadism either via the rare toxoplasma encephalitis or via mechanisms of severe acute illness. In addition, in oligosymptomatic infections, cytokine-induced inflammatory reaction of the hypothalamus may cause disrupted GnRH release leading to hypogonadotropic hypogonadism with normal response to exogenous GnRH (Oktenli et al. 2004).

Tuberculosis can lead to hypogonadotropic hypogonadism by disruption of the gonadal axis (Post et al. 1994; Kibirige 2014) and may infiltrate the genito-urinary tract, causing epididymitis and consequently infertility. Although

cases are mostly found in developing countries and countries in transition, the diagnostic work-up should consider this possibility, especially in migrant populations (Tzvetkov and Tzvetkova 2006; Al-Ghazo et al. 2005).

Sleeping sickness caused by parasitic infections with Trypanosoma brucei causes an only partially reversible hypogonadotropic hypogonadism, probably due to a combination of direct parasitic infiltration and secondary central effects of a severe systemic inflammatory reaction (Petzke et al. 1996).

Other tropical diseases may cause specific symptoms such as **filariasis,** which can cause hydrocele, or **schistosomiasis,** which can cause hemospermia (Richens 2004).

34.3.11 Cardiovascular Diseases

Throughout most of their lifespan, men are more prone to hypertension than women, except after menopause, when the sex difference is reversed possibly due to the depletion of susceptible men by their premature deaths (Gillis and Sullivan 2016)**. Hypertension** is associated with lowered circulating testosterone levels even after adjustment for age and BMI (Hughes et al. 1989) with an inverse correlation of testosterone to both systolic and diastolic blood pressure (Khaw and Barrett-Connor 1988). Antihypertensive medications are associated with even lower testosterone levels (Suzuki et al. 1988). Testosterone treatment in female-to-male transgender persons leads to small increase in blood pressure (Velho et al. 2017). The small decreases in blood testosterone levels are insufficient to account for the disproportionately high prevalence of erectile dysfunction in treated hypertensive men. This presumably reflects both more advanced atherogenesis and hemodynamic factors rather than hormonal effects of antihypertensive medication (Jaffe et al. 1996).

Observational epidemiological studies show consistently that low testosterone levels are associated with increased rates of cardiovascular disease, but the direction of causality remains unknown. Whether there are any additional factors beyond the nonspecific chronic disease (**ontogenic regression**) effects of moderate lowering of blood testosterone levels due to the presence of acute or chronic cardiovascular disease remains to be established. Some (Khaw et al. 2007; Laughlin et al. 2008), but not all (Araujo et al. 2007; Smith et al. 2005), recent prospective studies show that low serum testosterone levels can predict cardiovascular death. In conjunction with low testosterone levels being associated with some other cardiovascular risk factors, it remains to be elucidated whether low testosterone levels are more than just a universal epiphenomenon reflecting various aspects of chronic cardiovascular disease. At pharmacological concentrations, testosterone has dose-dependent vasodilator properties ex vivo on human and experimental animal resistance arteries (Malkin et al. 2006; Jones et al. 2004).

The question of whether testosterone treatment has an adverse effect on cardiovascular events in men without pathologic hypogonadism remains controversial even after the subject of numerous reviews and meta-analyses reviewing the same limited observational data, e.g., Gagliano-Juca and Basaria 2019; and Corona et al. 2020, (see also Chap. 36). The majority of systematic meta-analyses show small or no increased risk of testosterone treatment on cardiovascular events, but so far the question remains unanswered given the limited power of available studies and preclusion of defining causality from observational studies. One of the testosterone trials investigated the effects of 1 year of testosterone treatment on coronary plaque volume in 138 men. Finding a testosterone-induced increase in non-calcified coronary plaque but without difference in coronary events seen within the 1-year study created a powerful suspicion of an increased risk of testosterone treatment-related cardiovascular events (Budoff et al. 2017). A large safety study mandated by the FDA with a planned enrolment of 6000 men is underway and can hopefully clarify the safety of testosterone treatment in men without pathologic hypogonadism for major cardiovascular events (TRAVERSE study, NCT03518034) (ClinicalTrials n.d.).

There is a strong association between **atherosclerotic cardiovascular disease** and erectile dysfunction. In addition to the classical **Leriche syndrome** (an aortoiliac occlusive disease leading to erectile dysfunction, intermittent claudication, and absent or diminished femoral pulses), there is a high prevalence of known and undiagnosed cardiovascular disease (angina, ischemia, infarction, thrombotic stroke, and peripheral vascular insufficiency) among men with organic erectile dysfunction (see Chap. 16). It is now increasingly understood that the onset of erectile dysfunction is an early sentinel warning event for incipient, but usually still undiagnosed, cardiovascular disease. This forms an important pathological basis for vasculogenic, the most frequent form of organic erectile dysfunction. In addition, the interaction with nitrate therapy for cardiovascular disease creates the most serious adverse effect of **phosphodiesterase-5-inhibitors** in treatment of erectile dysfunction. There is also evidence that atherosclerosis is an important determinant of the testicular degeneration in aging men.

34.3.12 Dermatological Diseases

Skin diseases are linked to reproductive health by impacting testicular function and the HPG axis (Abdel-Naser and Zouboulis 2016). Besides genomic effects, this link can potentially be mediated via partial immunosuppressive effects of testosterone via suppression of the B-cell differen-

tiation and T-cell function (Gubbels Bupp and Jorgensen 2018; Trigunaite et al. 2015), which explain the aggravation of certain skin diseases in hypogonadal patients and higher frequency of autoimmune diseases in women. Sexual function can be impaired by functional and esthetic aspects, for example, in patients with scrotal eczema, hidradenitis suppurativa/acne inversa, or sexually transmitted diseases (STDs).

Systemic lupus erythematosus (SLE) is more prevalent in patients with Klinefelter syndrome (Seminog et al. 2015) and 7.7-fold more frequent in patients with androgen deficiency (Pakpoor et al. 2018). While low gonadotropins and low inhibin B levels are consistent with the **ontogenic regression** mechanism, SLE patients also show decreased sperm parameters and an increased prevalence of antisperm antibodies (Soares et al. 2007; D'Cruz et al. 1994). Patients with dermatomyositis show impaired semen parameters despite normal gonadotropins (Moraes et al. 2008, 2010).

Psoriasis is associated with sexual dysfunction, androgen deficiency, and impaired sperm quality showing a correlation with disease severity in some but not all studies (Caldarola et al. 2017; Eltaweel et al. 2018; Allam et al. 2019; Perez-Garcia et al. 2020a). A pilot uncontrolled study with 15 hypogonadal patients shows an apparent beneficial effect of long-term testosterone treatment on the disease course (Saad et al. 2016). Impaired spermatogenesis correlates with the extent and severity of the disease rather than with methotrexate or corticosteroid treatment (Grunnet et al. 1977). Nevertheless, it is wise to advise patients that immunosuppressive treatments might impair reproductive function (Finelli et al. 2021). The usual recommendation to stop MTX 3 months prior to conception lacks any sound evidence base on adverse outcomes (Gutierrez and Hwang 2017). A large number of often rare genetic syndromes with dermatological manifestations are linked to developmental anomalies of the genitals (Abdel-Naser and Zouboulis 2016).

34.3.13 Other Chronic Diseases

Hereditary angioedema (HAE) is an autosomal dominantly inherited disorder of the complement system caused by mutations of the C1-INH gene on chromosome 11q12, which may lead to deficiency (type 1 HAE) or impaired function (type 2 HAE) of C1 esterase inhibitor. The disease is characterized by episodic edematous attacks mostly of hands and feet, but sometimes also involving genitalia, trunk, face, tongue, and the wall of the bowel and the respiratory system, which can be fatal, if not treated adequately (Frank 2008). Synthetic androgens such as danazol and oxandrolone can reduce the incidence of swelling, but recombinant C1 esterase inhibitors are more effective preventive and acute treatments (Zeerleder and Levi 2016) although androgens remain cost-effective. Although this is a condition where synthetic 17-α alkylated androgens have proven beneficial effects on the clinical course of the disease (Banerji et al. 2008) by increasing circulating C1 inhibitor levels in vivo and in vitro (Falus et al. 1990), the underlying testicular function has been little studied and is probably normal (Thon et al. 2007). This illustrates that effective pharmacological androgen therapy of a disorder does not depend on any underlying testicular dysfunction from that disorder.

Amyloidosis of the testis is rare, occurring mostly as secondary systemic AA amyloidosis. However, massive primary testicular infiltration causing macro-orchidism is reported (Handelsman et al. 1983). Hypogonadism due to testicular amyloid deposition and abnormal semen parameters and infertility are described with spermatogenic impairment potentially occurring from direct amyloid obliteration of intratesticular canaliculi or hypoxia due to occlusion of vessels. The age-dependent and progressive impairment of sperm production can occur quickly with progression from normozoospermia to azoospermia within 24 months (Scalvini et al. 2008; Ozdemir et al. 2002; Delbarba et al. 2020).

Familial Mediterranean fever (FMF) is a disease characterized by recurrent episodes of fever, peritonitis, pleuritis, and arthritis with amyloidosis as one of the main complications. It is associated with testicular germ cell arrest and oligo- or azoospermia (Ben-Chetrit and Levy 2003; Mijatovic et al. 2003). Similarly, adverse effects on spermatogenesis are also reported in **Behçet disease,** a multisystemic inflammatory disease involving the urogenital system featuring genital aphthous ulcers as well as cystitis, urethritis, and epididymitis and with an onset after puberty and strong male preponderance suggestive of a role for androgens in its pathogenesis (Sakane et al. 1999). These effects are most likely attributable to the deleterious effects of recurrent fever on spermatogenesis (Mieusset et al. 1991). Potential additional effects of colchicine, an alkaloid commonly used for the prevention and treatment of arthritic episodes in gouty arthritis, FMF, and Behçet's disease, on the testis remain speculative (Haimov-Kochman and Ben-Chetrit 1998).

Chronic opioid consumption leads to opioid-induced androgen deficiency with decreased testosterone and gonadotropin levels proportionate to net opiate dose but independent of the route of administration (oral, transdermal, intrathecal) or source (prescribed, illicit). For example, intrathecal delivery for chronic pain produces near-castrate testosterone levels in ~90% of cases. The modifying effects of confounding factors such as chronic illness, pain, nutrition, and additional substance use remain difficult to separate or quantify but remain quantitatively small compared with opiate dosage. The pathophysiological mechanisms are predominantly due to μ-opioidergic receptor effects, which, on the one hand, have a strong influence on physiological

patterns of hypothalamic GnRH secretion, most probably leading to decreased GnRH pulsatility (Abs et al. 2000; Rajagopal et al. 2004; Hallinan et al. 2009). On the other hand, the application of the opioid antagonist naloxone leads to an increase in LH pulsatility (Veldhuis et al. 1984; Lofrano-Porto et al. 2007). While short-term application of opioids may cause only a brief suppression of the HPG axis, long-term use of opioids is associated with other effects related to hypogonadism such as sexual dysfunction and loss of bone mineral density. Hence, testosterone treatment warrants consideration; however, two well-designed randomized placebo-controlled clinical trials have shown no postulated benefits from testosterone treatment (Basaria et al. 2015; Glintborg et al. 2020). This clear finding presumably arises because testosterone may overcome the μ-receptor-mediated effects of opioids in suppressing endogenous testosterone, but may not reverse the other non-μ receptor effects of opiates.

Data about sperm production and fertility among chronic opiate users are scarce, but asthenozoospermia is reported among drug addicts and experimental data provide a basis for direct pharmacological opioid effects on spermatozoa, which express δ-, κ-, and μ-opioid receptors (Agirregoitia et al. 2006). Transdermal testosterone replacement in opiate-dependent men treated for nonmalignant pain is reported to improve quality of life, but placebo-controlled studies are lacking (Daniell et al. 2006).

The effects of **recreational drugs** such as marijuana, cocaine, or ecstasy on testicular function are not well understood; few studies have been reported, and none are well controlled for confounding effects of undernutrition, multiple drug usage, and psychological and socioeconomic factors. While the majority of these studies report adverse reproductive effects, clinical experience and some data suggest that recreational drug use has only little adverse impact on testicular function and sperm parameters (Hart et al. 2015).

Chronic heavy **alcohol** intake has multiple, cumulative effects on the male reproductive system due to central effects and irreversible testicular toxicity (La Vignera et al. 2013) as well as indirect toxic effects (e.g., nutritional and hepatotoxicity; see Sect. 34.3.2). Ethanol decreases testicular testosterone production through different modifications in the pituitary–gonadal axis even with moderate, but regular alcohol intake over 30 days (Emanuele and Emanuele 2001; Van Thiel et al. 1983), including an alcohol-induced decrease in LH secretion in adult men (Frias et al. 2002).

While moderate alcohol intake seems not to be associated with altered semen parameters (Jensen et al. 2014b; Ricci et al. 2017), habitual, more intense consumption is linked to deterioration of sperm count, concentration, and morphology in a dose-dependent manner (Jensen et al. 2014a; Ricci et al. 2017). The combination of heavy drinking with smoking has an even greater negative effect on sperm parameters than heavy drinking alone (Boeri et al. 2019).

In contrast to animal studies, **smoking** in men is correlated with higher testosterone levels than in ex-smokers or non-smokers in several studies and meta-analyses (Sartorius et al. 2012; Lotti et al. 2015; Trummer et al. 2002; Zhao et al. 2016; Svartberg et al. 2003). Whether high testosterone leads to the risky behavior of smoking (Blanco-Munoz et al. 2012) or whether this correlation is due to a nicotine-induced reduction in androgen inactivation needs further clarification (Zhao et al. 2016). Smoking has detrimental effects on spermatogenesis, sperm function (Wallock et al. 2001; Zavos et al. 1998), and male fertility in otherwise healthy men (Bolumar et al. 1996; Chia et al. 2000). This effect has been confirmed in several studies and meta-analyses (Li et al. 2011; Sharma et al. 2016; Bundhun et al. 2019). Smoking was also linked to decreased success rates in ART including IVF and ICSI (Zitzmann et al. 2003). Reversal of any male subfertility after smoking cessation is not well established.

Caffeine can cross the blood–testis barrier and is therefore found in the same concentrations in blood and semen (Beach et al. 1984). Whether caffeine consumption has an impact on reproductive hormones remains unclear with some studies showing no effect (Jensen et al. 2010) and others showing a positive correlation between caffeine intake and total testosterone (Svartberg et al. 2003; Ramlau-Hansen et al. 2008). Data about negative effects on sperm production remain controversial, with most of the studies showing no effect on sperm parameters (Karmon et al. 2017; Jensen et al. 2010; Ramlau-Hansen et al. 2008); however, a paternal intake of more than 272 mg/day (equivalent of 4–5 espresso) was linked to a decreased live birthrate in ICSI cycles (Karmon et al. 2017).

The effect of c**annabis**, with its major psychoactive substance THC on male reproductive hormones, remains controversially discussed. While some large studies show an increase in testosterone similar to that of tobacco smokers (Gundersen et al. 2015; Thistle et al. 2017), a recent systematic review did not support this effect at least in long-term consumption (Rajanahally et al. 2019). The effect of cannabis consumption on sperm parameters remains controversial, but a recent systematic review concluded that cannabis consumption has a negative effect on sperm parameters (Rajanahally et al. 2019).

Data about **cocaine** use is surprisingly scarce, but it seems to show no or a slightly negative correlation with low testosterone levels in chronic consumption (Wisniewski et al. 2007). Cocaine consumption was linked to oligozoospermia (Bracken et al. 1990), a finding that might be explained by a direct toxic effect on spermatogenesis as evidence in rodent studies suggests (Duca et al. 2019).

Anabolic steroids (Chap. 37), which are frequently abused by elite and recreational athletes to enhance physical

performance and body image, inhibit gonadotropin secretion by negative feedback mechanisms and act as hormonal contraceptives (Christou et al. 2017), which is usually reversible with 6 to 18 months after withdrawal of the steroids (Shankara-Narayana et al. 2020). However, reduced serum testosterone concentrations and an increased prevalence of erectile dysfunction and decreased libido and depressive symptoms persist for years after cessation (Rasmussen et al. 2016).

34.4 Therapeutic Implications

Teleologically, the evolutionary significance of reversible inhibition of reproductive functions during acute or chronic illness is not clearly understood. Many common features observed in the underlying physiological mechanisms employed by mammals during puberty, seasonality, undernutrition, catabolic states, and systemic illness have led to the term "**ontogenic regression"** to describe the common underlying mechanism (see Sect. 34.2.2), which may also be considered a functionally adaptive form of physiological regulation of reproductive function tailored to environmental circumstances similar to what apoptosis is for orderly cellular turnover.

In this setting, the long spermatogenic cycle time requires prolonged periods to regress to infertile levels. This may have necessitated a speedier inhibition of fertility, which is brought about by the inhibition of sexual function through withdrawal of androgen secretion. This latter adaptive mechanism, ideal for short periods, may, however, become deleterious during prolonged, nonfatal illness. In these circumstances, prolongation of ontogenic regression may lead to tissue effects of androgen deficiency superimposed on the effects of the underlying disease. This is analogous to the damaging effects of autoimmunity, which, in a corruption of necessary immune function, leads to damage to body systems. Although from an evolutionary point of view, transiently reduced fertility during severe illness can be considered usefully adaptive, the general issue of whether androgen deficiency caused by chronic disease is protective, harmful, or neutral cannot be readily answered and may depend on more specific circumstances of modern life.

The therapeutic implications of gonadal dysfunction during non-gonadal systemic diseases vary from minimal to potentially major. They depend on the exact manifestations of the gonadal dysfunction, its chronicity, and severity. At minimum, systemic illness may alter routine tests of testicular function, which may interfere with evaluation for infertility or other andrological disorders. The value of testosterone treatment to rectify the partial androgen deficiency associated with chronic medical illness is not well established. Certain specific instances of chronic disease-related androgen deficiency, such as bilateral orchidectomy or acquired hypogonadism due to iron overload, warrant androgen replacement as causes of classical androgen deficiency. In many transient illnesses such as febrile infectious episodes, acute accidental, or surgical trauma, the hypothalamic response to systemic illness with its attendant acute androgen deficiency has no known lasting effect on general health and well-being.

> Despite the transient reductions in androgen secretion in such settings, the presently available evidence does not justify testosterone therapy but would allow for appropriate well-controlled clinical studies. In more prolonged chronic medical illness leading to persistent and sustained lowering of androgen levels or during severe prolonged catabolic states, such as critical illness, there may be a plausible rationale for testosterone replacement therapy but which warrants rigorous evaluation in placebo-controlled clinical trials.

This would aim to reduce long-term morbidity from sustained androgen deficiency causing loss of bone and muscle mass. Convincing evidence from properly placebo-controlled randomized clinical trials to justify such treatment is, however, still lacking. Uncontrolled and short-term studies during the 1960s did suggest that androgen supplementation initially augmented anabolic status; however, this response was not sustained during prolonged treatment. Although testosterone treatment cannot always be recommended at present, well-designed clinical trials to evaluate such interventions are feasible and desirable.

▶ Infertility is an increasingly common but underrecognized problem presenting among men with chronic medical illnesses.

These include men with kidney transplants who can now expect prolonged survival with good quality of life and, increasingly, among men with heart, liver, or marrow transplants in whom prolonged survival is increasingly possible. Following many successful organ transplants, impaired reproductive function due to organ failure is often improved or normalized. In addition, most men now survive cancer treatment of testicular tumors, hematological malignancies, or sarcomata but are effectively cured of their malignancy at the cost of sustained, severe, and often irreversible testicular damage. In these men, counseling about the likelihood of spermatogenic recovery, contraceptive advice, and appropriate application of assisted reproductive techniques such as pretreatment sperm cryostorage, insemination, or male factor IVF/ICSI may be required and should be provided

sympathetically. Men with chronic viral infections such as HIV and hepatitis B and C facing prolonged life expectancy with a life-threatening infectious disease warrant careful and expert counseling for their reproductive health. Difficult psycho-social and ethical decisions may be faced by patients (rather than their doctors) regarding the responsibilities of parenthood with limited life expectancy, risks of paternally mediated malformations, and postmortem insemination. The advent of autologous germ cell transplantation may add further options for fertility insurance to the medical care of men facing impaired reproductive function as a predictable side effect of life-saving medical therapies.

Sexual dysfunction including impotence is a common feature of aging and most chronic diseases that accumulate in older men. Authentic androgen deficiency is an easily defined and rewardingly treated cause of sexual dysfunction but is relatively rare (see Chap. 14).

Key Points

- Systemic disease and its treatment often have an impact on reproductive health including fertility, androgenic status, and sexual function.
- The pathogenic mechanisms of disruption to reproductive health are only partially known but are often disease-specific and depend on the timing of disease onset relative to birth, puberty, or aging.
- Although not life-threatening, the diagnosis of impaired reproductive health is important as it might decrease the quality of life and worsen the course of disease. However, reproductive dysfunction including subfertility and/or androgen deficiency occurring in association with chronic disease is readily overlooked.
- Such underrecognition is particularly likely when the relatively subtle and nonspecific effects of reproductive dysfunction are overshadowed by a major systemic illness with more dramatic but similar nonspecific clinical features.
- The optimal clinical management of medical disorders should include knowledgeable consideration of the effects of illness and its treatment on male reproductive health (including fertility, androgenic status, and sexual function).
- Symptoms are nonspecific but might be reversible, depending on the pathophysiological origin.

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Environmental Influences on Male Reproductive Health

35

Martin H. Brinkworth and Jorma Toppari

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Abstract

The ever-increasing number of chemicals to which humans are exposed, together with a recognition that some of them are potentially harmful to the male reproductive system, have driven the development of male reproductive toxicology. This was given a boost in 1992 when a 50% decline in sperm counts over the preceding 50 years was reported (Carlsen et al., Br Med J 305:609–613, 1992), stimulating a huge amount of research into adverse effects on the male reproductive system. Somewhat contentious at the time, the claim has nevertheless been supported by similar, subsequent research (Levine et al., Hum Reprod Update 23:646–659, 2017) that was specifically designed to avoid the problems with the earlier study. Even so, no clear cause for the decline has yet been established. In general, adverse effects on the reproductive system have been found experimentally with a wide range of chemicals but relatively rarely under conditions comparable to human exposure. Population-wide monitoring bears this out with only very small effects on reproductive parameters detectable. The nematocide dibromochloropropane is the classic example of an industrial compound that affects the male reproductive system but there is little evidence that many other toxic agents (e.g. metals, complex organochlorine compounds, alcohol, electromagnetic radiation, heat) have comparable effects at normal exposure and adult susceptibility levels. Certain other agents, particularly smoking and ionising radiation, only show limited heritable effects under certain (usually chronic) exposures. The principal risk to sperm production is from anti-cancer therapies, although the available (albeit limited) evidence indicates no heritable effects, perhaps because the exposures are high dose and acute or sub-chronic. Nevertheless, there is considerable concern that unknown factors in the environment, possibly with endocrine disrupting characteristics, are capable of inducing a range of effects sometimes now grouped together as testicular dysgenesis syndrome. Although there is abundant experimental and environmental evidence that a wide range of specific toxins, especially endocrine-disrupting compounds, can induce such effects, epidemiological evidence in humans is mixed (Rodprasert et al., Front Endocrinol 12:706532, 2021). Consequently, the current legislative framework for regulating exposures that could harm the male reproductive system, has not substantively altered in recent years: with the one exception that guidelines have been introduced for the testing of endocrine disruptors. This is also because the roots of male reproductive toxicity in humans are still only poorly understood. Exciting developments over the last decade however, may be pointing the way towards a better understanding of the mechanisms involved.

35.1 Introduction

Humans are exposed to a vast array of chemicals from a wide range of sources. Innumerable environmental, toxic hazards arising from nature have always existed but the additional exposures to man-made chemicals have increased progressively since the Industrial Revolution. Regulatory agencies, themselves the product of public demand for drug regulation following thalidomide and other catastrophes, have developed licensing guidelines for new chemicals in order to protect human populations from damaging environmental exposures, while permitting the production and use of important new chemicals that are an integral part of our built environment. Over the last three decades, the scope and sophistication of testing requirements have increased greatly as the quantity and diversity of chemical entities to which humans may be exposed continue to increase. The earliest safety screening mainly concerned **acute toxicity**, **carcinogenesis** and **teratogenesis**, and subsequently, **reproductive and genetic toxicity** have become fully embedded in safety regulation. Although historically, reproductive effects have been most studied in females, it has been recognised for several decades now, that adverse effects on **male reproductive function** are also important. Indeed, the **Registration, Evaluation, Authorisation and Restriction of Chemical Substances** (**REACH**) regulation passed by the European Union in 2007 (EC 1907/2006) is a recognition of the myriads of industrial chemical hazards to which humans are exposed and has required the safety testing of hundreds of chemicals that have not been extensively tested before. Ironically, equally hazardous medical procedures like surgery and in vitro fertilisation are still not as rigorously regulated for safety and efficacy, unlike the drugs they require. Complex regulatory standards exist for all these categories of chemicals with the rigor of the testing and safety margin required varying pragmatically according to likely human exposure and risk. Scientific methodologies to evaluate human health risks, including those to reproduction, remain in their infancy while the task grows in scope and complexity. In principle, **human toxicology** is based on an amalgam of

- Limited **observational human data**, usually retrospective epidemiological studies
- More abundant **experimental studies in animals and cells**

The former lacks the rigorous control of randomised or controlled prospective studies, while the latter has uncertain applicability to humans. Neither approach is entirely satisfactory or even complementary, as species-specific variability can lead to contradictory findings. Nevertheless, these are the only tools currently available to bridge rationally the gulf

between, on the one hand, the essential task of providing some assurances of safety for current and future exposures to chemicals, while on the other, allowing for the timely introduction of new agents into medicine, industry and, inevitably, into the shared environment.

The contrast between observational data in humans and data from experimental studies exemplifies **an important distinction in toxicology**, that **between hazard and risk**.

Experiments aimed purely at determining whether an agent has **the potential to damage a biological system** are concerned with **hazard**. The concept of **risk combines hazard and biological context**. It incorporates not only the level of exposure but levels reaching the target tissue, the effects of toxifying/detoxifying metabolic systems, repair processes and any other factors modulating the final response. The ultimate objective of toxicological investigations is thus not just to identify particular compounds as theoretically hazardous but to provide the best possible assessment of the actual risks they pose to humans.

35.2 Potential Adverse Effects on Spermatogenesis

Spermatogenesis involves the continuous replication and complex metamorphosis of relatively undifferentiated, diploid, stem cells into highly specialised, motile, haploid cells. This entails a developmental progression through a series of different cell types, each of which differs from the others in terms of particular biochemical, cell biological and molecular processes occurring within them. Hence, each cell type has specific characteristics and, in consequence, potentially different responses to toxins. Therefore, detailed toxicological investigations designed to characterise germ cell toxicity precisely must take account of these differences. Furthermore, since spermatogenesis is a process of terminal differentiation with the fully differentiated cells ultimately eliminated from the body, any germ cell toxicity that affects cells other than the stem cells, may only have a transient effect.

The considerations above lead to two types of male germ cell toxicology study: acute/sub-acute and chronic/sub-chronic. In the former, a short-term exposure, usually on a single day (acute) or up to five consecutive days (sub-acute), is used and can target a particular germ cell type or types. They can do this by exploiting the fact that spermatogenesis is a tightly regulated process and under most circumstances, the time taken for each cell type to develop into the next, does not vary. Thus, it is possible to know precisely how long it will take before each germ cell type completes its development and differentiation into a mature spermatozoon in the cauda epididymis. Therefore, by waiting the same length of time between exposure and sampling (which can include mating as well as measurement of end-points in the sperm themselves), one knows exactly which germ cell type was affected when any effects are found. Chronic studies on the other hand, test all the different cell types together. Although they cannot identify in which cell type an effect occurs, chronic studies are useful for determining whether spermatogenesis can be affected per se. ‘Chronic’ in male reproductive toxicology studies refers to exposures that last for a period of time at least equivalent to the length of spermatogenesis, i.e. the development of a **spermatogonial stem cell** into a mature **spermatozoon** in the **cauda epididymis**. (This is rather shorter than the time implied by the term ‘chronic’ in other types of toxicology study.)

These haploid gametes must, furthermore, be capable of travelling through the female reproductive tract and fertilising an ovum. This highly complex process occurring in the reproductive tract is closely governed by hypothalamic-pituitary and testicular hormones, thereby making it also susceptible to influence on endocrine organs, notably the brain. It is, therefore, not surprising that environmental agents can adversely affect germ cell development at many different stages and by different means. Indeed, this may explain why huge numbers of sperm are produced (around 2×10^8 per day and 2×10^{12} in a lifetime), while only one is required for fertilising an ovum, an event usually occurring less than 10 times per lifetime.

Chemicals can potentially damage spermatogenesis at any stage from proliferating spermatogonia to mature spermatozoa. At least three, different and not mutually exclusive toxic effects are possible: **cell death**, **genetic change or epigenetic alteration**.

35.2.1 Cell Death

Lethally damaged cells either die within the epithelium or are shed into the lumen of the seminiferous tubule. Those dying in situ may do so by necrosis or apoptosis.

- **Necrosis**: An uncontrolled lysis and non-specific spilling of cellular contents, which may cause a local inflammatory reaction and can result in widespread damage to surrounding cells.
- **Apoptosis**: A physiological process of programmed cell death whereby a doomed cell breaks down into smaller, apoptotic bodies that are then phagocytosed by **Sertoli cell**s without bystander damage (Kerr et al. 1972). In the testis, this is the orderly mechanism by which spermatogenic cells die within the germinal epithelium without collateral damage to adjacent Sertoli or germ cells.

Since apoptosis is an important mechanism for deleting damaged cells from the germline, there has been interest in the consequences of an impairment of apoptosis on the persistence of genetic damage in the germline, and its implications for the transmission of mutation to the next generation. For example, in the development of a model system for the induction of male-mediated heritable abnormalities, it was found that administration of a **cyclophosphamide** treatment regimen to male rats that leads to the induction of foetal abnormalities among a proportion of their offspring (low-dose, chronic administration) (Trasler et al. 1985; Jenkinson and Anderson 1990), also reduces the level of apoptosis among their germ cells (Brinkworth and Nieschlag 2000). Thus, not only may apoptosis be a response to male reproductive toxicity but disturbances of apoptosis could also influence the vertical transmission of mutation (Brinkworth 2000).

Non-lethal germ cell damage will either be **repaired** or leave permanent effects on the structure or function of the mature spermatozoa, including the possibility of **transgenerational genetic effects**.

35.2.2 Genetic Change

Genetic damage in the male germline can either render the sperm infertile, cause pre-implantation losses, post-implantation losses (spontaneous abortion), cause the death of the foetus, or result in some sort of heritable, genetic abnormality. It has been suggested that **chronic, low-dose chemical exposures** can be more effective at inducing **paternally mediated effects in the offspring** (Brinkworth 2000; Anderson et al. 2014) than high, acute exposures. Originally, the effects observed most commonly were gross morphological abnormalities in the foetuses, which could often be shown to be associated with **gross chromosomal abnormalities** (Jenkinson and Anderson 1990) but there is evidence that certain, specific, dosing regimens can induce cancer in the offspring (Lord et al. 1998). For these effects to be experimentally detectable, a relatively very high proportion of sperm would presumably have to carry the necessary genetic lesions. It has been suggested therefore, that these genetic outcomes could be promoted either by a suppression of apoptosis in genetically damaged germ cells (see above), or the induction of **genomic instability** (the appearance of additional mutations in the unexposed generation) (Brinkworth 2000). Subsequently, it has been shown that there can also be epigenetic mechanisms involved, for example involving the transmission of particular RNA species (Paris et al. 2015). Clinical implications are far from clear due to the lack of evidence for similar effects in human populations.

35.2.2.1 DNA Repair in the Germline

Very little is known about the **repair of non-genetic damage** in germ cells. **DNA repair** on the other hand is the subject of enormous research effort, including a considerable amount in the testis (reviewed in Marjault and Allemand 2016). **DNA repair enzymes** are extremely important throughout spermatogenesis and participate in a multitude of functions, serving not just as a defence against exogenously induced damage. Different enzymes are expressed in different germ cells but many types also overlap throughout germ cell development. It is therefore difficult to generalise about the status of any one type of repair system in particular cell types. However, it is broadly true to say that **base excision repair** and **mismatch repair** systems are present throughout the mitotic and meiotic stages while **nucleotide excision repair** and **transcription coupled repair** systems are most important in spermatogonia. These systems are likely to be especially important in the repair of damage caused not only by **exogenous** but also **endogenous agents**, which include free radicals arising as a result of cellular processes such as respiratory oxidation. During meiosis, a host of other systems can be found, in particular **homologous repair** and **non-homologous** end-joining enzymes. These are likely to aid in the recombination events characteristic of meiosis. DNA repair capacity is believed to diminish during spermatid elongation and to be virtually non-existent in spermatozoa. This can make them more susceptible to the effects of hazards such as radiation and alkylating drugs, although some protection may be afforded by the progressive chromatin condensation that accompanies spermatid elongation. There is also growing evidence that DNA repair activity in the testis alters with age, which can influence the accumulation of mutations in the germline with age (Aitken 2022).

35.2.3 Epigenetics in Spermatogenic Cells

Epigenetics, a term first coined in the 1950s but only coming into its own in male reproductive biology in the last couple of decades, refers to the permanent or semi-permanent, heritable, alteration of gene activity without any alteration of DNA sequence. Its effects are mediated by **epigenetic 'marks' or signals**, that modify how the gene can interact with cellular machinery, particularly transcriptional machinery. The most common and best studied of these epigenetic signals are: **DNA methylation** (the addition of a methyl group to the fifth carbon in the carbon ring of the DNA base cytosine to make **5-methylcytosine**); **histone modification** (the addition of any of a wide range of chemical moieties, including methyl and acetyl groups, to recipient loci on histone molecules in chromatin); **non-coding RNAs** (which are generally single stranded and can bind

to the double-stranded DNA, creating a triplex structure that is then not recognised by DNA-interacting proteins). A classic example of gene silencing by epigenetic mechanisms is the shutting down of one of the two X-chromosomes in almost all cells in the female body. (This avoids the 'double dosage' of X-chromosome gene-products that would occur in female cells relative to male cells, which only contain one X chromosome.) Extensive DNA methylation, histone modification and the binding of **long non-coding RNAs** to the DNA, all occur along the full length of the chromosome, causing it to become transcriptionally silent. These **epigenetic modifications** are maintained, even after replication of the cell, so the inactivation is permanent throughout the lifetime of the individual.

There are other epigenetic mechanisms that seem to have important roles in male reproduction. The phenomenon of paramutation, first recognised in maize, is the transmission of RNA molecules via sperm, to the embryo and influencing the phenotype of the offspring (for useful reviews, see Rassoulzadegan and Cuzin 2015; Cullen et al. 2021). In mammals, this was first identified in a strain of mice carrying an allele of the c-myc gene that produces a white tail-tip that is not present in wild-type mice. By careful breeding studies, it was shown that the white tail-tip persisted in the offspring, even after the responsible allele had been bred out of the lineage. Thus, genotypically wild-type mice were phenotypically white tail-tip (Rassoulzadegan et al. 2006).

A further proposed epigenetic mechanism is the phenomenon of histone retention in sperm chromatin. During the process of spermiogenesis, by which spermatids transform morphologically into spermatozoa, histones are removed from the chromatin and replaced by protamines, smaller molecules than histone, which packages the DNA in a very different way, causing profound condensation of the chromatin and thus reducing the sperm nucleus to a size compatible with efficient motility. However, a small proportion of histones are retained and it has been shown that they are always retained in the same genomic locations (Hammoud et al. 2009; Arpanahi et al. 2009). This suggested that they were functional and led to the proposal that they constitute an epigenetic signal regulating gene expression from the paternal genome in the very early embryo (Miller et al. 2010; Jenkins and Carrell 2012).

A key property of epigenetic signals is that they can be influenced by the environment. A classic example of this, discussed in Jirtle and Skinner (2007), is the variant of the **Agouti mouse** that has an additional promoter region upstream from the agouti gene, which causes a yellow coat colour. Methylation of this additional promoter silences it and the coat colour becomes brown. **Dietary supplementation** of pregnant females with methyl donors such as **choline** or **betaine** allows this additional promoter to become methylated in the embryos, causing a change in coat colour among them compared with their mother.

Thus, an environmental stimulus can bring about a heritable effect on the phenotype of the offspring without affecting their genotype.

Genome-wide DNA methylation undergoes extensive remodelling both in somatic cells and the germ cells in the early embryo, and it is thought this provides **windows of susceptibility** in which **environmental agents** can alter methylation patterns. Alteration of DNA methylation in the germ cells will have effects that are then transmissible to subsequent generations beyond the F1.

In 2005, it was reported that administration of certain endocrine disrupting compounds (**vinclozolin** or **methoxychlor**) to pregnant rats, resulted in adverse effects on the male reproductive system not only in the F1 but successive generations up to ~fourth generation (Anway et al. 2005). These findings have since been extended to a wide range of toxic insults and other types of pathological outcome (Nilsson et al. 2022). Molecular analyses of these types of data show an association with DNA methylation changes, and indeed specific patterns of altered methylation (differentially methylated regions) have recently been associated with specific pathologies (Beck et al. 2022).

35.3 Common Threats to Male Reproduction

Testicular function can be impaired by a wide range of factors but it is important to remember that the timing of exposure may be as important as the type of exposure. This is because in many cases, the developing reproductive system in the male embryo/foetus is more vulnerable than the adult system.

The following mechanisms may not necessarily apply to the whole population equally, but could instead apply particularly to vulnerable subgroups, who may have **genetic polymorphisms** that enhance or reduce individual or group susceptibility to toxicological processes. Such systematic variation in susceptibility may result in apparent enhancement of effects in small, but vulnerable, subpopulations. Further toxicological studies of genetic polymorphisms in susceptibility to environmental agents are needed.

35.3.1 General

The contamination of the environment by **pollutants** comes from a variety of sources, including the burning of fuels and waste, widespread use of pesticides, release of products from industrial processes, leaching of chemicals from manufactured products and food, excretion of pharmaceuticals and metabolites into the water supply, etc. Efforts to reduce the risks of exposure include not only stringent **safety testing**, and tight regulation and monitoring of exposure levels during manufacturing, but also refinements in use, e.g. spraying techniques for pesticides. Analysis of the association between environmental contaminants and trends in fertility/infertility revealed no more than a weak association between exposure and fertility (Foster et al. 2008). Similarly, a survey of data on the most common environmental contaminants for which there is experimental evidence of effects on semen parameters, showed that they are relatively minor and in some cases contradictory (Phillips and Tanphaichitr 2008).

Thus, it would appear at first sight, that the various regulatory processes in place to limit the risks to human reproduction from environmental exposures are reasonably successful. However, it should be noted that: firstly, as will be shown later, some studies do report finding correlations between exposure and effect and any genuine effect, however small, is a concern. Secondly, globally there is wide variation in the levels of environmental protection applied. Thirdly, such evidence is principally collected from studies of actual or potential adult exposure, whereas it has long been known that the embryonic and foetal developmental phases can be much more susceptible to damage. Furthermore, if infants are born with a compromised ability to reproduce in the future, that could affect the baseline levels of, for example, sperm production. In the long-term, this could prove problematic for research, if it results in an alteration of so-called normal levels in the population. (See Sect. 35.4.4 on semen quality, later.)

Details of the commonest hazards thought to provide a potential risk to men's reproductive health are described below along with a consideration of relevant evidence. A significant challenge for the future will be the identification of new threats to human health, as yet unconsidered.

35.3.2 Smoking

Inhaled cigarette smoke contains many hazardous compounds, some of which (such as acrolein) are reproductive toxins. Furthermore, it can induce **oxidative damage** to macromolecules that is detectable in the testis. Human studies of the impact of smoking on reproductive outcomes are frequently compromised by **psychological self-selection**, i.e. smokers are likely to be the type of people more heavily exposed to other hazards that could cause any adverse effects seen (Vogt et al. 1984). Nevertheless, a number of human and animal studies have detected **chromatin disturbances** (Potts et al. 1999), sperm **DNA adducts** (Fraga et al. 1996), DNA adducts in the embryo (Zenzes et al. 1999), **disomy** (e.g. Robbins et al. 2005) and **Simple Tandem Repeat (STR) locus mutations** (Marchetti et al. 2011) in sperm, and genomic instability (Laubenthal et al. 2012).

The latter study (Laubenthal et al. 2012) is significant because it demonstrated for the first time, that genetic damage caused by paternal smoking, could be transmitted to the offspring.

This may help explain earlier findings of a positive association between paternal smoking and the incidence of cancer, especially leukaemia, among their offspring (Ji et al. 1997; Sorahan et al. 1997a, 1997b).

35.3.3 Ionising Radiation

The effects of ionising radiation on spermatogenesis have been thoroughly analysed over several decades. X-irradiation has direct toxic effects on spermatogenic cells, particularly on differentiating spermatogonia that are proliferating. Accurate data in humans are rare but some accepted parameters are shown in Table 35.1. Testicular exposure to higher than 4–6 Gy can produce permanent azoospermia, whilst recovery can occur during several years after lower doses. The recovery time is inversely correlated to the dose (Rowley et al. 1974). Exposure to lower doses of irradiation (down to 0.15 Gy) can produce a transient decline in sperm production.

Fractionated irradiation causes more damage than the same total dose administered as a single exposure.

Table 35.1 Summary of the effects of ionising radiation on the human testis by dose

Dose (cGy)	Effect	Reversibility
<10	Little effect evident at cellular level	–
10–50	Moderate oligozoospermia	6 months
50–75	Severe oligozoospermia	6 months
75–100	Azoospermia	6 months
200–300	Azoospermia	1–2.5 years
>300	Azoospermia	~5 years or none

Data from Rowley et al. (1974) and Jockenhövel (1993)

There is evidence that irradiation can cause heritable changes in the germline in humans. **Minisatellites** are a form of STR and are stretches of DNA containing tandemly repeated sequence motifs, distributed apparently randomly throughout the genome in humans. They are believed to be particularly prone to **rearrangement** during meiosis (Dubrova et al. 1998) and have **spontaneous mutation rates** up to 1000-fold greater than **protein-coding sequences**. Therefore, they may be suitable sentinel sequences for the determination of exposure to germ cell mutagens and of alterations in mutation rates. The children of men exposed to radiation at **Chernobyl** have been found to have elevated levels of minisatellite mutations in their sperm, demonstrating the inheritance of genomic instability (Dubrova et al. 1996, 2002) and similar findings exist for rodents (Barber et al. 2002). A large-scale study to examine heritable mutation among the offspring of people who experienced low-dose-rate exposures in the aftermath of the Chernobyl explosion is currently underway (Bazyka et al. 2020). Intriguingly, however, data from the sperm of men before and after chemotherapy or radiotherapy show no sign of a treatment-induced increase in the germline minisatellite mutation rate (Armour et al. 1999; Zheng et al. 2000). These data accord with the lack of effect on offspring following cancer treatment whilst being at odds with the Chernobyl findings and may highlight a difference between environmental and therapeutic exposures. Studies in mice indicate not only a dose-response relationship between damaging radiation exposures and genomic instability in F1 mice, but also that there may be a threshold below which genomic instability is not induced. Potentially, therapeutic exposures in humans never reach this threshold (Mughal et al. 2012).

35.3.4 Electromagnetic Radiation

Electromagnetic fields (EMF) are generated by domestic as well as industrial electrical equipment and vary in frequency, intensity and waveform. The frequency of electricity transmission is 50 Hz in Europe and 60 Hz in North America, both of which fall into the Extremely Low Frequency (ELF) range. The intensity of the magnetic component of these fields is measured in Teslas (T) and is a function of distance from, and strength of, the electric current. It is known that high intensity, low-frequency magnetic fields (>10 mTesla at 50–60 Hz) can be damaging, but normal environmental exposure to ELFs does not usually exceed 0.3 mT. Of particular concern however are portable (mobile) telephones, which can generate magnetic fields of moderate intensity but high frequency in the GHz range. The WHO has initiated a series of Systematic Reviews (SRs) to assess the health effects of EMF exposure and protocols for the assessment of risk to male fertility, pregnancy and birth outcomes posed by EMF, have recently been published (Pacchierotti et al. 2021). The literature in this area runs to hundreds of papers and this SR is yet to be completed and published. It should be noted however, that any conclusions need to deal appropriately with the varying quality of studies in terms of the design, conduct, or interpretation, of the work. This is why prior publication of peer-reviewed protocols for the conduct of the SRs is so important, in order that this may be conducted objectively.

35.3.5 Cancer Therapies

Cancer chemotherapy includes many drugs that are toxic to germ cells. **Alkylating agents**, such as cyclophosphamide, may cause permanent azoospermia, whereas **non-alkylating agents**, e.g. **vincristine** and **methotrexate** are less harmful in the long-term for spermatogenesis. Several new cancer drugs have been introduced during this century. Many of them act on receptor kinases and thereby interfere also with paracrine signaling in the testis and can influence spermatogenesis. However, human studies of their consequences on testicular function are still rather scarce.

As mentioned above (Sect. 35.3.3), there is very little evidence for the induction of heritable mutations in the germ cells of male patients following chemo- or radiotherapy, in contrast to environmental radiation exposures following nuclear disasters. This seems to be reflected by an absence of heritable effects in the children of cancer survivors (e.g. Sankila et al. 1998 and a number of smaller studies. See Table 35.2 for data from Byrne 1999, which are typical of such studies, in modest scale and lack of detectable effect). If the male germline is somehow relatively impervious to heritable consequences of cancer treatment, it could be enormously beneficial to understand the mechanisms. However, more data on this are always required, and on-going monitoring is still underway (see for example Sommerhäuser et al. 2021).

35.3.6 Heat

Heat can damage spermatogenic cells. In **undescended testes,** temperature is higher than in the scrotal gonad.

Table 35.2 Incidence of genetic disease in the offspring of parents surviving cancer and in sibling controls

	Birth defects in the offspring of:	
	Survivors	Sibling controls
Offspring with birth defects	74	142
Total number of offspring	2198	4544
Percentage (%)	3.4	3.1

Data from Byrne (1999)

Spermatogonia undergo apoptosis over time in an undescended testis (Kollin et al. 2012). Bathing in a **hot tub** at >36 °C for more than 15 min, or long working in very hot environments, can disturb spermatogenesis (Procopé 1965; Jung and Schuppe 2007). Results on **sauna bathing** at high temperatures are not clear (Jung and Schuppe 2007; Garolla et al. 2015). Variable results on the effects of tight underwear clothing have been reported (Wang et al. 1997; Jung and Schuppe 2007; Mínguez-Alarcón et al. 2018). Obesity, and seated working, have been associated with increased scrotal temperature and impaired sperm production; however, many other factors may have confounded these results (Jung and Schuppe 2007).

35.3.7 Ageing

An **increase in mutation rate in the sperm of older men** has been known for many years (Crow 1997, 2000; Kong et al. 2012). The sperm of elderly men is known to contain elevated levels of genetic damage (Sloter et al. 2007) and there is a close association between paternal age and the incidence in their children of certain genetic diseases such as **achondroplasia**, **Apert syndrome** and others (reviewed in Crow 1997, 2000; Kühnert and Nieschlag 2004). However, the mechanisms by which these phenomena arise are not solely a function of the increased mutation rates (Tiemann-Boege et al. 2002; Choi et al. 2008).

It has been established that a mutation in the FGFR2 gene is present in sperm at significantly higher levels in older compared with younger men, and appears to confer a survival advantage on testicular germ cells (reviewed in Maher et al. 2014). This mechanism could lead, over a man's lifetime, to a significant enrichment of the proportion of sperm bearing the respective mutation without the need to invoke an increase in the de novo mutation rate.

Such so-called, '**selfish mutations**' have now also been found in several other genes. Duplex sequencing has recently provided direct evidence that de novo mutations in regions associated with congenital disorders are much more common in the sperm of older men than in younger men (Salazar et al. 2022). However, the evidence of increased genetic damage and the multiplicity of diseases associated with advanced paternal age may suggest that other, as yet unknown, mechanisms could additionally be in operation.

35.3.8 Occupational Exposures

Occupational chemical hazards can present much greater risks than those in the general population. Exposure to **1,2,dibromo-3-chloropane** (**DBCP**) in a chemical production plant was associated with workmen's infertility in a dose-dependent manner (Whorton et al. 1977; Potashnik et al. 1978). Sperm production recovered in many men who were transferred to duties where they were no longer exposed to DBCP, but some men remained azoospermic (Eaton et al. 1986). Reproductive toxicity of this compound in animals had been reported much earlier (Torkelson et al. 1961). Many other pesticides such as **chlordecone**, **carbaryl** and **ethylene dibromide** have also been associated with reproductive toxicity (Giwercman and Bonde 1998). DDE however did not affect anogenital distance in newborns (Longnecker et al. 2007).

35.3.9 Toxic Mixtures

Chemical toxicity is usually tested in single compounds at a time, and each chemical is characterised by an exposure concentration that produces no harm to the animal, no adverse effect level, NOAEL. Usually only occupational or accidental exposures to single chemicals cause levels that exceed NOAEL. In real life, we are always exposed to multiple chemicals at the same time. Chemicals in mixtures can act additively, synergistically or opposing each other. Additive mixture effects are typical when several anti-androgens are tested together (Rider et al. 2008). Combining chemicals at levels below their NOAEL can produce effects in 100% of the exposed animals (Christiansen et al. 2008). The mechanism of action of the anti-androgens does not need to be the same (e.g. androgen receptor antagonism) to cause an additive effect. Furthermore, chemicals with a different signalling target, such as aryl hydrocarbon receptor for dioxins, can be components in the harmful mixtures (Rider et al. 2010). Mixture effects are a challenge for epidemiological studies and for regulatory agencies. Safety margins are built in to the daily allowable doses of chemicals, but uncertainty remains because the number of chemicals are very high and their NOAEL tends to decline when new research is emerging. Therefore, the current safety margins may not be large enough, because the mixtures of chemicals can cause adverse effects even when individual chemicals in the cocktail are present at lower concentrations than their NOAELs that are used for determination of daily allowable doses of exposure (Kortenkamp and Faust 2018). Since male reproductive birth defects, such as cryptorchidism and hypospadias, are affecting relatively large proportion of men (several percent) and anti-androgenic compounds can cause these disorders, recognition of harmful exposures in the environment should be a high priority to prevent reproductive health problems in the future.

35.4 Developmental Reproductive Toxicity

We have learned much of the development and function of male reproductive system by studying genetic disorders. **Gonadotropins** and testicular hormones regulate male-specific differentiation of sex organs. **Androgens** play a central role in the development of **external genitalia**. Defects in androgen biosynthesis or action lead to female-type sexual differentiation of external genitalia or under-masculinisation. These conditions are associated with impaired spermatogenesis and increased risk of testicular germ cell neoplasia. Hypospadias and cryptorchidism are common mild forms of under-masculinisation and present most often without recognisable genetic defects. The common co-occurrence of hypospadias, cryptorchidism, poor semen quality and testicular germ cell cancer gave a reason for Skakkebaek et al. (2001, 2016) to coin a term Testicular Dysgenesis Syndrome (TDS) which emphasised the developmental basis of these reproductive disorders and similarity of the underlying risk factors. Whilst we know several genetic causes of TDS, such as androgen insensitivity and steroidogenic enzyme defects, much less is known about possible environmental causes. However, developmental biology guides us to look for toxicants that could inhibit **steroid synthesis** or block androgen action. Indeed, we currently know hundreds of chemicals that have such properties. In animal experiments, **anti-androgens** cause **hypospadias**, **cryptorchidism**, **shortened anogenital distance**, **structural changes in testicular histology** and **impaired sperm production** (Sharpe 2020). The effects are additive, i.e. several anti-androgens together act according to their sum amount (Rider et al. 2008, 2010). Human exposure assessment has mostly been made on a single chemical at a time, and the accompanying uncertainty treated with safety margins.

35.4.1 Cryptorchidism

Cryptorchidism can be congenital or acquired, which has not been taken into account in many studies, and the two forms are often mixed together. Congenital cryptorchidism is a foetal disorder by definition, whereas acquired cryptorchidism may also have post-natal reasons. Ascertainment of the diagnosis is variable, which also adds to uncertainty of classification of the outcome. Exposure assessments are also variable. The **sample matrix** can be maternal serum, urine, amniotic fluid, or breast milk, placenta, or child's serum, urine, adipose tissue, hair, tooth, nail, or almost any tissue. Occupational and epidemiological studies may rely on **job matrix** analysis or regional data on exposure levels. No wonder that the results of the studies are also variable.

Some studies have reported positive associations between the levels of **organochlorine pesticides**, **polybrominated diphenyl ethers** (**PBDE**s; used as flame retardants), **polychlorinated biphenyls** (**PCBs**; used as flame retardants and insulators) and **dioxins** (pollutants from, e.g. incineration) in either breast milk or child's adipose tissue and the risk of cryptorchidism (Damgaard et al. 2006; Brucker-Davis et al. 2008; Koskenniemi et al. 2015; Main et al. 2007). Also some occupational and epidemiological studies have shown an association between pesticide exposure in utero and risk of cryptorchidism (e.g. Garcia et al. 2017; Weidner et al. 1998; Andersen et al. 2008), whereas some other studies have not confirmed these associations (reviewed in Virtanen et al. 2019). No significant association with cryptorchidism was found in studies where pesticide levels were measured in maternal serum or in cord serum (Longnecker et al. 2002; Trabert et al. 2012; Bhatia et al. 2005; Pierik et al. 2007; Brucker-Davis et al. 2008). Levels of PCBs in different matrices were not associated with the risk of cryptorchidism in most studies (Virtanen et al. 2019). In a combined analysis of 106 environmental chemicals in breast milk, PBDEs and dioxins were identified as risk factors for cryptorchidism (Krysiak-Baltyn et al. 2012). Levels of **perfluorinated compounds** (used in surface materials, such as cooking ware) in amniotic fluid or cord blood were not associated with the risk of cryptorchidism (Vesterholm Jensen et al. 2014; Toft et al. 2016). Most of the above-mentioned chemicals have been banned because of their persistence, bioaccumulation and toxicity. European countries have committed to phase out toxic chemicals listed in the Stockholm convention (UNEP 2008). Therefore, their levels in the environment have decreased over the last decades. However, due to their persistence they are still forming 'background' exposure that is measurable in all of us.

Exposure assessment of non-persistent chemicals is much more challenging than that of persistent compounds. This has limited the studies of non-persistent chemicals, although there is a lot of experimental evidence of their possible reproductive toxicity. Species differences further complicate interpretation of the findings and the conclusions that can be drawn from them. **Phthalates** (used as plasticisers) are well-characterised anti-androgens in rat studies and therefore tested in cryptorchidism studies. Phthalate levels in maternal urine during pregnancy were positively associated with the risk of cryptorchidism (Swan 2008), whereas no positive association was found when phthalates were measured in amniotic fluid, cord serum, or breast milk (Jensen et al. 2015; Main et al. 2006; Brucker-Davis et al. 2008). Serum levels of bisphenol A (used in plastics) were positively associated with the risk of cryptorchidism in one study (Komrowska et al. 2015), whereas no association was found when BPA was measured in cord blood (Fénichel et al. 2012; Chevalier et al. 2015).

A recent meta-analysis of case-control studies published between 1990 and 2020 reported an increased risk of cryptorchidism associated with prenatal exposure to phenol substances (Wu et al. 2022).

35.4.2 Hypospadia

Hypospadia is usually present as an isolated birth defect but cryptorchidism can accompany it (Thankamony et al. 2014; van der Horst and De Wall 2017). Exposure to pesticides has been associated with the risk of hypospadias in ecological and occupational studies. The **risk ratio** was 1.36 (95%CI 1.04–1.77) after maternal exposure to pesticides and 1.19 (95%CI 1.00–1.41) after paternal exposure in a meta-analysis (Rocheleau et al. 2009). A positive association between foetal exposure to pesticides and hypospadias was also reported in two later studies (Garcia et al. 2017; Kalfa et al. 2015), whereas no association was found in four other studies (Nassar et al. 2010; Morales-Suárez-Varela et al. 2011; Rocheleau et al. 2011; Carmichael et al. 2013).

Variable results from case-control studies have been reported for associations between hypospadias, and pesticides or the sum of PCBs in maternal serum or in the boy's serum (reviewed in Virtanen et al. 2019). When cryptorchidism and hypospadias were combined, a positive association of placental concentrations of pesticides, BPA and **benzophenone** (**UV screen**) with hypospadias was observed (Fernandez et al. 2007, 2016). A recent meta-analysis showed an association between exposure to endocrine disrupting chemicals and hypospadias (Wu et al. 2022), while an older meta-analysis reported no association (Bonde et al. 2016).

35.4.3 Testicular Cancer

Testicular cancer has a germ cell origin in more than 90% of cases, and therefore cancer statistics reflect the incidence of **testicular germ cell tumour**s (**TGCT**s) when testicular cancer is discussed (Rajpert-De Meyts et al. 2016). The incidence of testicular cancer peaks at young **adulthood** after **puberty** also affecting fertility. The incidence has increased rapidly globally (Trabert et al. 2015), and TGCTs are the most common cancers of young men in Western countries (Znaor et al. 2014). **Germ cell neoplasia in situ** (**GCNIS**) precedes TGCT. Since GCNIS cells share many features with foetal gonocytes, it is likely that the TGCTs have a foetal origin (Rajpert-De Meyts et al. 2016). Polymorphisms in some genes, such as *KIT* and *CDC27*, are associated with an increased risk of testicular cancer (Litchfield et al. 2015). Cryptorchidism and hypospadias are even more well-established risk factors for testicular cancer (Trabert et al. 2013).

Most studies on environmental effects on testicular cancer have analysed adult exposures. However, considering the foetal origin of the disease, this was clearly not a relevant exposure time, and most of the studies have reported negative results. However, some case-control studies have reported an association between testicular cancer and exposure to organochlorine pesticides, whilst the results of the association with exposure to PCBs are conflicting (Giannadrea et al. 2011; McGlynn et al. 2008, 2009; Purdue et al. 2009).

Foetal exposure has been extrapolated from measurements of persistent chemicals in mothers' serum samples collected years after pregnancy. Case-control studies of men with testicular cancer compared men's serum chemical levels and maternal serum chemicals, showing that mothers of cases had significantly higher serum levels of PCBs, **hexachlorobenzene**, PBDEs and cis- and **trans-nonachlordane**s than mothers of controls, whereas only **cis-nonachlordane** concentration was higher in serum of cases themselves, compared with controls. (Hardell et al. 2003, 2004, 2006).

35.4.4 Semen Quality

Sperm concentrations have declined in developed countries since 1940s according to many meta-analyses (Carlsen et al. 1992; Swan et al. 1997, 2000; Levine et al. 2017). Semen quality in the general population seems to be very similar in different parts of the world (Virtanen et al. 2017). The association of the current rather poor semen quality and its decline with environmental effects, has been controversial (Fisch 2008; Levine et al. 2017; Ravanos et al. 2018).

Spermatogenesis depends on the normal function of Sertoli cells that cease proliferation at puberty when they are needed to maintain development of germ cells. The number of Sertoli cells determine the maximum sperm production capacity. Therefore, sperm production capacity is established already before puberty and individual sperm numbers stay rather constant in adulthood (Perheentupa et al. 2016). Sertoli cells proliferate in at least three developmental phases, first during foetal life, then during '**mini-puberty**' at the first few months after birth and lastly during **pre-puberty** just before the onset of spermatogenesis (Mäkelä et al. 2018). In **hypogonadotropic hypogonadism**, hormone treatment with **gonadotropin**s or **gonadotropin-releasing hormone** can induce and improve sperm production, but otherwise we do not have means to influence sperm production capacity of adult men. However, we know several factors that can inhibit sperm production, and

recovery after such insult can lead back to normal spermatogenesis. Heat, irradiation, illnesses, many pharmaceuticals and toxicants can reduce sperm production. Semen quality reflects foetal and childhood testicular development, and these life stages are also windows for possible damage that affects life-long sperm production capacity (see for example van den Driesche et al. 2017).

Exposure to **2,3,7,8,-tetrachlorodibenzo-p-dioxin** (**TCDD**) at the three times of Sertoli cell proliferation (foetus, neonatal infant, 8–9-year-old boy) has been associated with reduced semen quality (Mocarelli et al. 2008, 2011; Minguez-Alarcon et al. 2017).

Foetal exposure to background levels of **perfluorooctanoic acid** (**PFOA**) was inversely associated with semen quality (sperm concentration, **total sperm count**), whereas no association with **perfluorooctane sulfonate** (**PFOS**), polychlorinated biphenyls (PCBs), or **p,p'-DDE** was observed (Vested et al. 2013, 2014b). PFOA can inhibit **testosterone** production (Eggert et al. 2019). While both dioxins and PFOA are persistent toxicants that have a long half-life in our body, phthalates have a short life, but exposure to them can be ubiquitous. Adult exposure to phthalates has been reported to be associated adversely with semen quality (Cai et al. 2015; Axelsson et al. 2015a; Thurston et al. 2015), whilst results on bisphenol A remain inconclusive (Minguez-Alarcon et al. 2016). Persistent organochlorine pollutant exposure of adults was only associated with changes in sperm motility and not concentration or morphology (Toft et al. 2006).

35.4.5 Hormone Levels

Toxic effects can target several endocrine organs that produce reproductive hormones. The **hypothalamus**, **pituitary gland** and testis act in good co-operation via functional feedback and feedforward circuits. Therefore, changes in reproductive hormone levels have to be considered always in relation with each other. For example, low **inhibin B** levels together with high **FSH** levels in adult men who were exposed to high levels of dioxins in early life in Seveso suggested direct testicular toxicity of dioxins (Mocarelli et al. 2011). Similarly, foetal exposure to PFOA was associated positively with adult FSH and **LH** levels, whilst no significant associations with gonadotropin levels were observed for PFOS, PCBs and p,p'-DDE (Vested et al. 2013; Vested et al. 2014a). Foetal phthalate exposure was also associated with increased serum concentration of gonadotropins in adult men (Axelsson et al. 2015b). Phthalate and PBDE concentrations in breast milk were positively associated with LH and LH-free testosterone ratio in boys at 3 months of age (Main et al. 2006, 2007).

35.5 Design and Interpretation of Toxicological Studies

35.5.1 Design of Non-Human Studies

The value of non-human studies rests on the balance between the strengths of experimental toxicology: the ability to make carefully controlled, reproducible observations on any relevant physiological variables, and its weaknesses—including the uncertainty of extrapolation from a test system to humans. **Randomisation** is essential to ensure **balance** between **known** as well as **unknown covariables in the control and treatment groups**. The conduct of the work should adhere to the protocols of validated assays, in which the investigators are blinded as to the identity of the control and test groups, which should be of an appropriate size, and should be analysed with suitable statistical methods. Precision, reproducibility and replication are all additional key features of the optimal experimental design expected in such studies. **Interpretation must consider** carefully the possibility of **species differences in** relevant factors such as **route of administration, distribution, metabolism, excretion and mode of action** of the chemical under consideration.

35.5.2 Design of Human Studies

Human toxicological studies usually yield retrospective epidemiological or clinical data relating to uncontrolled exposures so that controlling for covariables may be difficult or impossible. A major **source of bias** in human studies, particularly those requiring collection of semen samples, is the **participation rate**. Although the social acceptability of providing semen samples may have increased in recent years, most men are unwilling to participate unless they are concurrently concerned about their fertility (**entry bias** [the reproductive analogy of the 'healthy worker effect']) and the participation rates remain very low. This inevitably means that **participants** in such studies **are self-selected**, which introduces a systematic bias that is difficult to overcome and cannot be adjusted for.

As sperm counts are only a surrogate for male fertility, other surrogate measures of male fertility that avoid the need for semen analysis (e.g. time to pregnancy [Joffe et al. 1993, see Sect. 35.6.1.2, below], testis size, hormone levels) may prove more useful in practice for population studies. Typical end-points measured in human studies are derived from a medical history, a physical examination and samples of blood and semen. These will yield information on testicular volume, semen parameters (sperm count, motility, morphology, seminal volume, other special tests of sperm function) and blood FSH concentration. Serial observations are par-

ticularly useful in adjusting for the between-subject variability in many of these parameters. Normal-range values can provide some indication of whether an individual may have been intoxicated, especially if serial observations are not available.

35.5.3 Regulatory Testing for Reproductive Toxicity

35.5.3.1 Regulatory Reproductive Research Strategies

In regulatory terms, chemicals are regarded as **food chemicals, pharmaceuticals** or **compounds for environmental use**. The first category contains food additives and packaging components that come into contact with foodstuffs. The second covers drugs and compounds used in human and veterinary medicine, while the third includes any chemical whose use causes it to be released into the environment. This enormous range of chemicals and uses handled by independent regulatory agencies has further diversified testing requirements according to the proposed use of the compound and the country in which the application is made. The need to unify the safety-testing demands for chemical entities intended for international usage has resulted in agreement on **common strategies for drug toxicity testing** between the European Community, the United States and Japan. Through the mutual establishment of the International Committee on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, recommendations for assessment of reproductive toxicity have now been presented and adopted by the regulatory authorities in each domain. (Adopted as, 'ICH Harmonised Tripartite Guideline: Detection of Toxicity to Reproduction for Medicinal Products, June 1993'; and also as an Addendum: '… Detection of Toxicity to Reproduction for Medicinal Products and Toxicity to Male Fertility, November 2005'.) Based on the '3 segment' design first introduced by the US Food and Drug Administration (FDA) in 1966 (see Sullivan 1988), they involve a **fertility study in male and female rats**, **a pre- and post-natal study in pregnant female rats and a teratology study** in the rat and one other mammalian species. The Addendum recommends at least a 2-week pre-mating dosing period for males treated in regulatory studies since relying on mating alone is an insensitive means of detecting effects on spermatogenesis. Furthermore, since almost all compounds that have been found positive in such tests were shown to affect post-meiotic cells, a 2-week pre-mating dosing period is sufficient (Sakai et al. 2000). Only the first can identify direct effects on male reproduction, although any preliminary studies performed to set dose levels should obtain data on testicular histology, sperm motility and viability. If good data are available from such studies, **seminology** data are not required from the fertility study.

Food chemical and environmental chemical regulation is overseen by the FDA and the Environmental Protection Agency respectively, in the USA, by the Organisation for Economic Cooperation and Development in Europe and by the Japanese Ministry for Agriculture, Fisheries and Food in Japan. All these agencies require essentially the same testing for these chemicals, which involves

- **Teratology study in two species**
- **One-generation toxicity study**
- **Two-generation toxicity study**or
- **Extended one-generation toxicity assay**

Testicular pathology and **seminology data** are required from all generations.

The most significant new development in recent years is the recognition of the potential significance of endocrine disruptors in male reproductive toxicology, reflected by the huge body of literature currently accumulating on this topic. The **OECD** has published guidelines (OECD 2018) for the conduct and interpretation of all major assays that have been used for the toxicological testing of endocrine disruptors in reproductive systems across a wide range of species in both males & females. The document focusses not only on the procedures themselves, but also reflects on the assays and the interpretation of data arising from their use.

35.5.3.2 Regulatory Reproductive Research Strategies

35.5.3.2.1 Experimental Methods in Male Reproductive Research

Regulatory male reproductive toxicology is increasingly adopting techniques used in basic research. In addition to the basic techniques already in wide use, these include:

- Detailed **histological evaluation of the germinal epithelium**
- Recognising the characteristic associations and stages (Hess 1990)
- **Flow cytometry**, which quantitates both total sperm numbers as well as the proportions of haploid, diploid and tetraploid cells in the testis
- **Vital dye staining** to determine the numbers of cells with intact membranes as an estimate of viability
- **Computerised techniques for objective assessment of sperm motion** (Seed et al. 1996)
- **Transgenic animals**
- Novel in vitro assays

- A proliferation of 'omics' approaches, including **genomics, toxicogenomics, epigenomics, proteomics, transcriptomics, exposomics, metabolomics,** etc. These attempt to capture the totality, or a representative proportion, of data available within a biological system for a given type of end-point. One of the major challenges for the future is the integration of combinations of these data sets for a more complete understanding of the effect of exposures on biological systems.

A variety of in vivo genotoxicity assays have also been developed to detect potentially transmissible genetic mutations in germ cell DNA. These include tests for **dominant lethal mutations, specific-locus mutations, dominant skeletal mutations, heritable translocations** and **aneuploidy**. Other research techniques such as stereological quantitation of germ cell numbers and genetic evaluation of germinal epithelium are yet to be widely adopted. In part, this is due to the expense and time required to undertake animal studies. In the light of the increasing need for toxicity testing (see the Introduction to this chapter), increasing emphasis is being placed on the development of in vitro tests (Gura 2008). It should be noted however that the testing required under REACH does not involve any in vitro tests.

Critically, however, no single test provides an overall prediction of toxicity to the male reproductive system.

35.6 Future Perspectives

35.6.1 Experimental Studies

35.6.1.1 Non-Human Studies

The suggestion that intra-uterine exposure to endocrine-disrupting or other chemicals can affect future reproductive potential is a hypothesis that can only be tested in experimental animals. A variety of studies have reported that both potent and relatively weak xenoestrogens can affect the reproductive system in male rodents following pre−/neonatal exposure just as a number have reported negative effects. It is increasingly recognised that the timing of exposure is critical: there appear to be windows of susceptibility during early development that determine both whether an effect will be induced and of what type. These may go some way towards explaining the varied results reported among many studies. Studies are also needed to clarify the apparent greater sensitivity of the developing embryo/foetus to reproductive toxins compared with the adult, which could have an impact on regulatory testing strategies. The search for better predictors of human reproductive toxicity is acquiring greater urgency as public attention is increasingly focussed on potential adverse environmental influences on reproduction. In this respect, studies using chronic, low-dose regimens that mimic actual human exposures more closely than conventional acute dosing need to be developed and validated further together with more usable surrogate measures of male fertility.

Recent progress towards the understanding of how the level of mutations in sperm rises with age, should stimulate work to investigate whether environmental exposures can also influence these processes. In one of the first studies of this type, no effect of anti-cancer treatments on the incidence of certain 'selfish mutations' was found, although the highest levels of treatment seemed to depress the levels of one of the mutations (Maher et al. 2019). Whilst reassuring, similar investigations of other types of exposures are needed. In the same way, the coming years should see a much greater appreciation of the role of germline epigenetic factors in inheritance, including DNA methylation patterns, histone modifications and RNA among others. Once more is known about the basic biology of all these phenomena and their relevance to reproductive toxicology, tailored testing strategies can be developed to help protect the public.

35.6.1.2 Human Studies

An increasing awareness that many chemicals can adversely affect male reproductive function is an important starting point for future development in human reproductive toxicology. In future, more **systematic collections of reproductive information** from **exposed** and **unexposed workers** as well as more public availability of **pre-registration testing of new drugs** would greatly facilitate early and sensitive detection of previously unrecognised hazards to male reproduction. This might contribute to reducing the high proportion of infertile men in whom the diagnosis of male infertility remains unexplained but environmental causes are suspected. The last two decades, have failed to give clarity on the roles played by environmental exposures or lifestyle factors, while at the same time revealing a much greater complexity in the biological factors that may mediate toxic responses. Thus, progress on identifying environmental causes of male infertility have lagged behind the tremendous advances in understanding the genetic and epigenetic bases of spermatogenesis, and their defects in causing male infertility.

Analysis of semen samples for both clinical and research purposes should be standardised by universal adoption of the WHO *Laboratory Handbook for the Examination and Processing of Human Semen* (2021). Inter- and intra-laboratory variability in semen evaluation is increasingly being reduced by **quality control programs for semen analysis**. The establishment of **normal ranges**, however,

remains a difficult task. Data collected from any population, whether on numbers of children or sperm counts, may be difficult to interpret unless an appropriate control group can be identified.

The collection of sperm data from 'at risk' populations is fraught with practical difficulties including low and biased participation rates according to perceived risk, as well as deterministic interpretations of sporadic clusters of cases. The limited acceptability of semen collections makes it desirable that different monitoring systems less reliant on sexual function should be available. An effective alternative is the **Time-To-Pregnancy** (TTP) **questionnaire-based data collection system** developed by Joffe et al. (1993), which allows quantitative estimation of fertility performance of groups of men as a means of monitoring fertility in occupational or other settings. Thus, the routine collection of reproductive data in the workplace, whether from questionnaire or semen samples, would greatly improve the possibility for early warning of potential fertility problems.

35.6.2 Clinical Implications

35.6.2.1 Clinical Practice

In routine clinical practice, **the possibility of environmental and toxic factors being responsible for infertility and other andrological disorders should always be considered carefully**. The accurate identification of such influences is largely dependent upon an **index of suspicion** together with confirmation by a careful **history of occupational**, domestic, recreational and other potential **environmental exposures**. Laboratory confirmation of specific forms of intoxication may occasionally be helpful but is rarely possible. Although measurement of suspected toxins in body fluids (e.g. blood, seminal plasma) may be possible, its significance needs to be established by properly controlled clinical studies. Without these, interpretation can be impossible. A careful and thorough occupational history remains an essential part of proper evaluation of men for infertility or other andrological disorders. Specific attention should be paid to the type of work routinely performed, and compliance with and results of health monitoring, if applicable. In addition, domestic exposures from gardening (pesticide exposure), holidays (visiting farms or zoos) and hobbies should be considered. In addition to its value for counselling patients, such categorisation of life styles of patients may become useful for retrospective studies. In the case of patients who believe they have been intoxicated by various environmental agents, a full evaluation of reproductive function is usually necessary to dispel any concerns or identify the extent of damage.

Key Points

- Male germ cells can be adversely affected by factors causing either death of the cell, or genetic damage or epigenetic alterations, both of which can be heritable and affect gene function in the offspring.
- Factors that can cause these outcomes include: smoking, exposure to ionising or electromagnetic radiation, cancer therapies, heat, ageing or mixtures of toxins.
- Effects can be seen on the full range of male reproductive function, potentially leading to cryptorchidism, hypospadia, testicular cancer and a wide range of effects on sperm quality.
- In order to be sure of such observations, data must be gathered with rigorous attention to experimental design. For regulatory research, protocols and study design are mandated by legislation.
- It is noted that advances in reproductive biology in recent years have outstripped those in reproductive toxicology. Future research should utilise our increasing biological understanding to bring fresh perspectives on current toxicological problems.

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Part VIII

Andrological Therapy

Therapy with Testosterone

36

Eberhard Nieschlag and Hermann M. Behre

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Abstract

All forms of hypogonadism require therapy with testosterone. Even in secondary hypogonadism, testosterone substitution is indicated in the long-term, which is only interrupted for the duration of GnRH or gonadotropin therapy if the patient wishes to be fertile. For many decades, substitution with testosterone enanthate or cypionate was the leading form of testosterone substitution. In the 1970s, orally active testosterone undecanoate was added and in the 1990s, the first transdermal preparations in the form of membranes and patches were introduced. Later, testosterone gels became available allowing physiological serum testosterone levels to be reached. With injectable testosterone undecanoate, a true depot preparation came into clinical use. Regular monitoring of testosterone substitution must observe the positive effects and, due to possible undesirable side effects, especially monitor red blood count and prostate.

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36.1 Overview of Indications and Preparations

All forms of hypogonadism described in the previous chapters that are associated with Leydig cell insufficiency require therapy with testosterone. The prerequisite is a reliable diagnosis of hypogonadism based on symptoms of androgen deficiency and low-serum testosterone levels. Even in secondary hypogonadism, testosterone substitution is indicated in the long-term, which is only interrupted for the duration of GnRH or gonadotropin therapy if the patient wishes to be fertile.

Testosterone was first synthesized in 1935 (Butenandt and Hanisch 1935; Ruzicka and Wettstein 1935; Nieschlag and Nieschlag 2019) and was available for clinical use a year later. The main indication for testosterone is male **hypogonadism**. An overview of further possible applications is provided in Table 36.1. Some of these are dealt with in special chapters in this book, such as the use of testosterone in **constitutionally delayed puberty** (see Chap. 14), in **age-related hypogonadism** (see Chap. 25), in male hormonal contraception (see Chap. 48), and in abuse for doping in sports and by bodybuilders (see Chap. 37). The obsolete use in idiopathic infertility is also discussed (see Chap. 39). Furthermore, this chapter deals with use in cases of excessive height (Sect. 36.6).

Indication for therapy with testosterone is given when morning serum testosterone concentrations fall below a certain value accompanied by symptoms of androgen deficiency (Chap. 5, Table 5.1). In Germany, a value of 12 nmol/L has generally been regarded as lower limit of normal for many years. Surprisingly, this value was lower in other countries, for example in Spain (9.0), Great Britain (7.5–8.0), and France (7.5 nmol/L) (Nieschlag et al. 2004). A study in older hypogonadal patients designed to clarify these differences concluded that there is no single **threshold** for the occurrence of symptoms of hypogonadism, but that there are symptom-specific thresholds between 15 and 8 nmol/L (Fig. 36.1). Loss of drive and libido already occur below 15 nmol/L, while testosterone-deficiency-related erection loss occurs only below 8 nmol/L (Zitzmann et al. 2006). The other symptoms have their threshold values between these two values. A study of younger hypogonadal men came to a similar conclusion (Kelleher et al. 2004). The question at which testosterone level therapy should be initiated has thus been based more on the subjective perception of the diagnosis of hypogonadism by the medical profession than on patients' complaints. The fact that libido loss is also the most common symptom of testosterone deficiency reported by doctors surveyed (Gooren et al. 2007) indicates the low disease value that doctors attribute to sexuality. The international recommendations of age-related hypogonadism (LOH) also still give 8 nmol/L as the absolute limit for testosterone substitution and the range between 8 and 12 nmol/L as a relative indication (Nieschlag et al. 2004; Wang et al. 2009). In the course of time, the now numerous guidelines for testosterone therapy have also accepted this lower limit value of 12 nmol/L. In unclear cases, the calculated free testosterone below 225 pmol/L can also be used as an indicator for testosterone substitution (Table 36.2) (Chap. 7, Table 7.1).

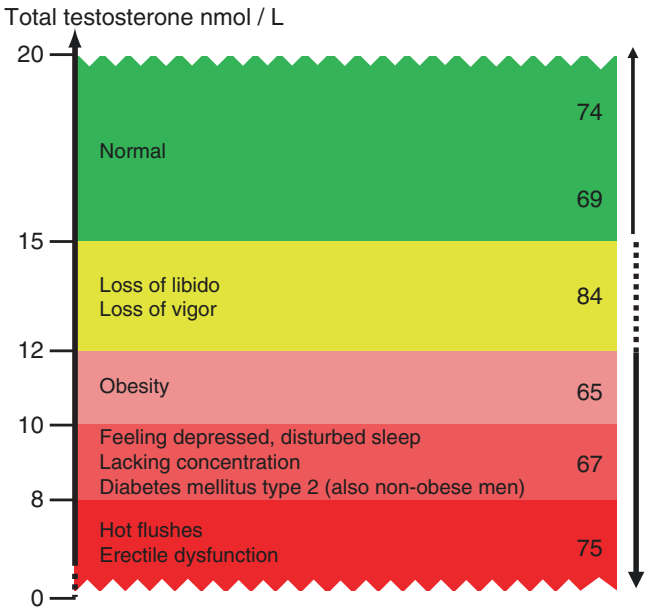


Fig. 36.1 **Threshold levels** of serum testosterone for various symptoms of late-onset hypogonadism (LOH) in 434 patients (Zitzmann et al. 2006)

Table 36.1 Use of testosterone in men

Clinical application	Hypogonadism
	Primary hypogonadism
	Secondary hypogonadism
	Late-onset hypogonadism (LOH)
	Delayed puberty
Off-label use	Excessive height
Experimental use	Male contraception
Obsolete use	Idiopathic infertility
Abuse	Sports and bodybuilding

Testosterone substitution must therefore be based on the symptoms of the patient and take into account symptom-specific thresholds. 12 nmol/L testosterone in serum is still a good orientation guide as a lower limit.

Table 36.2 Lower limits for total and free testosterone according to the guidelines of various organizations

Organization	Total T (nmol/L)	Free T (pmol/L)
ISA, ISSAM, EAU, EAA, ASA[a] Wang et al. (2009)	8–12	225
US endocrine society Bhasin et al. (2018)	9.2–10.5	
International consultation for sexual medicine Morgentaler et al. (2019)	12	225
European academy of andrology Corona et al. (2020)	12	
European association of urology Salonia et al. (2020)	12	225

[a] ISA International society of andrology, ISSAM International society for the study of the aging male, EAU European association of urology, EAA European academy of andrology, ASA American society of andrology

Since this is a substitution therapy, the effectiveness of the treatment can be read directly from serum testosterone concentrations.

According to international consensus (WHO 1992), which is still valid today, the immediate goal of **testosterone substitution** should be to achieve serum testosterone concentrations in the middle normal range. In addition, the **natural testosterone molecule** should be used for substitution, since this is the best way to guarantee the broad spectrum of testosterone effects. Available testosterone preparations must be judged according to these criteria.

In some target organs, testosterone acts directly, in others it must first be converted into 5-dihydrotestosterone or estradiol in order to become effective (Chap. 2). In order to achieve a physiological balance between testosterone and its effective metabolites, it makes sense to use natural testosterone for substitution and not synthetic androgens that are predominantly metabolized in one direction or the other (e.g., 19-nortestosterone) or are direct derivatives of a metabolite (e.g., mesterolone). Even the direct or even sole administration of estrogens to the hypogonadal man cannot replace testosterone substitution. No rational basis has yet been found for the use of testosterone precursors such as dehydroepiandrosterone (DHEA) or androstenedione. Past and current attempts by the pharmaceutical industry to replace testosterone in substitution therapy with **synthetic androgens** or **Selective Androgen Receptor Modulators (SARMs)** have not yet resulted in a substitute preparation. The use of natural testosterone is the safest way to induce or maintain all androgen-dependent functions and avoid possible side effects.

Testosterone was first synthesized in 1935 (Butenandt and Hanisch 1935; Ruzicka and Wettstein 1935) and was introduced into the clinic shortly afterwards, making it one of the oldest hormones in clinical use (Nieschlag and Nieschlag 2019). Initially, testosterone **implants** were used and, since the 1950s, testosterone **enanthate** or **cypionate** were preferred as intramuscular injections. In the 1970s, the orally active testosterone **undecanoate** was added and in the 1990s, the first transdermal preparation, a scrotal testosterone film, was introduced. Further patches for use on the rest of the skin followed and later testosterone **gels** were introduced. Finally, the injectable testosterone **undecanoate** was launched as a true depot preparation. A mucoadhesive **buccal testosterone tablet** completes the range of preparations available (Nieschlag and Behre 2012a).

Based on the clinical experience of the physician and the personal experience of the patient, the most suitable preparation for the individual patient according to clinical picture and phase of life must be selected from this spectrum. Often, the optimal choice for the individual patient is only found after testing different preparations.

36.2 Pharmacology of Testosterone Preparations

In chemical terms, testosterone is derived from the basic structure of all androgens, i.e., from androstane. Testosterone owes its specific biological activity to the keto group in position 3, the double bond in position 4, and the hydroxy group in position 17 of the basic androstane structure (see Fig. 36.2 and Chap. 2).

There are three ways to use testosterone in therapy:

1. Chemical modification of the molecule
2. Esterification in position 17
3. Different forms of application

Since the forms of application are of particular importance for clinical concerns, they are chosen here as the classification criterion for testosterone preparations. In addition to the routes listed here, testosterone can also be administered nasally, conjunctively or rectally; however, currently these forms of administration play only a minor role in clinical practice.

In this chapter, the clinically relevant aspects of androgen therapy are discussed. For further information, please refer to the relevant monographs (e.g., Nieschlag and Behre 2012b). Table 36.2 and Fig. 36.3 provide an overview of the pharmacokinetics of the testosterone preparations currently available in clinical practice.

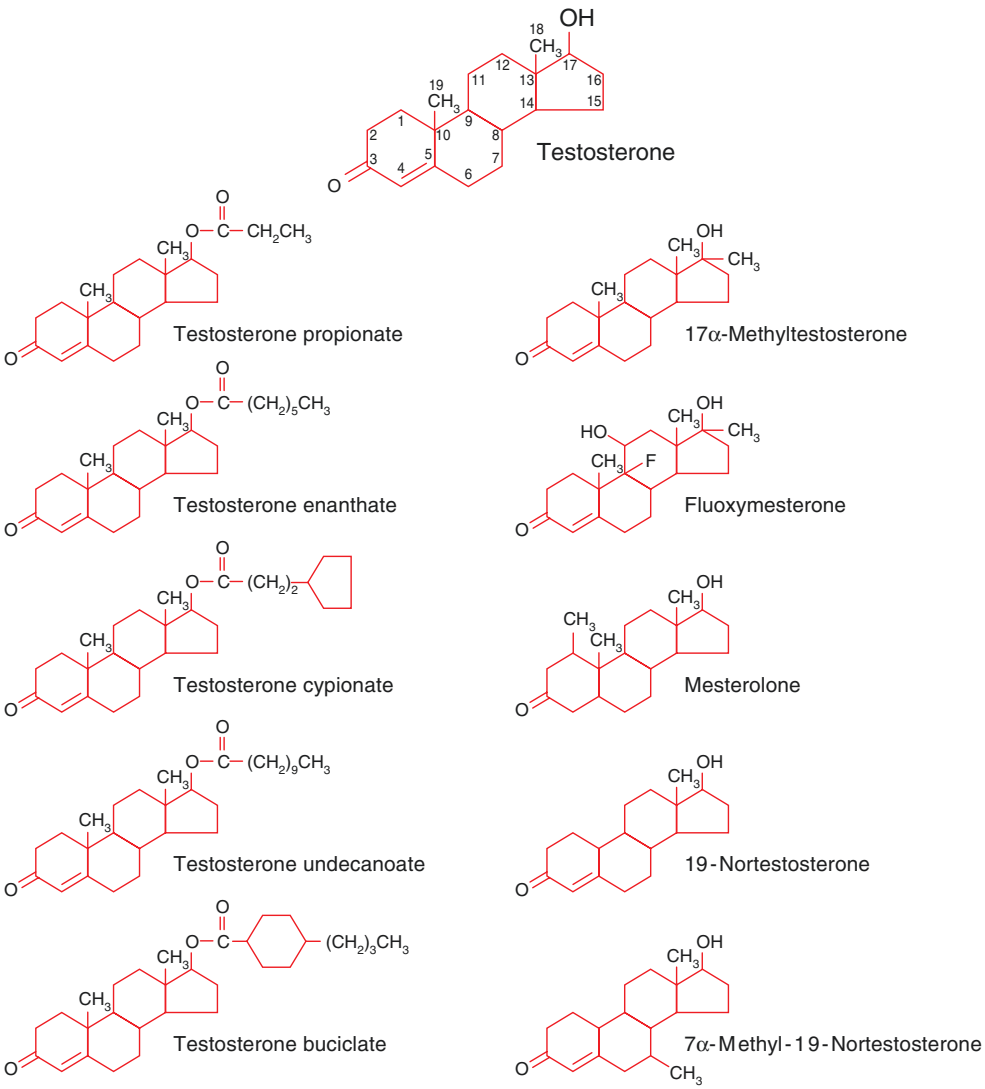


Fig. 36.2 Molecular structures of testosterone and various testosterone preparations

36.2.1 Oral Testosterone Preparations

The attempt to use natural testosterone, as secreted by the testes, for substitution therapy is obvious. In fact, orally administered free testosterone is also readily absorbed from the intestine, but it is completely metabolized in the liver in a **"first-pass effect"**so that it does not reach the target organs. Only when 400–600 mg of testosterone is administered orally, i.e., about 100 times more than is normally produced by the testes per day, is the testosterone-metabolizing capacity of the liver exceeded and peripheral serum levels in the normal range are achieved. Since administration of such large doses of testosterone seems uneconomical and long-term side effects are difficult to estimate, this form of therapy never went beyond the experimental stage (Nieschlag and Behre 2012a).

36.2.1.1 Testosterone Undecanoate

To avoid the "first-pass effect" of the liver after oral application, testosterone was esterified with undecanoic acid at position 17ß. This long aliphatic side chain directs the absorption of the molecule into the lymphatic system, so that testosterone enters the circulation through the thoracic ducts and via the subclavian vein and reaches the target organs before being metabolized in the liver. Resorption is further improved if a meal with fatty content is taken at the same time.

Testosterone undecanoate is offered dissolved in oil in 40 mg capsules. Since testosterone accounts for 63% of the molecular weight, one capsule contains about 25 mg testosterone. In order to achieve good absorption, it should be taken with a meal (Bagchus et al. 2003). Maximum serum peaks are observed 2–6 h after ingestion (Schürmeyer et al.

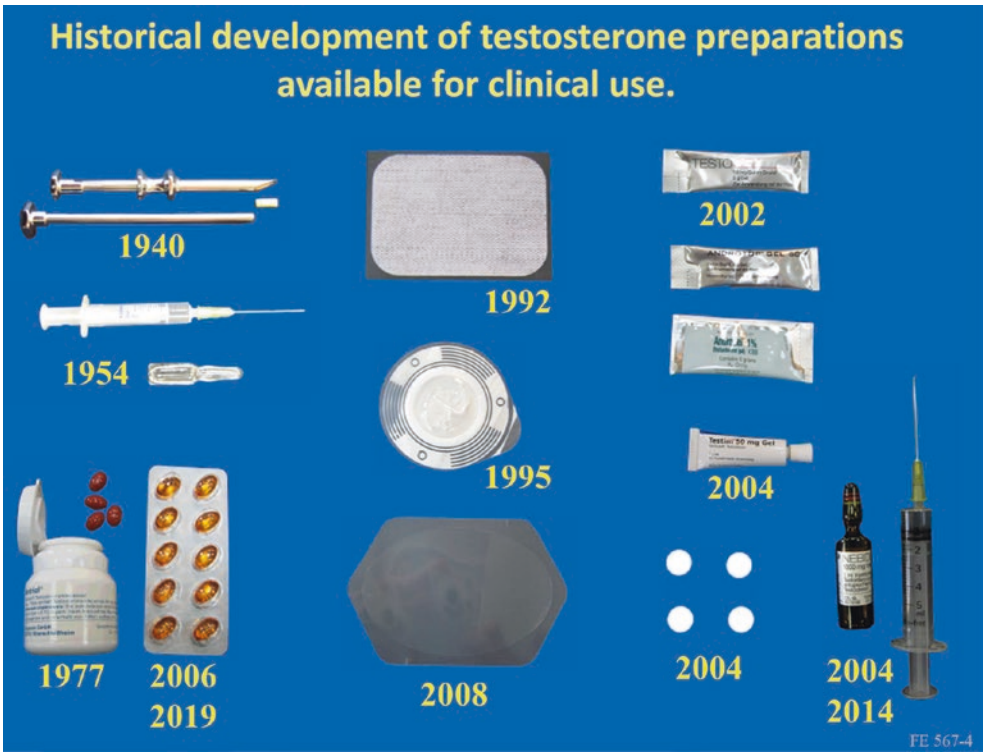


Fig. 36.3 Historical development of testosterone preparations for clinical use (T, testosterone)

1983; Behre et al. 2004). Therefore, 2–4 capsules spread over the day are required for substitution. Although relatively high concentrations of testosterone are administered, no long-term side effects or toxic effects have been observed (Gooren 1998).

Testosterone undecanoate is particularly suitable for substitution therapy if a certain amount of testosterone is still produced by the patient himself (e.g., Klinefelter's patient in the early stages of substitution) or if intramuscular injections cannot be administered due to coagulopathies (e.g., marcumarized patient) or if transdermal gels are not desired. The main disadvantages of this therapy are the short-term fluctuations in serum testosterone that do not correspond to physiological conditions and the poor predictability of individual absorption patterns (Fig. 36.4).

Although orally administered testosterone undecanoate at a dosage of 40 mg/capsule has been approved for clinical use in most countries, including Canada since 1985, it has not been launched in the United States. It was not until 2019 that testosterone undecanoate in a capsule with dosages of 158, 198, and 237 mg testosterone undecanoate in an emulsified solution was approved in the USA. The dosage is intended for oral use twice daily before breakfast and dinner. One 237 mg testosterone undecanoate capsule is equivalent to 150 mg testosterone. This recommended initial dose is approximately three times higher than that of the originally approved oral testosterone undecanoate, and in hypogonadal patients, serum levels of testosterone are usually within the normal range (Swerdloff et al. 2020).

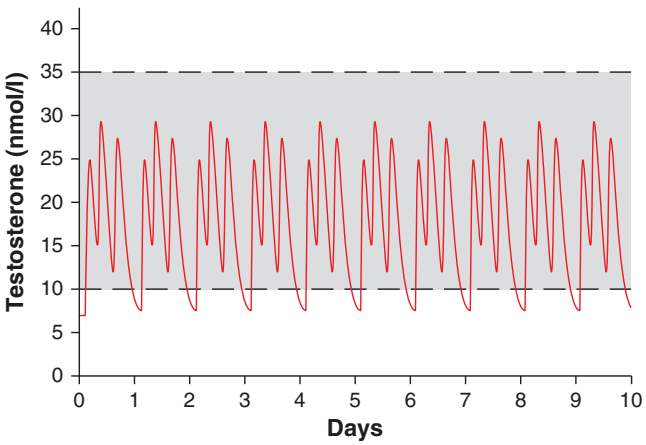


Fig. 36.4 Serum testosterone concentrations following oral application of 3 × 40 mg testosterone undecanoate to hypogonadal men (pharmacokinetic computer simulation assuming basal testosterone levels of 7 nmol/L). The gray area indicates the range of testosterone in normal men

36.2.1.2 Methyltestosterone and Fluoxymesterone

17-methyltestosterone resulted from experiments on the chemical modification of the testosterone molecule shortly after its initial synthesis in 1935 (Ruzicka and Wettstein 1935). The methyl group in the 17 α-position protects the testosterone molecule from metabolism in the liver, so that it can reach the target organs after oral administration. However, long-term use can lead to elevated liver enzymes,

cholestasis, and peliosis of the liver and is therefore no longer used clinically in Europe.

Fluoxymesterone also contains a methyl group in the 17α-position in addition to a fluoride atom and a hydroxyl group. This makes fluoxymesterone a highly effective oral testosterone preparation, but the 17α-methyl group also leads to liver toxicity. This is why this preparation has also disappeared from the market in Europe, first as single preparations and then also in combined preparations. However, since they are still on the market in other countries, a warning must be issued against their **toxic side effects.**

Finally, it should be mentioned that **17α-methylation** leads to liver toxicity in all androgens (including anabolic steroids) and these preparations should therefore be considered obsolete.

36.2.1.3 Mesterolone

Mesterolone is derived from the 5α-reduced testosterone metabolite 5-dihydrotestosterone (DHT) and is also protected from immediate metabolism in the liver after oral administration. As a DHT derivative, however, it can only compensate for functions of DHT-dependent functions and not, for example, the direct effects of testosterone and those after aromatization to estrogens. Therefore, it does not unfold the full spectrum of action as required for substitution therapy and is not suitable for substitution in hypogonadism. Since a number of testosterone preparations are available, mesterolone is hardly ever prescribed, but is still traded for doping purposes.

36.2.2 Buccal Forms of Administration

By incorporating testosterone into **polyethylene matrices** with limited water solubility, it has been possible to develop buccal applications. The mucoadhesive tablets adhere to the gingiva above the incisors for many hours and slowly release testosterone into the circulation. When taken twice daily for equal periods of time, the result is uniform serum levels (Korbonits et al. 2004; Nieschlag et al. 2004; Dinsmore and Wyllie 2012).

36.2.3 Intramuscular Testosterone Preparations

36.2.3.1 Testosterone Enanthate

If natural testosterone is administered intramuscularly, it has a very short half-life. In order to extend its duration of action, testosterone was esterified with aliphatic side chains in position 17. The duration of half-life depends on the length and structure of the side chain.

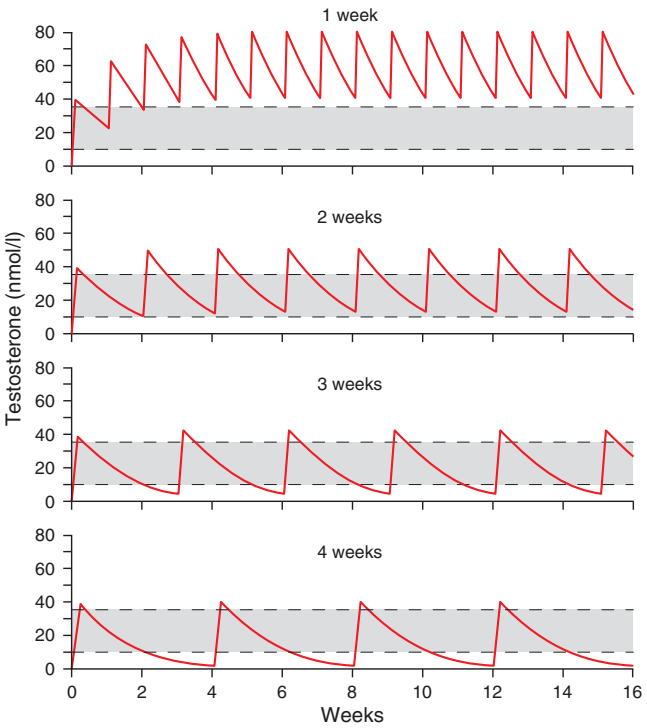


Fig. 36.5 Testosterone serum levels in hypogonadal men following intramuscular injections of 250 mg testosterone enanthate given at intervals of 1, 2, 3, or 4 weeks. The gray area indicates the normal range of testosterone

Testosterone **enanthate** was introduced for substitution therapy in 1952 and is administered intramuscularly. Testosterone enanthate has a terminal half-life of 4.5 days (Table 36.2). The standard dosage is 250 mg testosterone enanthate. As shown by pharmacokinetics (Fig. 36.5), supraphysiologically high concentrations of testosterone serum are reached very quickly and persist for several days (Behre and Nieschlag 2012). Afterwards, serum levels gradually drop to pass the lower normal limit on about day 12. Repeated injections, as required for substitution therapy, thus create a "sawtooth profile" in which phases with supraphysiological, physiological, and infraphysiological values follow one another, depending on the injection interval (Fig. 36.5).

While this form of substitution is sufficient to maintain the biological effects of testosterone, the patient perceives the strong fluctuations as disturbing, since general well-being, mood, and sexual activity follow these ups and downs. Nevertheless, testosterone enanthate was the most widely used preparation for a long time because there were no suitable alternatives.

In 2019, testosterone enanthate was approved for **subcutaneous injection** in the USA. The preparation is offered as an autoinjector in doses of 50 or 100 mg, which are administered once a week. The pharmacokinetics are similar to those of intramuscularly injected testosterone enanthate, but the

Table 36.3 Pharmacokinetic characteristics of various testosterone preparations

Preparation	Application	Mean residence time (MRT) (t)	Terminal half-life (t ½)
Testosterone undecanoate	p.o.	3.7 h	1.6 h
Testosterone propionate	i.m.	1.5 days	0.8 days
Testosterone enanthate	i.m.	8.5 days	4.5 days
Testosterone undecanoate			
In tea seed oil	i.m.	34.9 days	20.9 days
In castor oil	i.m.	36.0 days	33.9 days

majority of patients report pain for 24 h at the injection site (Kaminetsky et al. 2015).

Testosterone cypionate and **testosterone cyclohexane carboxylate**, which are on the market in some countries, have the same pharmacokinetics and thus the same advantages and disadvantages as testosterone enanthate (Behre and Nieschlag 2012).

36.2.3.2 Testosterone Propionate

Testosterone propionate has a short half-life corresponding to the short side chain (Table 36.2). The initial serum concentrations after intramuscular injection can be similar to those of testosterone enanthate, while due to the short half-life, injections must be repeated every 2–3 days to achieve full substitution. Testosterone propionate is therefore not suitable for long-term therapy because of the need for frequent injections. Even a combination of testosterone propionate and testosterone enanthate does not öffer any advantages for therapy (Behre and Nieschlag 2012).

36.2.3.3 Testosterone Undecanoate

Testosterone undecanoate (see Sect. 36.2.1.1), which was introduced in Europe as an oral preparation as early as the 1970s, was first developed in China as an injectable preparation. In China, testosterone undecanoate was dissolved in tea seed oil. When castor oil is used as a vehicle, the half-life can be extended even further (Behre et al. 1999a, Table 36.3). In addition to the longer duration of action, the absence of the initial supraphysiological peak values compared to testosterone enanthate can be considered an advantage (Fig. 36.6).

For substitution, 1000 mg testosterone undecanoate in 4 mL castor oil is injected intramuscularly about four times a year. In order to achieve a steady state at the beginning of substitution, the second injection is given 6 weeks after the first, the further injections then after 10–14 weeks each (Fig. 36.7). The individual interval depends on the serum testosterone levels measured immediately before the next injection. These measurements are then repeated at annual

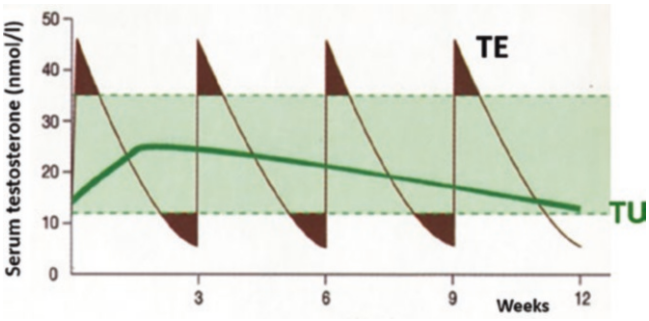


Fig. 36.6 Comparative pharmacokinetics following four intramuscular injections of 250 mg testosterone enanthate at 3weekly intervals (**TE = black line**) and one single injection of 1000 mg testosterone undecanoate (**TU = green line**) in hypogonadal men. The dotted lines represent the normal range of testosterone

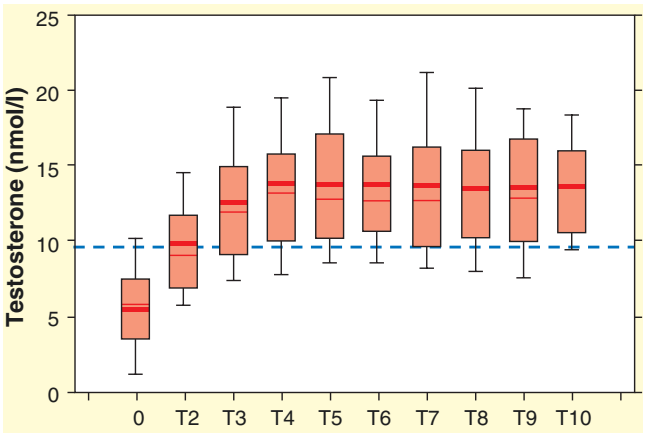


Fig. 36.7 Serum testosterone concentrations before and during long-term therapy with intramuscular testosterone 1000 mg (Nebido®, injection interval between the first and second injections lies between one and two 6–10 weeks, thereafter every 10–14 weeks). Serum levels are shown as box and whiskers plots (red line in box: mean value; black line in box: medium value: 15% of single determinations within box; 10th and 19th percentile shown by whiskers). 0 = basal testosterone concentrations before therapy; T2 = testosterone concentration immediately before 2nd Nebido® injection, etc. Dotted line indicates lower level of normal

intervals. If the values are too high, the injection intervals will be extended, if they are too low, they will be shortened.

After 1000 mg testosterone undecanoate for intramuscular injection was approved in most countries shortly after 2004, testosterone undecanoate for intramuscular injection was not approved in the USA until 2015. This US preparation contains 750 mg in 3 mL and must be injected at shorter intervals (Krakowsky et al. 2017).

Due to the relatively large volume, the intergluteal injections should be administered slowly. Even after many years of use, no serious side effects have been reported (Zitzmann and Nieschlag 2007). In rare cases, the oily solution may enter the venous system and cause microemboli in the lungs,

which manifest themselves as coughing and irritation of the throat (Middleton et al. 2015; Pastuszak et al. 2020). Correct injection technique is imperative.

36.2.4 Transdermal Testosterone Preparations

36.2.4.1 Testosterone Patches

Transdermal application of pharmaceuticals has gained in popularity in recent years due to numerous advantages over conventional forms of application. In the field of endocrinology, transdermal application of estrogens for treatment of climacteric deficiency symptoms in women has become particularly popular. However, development of a transdermal system for substitution therapy of hypogonadal men has met with difficulties, since in men, doses in the range of the normal self-production of testosterone, i.e., about 6 mg/day, have to be transported through the skin, whereas the corresponding dose for estradiol in women is in the microgram range. However, different skin types have different absorption capacities. Because of the physiological task of temperature regulation, scrotal skin, extending to the uppermost epithelial layers, is particularly well supplied with blood, and has a particularly high absorption capacity (about 40 times higher than the skin of the forearm). This special feature was exploited in the development of a transscrotal application system. Although this preparation is currently no longer used, it was the first transdermal preparation and deserves mention.

The **transscrotal therapeutic system** consists of 40 or 60 cm^2 polymeric membranes loaded with 10 or 15 mg of pure natural testosterone. When these membranes are applied to the scrotum, they release enough testosterone into the circulation to guarantee physiological serum testosterone levels for 1 day. If the membrane is applied in the morning hours, even the physiological daily rhythm of testosterone can be imitated (Bals-Pratsch et al. 1986). Daily renewal results in constant serum testosterone levels within the normal range. Patients who were treated in this way for up to 10 years showed very good results of the substitution therapy (Behre et al. 1999b). Since an "enhancer" does not have to be used, skin irritations are rare. To ensure close skin contact, the scrotum must be freed from hair from time to time (scissors or shaving).

Following transscrotal forms, a **transdermal testosterone patch** was also developed which can be applied to non-scrotal skin (e.g., abdominal skin, upper arm). In order to be able to transport the required amount of testosterone through the skin, these systems are equipped with an "enhancer," which can lead to skin irritation. For substitution, two systems must be applied in the evening and worn for 24 h. For replacement therapy, this preparation also requires daily renewal. Similar to the transscrotal system, testosterone levels can be achieved in the normal range and with a physiological daily profile, with DHT and estradiol also remaining in the normal range. Although today the previously mentioned testosterone patches are hardly ever used, another testosterone patch was developed that caused hardly any skin irritation and only needed to be changed every 2 days, but two systems with either 1.8 or 2.4 mg resorption amount/day had to be used (Raynaud et al. 2010). However, in view of other modalities, the manufacturer withdrew the preparation from the market in 2015.

36.2.4.2 Testosterone Gels

A further dermal application option is the use of hydroalcoholic **testosterone gels** containing 1% to 1.6% testosterone, which are applied to larger areas of skin to ensure sufficient testosterone absorption. These gels are applied to the upper arms, shoulders or abdomen in the morning and left there to dry for about 5 min. During this time, contact with women and children must be avoided due to the risk of transmission. After this short period, the risk of contamination is negligible, especially if the skin is washed after evaporation of the alcohol (Rolf et al. 2002a, 2002b). If these gels are applied in the morning, physiological serum values will result in normal serum levels for several hours (Swerdloff et al. 2015) (Fig. 36.8). Recently, gels with higher testosterone concentrations of 2% and 2.5% were introduced without any risk of skin irritation (Arver et al. 2018; Kühnert et al. 2005). As smaller areas of skin are required for absorption, a scrotal

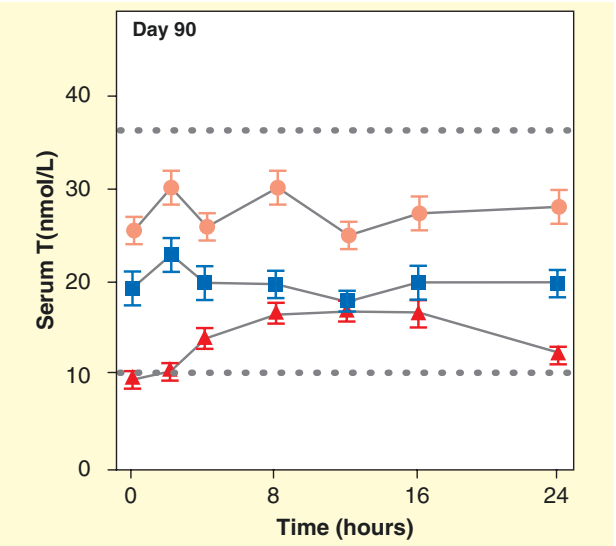


Fig. 36.8 Serum testosterone concentrations (mean ± SEM) after 90 day therapy with transdermal testosterone gel (Testogel® or Androtop® Gel) at a dose of 50 mg/day (squares, one package/day), oder 100 mg/day (circles, 2 packages/day) compared to transdermal therapeutic system (Androderm®, triangle, 5 mg/day). Serum levels are shown over 24 h (0 = 8 a.m.). Dotted line represents the normal range (modified from Swerdloff et al. (2020) with permission from the Endocrine Society, USA)

application is also being considered, which requires less gel overall, as scrotal skin has a much higher absorption capacity than the rest of the integument, allowing testosterone to be used more sparingly and in a more environmentally friendly manner (Kühnert et al. 2005).

Overall, the different gels show good results when used over a long period of time over several years.

36.2.4.3 Transdermal Dihydrotestosterone

In France, another transdermal preparation is on the market containing **5α-dihydrotestosterone** in an ointment or gel. The DHT is incorporated in a concentration of 2.5% in a hydroalcoholic gel from which dihydrotestosterone penetrates the skin when applied to sufficiently large areas, e.g., the chest and abdomen. Supraphysiological DHT values are measured in serum. Hypogonadal patients treated in this way show satisfactory short-term substitution results (Schaison and Couzinet 1998). However, the same criticism applies to this form of substitution as to mesterolone, since DHT cannot exert the direct effects of testosterone and the estradiol-mediated effects. Therefore, this preparation is used in individual therapeutic trials for the treatment of a micropenis in boys or gynecomastia in adolescence. As with testosterone gels, attention must be paid to possible transferability to contact persons.

36.2.5 Testosterone Implants

Testosterone implants are among the oldest testosterone preparations. They consist of pure testosterone, molded into cylindrical forms of 12 mm length and 4.5 mm diameter and heated. One implant contains 200 mg. The implants are inserted under the abdominal skin under sterile conditions using a trocar through a 0.5–1 cm long incision. The wound is closed with plaster or suture. Patients who are prone to infections are given antibiotics prophylactically. When 3–6 implants are applied, slowly decreasing serum testosterone levels in the normal range are achieved for 4–6 months (Behre and Nieschlag 2012; Pastuszak et al. 2012). Despite the long depot effect and favorable serum testosterone levels, only one preparation is on the market in Great Britain, Australia, and South Africa. The small surgical procedure required for implantation, the extrusion of the implants observed in 8.5% of applications (n = 973 in 221 patients over 13 years of age), and occasional bleeding (2.3%) and infection (0.6%) are limiting factors (Kelleher et al. 1999).

36.2.6 Nasal Testosterone Preparations

As early as the 1970s, a nasal application of testosterone propionate was tested experimentally. Positive effects on symptoms of androgen deficiency were reported in pilot studies (Danner and Frick 1980). Recently, a testosterone gel has been approved for nasal use in the USA and Canada. The gel containing 5.5 mg of testosterone in one dose is inserted into each nostril four times daily. Even when sufficient testosterone is absorbed, patients find frequent applications irritating, and side effects have been observed in sinusitis and pharyngitis (Rogol et al. 2016).

36.3 Contraindications for Testosterone Therapy

Before testosterone therapy is initiated, contraindications must be excluded or therapeutically eliminated. An existing **prostate carcinoma** is an absolute contraindication, since testosterone does not cause prostate cancer, but can promote growth of an existing one. A suspicion must be clarified in each case. **Breast cancer** must also be ruled out, since this carcinoma in men is usually androgen and estrogen receptor-positive and therefore testosterone would promote its growth.

Chronic, severe **heart failure,** and **polyglobuly** with hematocrit >54% are reasons to refrain from testosterone substitution.

In some countries, **sexual delinquents** are protected from relapses by antiandrogens or even castration. In these cases, it would be wrong to treat these individuals with testosterone. The prescribing doctor may even be held legally responsible.

Since testosterone suppresses spermatogenesis and is therefore being tested experimentally as a contraceptive for men, testosterone therapy is contraindicated in patients who wish to have children. Therefore, any acute or foreseeable desire for **paternity** must be clarified with each patient. This question is particularly important in patients with secondary hypogonadism, for whom therapy with gonadotropins or GnRH would then be preferable. In Klinefelter patients, too, the question of fertility must be clarified prior to testosterone therapy.

If hypogonadism is detected in a participating **athlete** being controlled for doping, testosterone may only be given if the athlete has been granted a Therapeutic Use Exemption by the national sports organization and the World Antidoping Agency (WADA).

Finally, it should again be stressed that testosterone should only be given when **hypogonadism** is confirmed by symptoms and low testosterone (Table 36.4).

Table 36.4 Contraindications for testosterone therapy

Existing prostate cancer
Virile breast cancer
Hematocrit >54
Chronic, severe heart failure
Active desire to have children
Lack of diagnosis of hypogonadism

36.4 Monitoring Testosterone Therapy in Hypogonadism

All forms of therapy listed here pursue the same purpose, namely to provide the hypogonadal patient with an optimal supply of testosterone. The physiological effects of testosterone, pharmacokinetics, and pharmacodynamics result in a wide variety of parameters with which the effectiveness of testosterone therapy can be tested. These parameters are also the subject of the various guidelines (e.g., Wang et al. 2009; Bhasin et al. 2018; Morgentaler et al. 2019; Corona et al. 2020; Salonia et al. 2020) and are discussed in the following sections (Table 36.5).

36.4.1 Psyche and Sexuality

The patient's **general well-being** and **activity level** is a good parameter to check the effectiveness of substitution therapy. With adequate testosterone therapy, the hypogonadal patient feels physically and psychologically more active, alert, and in good spirits, while inadequate testosterone levels are accompanied by **inactivity, lethargy,** and **depressive moods** (Barratt-Connor et al. 1999; Christiansen 2004; Wang et al. 2004). Elements of **aggression** such as **tension**, **irritability,** and anger are also favorably influenced by testosterone substitution (O'Connor et al. 2002). This improvement in the aggressiveness of hypogonadal men through testosterone substitution must be clearly differentiated from the undecided question of whether supraphysiological testosterone doses increase aggressiveness in normal men (Christiansen 2004). In hypogonadal men, normalized testosterone levels also result in better **socialization** and **integration** into the community. Whether testosterone administration in hypogonadal men improves **cognitive performance** has not yet been conclusively demonstrated. In a large-scale, placebo-controlled, randomized study in 493 men aged ≥65 years and with memory impairment, testosterone therapy did not show significant improvements in verbal or visual memory, spatial awareness, or other cognitive variables compared to placebo (Resnick et al. 2017).

Table 36.5 Monitoring testosterone replacement therapy

Psychological and sexual parameters	General well-being
	Mental and physical activity
	Mood
	Libido
	Erections
	Sexual activity
Somatic parameters	Body proportions
	Body weight
	Muscle mass and strength
	Fat mass and distribution
	Hair (beard, pubes, frontal hairline)
	Sebum
	Voice change
	Local side effects at application site
Laboratory parameters	Testosterone in serum
	Possibly free testosterone
	Gonadotropins (LH, FSH)
	Erythropoiesis (Hk, Erys, Hb)
	Possibly liver enzymes, lipid values, HbA1c
Prostate/seminal vesicles	Ejaculate volume
	Prostate size (palpation and TRUS)
	PSA in serum
Bones	Bone density (DXA, X-ray)

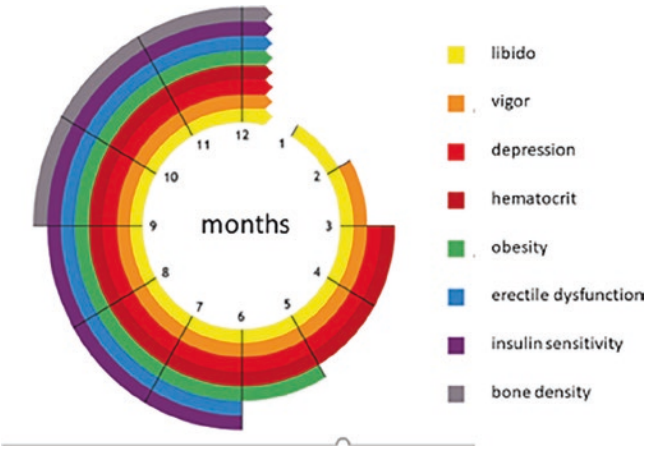


Fig. 36.9 Effectiveness of testosterone substitution in hypogonadal patients in relation to time in months and symptoms (modified from Saad et al. 2011)

While loss of **libido** and **sexual appetite** are signs of decreased testosterone levels, sufficient substitution is associated with sexual thoughts and fantasies and the frequency of these thoughts correlates with testosterone levels up to normal. The onset or regained libido is one of the first signs of effective testosterone substitution (Saad et al. 2011) (Fig. 36.9). Spontaneous nocturnal or morning **erections** are another sign of good substitution. Even when testosterone levels are still low, erections can be triggered by visual and tactile stimulation. Overall, testosterone substitution has a positive effect on the frequency and quality of erections (Ponce et al. 2018). In the normal to slightly subnormal range, the frequency of ejaculations and **sexual intercourse** correlates with testosterone levels. However, such a correlation no longer exists above the lower normal range, so that a further increase in testosterone serum levels does not lead to increased sexual activity.

These correlations indicate that detailed exploration and a sex diary kept by the patient from time to time provide useful information for the assessment of testosterone therapy. Sexual questionnaires, registering sexual thoughts and fantasies, sexual desire, satisfaction with sexuality, frequency of erections and ejaculations, can help to objectify therapy

results. When testosterone was lowered pharmacologically with GnRH antagonists, it was found that adequate sexual functions are still found at relatively low and already slightly subnormal testosterone levels (Behre et al. 1994a), while other functions require higher testosterone levels. Thus, although the patient's sexuality provides an important parameter for therapy monitoring, it must not be the only one.

36.4.2 Somatic Parameters

Muscle mass and **strength** of the hypogonadal patient increase under testosterone treatment and the patient develops a more virile phenotype (Bhasin et al. 2012). The anabolic effect of testosterone causes an increase in **body weight** of about 5%. Thus body weight, easily measured, is part of routine monitoring. The relative increase in muscle mass at the expense of fat can be measured, but is not part of standard monitoring of testosterone therapy. The distribution of **subcutaneous fat** in the lower abdomen, hips, and buttocks, which tend to have more feminine features in hypogonadal patients, also assumes the male phenotype under testosterone therapy (Allan et al. 2008).

In addition to weight loss, testosterone therapy also has metabolic effects. For example, in a nonrandomized study it was shown that testosterone can prevent the development of a metabolic syndrome and type 2 diabetes in hypogonadal men (Yassin et al. 2019). Furthermore, in existing type 2 diabetes and testosterone deficiency, substitution can have a life-prolonging effect (Hackett et al. 2019). This does not mean that conventional therapy can be dispensed with, but testosterone can be considered as an adjuvant therapy for hypogonadal type 2 diabetes.

The development and maintenance of **male hair pattern** is a good parameter for monitoring testosterone therapy (Randall 2012). In particular, **beard growth** and frequency of shaving can be easily measured. Hair growth in the upper pubescent triangle is an important indicator of adequate testosterone replacement. While women, prepubertal boys, and untreated hypogonadal patients have a straight forehead **hairline**, androgenization is associated with the formation of receding hairline corners and, if genetically predisposed, with baldness. This is perceived by some patients as an unpleasant effect about which they should be informed before the start of therapy and which may require special guidance of the patient with information about this symptom of masculinity. The pattern of male hair is of greater diagnostic importance than its intensity. Patients with long CAG-repeats of the androgen receptor may need a higher dose of testosterone to achieve satisfactory beard growth. A well-substituted patient will report the need for daily shaving or may develop a good full beard. However, in some patients, even normal serum testosterone levels do not result in beard growth. In these cases, the androgen receptor polymorphism may be important.

Hypogonadal patients have prepubertal **dry skin**. Testosterone substitution also stimulates **sebum production** and activity of the **sweat glands**. In the early stages of therapy, some patients complain about the increasing greasiness of their skin, especially on the head, which makes more frequent hair washing necessary. Education about this normal symptom of masculinity is required. **Acne** may occur at the beginning of testosterone substitution in adolescents as a sign of the onset of puberty, but is also occasionally observed in adults under substitution. This occurs mainly when preparations such as testosterone enanthate are used, which produce supraphysiological testosterone serum levels, and is rarely observed with gels and testosterone undecanoate i.m. Changing and/or reducing the dose may be necessary to improve acne.

Gynecomastia may occur under overdosage of testosterone therapy, especially testosterone enanthate, and may require dose reduction. Preexisting gynecomastia in Klinefelter patients is usually hardly affected by testosterone substitution (see this chapter).

In patients who have not yet reached puberty, a **voice change** occurs shortly after initiation of testosterone substitution (Akcam et al. 2004). This phenomenon significantly strengthens the patient's self-confidence and helps him to adapt socially, as the gap between chronological and biological age is closed. It is of enormous importance for the patient's self-confidence to be recognized as a man by the voice, this is particularly evident during telephone calls. Once the voice breaks, it no longer provides a parameter for monitoring testosterone therapy, since the size of the larynx and vocal cords, and thus vocal pitch, once reached, is maintained even without further testosterone substitution.

Patients with prepubertal hypogonadism develop eunuchoid **body proportions** over the years, as the epiphyseal joints of the long tubular bones close more slowly than in normal growth. With the onset of testosterone substitution, a short growth spurt occurs, but then the epiphyseal fissures close quickly and growth comes to a halt. In these patients, bone age of the left hand should be determined before the onset of puberty and regular checks of bone age will show whether bone maturation has been completed. The ratio of arm span to height and trunk height to leg length should also be monitored until the proportions are established. A further increase in arm span indicates inadequate testosterone therapy.

36.4.3 Laboratory Parameters

When using **serum testosterone concentrations** to evaluate substitution therapy, the pharmacokinetic profiles of the different preparations must be taken into account. Furthermore,

for long-term assessment of testosterone therapy by determining serum concentrations, methods of determining must be subjected to strict quality control, so that reliable values are delivered over longer periods of time (see Chap. 7).

In principle, serum testosterone should be measured immediately before the next application of a testosterone preparation. Especially in the case of oral or transdermal preparations, the exact time of the last application should be noted.

- If values are below normal 3 weeks after injection of 250 mg **testosterone enanthate,** the injection interval should be reduced. However, if the values at this time are still in high physiological ranges, the interval can be extended.
- Six weeks after the first injection of 1000 mg **testosterone undecanoate** i.m., the second injection is given; the third injection follows 12 weeks later. Twelve weeks later, immediately prior to the fourth injection, testosterone is measured in serum. If the value is still high, the injection interval is extended to >12 weeks; if the value is low, the interval is shortened to 10 weeks. These measurements and adjustments should then be made annually.
- Low-serum testosterone levels measured 2–4 h after taking **oral testosterone undecanoate** should remind the patient of the need to take it with a meal for better absorption. However, because of the considerable intra- and interindividual variation in absorption patterns, it is difficult to monitor oral testosterone undecanoate therapy by serum testosterone levels and other parameters should be considered additionally.
- If unsatisfactory serum levels are measured after **transdermal testosterone** application using a gel, testosterone should be measured approximately 4 to 6 h after application and immediately prior to the next application in order to get an idea of the patient's pharmacokinetics. If the values are too low, the dose of the gel may be increased, and if the values are too high, the dose may be reduced or the interval extended.

Until the correct form of substitution is found, repeated testosterone measurements may be necessary. Once good substitution is established, checks at 6- to 12-month intervals are sufficient. The patient should then be scheduled to have blood drawn at the end of each therapy interval.

Basically, the determination of **total serum testosterone** is sufficient and measuring free testosterone not bound to sex hormone-binding globulin **(SHBG)** is not necessary, since free and total testosterone are closely correlated. However, hyperthyroidism and antiepileptic drugs may increase SHBG, so that total testosterone also increases. Conversely, severe obesity leads to a reduction in SHBG and thus also to a reduction in total testosterone. In these cases, determination of SHBG is indicated to clarify the situation (see Chap. 7).

In patients developing gynecomastia under therapy, the simultaneous determination of estradiol and testosterone is indicated to detect increased conversion to **estradiol**. If this is the case, the dose should be reduced or switched to a preparation producing physiological serum levels.

While **gonadotropins** are crucial to differentiate between primary and secondary hypogonadism, they play a less important role in monitoring testosterone therapy of (primary) hypogonadism. In some forms of primary hypogonadism, such as anorexia, there is a relatively good correlation between testosterone serum levels and LH, but also FSH. In these cases, normalization of the gonadotropins may occur. However, in the most common form of primary hypogonadism, Klinefelter syndrome, LH and FSH levels often show no suppression correlating with testosterone serum levels, although all other parameters indicate good testosterone levels. Furthermore, oral and transdermal testosterone therapy has little effect on gonadotropins. Therefore, gonadotropins can only be used to a limited extent as parameters for monitoring testosterone therapy—and then only in primary hypogonadism.

The mild **anemia** characteristic of the hypogonadal patient normalizes under testosterone therapy. Therefore **hemoglobin**, **erythrocyte count,** and **hematocr**it are good parameters for monitoring therapy. If the testosterone dose is too high, the values for hemoglobin, erythrocyte count, and hematocrit move into the supraphysiological range and polycythemia can develop; a hematocrit value >54% must be considered an alarm signal (Ponce et al. 2018). This is mainly observed under testosterone enanthate (Calof et al. 2005). In this case, the testosterone dose must be reduced. If hematocrit rises above 54%, bloodletting may be necessary. Especially older and obese patients and those with short CAG repeats in the androgen receptor are more likely to react by developing polycythemia (Zitzmann and Nieschlag 2007) (Fig. 36.10).

Since testosterone has a clear effect on erythropoiesis, in many cases testosterone therapy resolves previously unexplained anemia (Roy et al. 2017). However, if no improvement in anemia can be observed despite apparently sufficient testosterone therapy, other causes, e.g., iron deficiency, must be considered, which then require additional treatment.

At the beginning of therapy, **red blood counts** should be performed every 3 months, and later at annual intervals.

A case-control study of 19,215 patients in the UK found a minimally increased risk of **thromboembolic events** in testosterone-treated patients, primarily within 6 months of initiation of therapy, with no differentiation between the different preparations used for treatment (Martinez et al. 2016). However, a later analysis concluded that the affected patients

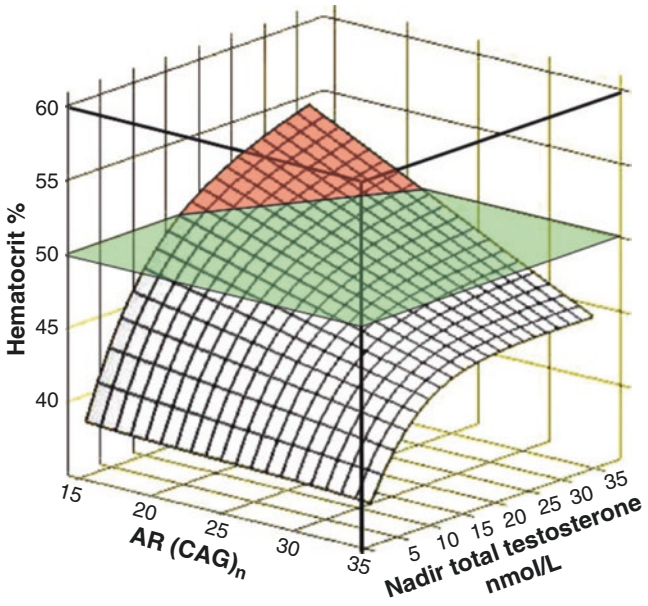


Fig. 36.10 Hematocrit in 66 hypogonadal men in relation to serum testosterone nadir values and androgen receptor CAG repeats. In total, 515 intramuscular injections of testosterone undecanoate (1000 mg Nebido®) were administered at 10–14 week intervals over several years. The green area signifies the upper limit of normal, the red area the pathologically elevated hematocrit (Zitzmann and Nieschlag 2007)

were suffering from thrombophilia and hypofibrinolysis and therefore, like the European Medicines Agency (EMA), recommends that thrombophilia be excluded before starting therapy (Corona et al. 2017; www.ema.europa.de).

The testosterone preparations recommended here do not have a negative effect on **liver function** even with long-term use, although this opinion still prevails among some physicians (e.g., Gooren et al. 2007). This is due to the obsolete administration of 17-methyltestosterone and 17-alkylated anabolic steroids, which are actually toxic to the liver. However, this is not true for natural testosterone. Monitoring liver function is, however, of particular importance in patients with diseases that simultaneously affect the liver or in hypogonadism caused by general diseases (see Chap. 34). In these cases, additional medication can influence liver function and thus slow down testosterone metabolism, e.g., by increasing SHBG concentrations. This must then be taken into account when evaluating testosterone levels. Liver values should be routinely checked at the annual follow-up.

Testosterone influences **lipid metabolism**. Although it is difficult to find bioequivalent doses when comparing different preparations, the effects are different for individual preparations. However, influencing these parameters in hypogonadal patients under testosterone therapy should only lead to a shift into the normal male range, and it remains unclear whether a decrease in HDL and an increase in LDL within the normal range have any biological effect on the vascular system. Overall, the pro- and antiatherogenic effects of testosterone appear to be balanced (Channer and Jones 2012). A meta-analysis found no association between testosterone therapy and increased cardiovascular risk in 7/8 studies (Clavell-Hernández and Wang 2018). Instead, subnormal testosterone levels appear to increase cardiovascular risk and mortality (Kirby et al. 2019). Patients at-risk should be given the drug least likely to cause pathological changes of these parameters.

36.4.4 Prostate and Seminal Vesicles

Under testosterone therapy, the prostate and seminal vesicles of the hypogonadal patient grow into the normal range and achieve normal function. The easiest way to document this is by increasing the **ejaculate volume** into the normal range. A normal ejaculate volume (≥1.5 mL) is therefore a good parameter for testing the efficacy of testosterone therapy.

Testosterone therapy does not stimulate prostate volume beyond the normal range—not even testosterone enanthate therapy with intermittent supraphysiological testosterone serum levels (Fig. 36.11). PSA (prostate-specific antigen) levels increase slightly but remain in the normal range and uroflow is not negatively affected (Behre et al. 1994b).

In recent years, it has increasingly been recognized that testosterone is not the cause of **prostate cancer**, as several epidemiological and therapeutic studies have shown (e.g., Shores et al. 2012; Debruyne et al. 2017). Nevertheless, an

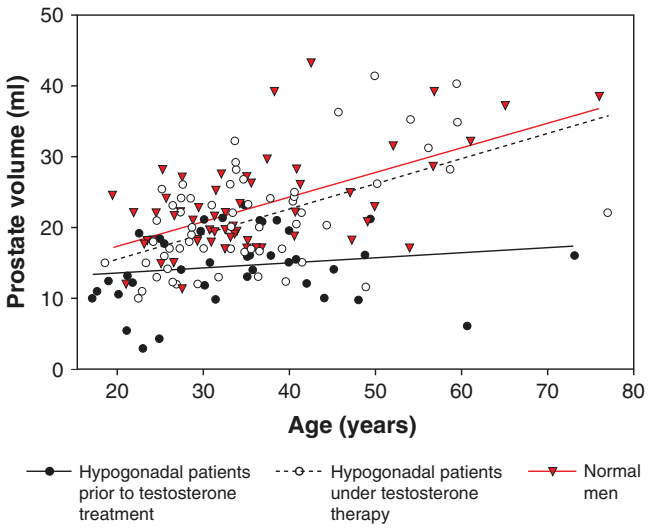


Fig. 36.11 Prostate volume (planimetric determination by transrectal ultrasound) in hypogonadal patients prior to testosterone therapy, in hypogonadal men receiving long-term effective testosterone substitution, and in age-matched normal men

existing prostate carcinoma can be stimulated by testosterone.

Since **benign prostatic hyperplasia (BPH)** and **prostate cancer** increase with age and thus the risk of stimulating an existing prostate cancer increases, every patient should be thoroughly examined before testosterone therapy and at least one annual checkup of the prostate should be performed in patients over 45 years of age.

Rectal examination of the prostate for size, surface area, and consistency is part of routine monitoring of testosterone therapy. If possible, rectal palpation should be supplemented by **transrectal ultrasonography (TRUS)**, as it allows non-invasive assessment of the entire organ. Precisely measured volume is a parameter for the efficiency of testosterone substitution and depends not only on serum testosterone levels but also on the polymorphism of the androgen receptor (Zitzmann et al. 2003). **PSA (prostate-specific antigen)** in serum is part of routine monitoring of the prostate. Increases above 4 ng/mL or an increase of more than 0.4 ng/mL/year under long-term therapy are alarm signals for the possibility of carcinoma. However, carcinoma may also be present at low PSA levels, which is unmasked by testosterone substitution. Therefore, checkups are required 3–6 months after starting testosterone substitution and then annually (Wang et al. 2009). If a carcinoma is suspected, the patient must be referred to a urological consultation, where diagnosis may be supplemented by prostate biopsy.

Urological oncologists have cautiously approached the question of whether a patient should ever receive testosterone again after successful treatment of **prostate cancer**. As the consequences of complete androgen deprivation became increasingly clear and unbearable for the patient, the guidelines initially used an "appropriate interval" with no increase in PSA as a criterion for testosterone substitution. Then, surprisingly, a high-dose interval therapy was recommended for castration-resistant prostate cancer (Isaacs and Denmeade 2012). In the meantime, it has been established that testosterone therapy after radical prostatectomy prolongs the time to recurrence and life expectancy (Ahlering et al. 2020). This renders obsolete, Charles B. Huggins' (1901–1997) postulates that testosterone causes prostate cancer and patients can be treated with counter sex hormones (= estrogens) for which he received the Nobel Prize in 1966 (Morgentaler and Caliber 2019).

36.4.5 Bone and Muscle

Reduced bone mass in hypogonadal patients can be normalized by testosterone therapy (Behre et al. 1997; Snyder et al. 2017) (Fig. 36.12). With good therapeutic settings, both cortical and trabecular bone mass increase, while the vertebrae area (in QCT) remains unchanged. However, if testosterone therapy is started very late, the increase in cortical bone mass will be predominant. Vertebral bone density may also increase and even normalize in patients who do not require

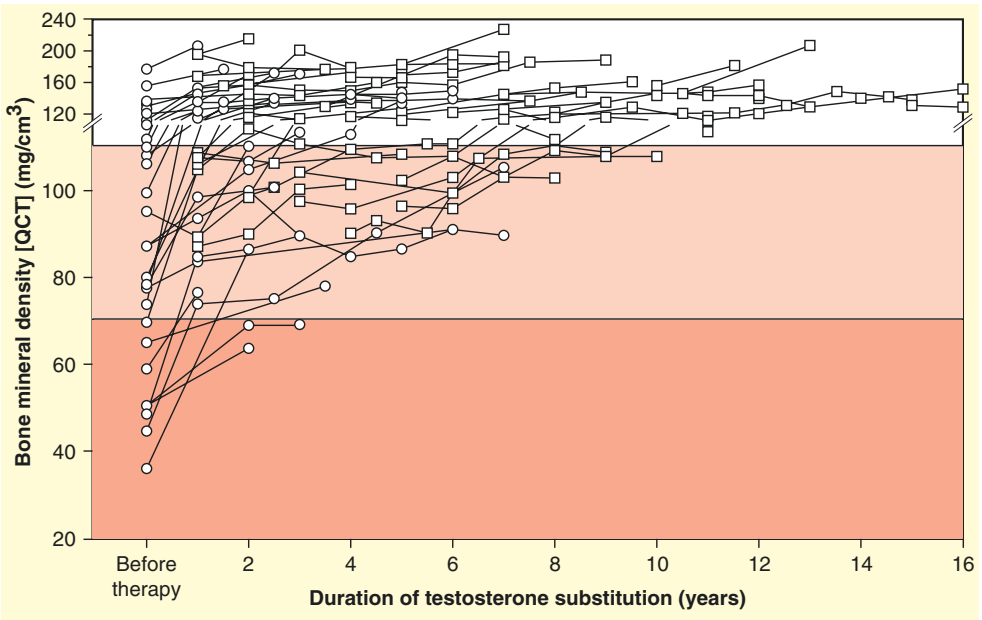


Fig. 36.12 Bone mineral density (BMD), measured by QCT of the lumbar vertebrae during long-term testosterone substitution therapy up to 16 years in 72 hypogonadal patients. Circles indicate hypogonadal patients with first QCT measurement before initiation of testosterone substitution therapy, squares show those patients already receiving testosterone therapy at the first QCT. The dark pink area indicates the range of high fracture risk, the white area shows the range without significant fracture risk, and the light pink area indicates the intermediate range where fractures may occur

therapy until advanced age (Behre et al. 1997; Snyder et al. 2017).

The European Academy of Andrology (EAA) Osteoporosis Guideline (Rochira et al. 2018) states that every hypogonadal patient should undergo a DXA bone density assessment prior to testosterone therapy. If osteoporosis is detected, it should be verified by x-ray examination of the vertebrae and hips. If osteoporosis is manifest, testosterone therapy should be supplemented with vitamin D and calcium. If there is a high risk of fracture, antiresorptive drugs should be added (e.g., bisphosphonates). An increase in bone density can be observed at the earliest 6 months after the start of therapy (Snyder et al. 2017). As a general rule, hypogonadal patients on testosterone substitution therapy should undergo a DXA examination of bone density every 2 years.

Under testosterone substitution, muscle mass increases while fat mass decreases (Bhasin et al. 2012). The increased muscle mass results from hyperplasia of the existing muscle cells and simultaneous new formation of muscle cells from mesenchymal cells. The increase in muscle mass in turn has a positive effect on bone density. Subjectively, increased muscle formation is evaluated by the patient as a visible therapeutic success that increases self-esteem.

36.5 Evaluation of Testosterone Replacement Therapy in Hypogonadism

Testosterone deficiency does not pose an immediate threat to life, but it leads to a drastically reduced quality of life and various health problems. The often expressed assumption that testosterone is responsible for the shorter life expectancy of men compared to women can hardly be verified experimentally. However, the equally long life expectancy of prepubertally castrated and intact men (Nieschlag et al. 1993) suggests that factors other than testosterone are responsible for the different life expectancies of the sexes (see Sect. 16.1.2.4). Low-serum testosterone levels have even been described as predictors of shorter life expectancy (Shores et al. 2006). At the very least, there is no reason to withhold testosterone substitution from a patient.

Testosterone is characterized by a high degree of **therapeutic safety**, which is demonstrated not least by the absence of serious long-term side effects after high-dose application, such as for excessive height (see Sect. 36.5) and illegal use in sports (see Chap. 37). The most important contraindications are a manifest **prostate carcinoma** or a very rare **breast carcinoma**.

The immediate benefit of testosterone replacement therapy is difficult to quantify. There is no doubt, however, that testosterone significantly improves the patient's **quality of life**. Elevated self-esteem and confirmation through sexual activity and satisfaction are factors not to be underestimated in the social integration of a patient. By promoting physical and mental activity, the patient's performance is increased or maintained, and disability and infirmity are prevented by eliminating anemia, strengthening the musculoskeletal system and reducing the risk of fracture.

> Through adequate substitution therapy, patients become integrated members of society with a satisfactory quality of life.

Although some patients may initially have to be convinced of the benefits of the planned therapy because they lack the imagination to see its advantages, in the long-term hardly any patient would forego substitution therapy. Increased public discussion of the effects of testosterone and suggestions from increasing numbers of self-help groups of patients, make it easier for patients to formulate their own ideas about adequate therapy. It remains the task of the physician to guide the patient, monitor the various parameters, and find an optimal therapy for the individual patient.

36.6 Testosterone Therapy for Excessively Tall Stature

Testosterone deficiency occurring at the time of puberty causes eunuchoidal height, while testosterone normally leads to proportionate body growth by closing the bone epiphyses in time. Testosterone can cause the epiphyses to close before puberty and thus lead to reduced growth, e.g., in pubertas praecox. These facts are used to achieve a reduction in final height in boys with expected **excessively tall stature**

(over 2 m height). This is because early high-dose testosterone therapy can lead to growth arrest.

In most cases, 250 mg of testosterone enanthate is administered weekly at the age of 12–16 years or 500 mg of testosterone enanthate every 2 weeks intramuscularly for about 1 year. The dose to be used is therefore at least twice as high as would be used for substitution. Too short therapy phases with discontinuation before complete epiphyseal closure destroys the desired therapeutic success. Before growth stops, the possible success of therapy can be read from the development of bone age. The earlier therapy is started, the more effective is the desired size reduction. If bone age is over 14 years, the use of testosterone is usually futile (Drop et al. 1998). For psychosocial reasons, the early use of therapy is prohibited because of induced early puberty development.

Even though testosterone is not approved for these indications, such treatment in the sense of an attempt to cure is relatively common and has been used since the early 1950s. It is noteworthy that no controlled studies have been conducted to date to verify the actual therapeutic effect. Therefore, it seems important to deal with the question of possible long-term effects.

During treatment with pharmacological doses of testosterone, the development of the testes is suppressed (see Chap. 48), and the question arises as to whether this temporary suppression of the testes and high-dose testosterone administration in (early) pubertal age have any consequences. A similar question arises in testosterone-based studies on male contraception, but with the difference that growth leading to final height is to be suppressed in the immature organism. After discontinuation of therapy, the endocrine pituitary-gonadal axis normalizes rapidly.

Nor have long-term follow-up examinations of boys treated in this way compared to control collectives revealed any abnormalities that could be considered a consequence of the treatment (de Waal et al. 1995; Lemcke et al. 1996). In particular, there was no evidence of changes in the cardiovascular system and lipids in the serum or prostate (measured by volume, internal echoes, and PSA). The abnormalities of ejaculate parameters found more frequently in the follow-up examinations are more likely to be due rather to the higher incidence of maldescended testes or varicoceles than to testosterone therapy of these patients (Lemcke et al. 1996). A study conducted on average 21 years after treatment showed that the probability of conception within 1 year was as high in 36 treated patients as in 30 untreated controls (Hendriks et al. 2010). With regard to the therapeutic safety of testosterone, previous follow-up studies have shown that the use of relatively high doses during puberty remains without long-term detectable side effects.

Thus, although the long-term follow-ups, as far as they have been conducted so far, do not provide any objections to such a therapy, it must be remembered that this testosterone therapy leads to an accelerated development of puberty with all its psychological and physical consequences. Furthermore, since there are hardly any medical, but mainly psychological reasons for this therapy, it should be carefully considered in each individual case and, if possible, should only be used in extreme cases of the expected body size. Since the boys concerned (and their parents!) suffer from the excessive body size mainly in the growth phase, but are later quite well-integrated, adequate medical or psychological care is indicated for better adaptation. Often such guidance of the patient will be sufficient and make additional testosterone therapy unnecessary.

Key Points

- All forms of hypogonadism require therapy with testosterone.
- Even in secondary hypogonadism, testosterone substitution is indicated in the long-term, which is only interrupted for the duration of GnRH or gonadotropin therapy if the patient wishes to be fertile.
- For many decades, substitution with testosterone enanthate or cypionate was the leading form of testosterone substitution.
- In the 1970s, the orally active testosterone undecanoate was added and in the 1990s, the first transdermal preparations were introduced in the form of membranes and patches.
- Later, testosterone gels were also added, with which physiological serum testosterone levels can be reached.
- With injectable testosterone undecanoate, a true depot preparation came into clinical use.
- Regular monitoring of testosterone substitution must observe the positive effects and, due to possible undesirable side effects, especially monitor red blood count and prostate.

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Abuse of Anabolic Androgenic Steroids (AAS) for Doping

37

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Abstract

Anabolic androgenic steroids (AAS) are the preferred drugs used in competitive and recreational sports and by bodybuilders to improve appearance and performance (Appearance and Performance-Enhancing Drugs, APED), commonly known as doping. Many AAS, often obtained via the Internet and from dubious sources, are not properly tested, and are consumed in extremely high doses and in irrational combinations with other drugs. Controlled clinical trials examining adverse effects are lacking because ethical restrictions prevent study subjects from being exposed to potentially toxic therapies, obscuring a causal relationship between AAS abuse and potential consequences. This chapter summarizes the side effects of AAS abuse, particularly the impact on reproductive system functions, based on detailed case reports and small clinical trials.

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37.1 Dimension of the Problem/ Epidemiology

Although doping has been practiced since ancient times, often with placebo or toxic effects, truly effective Appearance and Performance-Enhancing Drugs (APEDs) became available only with the rise of modern pharmacology, particularly after the isolation and synthesis of testosterone and anabolic androgenic steroids (AAS). Testosterone was used clinically shortly after its synthesis in 1935 (Nieschlag and Nieschlag 2019), and its first documented use for doping was by

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German rowers in 1952 (ostensibly to maintain their marital duties during strenuous training); Russian weightlifters soon followed in 1954 to improve their strength.

After various approaches to curb doping, a global anti-doping control network, the **World Antidoping Agency (WADA)**, was established in 1999. Under the motto "Play true," the World Antidoping Code strives to keep sports "clean" and protect each athlete (www.wada-ama.org). The **WADA Prohibited List,** renewed annually, summarizes all pharmacological substances and medical procedures that are prohibited in training and competition. Since then, AAS have been detected most frequently in positive samples by the 30 WADA-accredited laboratories worldwide (Handelsman 2020) or among black market substances seized by customs and police (Krug et al. 2014). By a rigorously administered **Therapeutic Use Exemption (TUE),** WADA may allow the use of testosterone in therapeutic doses for athletes suffering from *organic*, but not from *functional* hypogonadism.

Since all approved testosterone and AAS preparations are only available by prescription, the drug sources remain unclear. In some cases, these substances are no longer or have never been on the official market. There have been cases of physicians prescribing AAS, especially under pressure from bodybuilders who risk becoming champions by doping with AAS. Surveys of fitness center customers found that up to half of AAS users received the drugs with or without a prescription from physicians or pharmacies (Striegel et al. 2006; Raschka et al. 2013). Laboratories in Eastern Europe, Asia, and South America that manufacture a variety of AAS offer them for sale on the Internet, which, along with gyms, have become the main source of AAS. The **black and internet market for AAS** appears to be growing. In December 2011, there were 328,000 results on the Internet generated by the search term "steroids for sale" (Brennan et al. 2013) through the Google search engine; in December 2020, there were 4,130,000 for English-language sources (own research)! A qualitative analysis of the products provided to 100 subjects in the HAARLEM study showed that only about half of the samples did not contain the AAS declared on the package (De Ronde and Smit 2020).

In addition, AAS can be added **undeclared to dietary supplements** (Van Thuyne et al. 2006; Geyer et al. 2008; Rahnema et al. 2015) or are contained in **natural medicine** preparations made from **animal organ extracts**. For example, musk glands used in traditional Chinese medicine contain 16 different AAS, as detected in doping tests (Thevis et al. 2013).

Finally, secret but official **programs of sports organizations or states** can provide their athletes with AAS and other APEDs, as shown by the systematic doping program of the former German Democratic Republic (GDR) in the 1970s and 1980s, which became public knowledge after its regime collapsed in 1989 (Franke and Berendonk 1997). Nonetheless, state-organized doping in high-performance sports continues to flourish as shown by the example of Russia, where large-scale doping fraud was uncovered by WADA at the 2014 Winter Olympics in Sochi (Makarychev and Medvedev 2019).

AAS, like all other APEDs, can have **adverse side effects** in addition to the desired ones, resulting from the combination of various AAS in extremely high doses with other drugs and from the duration of administration over a period of months to many years. Due to the clandestine nature of this drug abuse, dosage and duration are largely unknown and there are understandably **no properly controlled clinical trials.** Therefore, scientific evaluation of the consequences of AAS abuse relies on case reports and some retrospective research, making a review of this area extremely difficult and frustrating in the age of evidence-based medicine. Nevertheless, this chapter aims to provide information on symptoms and diseases caused by AAS that can be misinterpreted without specific knowledge when searching for their origin. Proper diagnosis is further hampered by patient reluctance to admit to AAS use and ignorance of their potential serious side effects.

37.2 Chemistry and Detection

Testosterone and AAS (including designer steroids) are collectively referred to as AAS, although both the chemical structure and biological profiles of each differ (Fig. 37.1).

In general, both effects and side effects of specific AAS depend on their chemical structure. The full spectrum of biological effects requires that the androgen can be aromatized to estradiol and reduced to 5-alpha-dihydrotestosterone. As indicated in Table 37.1, the most commonly used AAS, testosterone, boldenone, Metandienone, and nortestosterone can be aromatized as well as 5-alpha-reduced, whereas fluoxymesterone and formebolone can be 5-alpha-reduced but not aromatized. Some AAS can neither be aromatized nor 5-alpha-reduced; in particular, the dihydrotestosterone derivatives are among them (Fig. 37.1 and Table 37.1). In addition, the genetic disposition of the individual athlete may modify the response to androgenic substances, as exemplified by the androgen receptor polymorphism that modulates testosterone activity (Zitzmann and Nieschlag 2007).

However, distinguishing them is difficult due to the different combinations and doses of additional commonly practiced doping polypharmacy (Skarberg et al. 2009; Dodge and Hoagland 2010), including erythropoietin, insulin, IGF-1, L-thyroxine, clenbuterol, amphetamines, diuretics, and so on. The characteristic of alkylation in the 17α-position of the androgen molecule should be noted, as these AAS may be

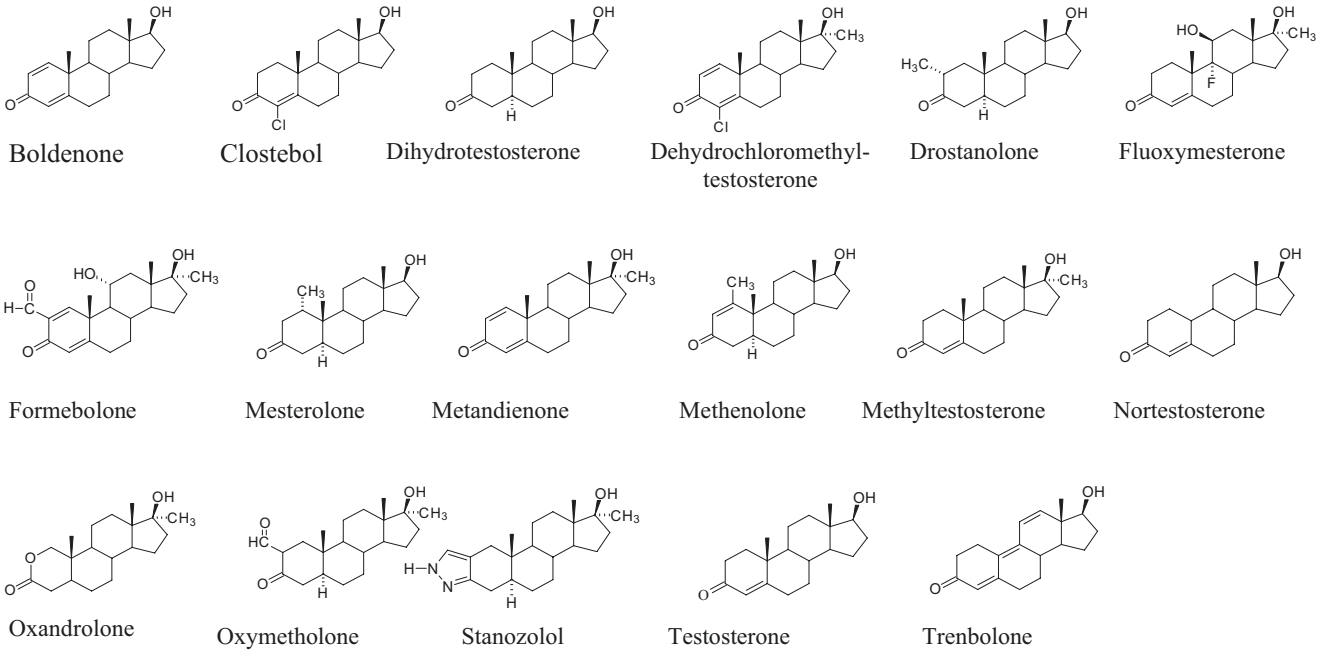


Fig. 37.1 Structural formulae of anabolic androgenic steroids (AAS) frequently detected in doping control samples (modified from Schänzer and Thevis 2012)

Table 37.1 Androgen anabolic steroids (AAS), metabolism, and hepatotoxicity

	Aromatization	5α-reduction	17α-alkylation (hepatotoxic)
Testosterone	x	x	–
19-nortestosterone	x	x	–
Boldenone	x	x	–
Dihydrotestosterone	–	x	–
Mesterolone	–	x	–
Methenolone	–	x	–
Trenbolone	–	x	–
17α-methyltestosterone	–	x	x
Fluoxymesterone	–	x	x
Dehydrochloromethyl testosterone	–	x	x
Formebolone	–	x	x
Oxandrolone	–	x	x
Oxymetholone	–	x	x
Stanozolol			
Metandienone	x	x	x
Closteboll	–	–	–
Drostanolone	–	–	–

highly toxic to the liver (Table 37.1). AAS abuse is also characterized by "stacking and cycling," i.e., increasing doses over time and changing preparations and their combinations in alternation with AAS-free periods to maximize desired effects and minimize side effects. Whether these therapies actually serve their purpose cannot be judged because they are based on trial and error and no evidence-based studies are available.

37.3 Side Effects on Reproductive Functions (Table 37.2)

Table 37.2 Side effects of high-dose steroids on reproductive and sexual functions/organs

Male reproductive functions
Decreased testicular volume
Reduced spermatogenesis
Infertility
Loss of libido
Erectile dysfunction
Gynecomastia
AAS-induced hypogonadism (ASIH)
Female reproductive functions
Anovulation
Amenorrhea
Dysmenorrhea
Infertility
Breast atrophy
Clitoral hypertrophy
Dysphonia
Deepening of the voice

37.3.1 Specific Side Effects in Men

Due to negative feedback regulation of the hypothalamic-pituitary-gonadal axis, AAS can cause reversible **suppression of spermatogenesis**, including azoospermia (Nieschlag and Vorona 2015;, Rolf and Nieschlag 1998). Since sperm-producing tissue accounts for up to 80% of the testes, its

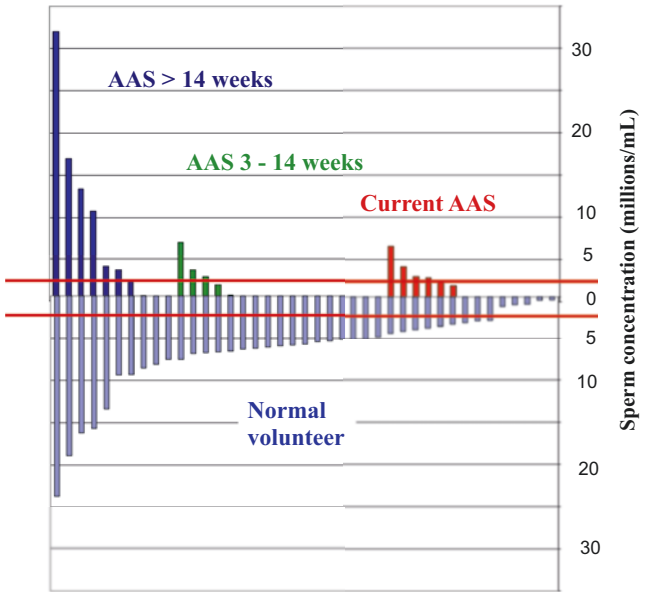


Fig. 37.2 Sperm concentrations in 41 bodybuilders currently using anabolic steroids, 3–14 weeks ago or more than 14 weeks ago (upper part) and in 41 drug-free volunteers (lower part). The bars represent sperm concentrations from individual bodybuilders (upper panel) and from normal volunteers (lower panel). The horizontal lines indicate a concentration of 20 million/mL as lower limit of normal (modified from Knuth et al. 1989)

functional impairment is followed by **atrophy of the testes**, which correlates with the dose and duration of AAS abuse (Rasmussen et al. 2016). After cessation of AAS intake, spermatogenesis and testicular volume recover within months (Fig. 37.2).

During intake, users may be infertile to varying degrees and are often unaware of the causal relationship. Proper diagnosis may be hampered by the fact that these men may not want to admit abuse to either their physician or their partner, and persistent follow-up is required. Low LH, FSH, and testosterone (in cases where endogenous testosterone is not used as an AAS) indicate suppression of the pituitary-testicular axis (Fronczak et al. 2012). The time required to **restore spermatogenesis** is significantly longer (10–14 months on average) than that required to normalize testicular steroidogenesis (7–9 months) (Shankara-Narayana et al. 2020). If spermatogenesis does not recover after cessation of use, a preexisting fertility disorder is more likely than AAS-induced damage. To accelerate recovery, hCG is sometimes prescribed without evidence of efficacy. It should be mentioned here that the suppression of the pituitary gland and spermatogenesis by testosterone is exploited in approaches to male hormonal contraception, with the complete recovery period taking an average of 3 months (see Chap. 48).

Given the large number of **teenagers using AAS**, the question arises whether the use of AAS in boys during puberty may be permanently detrimental to spermatogenesis. Although systematic studies in pubertal AAS users are lacking, the treatment of tall boys with high doses of testosterone to reduce final body size provides an analogy. Initially, it was suspected that this treatment would harm the testes and cause permanent damage. However, when appropriate control groups were co-sampled, the incidence of subnormal semen parameters was the same in both groups (Lemcke et al. 1996), indicating that at this age the testes do not differ from adult males in their ability to recover from suppression.

With high-dose use of aromatizable AAS, **bilateral gynecomastia** may develop in males with a prevalence of 20–30% (O'Sullivan et al. 2000). Concomitant application of estrogen receptor or aromatase inhibitors has been used to counteract this development. In cases of persistent, refractory gynecomastia, liposuction with mastectomy may be required (Babigian and Silverman 2001). After abrupt discontinuation of AAS abuse, athletes may exhibit **transient signs of hypogonadotropic hypogonadism,** such as decreased libido, erectile dysfunction, and depression (Basaria 2010).

An isolated case of a former GDR weightlifter who used oral turinabol in high doses (up to 20 tablets per day) between the ages of 18 and 23 years has been reported. He developed gynecomastia while on treatment and underwent surgery for unilateral **intratesticular leiomyosarcoma** at age 32 (Froehner et al. 1999). Because these tumors are extremely rare and have been described in hamsters after treatment with testosterone propionate and diethylstilbestrol, the authors suspected a causal relationship between AAS abuse and sarcoma. Since this is the only reported case, the pathogenesis of the tumor remains unclear.

37.3.2 Specific Side Effects in Women

Because the effects of testosterone also have a clear performance-enhancing effect in women (Bermon and Garnier 2017; Lindén Hirschberg et al. 2020), more and more male-to-female transgender athletes and 46,XY-DSD patients are entering women's sports, raising ethical, social, and legal issues, specific side effects of AAS in women will be discussed here.

37.3.2.1 Hypothalamic-Pituitary-Gonadal Axis

In women, small differences in endogenous testosterone levels appear to affect athletic performance. For example, female 400 and 800-m runners have been shown to have higher free testosterone in the upper third among female competitors (Bermon and Garnier 2017). Therefore, female athletes expect strong effects of AAS abuse, but pay through consequences on reproductive functions. **Dysmenorrhea, secondary amenorrhea with anovulation, and conse-**

quently infertility are the changes most commonly caused by AAS abuse.

A large study aimed at evaluating the side effects of therapeutic doses of testosterone administration in women showed that there were no significant differences in the incidence of cerebrovascular disease, coronary heart disease, breast carcinoma, deep-vein thrombosis/pulmonary embolism, and diabetes mellitus, or acute hepatitis between women receiving testosterone therapy and the control group (van Staa and Sprafka 2009).

Changes in the reproductive system due to suppression of the hypothalamic-pituitary-gonadal axis such as dysmenorrhea, secondary amenorrhea with anovulation, and **reduction in breast size** are reversible. It may take weeks or months for the axis to fully recover. **Clitoral hypertrophy** is among the irreversible effects of AAS abuse, but the incidence is not well documented.

37.3.2.2 Hirsutism

Hirsutism is the most common and reversible side effect of AAS use in women. The degree of increased facial or body hair growth depends on dose and duration of AAS excess and can be described according to the Ferriman-Gallwey hirsutism score (Ferriman and Gallwey 1961), which is based on the intensity of hair growth in nine facial/body areas. In some cases, it has been reported that it may take up to 2 years for serum testosterone concentrations to decrease to normal levels and for hirsutism to disappear after AAS administration in women (Urman et al. 1991).

37.3.2.3 Changes in the Voice

Deepening of the voice is part of the virilization that AAS can cause in women. Unlike acne, hirsutism, alopecia, and breast atrophy, deepening of the voice is irreversible. These effects of androgens in women have been repeatedly described (Strauss et al. 1985; Baker 1999). Lowering of the voice is caused by growth of the larynx in girls and by thickening of the vocal cords in women after puberty. The voice change can be so pronounced that women may be mistaken for men on the telephone. It is accompanied by hoarseness, which may increase with prolonged use of the voice. This dysarthria can become a problem for teachers, actors, and singers who depend on their voices for work. Such voice changes are also observed with endogenous elevation of testosterone levels, such as in congenital adrenal hyperplasia (Nygren et al. 2009) or in women who are sensitive to the androgenic effects of some oral contraceptives. Because voice changes are usually irreversible, use of AAS or other steroids must be suspended at the earliest sign of symptoms. Although no studies of women abusing AAS are available, in **female-to-male transsexuals** receiving testosterone treatment for virilization, a decrease in baseline voice frequency occurred within a few weeks, and attainment of full male frequency was documented within 6 months (Deuster et al. 2016).

37.4 Effects on Nonreproductive Organs (Table 37.3)

Table 37.3 Summary of the consequences of doping with AAS on nonreproductive organs and functions

Hematopoiesis and coagulation	Liver
Erythrocyte count increase	Arrhythmia
Hemoglobin increase	Cholestasis/ hyperbilirubinemia
Hematocrit increase	Steatosis
Polycythemia	Peliosis
Hypercoagulation	Adenomas
Venous thromboembolism	Hepatocellular carcinoma
Arterial thromboembolism	Liver failure
Stroke/apoplexy	
	Kidney
Musculoskeletal system	Creatinine increase
Premature epiphyseal occlusion (in adolescents)	Glomerulosclerosis
Rhabdomyolysis	Cholemic nephrosis
Tendon rupture	Renal failure
Ligament injuries	
Herniated disc	**Psyche and behavior**
	Irritability
Cardiovascular system	Nervousness, restlessness
HDL ↓ LDL ↑, ApoA1 ↓	Aggressiveness
Coronary heart disease	Reckless behavior
Myocardial infarction	Self-aggressiveness
Hypertension (?)	AAS dependence
Abnormal ECG (QRS > 114 ms)	AAS withdrawal syndrome
Arrhythmia	Depression
Left ventricular hypertrophy	Suicidal thoughts
Hypertrophic cardiomyopathy	
Dilated cardiomyopathy	**Skin**
Heart failure	Acne
Sudden cardiac death	Striae distensae
	Heavy sweating
	Alopecia
	Hirsutism

37.4.1 Hematological Side Effects

Stimulation of hematopoiesis (stem cells, reticulocytes, erythrocytes, hemoglobin, and hematocrit) is one of the important effects of testosterone and AAS used by athletes for higher performance. The increase in hemoglobin and hematocrit during puberty in boys results from an increase in testosterone (Hero et al. 2005), and the higher testosterone levels remain responsible for the differences between eugonadal males and females throughout life. In healthy and hypogonadal men, testosterone has a linear dose-dependent effect on hematopoiesis. Older men and those with higher BMI are more sensitive to testosterone stimulation than younger and leaner men (Zitzmann and Nieschlag 2007). This must be considered when treating patients with late-onset hypogonadism (Wang et al. 2008).

The hematopoietic effect of testosterone does not require aromatization, as shown in men with aromatase deficiency (Rochira et al. 2009). DHT has a similar effect on hematopoiesis as testosterone itself, indicating that 5α-reduction does not affect the hematopoietic effect of testosterone and other androgens (Sakhri and Gooren 2007). It is possible that, in addition to direct stimulation of bone marrow erythroid progenitor cell proliferation, the hematopoietic effects of testosterone and AAS are also mediated by erythropoietin and by increases in iron utilization due to hepcidin suppression, so that androgens may have three stimulatory pathways for hematopoiesis (Cheung and Grossmann 2018). Before erythropoietin and its analogs were available for clinical use, testosterone was widely used to treat aplastic and nephrotic anemia.

Androgens not only stimulate hematopoiesis but also increase 2,3-diphosphoglycerate in erythrocytes, thereby decreasing **hemoglobin oxygen affinity**, facilitating the release of oxygen from hemoglobin and improving oxygen delivery to tissues (Shahidi 2001). Androgens also appear to stimulate **granulopoiesis and thrombopoiesis** in vitro and in vivo (Inamdar Doddamani and Jayamma 2012; Roşca et al. 2021). High doses of AAS, as used in doping, cause significant increases in erythrocyte and hemoglobin concentrations (Kanayama and Pope Jr 2018), which is part of the intended effects as they increase oxygen transport. However, an increase in hematocrit above 52% can lead to **thromboembolism, intracardiac thrombosis, and stroke** (Lippi and Banfi 2011). Stroke may be associated with **left ventricular thrombus** and **cardiomyopathy** (Youssef et al. 2011) or fatal **massive myocardial infarction** (Shamloul et al. 2014).

Administration of testosterone and AAS to healthy men causes transient **activation of the coagulation system and fibrinolysis**. Both changes were reversible after discontinuation (Kahn et al. 2006). For example, testosterone can increase thromboxanA2 receptor activity and platelet aggregation and thus increase the risk of thrombosis. At the same time, the activity of the fibrinolytic system, particularly antithrombin III and protein S, increases (Shapiro et al. 1999). Levels of plasmin-α2-antiplasmin complex (PAP, terminal marker of fibrinolysis), factor XIIc, and antithrombin decreased significantly in men receiving testosterone undecanoate as depot injections (Zitzmann et al. 2002). Short-term low-dose administration of AAS-oxandrolone to healthy subjects resulted in an increase in blood coagulation factors and plasminogen, leading to a state of hypercoagulability (Kahn et al. 2006). Androgen-containing hormone replacement therapy decreased plasminogen activator inhibitor-1 (PAI-1) in premenopausal women, resulting in enhanced fibrinolytic activity (Winkler 1996).

Changes in the hemostatic system during testosterone therapy were also studied in female transsexuals (female-to-male) who received 250 mg testosterone enanthate injections every 2 weeks for a prolonged period (Toorians et al. 2003). This therapy had a mild **antithrombotic effect**. To what extent the AR polymorphism, which alters the erythropoiesis-stimulating effect of testosterone in substituted patients, is of influence in athletes is not known (Zitzmann and Nieschlag 2007).

37.4.2 Side Effects on the Cardiovascular System

37.4.2.1 Arrhythmias

Long-term AAS users show altered electrophysiological capacity of the myocardium with a significantly higher incidence of abnormal electrocardiograms (e.g., **prolongation of the QRS complex, arrhythmias, including atrial fibrillation, ventricular fibrillation, ventricular tachycardia, supraventricular, and ventricular extrasystoles**) after exercise compared to controls (Achar et al. 2010). Also, chronic consumption of supraphysiological doses of AAS increased interatrial and intra-atrial electromechanical delay and prolonged repolarization dispersion with significantly increased Tp-e interval, Tp-e/QT ratio, and Tp-e/QTc ratio compared with bodybuilders without AAS abuse (Alizade et al. 2015).

37.4.2.2 Myocardial Hypertrophy

AAS can cause **concentric left ventricular myocardial hypertrophy**, the extent of which appears to be dose-dependent (Dickerman et al. 1998). In one study, AAS was shown to exert a long-standing hypertrophic effect on the myocardium. Here, there were no significant differences between current and former AAS users (Di Bello et al. 1999). AAS does not appear to affect systolic cardiac function. However, because anabolic steroids affect left ventricular diastolic function, this serves as a criterion for distinguishing physiological exercise-induced hypertrophy from pathological myocardium (Caso et al. 2006; Kindermann 2006). A recent study found no differences in previous users who discontinued androgens for at least 3 months compared with nonusers in terms of left and right ventricular dimensions and systolic and diastolic functions (Shankara-Narayana et al. 2020).

The athlete's heart is characterized by moderately proportional myocardial hypertrophy without functional limitations. Pathological **left ventricular myocardial hypertrophy** that develops with AAS use is often associated with impaired diastolic function of the affected ventricle, likely caused by increasing myocardial fibrosis. The second diagnostic criterion is the thickness of the left ventricular myocardium, which can be determined during echocardiography. A ventricular wall thickness greater than 13 mm is suspected of pathological myocardial hypertrophy or AAS abuse

(Dickerman et al. 1998; Kindermann 2006). Left ventricular hypertrophy can be detected echocardiographically several years after AAS withdrawal (Achar et al. 2010). Although myocardial hypertrophy appears to be reversible, impaired left ventricular diastolic function and decreased inotropic capacity of the myocardium are irreversible (Turillazzi et al. 2011; Baggish et al. 2017). A cardiac magnetic resonance imaging (MRI)-assisted study described not only an increase in LV wall mass but also in the main volume of both cardiac ventricles (Luijkx et al. 2013). **Myocardial scarring** with severe left ventricular hypertrophy may occur in patients with normal coronary arteries after AAS abuse (Baumann et al. 2014), possibly due to an apoptotic testosterone effect on cardiomyocytes, as shown in cell culture studies (Nascimento et al. 2015).

In cases of **acute advanced heart failure** due to AAS abuse, maximal improvement in left ventricular ejection fraction was achieved within 6 months after discontinuation of AAS uptake and initiation of treatment with angiotensin-converting enzyme (ACE) inhibitors and beta-blockers. In severe cases, left ventricular assist device (LVAD) implantation and heart transplantation were required (Sondergaard et al. 2014).

37.4.2.3 Sudden Cardiac Death

AAS abuse can cause a significantly higher incidence of **sudden cardiac death** in apparently healthy young athletes. This mainly affects weightlifters and bodybuilders who take very high doses of AAS, often as a mixture with other drugs. The effects of AAS abuse based on autopsy data from ten young bodybuilders who had suffered sudden cardiac death and had taken unsupervised drug mixtures for performance enhancement were described (Kistler 2006). In all cases, the mean heart weight was significantly higher than the mean physiologic heart weight and histologically **chronic ischemic changes** of the myocardium were found. In almost all cases, **atherosclerosis of the coronary arteries and atheromatosis of the carotid and aortic** arteries were found despite the relatively young age of the athletes.

The most common cause of sudden death in young competitive athletes was **hypertrophic cardiomyopathy**, which occurred in one-third of cases (Maron et al. 2016) and previously undiagnosed **congenital heart failure** (Sullivan et al. 1998). Other potential causes of cardiac death in AAS users discussed include the following: **coronary artery spasm** due to inhibition of NO release, premature coronary atherosclerosis due to increased atherogenesis, thrombotic coronary artery occlusion due to increased platelet aggregation and/or increase in hematocrit and blood viscosity, and direct **cardiotoxic effects** with impairment of mitochondria and myofibrils and associated destruction of cardiomyocytes and their replacement by fibrous tissue (Kistler 2006; Sullivan et al. 1998; Dickerman et al. 1995; Fineschi et al. 2001).

37.4.2.4 Dilated Cardiomyopathy (DCM)

Some cases of **dilated cardiomyopathy (DCM)** have been described in healthy young bodybuilders while taking AAS. All cases involved uncontrolled high-dose AAS abuse, especially in combination with other drugs (Clark and Schofield 2005). In patients with a genetic predisposition to dilated cardiomyopathy using AAS, it becomes particularly difficult to disentangle causal relationships. Approximately 30% of DCM is thought to have familial accumulation. In most cases, inheritance is autosomal dominant, rarely X-linked or autosomal recessive. Because there is high variability in the probability of manifestation and gene expression, some other risk and environmental factors (e.g., viral infections or stress) may be responsible for the development of cardiomyopathy (Maisch et al. 2005).

37.4.2.5 Arterial Hypertension

It is not clear whether AAS cause arterial hypertension. In some cases, AAS abuse resulted in long-term (up to 1 year) elevation of blood pressure (Achar et al. 2010). AAS abuse-induced arterial hypertension may persist for up to 1 year after drug discontinuation. Activation of the sympathetic autonomic nervous system, as well as depression of parasympathetic modulation and structural cardiac changes such as interventricular septal hypertrophy, left ventricular wall hypertrophy, and diastolic ventricular wall thickness, appear to be a possible cause of arterial hypertension in AAS users (Barbosa Neto et al. 2018). Some AAS in high doses cause water retention, which may be associated with high blood pressure.

37.4.2.6 Atherosclerosis

High doses of AAS, especially when taken concomitantly with multiple preparations, can lead to a **decrease in high-density lipoprotein (HDL) cholesterol fraction, and an increase in low-density lipoprotein (LDL)** cholesterol (Kindermann 2006; Hartgens et al. 2004). These effects on lipoprotein levels can be seen approximately 2 months after the onset of ASA abuse. Lipid status returns to normal only a few months after discontinuation of administration. After long-term, high-dose AAS abuse, atherosclerosis and resulting coronary artery disease, cerebral vascular disease, or peripheral arterial disease (CAD) may develop. While no association was found between current AAS abuse and atherosclerotic plaque volume, the degree of atherosclerosis appears to be dependent on the duration of AAS exposure (Baggish et al. 2017).

37.4.3 Liver Disease

Changes in liver structure have been described, mainly in cases of chronic abuse of **17α-alkylated AAS**, e.g., methyl-

testosterone, methandrostenolone, oxandrolone, stanozolol (Turillazzi et al. 2011). 17α-alkylated AAS are considered obsolete for clinical use (at least in Europe) because of their liver toxicity (Rolf and Nieschlag 1998), but they are still available illegally for doping purposes. They may even be hidden undeclared in dietary supplements, as shown by two cases of severe hepatotoxicity after ingestion of the dietary supplement "Celtic Dragon" containing 2α-17α-dimethyl-etiocholan-3-one, 17β-ol (El Sherrif et al. 2013).

AAS are thought to play a key role in the development of **steatosis hepatis**, inhibiting the normal process of steroid biosynthesis and leading to the storage of cholesterol (Turillazzi et al. 2011). A slight increase in transaminases is usually completely reversible a few weeks after discontinuation of AAS (Basaria 2010).

As a direct toxic effect on hepatocytes with ultrastructural cell damage, oxidative stress leading to increased reactive oxygen species (ROS) production could play a role in the hepatotoxicity of AAS. Commonly observed changes include **intrahepatic cholestasis, peliosis hepatis** (lacunar blood-filled cavities originating from central veins or from focal necrosis of hepatocytes), and **proliferative changes in liver structure** such as focal nodular hyperplasia and hepatic adenomas (Rolf and Nieschlag 1998; Nakao et al. 2000). The appearance of adenomas can be detected as early as 6 months or after 15 or more years of AAS abuse, as described in two cases (Socas et al. 2005). Both bodybuilders had taken five different AAS in high doses, including stanozolol and oxymetholone. After cessation of AAS intake, the sonographically detected adenomas slowly disappeared without surgical intervention despite considerable initial size.

A causal relationship between AAS abuse and **hepatocellular carcinoma (HCC)** has been described mainly in patients with other severe liver diseases (Giannitrapani et al. 2000). Liver damage appears to be AAS dose-dependent (Schwingel et al. 2015).

37.4.4 Nephropathies

Renal disorders have been described mainly after prolonged use of AAS and range from a slight increase in serum creatinine to **acute renal failure as a complication of rhabdomyolysis**. It has been hypothesized that interindividual differences in the magnitude of adverse effects depend on the genetically determined function of the **uridine diphosphate glucuronosyltransferase (UGT)** enzyme, which enables glucuronidation of steroids, the first phase of the deactivation and elimination pathway of AAS (Deshmukh et al. 2010). Due to the UGT 2B17 deletion polymorphism, large interindividual variations in urinary testosterone metabolite concentrations are explained (Strahm et al. 2015). In vivo measurements of UGT 2B17 activity showed that low activity as a result of UGT 2B17 deletion was strongly associated with lower body mass index (BMI) in men, likely as an effect of higher serum testosterone concentration (Zhu et al. 2015).

Histologically, **focal segmental glomerulosclerosis** with tubular atrophy and interstitial fibrosis may be found in long-term AAS abuse. Mild forms of renal dysfunction with elevation of serum creatinine, blood urea nitrogen, and uric acid without sclerotic/fibrotic morphologic changes often return to normal range after discontinuation of AAS (Turillazzi et al. 2011).

37.4.5 Influence on the Musculoskeletal System

AAS in childhood or adolescence cause an acceleration of bone maturation in young athletes. At the end of puberty, activation of endochondrial bone formation leads to premature closure of growth zones with growth retardation, so early administration of testosterone or AAS can lead to growth arrest below expected levels (Kanayama and Pope Jr 2018; Przkora et al. 2005). AAS including testosterone support radial bone growth and periosteum formation. This also explains the larger cross-sectional size of male compared to female bone (Vanderschueren et al. 2012). The effect of testosterone on bone is mediated via the androgen receptor (AR) and via estrogens converted from testosterone through stimulation of osteoblasts and suppression of osteoclasts via the RANKL-OPG system (Vanderschueren et al. 2012). AAS that cannot be aromatized may therefore have little effect on bone.

Athletes often place extreme stress on their musculoskeletal system over long periods of time, resulting in a high incidence of **joint, tendon, bone, and muscle discomfort, injury, and dysfunction**. These can become chronic, causing the former athlete to suffer long after they have stopped playing high-performance sports and abusing AAS. However, there are no conclusive studies documenting a negative effect of AAS on the musculoskeletal system, and it is even suggested that AAS may prevent more severe damage. Synergistic effects of testosterone treatment and resistance training on muscle have also been outlined (Cheung and Grossmann 2018).

The anabolic effects of AAS on skeletal muscle are mediated by the androgen receptor (AR) and growth hormone (GH) and insulin-like growth factor-1(IGF1) mechanisms. Activation of the androgen receptor induces hypertrophy of type I as well as type II muscle fibers and an increase in the number of myonuclei and capillaries per fiber (Yu et al. 2014). These effects are mediated by stimulation of muscle protein synthesis, the GH/IGF-1 axis, and muscle mesenchymal progenitor cells (Bhasin et al. 2012).

As with other androgen effects, muscle mass under physiological conditions is determined by **androgen receptor (AR) polymorphism**. Shorter CAG repeats in exon 1 of the receptor are associated with higher muscle mass (Nielsen et al. 2010). This most likely also plays a role in the response to supraphysiological doses of AAS. There are also ethnic differences in AR polymorphism, for example, sub-Saharan Africans have shorter CAG repeats than Caucasians and East Asians (Ackerman et al. 2012). How this may contribute to performance differences and response to AAS is not yet known. Experiments in mice suggest that once muscle fibers are exposed to high doses of AAS, they respond more rapidly to further AAS treatment, even after a drug-free interval. This cellular memory appears to reside in myonuclei, whose numbers do not decrease after cessation of AAS ingestion (Egner et al. 2013).

Rhabdomyolysis has been observed with acute ingestion of AAS, with acute renal failure a possible complication. Tendon rupture and disc herniation may occur due to massive increases in muscle mass and strength without parallel increases in tendon strength and cartilage resistance.

37.4.6 Dermatological Side Effects

AAS act via the androgen receptor present in epidermal and follicular keratinocytes, sebocytes, sweat gland cells, dermal papilla cells, dermal fibroblasts, endothelial cells, and genital melanocytes. The use of AAS can rapidly lead to skin alterations in previously unaffected athletes, such as disruption of sebaceous gland growth and differentiation, hair growth, epidermal barrier homeostasis, and wound healing. AR polymorphism appears to play a role in the severity of symptoms (Zouboulis et al. 2007). The most common skin manifestations are **acne vulgaris, oily skin, seborrhea, striae, hirsutism, and androgenetic alopecia** (Walker and Adams 2009). Over 50% of athletes who participated in a questionnaire to identify unsupervised AAS therapies and side effects of AAS reported acne (Evans 1997).

After cessation of AAS use, these changes are usually reversible. To accelerate recovery, antiandrogenic therapy with cyproterone acetate or spironolactone could be tried (Zouboulis et al. 2007). However, severe forms of AAS-induced **acne conglobata** leave severe scars on the affected skin areas (Gerber et al. 2008). After acne, **striae distensae** resulting from rapid muscle hypertrophy supported by AAS ingestion are the most common skin side effect in athletes, especially bodybuilders. Over 40% of athletes complained of **stretch marks** of the skin (Parkinson and Evans 2006) with typical localization in the pectoralis muscle or upper arm area. After discontinuation of drug abuse, striae may persist as white streaks (Wollina et al. 2007).

37.4.7 Neoplasms

There is no evidence that testosterone at substitution doses has any effect on tumor development or growth, except in the prostate, where it stimulates the growth of an existing carcinoma. However, there are no reports of an association between current or past AAS abuse and **prostate carcinoma**. This lack of accumulation of case reports despite massive AAS abuse supports the hypothesis that androgens may protect against rather than cause prostate carcinoma.

The most feared malignancy after long-term AAS use is **hepatocellular carcinoma (HCC)**. The possible cause of tumor development in abuse of 17α-alkylated AAS is direct hepatotoxicity. In the case of aromatization of AAS (e.g., endogenous testosterone), a toxic effect of estrogens on liver tissue is discussed. It has been observed that human HCC tissue has increased aromatase activity. However, attempts to treat HCC with aromatase inhibitor tamoxifen did not yield positive results (Giannitrapani et al. 2006).

37.4.8 Side Effects on the Psyche

Headache, insomnia, increased irritability, depressed mood status after AAS abuse have been described (Turillazzi et al. 2011). Individuals exposed to such dangerous regimens may already be predisposed to irrational actions prior to AAS abuse (Piacentino et al. 2015). Dissatisfaction with one's body (e.g., muscle dysmorphia and dysphoria) appears to be common among men who use AAS, similar to men with eating disorders (Björk et al. 2013). Both groups share severe psychiatric symptoms such as **anxiety, depression, obsessive-compulsive behavior, and interpersonal sensitivity** and may be at **risk for suicide**. However, they differ in terms of self-image. The eating-disorder group had lower scores for self-emancipation and active self-love and higher scores for self-blame and self-loathing than former AAS users. There were no differences between these two groups in terms of psychiatric symptoms.

In a study of 17,200 adolescent boys in the United States, the lifetime prevalence for AAS abuse was five times higher among 635 homosexuals (21% vs. 4%) than among heterosexual boys. The homosexual youth showed a higher incidence of **depressive symptoms/suicidality, substance use, and victimization.** But whether these symptoms were precursors or outcomes of AAS abuse could not be determined (Blashill and Safren 2014).

As with other drug addictions (amphetamines, hallucinogens, narcotics), AAS abuse can lead to neurotoxicity and cause encephalopathy, which can present as altered mental status with memory loss and cognitive problems (Pomara et al. 2015).

AAS withdrawal may also be accompanied by **depression or symptoms such as depressed mood, loss of interest, loss of libido, sleep disturbances, and suicidal thoughts** (Turillazzi et al. 2011; Pope Jr et al. 2014). In some former AAS users, depression, anxiety, and melancholy persisted for many years, as noted by a 30-year follow-up of 683 Swedish strength athletes (Lindqvist et al. 2013). Up to 30% of AAS abusers may develop substance-dependence, often combined with alcohol and other drug addictions (Basaria 2010; Lundholm et al. 2015). At least, three etiological mechanisms may lead to AAS dependence:

- Body image disturbances such as muscle dysmorphia
- Dysphoria or depression following attempts to discontinue abuse
- Possible hedonic effects of AAS

Another attempt to explain the psychological causes of doping detects two predictors of doping abuse: fear of competitive failure, which may be associated with low self-esteem, and ego-oriented perspective (Blank et al. 2016).

Key Points

- Due to negative feedback in the regulation of the hypothalamic-pituitary-gonadal axis, AAS cause anabolic steroid-induced hypogonadism (ASIH) in men, characterized by reversible suppression of spermatogenesis, testicular atrophy, infertility, and erectile dysfunction. If spermatogenesis does not recover after AAS abuse, there may be an underlying preexisting fertility disorder. Other common side effects include gynecomastia and acne.
- In women, hirsutism, irreversible deepening of the voice, dysmenorrhea, secondary amenorrhea with anovulation, and infertility are the most common changes caused by AAS abuse.
- High doses of AAS cause significant increases in erythrocyte and hemoglobin concentrations in both sexes, which can lead to thromboembolism, intracardiac thrombosis, and stroke. Long-term AAS abusers have a higher incidence of arrhythmias, atherosclerosis, concentric left ventricular myocardial hypertrophy with impaired diastolic function, and sudden cardiac death. Alterations in liver function and structure, up to and including hepatocellular carcinoma, are mainly caused by chronic abuse of 17α-alkylated AAS. Insomnia, increased irritability, and depressed mood status are commonly observed with AAS abuse and may persist for many years after AAS discontinuation.
- The adverse effects of AAS described above pose a serious risk to individuals who participate in competitive sports, bodybuilding, or recreational sports, as well as to individuals who abuse these substances to enhance performance and/or appearance.
- Adverse effects are caused by supraphysiological doses of AAS and steroids that are known for their toxicity and have never been approved for clinical use or taken out of clinical use. Adverse effects of high doses of AAS may be exacerbated by concomitant use of a variety of other drugs in inappropriate doses and combinations.
- Detection of AAS abuse through the World Antidoping Agency (WADA) control network aims not only to ensure fair conditions for athletes, but also to protect them from health consequences of AAS abuse.
- Regardless of abuse, under physiological conditions testosterone remains the most important hormone for turning boys into men and maintaining adult masculinity. Deficiency of testosterone leads to clear symptoms of hypogonadism, which deserves proper diagnosis and treatment, testosterone substitution being the most important component.

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Treatment of Hypogonadism of Hypothalamic or Pituitary Origin

38

Julia Rohayem and Eberhard Nieschlag

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Abstract

There are different options available for treatment of males with central hypogonadism (i.e., with hypogonadism of either hypothalamic or pituitary origin).

The choice of a therapeutic measure should be guided by both the **underlying origin** of functional impairment and the **intention of treatment**.

- If **initiation (or maintenance) of virilization** is the exclusive intention of treatment, **testosterone** may be used for substitution. However, by this treatment modality pubertal maturation of testes is not initiated. Consequently, neither endogenous testicular testosterone secretion, nor spermatogenesis is stimulated.
- If the goal of treatment is to **induce puberty, including testicular maturation and induction of fertility**, then the hormones of the central hypothalamic-pituitary-gonadal (HPG) axis have to be replaced.
- Hypogonadotropic hypogonadism originating from **hypothalamic GnRH deficiency** can be replaced by both, **pulsatile GnRH or gonadotropins**. This is not successful if hypogonadism arises from **deficient GnRH action** on gonadotropic cells of the anterior pituitary or if **pituitary function is** otherwise **compromised**. In this latter case, only **gonadotropin** substitution is effective.

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- Hormone replacement dosages have to be adapted according to the degree of pubertal maturation that has occurred previously, in order to avoid undesirable side effects of too rapid androgenization.
- In the case of **functional hypogonadism**, treatment should to aim at **removing the cause of central axis suppression or of its downregulation**. If this is successful, hypogonadism may be fully reversible.

38.1 Hormonal Treatment of Hypogonadotropic Hypogonadism (HH)

The aim of hormone replacement in males with central gonadotropic axis dysfunction is either to **fully induce pubertal maturation**, including gonadal maturation (with testicular growth due to initiation of spermatogenesis) or to solely **initiate or maintain secondary sexual characteristics** (=pubertal virilization), without concomitant induction of fertility.

Ideally, replacement of hormones should be initiated as soon as clinical symptoms of hypogonadotropic hypogonadism appear,

- I.e., during adolescence, when puberty has not started spontaneously or when it remains arrested at an immature stage
- During adulthood, as soon as hypogonadism manifests by symptoms of androgen deficiency and/or infertility

If the choice of treatment is to induce or maintain virilization with testosterone, this does not preclude the option to replace central hormones later, at any time, in order to enable fertility.

If HH is of hypothalamic origin, either GnRH or gonadotropins may be substituted. However, if the origin of hypogonadism lies within the pituitary, GnRH is not effective; thus, gonadotropin replacement is the treatment of choice.

38.1.1 Comparison GnRH Versus Gonadotropins

Pulsatile GnRH replacement stimulates the release of LH and FSH from gonadotropic cells within the anterior pituitary.

Gonadotropin replacement uses highly purified urinary **human chorionic gonadotropin (hCG**) for substitution of deficient LH. HCG is administered parenterally twice a week. It acts similarly to LH on LHCG receptors of Leydig cells, stimulating them to synthesize and to secrete testosterone. In addition, FSH is replaced using recombinant FSH (rFSH) injections. These are applied subcutaneously thrice weekly. rFSH acts on FSH receptors of Sertoli cells, stimulating them to support spermatogenesis.

Although replacement of pulsatile GnRH is a therapeutic strategy that strongly imitates physiologic processes in males with hypothalamic GnRH deficiency, the efficacy in terms of spermatogenic induction is comparable to that achieved by gonadotropin replacement (Schopohl 1993; Delemarre-van de Waal 2004).

38.1.2 Replacement of GnRH

GnRH replacement is effective for treatment of men affected by hypothalamic hypogonadism under the condition that **pituitary function**, specifically function of **gonadotropic cells** within the pituitary and that **GnRH receptor function** are uncompromised (Morris et al. 1984; Liu et al. 1988; Schopohl 1993; Büchter et al. 1998). **GnRH insensitivity** cannot be distinguished clinically from absolute GnRH deficiency, unless a disease-causing *GnRHR* mutation is demonstrated (Caron et al. 1999).

The decapeptide GnRH has to be administered in a **pulsatile manner**, in order to display its activating effects on LH and FSH secretion in the gonadotropic cells of the anterior pituitary and thereby indirectly stimulate both testicular testosterone secretion and spermatogenesis. Continuous application of GnRH would lead to downregulation of pituitary GnRH receptors and blockade of signal transduction, with the consequence that LH and FSH pulses would dry up after an initial flair-up (Belchetz et al. 1978; Conn and Crowley Jr 1991).

GnRH substitution is costly. In addition, it is uncomfortable for patients, as a pump has to be worn night and day over the treatment period. The subcutaneous catheter causes irritation of the abdominal skin, and regular catheter changes are required. Therefore, this treatment modality, although effective has hardly gained acceptance in practice (Delemarre-van de Waal 2004).

38.1.2.1 Treatment Protocol for GnRH Replacement

The needle of a catheter (connected to the GnRH ampoule in a portable **mini-pump)** is inserted into the patient's abdominal subcutaneous tissue. The pump is programmed to deliver a GnRH bolus every 120 min (Whitcomb and Crowley Jr 1990). An initial dose of 4 μg per pulse is programmed; on follow-up, the dose may be adapted according to individual

needs, with increases of 2 μg every 4 weeks. The goal is to achieve a rise of serum testosterone concentrations into the adult normal range within 3–12 months. The response of Leydig cells regarding testosterone secretion depends on the degree of testicular maturity previously achieved, either spontaneously or by pretreatment with GnRH or gonadotropins.

The dosages of GnRH required for induction of spermatogenesis vary considerably among individuals with hypogonadotropic hypogonadism, ranging from 5 to 20 μg GnRH per pulse (or 25–600 ng/kg GnRH per pulse). If paternity is desired, treatment is continued until induction of pregnancy has proven successful. In patients with previously undescended testes, the time to pregnancy is generally longer than in men with ectopic testes (Büchter et al. 1998).

38.1.3 Replacement of Gonadotropins

Gonadotropin substitution is effective in stimulating endocrine and spermatogenic testicular functions, as well as in stimulating growth of previously immature testes in patients with hypogonadism of both hypothalamic and pituitary origin.

Although the drug approval for hCG specifies **intramuscular** injection, effective regimens for **subcutaneous** injections of hCG (and FSH) have been established in recent decades, with the advantage that they can be performed by the patients themselves.

Current standard treatment protocols for gonadotropin substitution provide that hCG therapy is started for Leydig cell stimulation and that FSH substitution is added as soon as serum testosterone levels have been shown to rise. It has not yet been conclusively clarified whether a treatment regimen in which combined gonadotropin replacement is preceded by a phase of FSH stimulation has advantages in terms of achievable semen quality (Dwyer et al. 2013). With the latter regimen, the hope is that FSH induces **expansion of Sertoli cells** before the definite number of cells are attained at puberty. This could have a positive effect on testicular spermatogenic capacity.

The following hormonal preparations are available:

- **Human chorionic gonadotropin (hCG),** purified from the urine of pregnant women. HCG has been used as a substitute for LH since 1952 (Maddock and Nelson 1952). It has almost exclusively LH-like bioactivity and effectively stimulates testosterone synthesis in Leydig cells and its secretion into the bloodstream (Siris et al. 1978).
- **Human menopausal gonadotropin (hMG)**, extracted from the urine of postmenopausal women. HMG has been used as a source of FSH since 1966 (Lytton and Kase 1966). FSH is required for spermatid maturation (spermiogenesis) and, together with testosterone, for the maintenance of quantitatively and qualitatively normal spermatogenesis (Matsumoto et al. 1986; Tapanainen et al. 1997; Nieschlag et al. 1999).
- **Highly purified urinary FSH** has been available since 1997/98 (Burgues and Calderon 1997; EMHSG 1998).
- The feared risks of prion disease transmission from urinary gonadotropin preparations have not been confirmed in over 50 years of use.
- **Recombinant FSH (rFSH)** has become available since 1995. rFSH has been used as an FSH substitute (Kliesch et al. 1995; Bouloux et al. 2003; Matsumoto et al. 2009; Warne et al. 2009).
  Recombinantly produced FSH (rFSH) also carries a minimal residual risk of infection transmission because fetal calf serum is used in the cell culture media during production.
- **Corifollitropin alfa** ("FSH-CTP"; Org 36,286) is a long-acting recombinant FSH-like substance that is injected every 2 weeks to achieve therapeutically effective FSH serum concentrations. Corifollitropin alfa is composed of an alpha subunit of human FSH and a beta subunit, which in turn is composed of the beta subunit of human FSH and the C-terminus (CTP) of the beta subunit of hCG to extend the half-life.

  The compound is approved for use in women (Pouwer et al. 2015).
  In a phase III study in azoospermic men with hypogonadotropic hypogonadism (HH), corifollitropin alfa (at a dose of 150 μg every 14 days s.c.) in combination with hCG was effective in inducing spermatogenesis in 75% of patients (Nieschlag et al. 2017).
  In a recent study with adolescent males with HH, corifollitropin alfa subcutaneously applied alone for 3 months and then combined with hCG for 1 year was successful in inducing testicular growth and pubertal development (Shankar et al. 2022).

Currently, there are no data on the efficacy of **recombinant LH (rLH) or recombinant chorionic gonadotropin (rCG)** to stimulate spermatogenesis in male HH patients, as these substances are authorized only for use in assisted reproductive techniques in females.

Gonadotropin preparations currently approved in Germany are listed in Table 38.1. Recombinant and biosimilar FSH preparations are approved by European authorities (EMA) for use in males from age 18 years onwards.

Table 38.1 Preparations with approval for puberty induction and for stimulation of spermatogenesis in men with hypogonadotropic hypogonadism

Hormone preparation	Trade name	Application	Dose
Human chorionic gonadotropin (hCG)	Brevactid®	i.m. (s.c. off label)	250–2500 IU twice weekly
Recombinant FSH (rFSH)	Gonal F®	s.c.	75–150 IU thrice weekly
	Puregon®		
	Biosimilars:		
	Bemfola®		
	Ovaleap®		
Pulsatiles GnRH	Lutre pulse®	Mini pump s.c.	4–20 µg per pulse every120 min

38.1.3.1 Treatment Protocol for Gonadotropin Replacement in Testosterone-Naïve Prepubescent Adolescents with Congenital Hypogonadotropic Hypogonadism (CHH)

Testosterone-naive adolescents have not yet undergone any virilization by endogenous testosterone or exogenously applied androgens. They are also not yet mature in terms of longitudinal growth. Target cells of the adolescent organism are highly sensitive to androgenic effects. Therefore, too rapid an increase in serum testosterone concentrations should be avoided. Prolonged spontaneous erections, potentially resulting in **priapism** may occur as **an undesired effect** of too high initial doses of parenterally applied **hCG** in previously testosterone-naïve boys. Increased **water retention** with remarkable body **weight gain,** an **increase in blood pressure,** development of **severe acne,** and **gynecomastia** are further adverse consequences of inadequate hCG dosing. Further, enhanced aromatization of testosterone to estradiol in adipose tissues may cause premature closure of the epiphyseal joints, limiting **final height**.

A gradual increase in the dosage of hCG, imitating the physiology of the slow rise in endogenous gonadotropin activity during puberty avoids all abovementioned undesired effects of gonadotropin substitution.

The following protocol has proven successful:

- An initial dose of **(250–) 500 IU hCG** is injected s.c. or i.m. on mondays and fridays. The testosterone response of testicular Leydig cells is explored after 3 months and, depending on this, the hCG dose is increased by 250 IU per injection. If necessary, the dose is further increased after 3–6 months; it can be increased up to a maximum of 3 × 2500 IU hCG/week. This is necessary only if testes are predamaged and therefore do not respond with adequate testosterone secretion. The goal of hCG stimulation is to achieve pubertal serum testosterone levels ≥5.2 nmol/L (≥1.5 ng/mL) after around 6 months and testosterone levels in the mid-normal range for adults ≥12 nmol/L (>3.5 ng/mL) after 1 year.
- **rFSH 3 × (75–)150 IU s.c./week** is additionally injected on mondays, wednesdays, and fridays as soon as pubertal serum testosterone levels (around 5 nmol/L) are reached. Subsequent rFSH dose increments beyond a dose of 3 × 150 IU/week are not recommended, as this will not further increase spermatogenesis.

If the adolescent with CHH has passed puberty and has reached a Tanner stage V with full testicular maturation, replacing the costly gonadotropin therapy by long-term substitution of testosterone is recommended. To verify whether all important points have been addressed previously, the following checklist may be helpful:

- Tanner stage V completed.
- No further longitudinal growth observed during the last outpatient presentation.
- No further increase in testicular volumes observed during the last outpatient presentation.
- No further increase in sperm concentration found in the ejaculate in two consecutive examinations 3 months apart.
- Information on the possibility of cryostorage of semen has been given.
- The possibility of omitting the hormonal treatment for 6 months to reevaluate spontaneous HPG axis function has been discussed with the patient who has also been informed about the chance for reversal in around 7%.
- The necessity of lifelong substitution with testosterone has been addressed, in case that central HPG axis function remains inactive.
- The different testosterone replacement modalities have been presented to the patient.
- Information has been given on the necessity of stopping testosterone and resuming gonadotropin substitution if fatherhood is desired in the future (a rapid awakening of spermatogenesis after 3–9 months to the previously achieved level is to be expected by a second cycle of hCG/rFSH treatment).
- Information on the possibility of microsurgical testicular sperm extraction (mTESE) in case of persistent azoospermia, despite combined hCG/rFSH for 3 years has been given; the patient has been informed that the chances of finding testicular spermatozoa by this procedure are high.

38.1.3.2 Treatment Protocol for Gonadotropin Replacement in Adolescents with HH, Previously Virilized by Testosterone

Adolescents with hypogonadotropic hypogonadism who have already experienced either spontaneous virilization or virilization through exogenously applied testosterone will respond with lower sensitivity to hCG stimulation. Therefore, when initiating gonadotropin substitution, a higher initial **hCG dose of 750–1500 IU** s.c. may be chosen. The dose of 2 × 1500 IU hCG/week corresponds to a full substitution dose in the adult male. **Reduction of the hCG dose** is recommended if polyglobulia or gynecomastia occurs, or if excessive acne develops. If testosterone levels remain below the normal adult range (<12 nmol/L) after 6–9 months of hCG stimulation, the hCG dose may be increased in increments of 500 (–1000) IU per injection every 3–6 months, up to a maximum of 3 × 2500 IU/week.

rFSH 3 × 150 IU is added with thrice weekly subcutaneous injections (on mondays, wednesdays, fridays) about 3 months after starting hCG therapy. Subsequent dose changes are not required.

38.1.3.3 Hormone Replacement in Men with Postpubertally Acquired HH

In patients with postpubertally acquired HH, the testes have matured spontaneously during puberty, as indicated by adult testicular sizes. If secondary hypogonadotropic hypogonadism remained previously undiagnosed and was therefore untreated for a prolonged period or if testosterone replacement has been started previously, paused spermatogenesis may have led to a reduction in testicular volumes in the meantime.

The therapeutic approach to postpubertally acquired HH depends on whether the affected individual desires to father a child. If this is the case in the near future, the therapeutic investment of a costly gonadotropin substitution is justified. In all other cases, testosterone replacement is the preferred therapeutic modality (see Chap. 36).

In patients with postpubertally acquired HH, Leydig cells and germ cells respond rapidly to gonadotropin substitution: serum testosterone levels return to the adult normal range within a few weeks after starting hCG therapy. If there is residual endogenous FSH secretion, **hCG monotherapy** may be sufficient to reinitiate spermatogenesis (Finkel et al. 1985; Vicari et al. 1992). However, prolonged treatment with hCG alone also provides the undesired effect of suppressing residual endogenous FSH secretion (Depenbusch et al. 2002). For this reason, the addition of FSH is often required to fully exploit the spermatogenetic potential of both testes. Elongated spermatids are usually detectable in semen 3–9 months after the reinitiation of combined hCG/rFSH. The **individual spermatogenetic capacity** is reflected by the **best achievable sperm concentrations in the ejaculate**. Gonadotropin therapy is continued until pregnancy of the partner is achieved. After birth of the offspring, sperm production may be maintained over several months by hCG replacement, without additional rFSH. This indicates that residual endogenous FSH secretion is present in some men. However, spermatogenesis will be active at a lower level than on combined substitution (Johnsen 1978; Depenbusch et al. 2002). Remarkably, in some males with residual endogenous FSH activity, spermatogenesis may also remain partially active on transdermal testosterone replacement.

There are concerns that long-term hCG therapy could induce production of **hCG antibodies** with consequent loss of hCG bioactivity (Sokol et al. 1981; Claustrat et al. 1983; Thau et al. 1988). This is the reason why continued treatment with hCG to maintain adequate testosterone levels has not prevailed in clinical practice. However, loss of hCG efficacy due to allergy is rare. Recombinant LH (rLH) (although not approved for this indication) may be considered a rescue strategy.

The following protocol has proven successful:

- A full **hCG starting dose of 1500 IU** is administered s.c. or i.m. twice weekly. In cases of polyglobulia, gynecomastia, or excessive acne, the hCG dose is reduced. If testosterone levels are still below the normal adult range (<12 nmol/L) after 3–6 months, the hCG dose may be increased in increments of 500–1000 IU per injection every 3–6 months up to a maximum of 3 × 2500 IU/week.
- In the vast majority of patients who remain azoospermic after 3–6 months on hCG alone, **rFSH 3 × 150 IU s.c.** is added thrice weekly, with no subsequent changes in dose.
- Combined gonadotropin replacement is continued until conception of the partner has been evidenced. It may be reasonable to maintain replacement until the end of the first 12 weeks gestation, during which spontaneous abortions are frequent.

38.2 Monitoring of Hormone Replacement and Cryostorage of Semen

During gonadotropin substitution, serum **LH, FSH, and testosterone** concentrations in serum should be determined every 3–6 months. Serum testosterone not only allows monitoring Leydig cell response but also, along with FSH concentrations, assessing adherence to treatment.

Testicular volumes are measured by Prader orchidometry and/or ultrasound imaging.

In adolescents, **height, body weight, and blood pressure** are monitored regularly.

Note: Special attention must be given to serum LH concentrations while patients are undergoing hCG/rFSH therapy, as an increase into the normal adult range would indicate spontaneous activation of the central gonadotropic axis. In adolescents, the differential diagnosis of constitutionally delayed pubertal development (CDGP) in this situation is probable, in adults "reversal" of CHH has to be considered.

If serum LH rises to normal, interruption of gonadotropin substitution and reassessment of testosterone serum concentrations (after a few weeks) are justified, in order to test whether central HPG-axis activation provides sufficient stimulation of the testes (see also Sect. 38.7).

In younger adolescents, especially those with short stature, bone age assessments are made by **left hand X-ray**, to evaluate the necessity of dose reduction of hCG in the case that bone maturation has advanced too rapidly. A bone age of 16–17 years (according to Greulich and Pyle 1959) indicates that the epiphyseal joints are closed and that final height has been reached.

Once patients are mature enough to provide a semen sample, **ejaculates** can be analyzed after at least 48 h of sexual abstinence, according to WHO guidelines (WHO 2010). Analyses are repeated every 3 months, until a plateau in sperm concentration is documented in at least two consecutive examinations or until pregnancy is achieved. In either case, cessation of gonadotropin substitution is indicated.

Cryopreservation of sperm (see Chap. 42) may be offered before gonadotropin replacement is replaced by long-term exogenous testosterone, as spermatogenesis will then no longer be stimulated. Sperm banking represents a kind of "fertility insurance"; therefore, this measure is explicitly recommended only to patients with **poor therapeutic response**. Cryopreservation of semen may also be useful in cases where assisted reproductive measures will be necessary to achieve pregnancy as a consequence of **female fertility impairment**.

Replacement with testosterone following completion of gonadotropin substitution is less expensive and sufficient to compensate for the androgen deficit. It provides long-term maintenance of secondary sexual characteristics and androgen-mediated psychosexual effects.

- As spermatogenesis enters the "dormant phase" upon switching to androgen substitution, in case of (renewed) desire for children, gonadotropin therapy has to be resumed to restore fertility. Restimulation of the testes with gonadotropins as part of a second treatment cycle activates spermatogenesis more rapidly than during the first cycle, even after a long period of intermittent testosterone substitution (Büchter et al. 1998; Liu et al. 2002). Sperm concentrations similar to those achieved during the first cycle can be expected.

38.3 Success Rates

Virilization is **successfully induced and maintained without exception** with the use of central hormone replacement strategies, provided that **adherence to treatment** is assured.

Induction of testicular growth to pubertal sizes is also **usually successful**, if the testes have not been irreversibly damaged by previous surgery, which is not always clearly ascertainable prior to initiation of therapy. Normal **adult testicular volumes** are achieved **in up to 75%** of patients.

Spermatogenesis can be successfully **induced in 90%** of affected individuals, although at **highly variable levels** (Rohayem et al. 2017). Normal sperm concentrations are achieved only by a minority of patients. Nevertheless, even with **sperm counts below the third centile of the WHO 2010 references, spontaneous conception** is possible in a large proportion of treated men, provided the partner's fertility is not impaired (Burris et al. 1988a; Büchter et al. 1998; Liu et al. 1999; Rohayem et al. 2016).

Studies reporting therapeutic responses achieved in male adolescents and adult men with hypogonadotropic hypogonadism are listed in Tables 38.2 and 38.3.

In patients with CHH or Kallmann syndrome and with **noninitiated puberty**, pubertal testicular growth and full activation of spermatogenesis are achieved less rapidly and less effectively than in patients with **arrested puberty** (Rohayem et al. 2016). HH patients whose central gonadotropic axis function dropped **after spontaneous completion of puberty** respond even faster and better to central hormone replacement (Rohayem et al. 2016).

Both **testicular volumes** and serum concentrations of the Sertoli cell marker **inhibin B at treatment initiation** reflect the degree of testicular tissue development and maturation that has previously occurred. Thus, low-serum inbibin B concentrations and low testicular volumes prior

Table 38.2 Studies on gonadotropin substitution using hCG/FSH in adult men with hypogonadotropic hypogonadism (HH)

Studies using hCG/FSH treatment in more than 25 adult HH patients	Number of adult HH patients	Age (years)	Gonadotropin preparations and sequence of applications	Duration of substitution (months) mean or median (range)	Previous gonadotropin exposure (endogenous or exogenous) or previous TRT	Mean± median (range) final single TV (mL) reached	Spermatogenesis achieved	Time to first sperm (months) from start FSH	Sperm concentration achieved (mill/mL) mean ± SD or median (range)	Time to sperm plateau (months)
Burgues and Calderon (1997)	60	18–45	hCG → hCG+ Purified FSH	hCG: 1 hCG + FSH: 6 (+3)	47 (78%) previous TRT 8 (13%) postpubertal onset HH 36 (60%) previous gonadotropins	11 ± 1	48/60 (80%)	47% sperm positive after 6 months combined tx.	25 ± 8	n.a.
Büchter et al. (1998)	39	21–48	hCG + hMG	12–24	n.a.	6–20	90%	1–16	n.a.	n.a.
European Metrodin HP study group (1998)	28	17–42	hCG+ purified FSH	hCG: 3–6 hCG + FSH: 18	11 (39%) Previous TRT 4 hCG 1 hCG/MHG	10 ± 4	89% after 18 month FSH	Ca. 20% sperm+ after 6 months combined tx	16 ± 21	n.a.
Liu et al. (2002)	26	26–52	hCG→ hCG + rFSH or uFSH (+further cycles)	hCG: 3–6 hCG + FSH: 4 (0–10)	26 no previous gonadotropins 9 postpubertal HH 19 no TRT 6 with sperm at start of hCG 16 with more than one cycle	n.a.	n.a.	Median in first cycles: 7 Median in further cycles:3.5	12 (0–238)	Median (±SE): 29 ± 2
Bouloux et al. (2003)	49	31 ± 7 (18–60)	hCG, hCG→hCG+ rFSH	hCG: 4 hCG + FSH: ≥11	7 GnRH 19 hCH/FSH 8 no previous tx	12 ± 7	23/49 (47%): 9/49 (18%) on hCG alone 14/30 (47%) after additional 6 months FSH	Median: 5.5	n.a. (ca. 1–10)	Ca. 10
Matsumoto et al. (2009)	36	18–55	hCG→ hCG + rFSH	hCG: 3–6 hCG + FSH: 18	5 hCG 2 hCG/FSH 5 GnRH 32 TRT	15 ± 4 13 (7–28)	22/29 (76%)	n.a. 19/29 (65%) at 6 months	10 ± 15 5.2 (0–70)	n.a.
Warne et al. (2009)	100	16–48	hCG→ hCG + rFSH	hCG: 3–6 hCG + FSH: 18	n.a.	12 (15) ± 5	68/81 (84%)	Median: 6–9 (3–18)	n.a. (69% > 1.5)	n.a.

(continued)

Table 38.2 (continued)

Studies using hCG/FSH treatment in more than 25 adult HH patients	Number of adult HH patients	Age (years)	Gonadotropin preparations and sequence of applications	Duration of substitution (months) mean or median (range)	Previous gonadotropin exposure (endogenous or exogenous) or previous TRT	Mean± median (range) final single TV (mL) reached	Spermatogenesis achieved	Time to first sperm (months) from start FSH	Sperm concentration achieved (mill/mL) mean ± SD or median (range)	Time to sperm plateau (months)
Sinisi et al. (2010)	34	20–44	hCG→ hCG + rFSH or hCG + uFSH	hCG: Several months	5 with previous hCG/rFSH	9 (4–15)			n.a.	
				hCG + FSH: 12	7 with previous TRT				Total sperm count	
									rFSH: 29 (0.6–96)	
									uFSH: 10.5 (0–72)	
Rohayem et al. (2016)	51	22–47	hCG→ hCG+ FSH (urinary derived or purified or rFSH)	hCG: 11 ± 8	10 with pubertal arrest	27 ± 15	50/51 (98%)	9 ± 7	15 ± 29	hCG/FSH: 24 ± 16
				hCG + FSH: 9 ± 7	10 postpubertal HH				2.5 (0–156)	
					0 with previous hCG/rFSH					
					42 previous TRT					
Liu et al. (2016)	223	22.4 ± 3	hCG + rFSH	hCG + FSH: 23 ± 13	Previous TRT:60%	8.1 ± 4.6	143/223 (64%)	15	11.7 (2.1; 24.4)	For sperm conc >10 mill/mL
					Previous hCG/FSH: 11%					39 (95%CI 19.7–34.4)

Table 38.3 Studies on gonadotropin substitution using hCG/FSH in adolescents with hypogonadotropic hypogonadism (HH)

Studies using hCG/FSH in adolescent patients with HH, with assessment of puberty (incl. increase in TV) and spermatogenesis	Number of adolescent HH patients	Age (years)	Gonadotropin preparations and sequence of applications	Duration of substitution (months)	Virilization/ adult T levels achieved (%)	Mean ± median (range) final single TV (mL) reached	Spermatogenesis achieved	Time to first sperm (months) from start FSH	Sperm concentration achieved (mill/mL) mean ± SD median (range)	Time to sperm plateau (months)
Liu et al. (1988)	3 (subset of cohort)	16–17	hCG/hMG		100	9 ± 1	n.a. Total cohort: 80%		<5	
Schopohl (1993)	9 (subset of cohort)	18–24	hCG/MHM	20 ± 2	100	n.a. (8–30)	n.a. Total cohort: 47%		n.a. Total cohort: n.a. (2–26)	20 ± 2
Barrio et al. (1999)	14 (IHH: 7 panhypopit: 7)	13–21	hCG + rFSH	31	100	IHH: 10 ± 4 Panhypo: 15 ± 5	7/8 (87%) IHH: 4/5 Panhypo: 3/3		n.a. (1.5–80)	
Raivio et al. (2007)	14	10–18	rFSH→ rFSH+hCG	rFSH: 2–34 hCG + FSH: n.a.	100	6 (2–37)	6/7 (86%)		8.5 (2.9–92)	
Sinisi et al. (2008)	10 (subset of cohort)	11–25	hCG→ hCG + rFSH	hCG/rFSH: 12 (−24)	100	10 (7–15)	n.a. Total cohort: 81%		29 (2.6–96)	
Zacharin et al. (2012)	7 (subset of cohort)	16–22	hCG→ hCG + rFSH	hCG/rFSH: 9	100	12 ± 7 10 (5–27)	7/7(100%)		1.2 (0.2–15)) 4.6 ± 6 after 9 months	
Rohayem et al. (2017)	60	14–22	A: hCG→ hCG + rFSH	A: hCG:31 ± 6	A: 100	A: 17 ± 3	A: 21/23 (91%)	A: n.a.	A: 17 (0.2–337)	A: 31 ± 6
	A: 34		B: Testo→ hCG→ hCG + rFSH	hCG/FSH: 25 ± 9	B: 100	15 (8–40)	B: 18/19 (95%)	B: 11 ± 6	40 ± 73	B: 30 ± 7
	B: 26			B: hCG:30 ± 7 hCG/FSH: 25 ± 9		B: 16 ± 3 17.5 (2–30)			B: 3.5 (0.1–158) 19 ± 38	

to the initiation of therapy are indicative of prior impairment of central HPG axis function during two decisive phases of Sertoli cell differentiation and expansion in life: in utero and during mini-puberty (Burris et al. 1988b; Pitteloud et al. 2002; Rohayem et al. 2016; Rohayem et al. 2017).

A history of **undescended testes** at birth entrains a limited therapeutic response in terms of achievable testicular growth and sperm counts in semen (Pitteloud et al. 2002; Bouloux et al. 2003; Miyagawa et al. 2005; Liu et al. 2009; Rohayem et al. 2017). Cryptorchidism causes changes in the germinal epithelium (Mengel et al. 1974). Nonetheless, a history of undescended testis does not preclude finding sperm in semen samples at the completion of gonadotropin replacement, although testicular response requires prolonged stimulation. Patients with Kallmann syndrome have a high prevalence of maldescended testes at birth (Rohayem et al. 2017). Patients with congenital multiple pituitary hormone deficiencies also tend to have an impaired response to therapy, especially those with a history of cryptorchidism (Rohayem et al. 2016; Rohayem et al. 2017).

38.4 Therapeutic Options in the Case of Persistent Azoospermia

In around 10% of all adolescents and men undergoing gonadotropin therapy, sperm fail to appear in the ejaculate, even after combined replacement for more than 3 years. In the majority of these males, there is a history of bilaterally undescended testes at birth. If orchidopexy has been performed, the ductus deferens may have been occluded accidentally. Alternatively, it is possible that the testes have been irreversibly damaged by cryptorchidism and/or surgery. In both cases, there is nevertheless a chance for successful surgical sperm retrieval via **testicular sperm extraction (TESE)**, before switching to permanent therapy with testosterone (Fig. 38.1). Tissue spermatozoa can be cryopreserved and used to perform **ICSI** in the future, whenever the patient wishes to father a child. A repeated gonadotropin cycle is not useful in these cases.

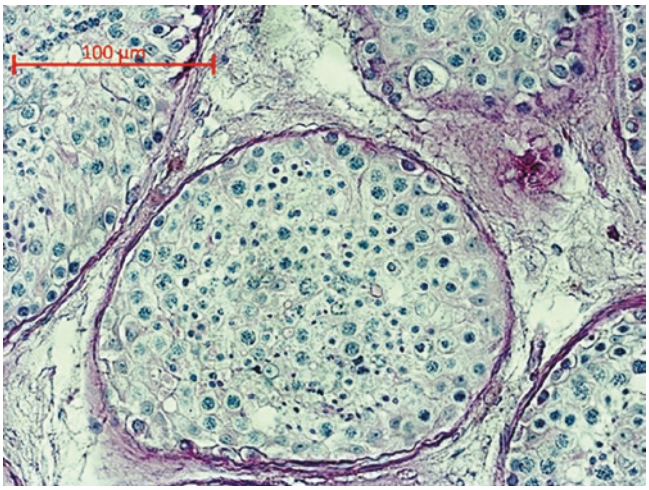


Fig. 38.1 Testicular biopsy (with PAS staining) from a right solitary testis (after previous surgical removal of the left testicle due to atrophy following testicular torsion) in a young man with persistent azoospermia despite 3 years of combined gonadotropin replacement for hypogonadotropic hypogonadism. The incised tubule shows full spermatogenesis. Sperm were evidenced in all other testicular biopsies. This proves the obstructive origin of azoospermia

38.5 Pregnancy Rates

In hypogonadotropic hypogonadal men wishing to father offspring, and therefore undergoing central hormone replacement, **pregnancies** are achieved in **40–55%** of partners (Ley and Leonard 1985; Büchter et al. 1998; Liu et al. 2009; Rohayem et al. 2016). Remarkably, **spontaneous** pregnancies may be induced by these men, even with subnormal sperm counts in semen. Obviously, the spermatozoa have an excellent fertilization potential (Burris et al. 1988a; Büchter et al. 1998; Liu et al. 2009; Rohayem et al. 2016) (Fig. 38.2). However, in **1/5 to 1/8 of patients, assisted reproductive techniques (ART)**, specifically intracytoplasmic sperm injection (ICSI) is required to achieve conception (Liu et al. 2002; Bakircioglu et al. 2007; Rohayem et al. 2016).

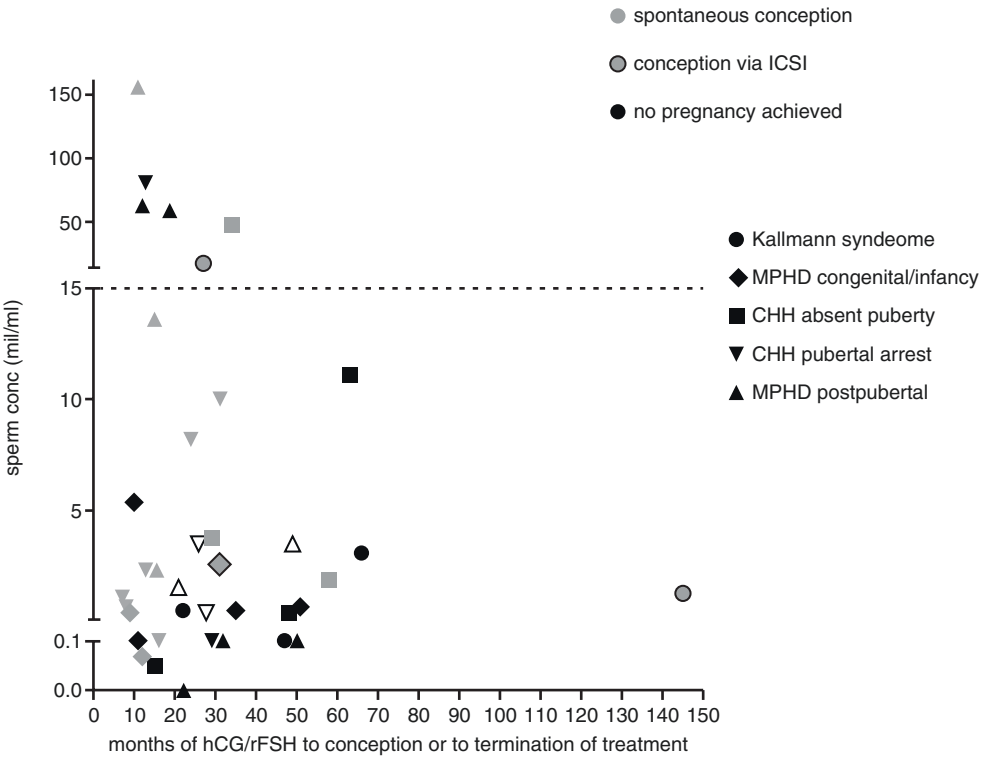


Fig. 38.2 Sperm concentrations in semen at the time of achievement of pregnancy (spontaneous or with ICSI) in different HH subgroups, plotted over months of hCG/FSH treatment to conception in successful patients. In the unsuccessful, maximal sperm concentrations are plotted over months of hCG/FSH till termination of treatment. MPHD multiple pituitary hormone deficiencies, CHH congenital hypogonadotropic hypogonadism, ICSI intracytoplasmic sperm injection

38.6 Inheritance of Congenital Hypogonadotropic Hypogonadism

Patients with congenital central hypogonadism are infertile without therapy. This is the reason why most cases of CHH appear sporadically, as a result of de novo mutations or, in the case of recessive inheritance, as offspring of (often consanguineous) parents, carrying a heterozygous mutation. Familial CHH cases also occur in dominant inheritance with incomplete penetrance. Successful fertility induction may increase **vertical transmission**, especially in dominant inheritance. Human **genetic counseling** is therefore recommended to all patients with identified mutations.

38.7 Misdiagnosis of Constitutional Delay of Growth and Puberty (CDGP) as CHH and "CHH Reversal"

Males with CHH require lifelong hormone replacement. By contrast, in adolescents with CDGP, the activity of the central HPG axis is only delayed. If gonadotropin replacement is started, the GnRH pulse generator will be suppressed, which prevents **spontaneous central activation of the axis**. Therefore, it is important to distinguish both conditions and not to misdiagnose constitutional delay of growth and puberty (CDGP) as CHH.

Nevertheless, a subset of subjects with CHH experience spontaneous resolution of hypogonadism during life in approximately 7%. This phenomenon, termed "CHH reversal," has been described in patients with *CHD7, FGFR1, GNRHR*, and *TACR* mutations (Quinton et al. 1999; Raivio et al. 2007; Laitinen et al. 2012; Tommiska et al. 2013; Sidhoum et al. 2014).

Continuous attention should therefore be paid to LH concentrations in serum, as this parameter reflects the endogenous activity of the patient's gonadotropic axis during hCG/rFSH treatment. Endogenous activation of the GnRH pulse generator on gonadotropin substitution is likely to occur only in patients who regularly skip injections, due to the central suppressive effects of hCG.

"CHH reversal" may not be sustained (Tommiska et al. 2013; Sidhoum et al. 2014). This vulnerability and plasticity of the central gonadotropic axis underscore the need for lifelong patient attention to symptoms of androgen deficiency. In addition, monitoring of gonadotropic axis hormones at regular interval seems reasonable.

38.8 Treatment of Functional HH

38.8.1 Treatment of Functional HH Due to Excessive Weight Loss or Obesity

Functional hypogonadotropic hypogonadism results from a disturbance of central GnRH secretion, as a consequence of severe changes in body weight within a short period of time. As the disorder is potentially fully reversible, its treatment consists of restoring weight to a level appropriate for body size and constitution.

38.8.2 Treatment of Functional HH Resulting from Drug-Induced Suppression of the Hypothalamic-Pituitary-Gonadal (HPG) Axis

Prolonged abuse of **anabolic androgenic steroids (AAS)** suppresses the central HPG axis (see Chap. 37). The same is true for the application of testosterone preparations. Suppression is reversible if androgen administration is stopped; however, reactivation of the GnRH pulse generator may require several (3–24) months (Gazvani et al. 1997; Liu et al. 2006; Nieschlag and Vorona 2015).

In clinical practice, different approaches to overcome AAS-induced hypogonadism have been described. The use of hCG, with or without combined hMG (Turek et al. 1995; Menon 2003) and the use of antiestrogens have been suggested to overcome the symptoms of androgen deficiency (Tan and Vasudevan 2003). However, with hCG/hMG, the central gonadotropic axis is further suppressed, and with antiestrogenic preparations, undesirable side effects such as gynecomastia and liver dysfunction may be induced. Therefore, it is best to wait for **spontaneous recovery of HPG axis function** after cessation of abuse.

GnRH secretion is suppressed if **morphine, heroin,** and **methadone** are applied, since opioids activate hypothalamic inhibitory neurons (Cicero et al. 1974; Daniell 2002; Rajagopal et al. 2004). This form of hypogonadism is reversible after cessation of opioid intake. The speed of recovery of central axis function depends on the duration of suppression.

Key Points

- In males with hypothalamic-pituitary dysfunction of the gonadotropic axis, failed or arrested testicular maturation can be fully induced by GnRH or gonadotropin replacement. Both pubertal virilization and activation of spermatogenesis are achieved by this treatment.
- Replacement of the central hormone deficiency should ideally be started following diagnosis of CHH during adolescence. However, treatment is also effective regarding spermatogenic induction if performed later in life.
- Activation of spermatogenesis as part of gonadal maturation may enable natural conception, even with low sperm concentrations in the ejaculate. Assisted reproductive procedures (ICSI) may enable biological paternity if conception is not successful by natural means.
- Optionally, spermatozoa can be cryostored after a first cycle of central hormone replacement.
- Spermatogenesis will no longer be stimulated as soon as gonadotropin replacement is switched to testosterone replacement. However, in some males with residual endogenous FSH activity, spermatogenesis may remain partially active during testosterone or hCG monotherapy.
- Spermatogenesis can be reinitiated at any time by an additional cycle of gonadotropin replacement. Similar sperm concentrations to those achieved during the first cycle can be expected.
- Human genetic counseling is recommended to all patients with identified mutations that are considered causative of CHH.

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Therapeutic Attempts in Idiopathic Infertility

39

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Abstract

About 30–60% of men with impaired fertility suffer from idiopathic infertility for which there are no rational therapeutic approaches. Nevertheless, numerous pharmaceuticals and procedures have been and are still being used, often over long periods of time and frequently in combination or in sequence, which are summarized as empirical therapy. Here, the postulate of evidence-based medicine for randomized controlled trials proves to be particularly important, and it is according to these strict standards that the various therapeutic procedures are critically evaluated in this chapter. While results in individual studies are often promising, further evaluation in meta-analyses is usually less clear. In summary, none of these drugs or procedures is evidence-based and fail to be recommended in guidelines for male infertility.

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39.1 Definition and Incidence of Idiopathic Infertility

About 30–60% of men with impaired fertility suffer from **idiopathic infertility** (Tüttelmann and Nieschlag 2009; Punab et al. 2017). Idiopathic infertility represents a diagnosis of exclusion and may be made only after other fertility-disrupting causes have been ruled out. Seminal parameters may be subnormal and elevation of FSH may indicate spermatogenic disorder. However, testicular biopsy does not provide further diagnostic information. Histologically, incomplete spermatogenesis or partial SCO syndrome is often found. These patients form the largest group of men attending the fertility consultation.

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The collective diagnosis of **idiopathic infertility** conceals a multitude of different pathogenetic mechanisms. Their elucidation is one of the most important and exciting tasks of andrology and may finally allow rational therapy to overcome the causative disorders of idiopathic infertility. New approaches can be expected mainly from research on the genetic and biological control of spermatogenesis, the action of gonadotropins and sex hormones at the molecular level, and the biology of gametes. Examples of such research areas include microdeletions on the Y chromosome, CFTR mutations in congenital bilateral aplasia of the vas deferens (CBAVD), and androgen receptor pathology.

It should be emphasized that the term idiopathic infertility is used differently in andrology and gynecology. The gynecologist speaks of idiopathic infertility of a woman when, despite intensive diagnostics, no pathological findings can be found in the patient explaining the unfulfilled desire for children. In this case, it is rather a case of unexplained infertility (see Chap. 41). In idiopathic infertile men, on the other hand, while a pathological condition can be found, there is (as yet) no causal explanation for it and consequently, no rational therapy.

Even though there are no clear pathophysiological concepts as to why a particular drug should be effective in idiopathic infertility, numerous pharmaceuticals have been and continue to be used, often over long periods of time, often in combination or in sequence, which are summarized here as **"empiric therapy."** Here, the postulate of **Evidence-Based Medicine for randomized controlled trials** proves particularly appropriate, and it is according to these strict standards that the drugs below are critically evaluated.

Empiric therapies were long practiced in andrology until their lack of effectiveness was verified in randomized controlled trials. Since temptations to use these forms of therapy flare up time and again, previous results have been summarized here to discourage the reader from unnecessary attempts at therapy. For this reason, older work is also listed. However, the number and types of empirical therapeutic approaches have changed since ICSI has partially taken over the role of empiricism (see Chap. 1).

39.2 Empirical Therapy

39.2.1 Gonadotropins: hCG/hMG/rFSH

FSH and LH are critical regulators in human spermatogenesis. In hypogonadotropic hypogonadism, administration of these gonadotropins (or FSH and hCG) is indicated and their effect on spermatogenesis is scientifically proven and appropriately recognized. But what is the **effect of FSH and/or hCG in men with impaired spermatogenesis** and normal FSH levels? In Germany, the application of FSH at physiological FSH levels is not approved.

Although in some single studies, an improvement of sperm parameters, DNA fragmentation index (DFI) or pregnancy rate after FSH treatment could be shown in idiopathic infertile men, in other older studies this could not be confirmed (Kamischke et al. 1998; Foresta et al. 2005; Paradisi et al. 2006; Simoni and Santi 2020). The **quality of studies is very heterogeneous** and comparisons between papers are difficult, mainly due to different: selection criteria in the study population, type of gonadotropins (rFSH vs. urinary-derived), duration of therapy and endpoints (pregnancy vs. sperm parameters).

A **2013 Cochrane analysis** analyzed six randomized controlled trials comparing the effect of gonadotropins to placebo or to no treatment in idiopathic infertility (Attia et al. 2013). Initially, there was a significant increase in the occurrence of spontaneous pregnancies (OR 4.94 [2.13–11.44; moderate level of evidence] and live births (OR 9.31 [1.17–73.75; very weak level of evidence]). However, the authors themselves concluded that due to the small study size and small number of participants ($n = 456$), the evidence is very weak and no definitive recommendations could be derived (Attia et al. 2013). In a 2015 meta-analysis that analyzed 15 studies (randomized and nonrandomized) for the effect of FSH or hMG (human menopausal gonadotropin) treatment, the authors came to a similar conclusion: partner pregnancy rates were higher among idiopathically infertile men who received FSH (Santi et al. 2015). This observation held true for spontaneous pregnancies (OR 4.5 [2.17–9.33]) as well as pregnancies after ART (OR 1.6 [1.08–2.37]). The results were independent of the type of FSH and duration of treatment. Effect size calculation showed that ten couples would need to be treated to achieve pregnancy. Interestingly, in a sub-analysis concerning sperm parameters, only sperm concentration showed a significant increase, other parameters remained unchanged. In the most recent meta-analysis, which included a total of five studies investigating the effect of FSH in oligozoospermic males with FSH serum levels <12 IU/L, a dose-dependent effect was found with respect to sperm concentration, total sperm count, and motility. This effect was independent of the preparation of FSH (hpFSH vs. rFSH) (Cannarella et al. 2020).

Overall, these are promising results, yet sensitive parameters such as FSH serum level and total sperm count are not sufficient to predict FSH treatment response.

Predictive markers are needed to target FSH therapy and to show the positive impact on the properly selected group (Santi et al. 2015; Behre 2019; Schubert et al. 2019). Here, pharmacogenetic approaches seem to be the most promising (Simoni and Casarini 2014; Busch et al. 2015; Schubert et al. 2019; Casarini et al. 2020; Simoni and Santi 2020). There are a few studies that have taken an FSH-based pharmacogenetic approach in idiopathic infertile men (Ferlin et al. 2011; Selice et al. 2011; Simoni et al. 2016; Casamonti et al. 2017). Patient

cohorts were subgrouped with respect to a genetic variant (single-nucleotide polymorphism—SNP) in the FSH receptor or in the FSHB gene and assigned to gonadotropin therapy accordingly. However, again, due to divergent selection criteria, treatment duration, and different endpoints, no reliable conclusions or consequences can be derived (Schubert et al. 2019). Fundamentally prospective, randomized, and placebo-controlled studies will be needed to clarify the question of FSH efficacy on a subgroup of idiopathically infertile men.

In the guideline on the management of oligoasthenozoospermia, the European Academy of Andrology (EAA) **recommends FSH administration as a potential therapeutic option** in normogonadotropic patients with idiopathic oligozoospermia or OAT, but the level of evidence is reported as very low (Colpi et al. 2018). In the European Association of Urology (EAU) Guidelines on Male Infertility, the authors stipulate that no clear recommendation for gonadotropin treatment can be made to men with idiopathic infertility (Jungwirth et al. 2012).

Given the good success rate of hCG/hMG therapy in achieving pregnancy in hypogonadotropic hypogonadism, since the early 1960s this treatment has also been tried in patients with normogonadotropic fertility disorders. A **placebo-controlled, prospective, randomized study** then showed that in patients with normal serum concentrations of LH, FSH, and testosterone and spermatozoa concentrations below 10 million/mL, treatment with hCG/hMG did not improve ejaculate parameters or increase pregnancy rates compared with the placebo group (Knuth et al. 1987). Moreover, this study highlighted the placebo effect, since improvements in ejaculate parameters were also observed in the double-blind treated placebo group. Accordingly, normogonadotropic idiopathic infertility is not an indication for hCG/hMG therapy.

39.2.2 Antiestrogens and Aromatase Inhibitors

The **antiestrogens (clomiphene citrate, tamoxifen**) cause an increase in GnRH via blockade of estrogen receptors in the hypothalamus and pituitary. **Aromatase inhibitors (anastrozole**) inhibit the conversion of androgens to estrogens. Assuming that the increase in LH and FSH leads to an improvement in spermatogenesis, antiestrogens and aromatase inhibitors are also used in idiopathic infertility.

39.2.2.1 Antiestrogens

Regarding the benefit of **tamoxifen** when used in idiopathic infertile men, there are some older meta-analyses and reviews. The earlier analyses failed to show a significant effect of antiestrogens on pregnancy rates (Kamischke and Nieschlag 1999; Vandekerckhove et al. 2005). A 2013 meta-analysis showed that SERMs (Selective Estrogen Receptor Modulators) increased sperm concentration, motility, and pregnancy rate (Chua et al. 2013). The drawback of this meta-analysis, which also chose pregnancy rate as the primary endpoint, is the relatively small number of studies (n = 11) and the associated small cohort size (n = 903). The pooled OR showed a 2.4-fold increased probability of pregnancy occurrence when the male idiopathic infertile partners were treated with antiestrogens compared with the control group. The calculation showed a **number needed-to-treat of n = 10 couples**. In a subgroup analysis comparing clomiphene 50 mg with tamoxifen 20–30 mg/day, no drug was shown to be more effective in comparison (Chua et al. 2013).

The low side effect profile and comparatively low cost may, according to some authors, support therapy for 3–6 months, but not longer than 12 months. The effect on FSH and testosterone was also significant, leading to the conclusion that the therapy can be used in idiopathic infertile men with testosterone deficiency (Whitten et al. 2006). It is an off-label use. The authors of the EAA guidelines on the management of OAT are not specific about recommending therapy with antiestrogens or aromatase inhibitors, and the **level of evidence is considered very low** (Colpi et al. 2018). Similarly, the authors of the EAU guideline on male infertility refrain from a clear recommendation regarding the use of antiestrogens (Jungwirth et al. 2012).

39.2.2.2 Aromatase Inhibitors

Among aromatase inhibitors (AIs), **testolactone** (steroidal; no longer approved in the U.S.) and **anastrozole** and **letrozole** (nonsteroidal) are the main drugs found in the literature. AIs have the advantage of increasing endogenous testosterone without altering estrogens. These drugs are approved for metastatic breast cancer; their use in idiopathic infertile men is an off-label treatment. Some men with severely impaired spermatogenesis have an increased testosterone/estradiol (T/E2) ratio due to increased aromatase activity, so the use of inhibitors would be a consistent approach (Pavlovich et al. 2001).

In a recent systematic review and meta-analysis, randomized and nonrandomized studies from the past 30 years were examined for the efficacy of AI on testosterone concentrations, T/E2 ratio, and sperm concentration in hypogonadal OAT patients. With regard to testosterone levels, all studies analyzed showed an increase of 48.5% (del Giudice et al. 2020). In the effect on T/E2 ratio, an increase of 227% (five studies included) and in the analysis of sperm concentration, an increase of 116% (three studies included) was observed. No improvement was detected in patients with azoospermia (del Giudice et al. 2020). Another study compared the efficacy of anastrozole and letrozole; no significant differences in the effect on testosterone level and sperm concentration were detected between the two AIs (Raman and Schlegel 2002).

Because of the divergent statistical evaluations of different sperm parameters (the main conclusion of the meta-analysis is that AIs have an effect on testosterone levels (del Giudice et al. 2020). Therefore, the EAA guidelines for the management of OAT patients cannot currently recommend the use of aromatase inhibitors (Colpi et al. 2018). A statement on pregnancy rates cannot be made. Overall, AIs could be considered as well-tolerated, apart from a temporary increase in liver enzymes. It can be concluded that men with a low testosterone level and a low T/E2 ratio might benefit from an AI, presumably rather than from a preparation from the SERM group (Schlegel 2012).

39.2.3 Antioxidants, Diets, and Supplements with Antioxidant Effects: Vitamins, Folic Acid, Zinc, Carnitine, and Others

As in other fields of medicine, pathological phenomena in andrology are associated with oxygen-free radicals. **Oxidative stress** is discussed as a **cause of fertility disorders** (Tremellen 2008) and antioxidants such as some vitamins are used for therapy. While oxidative stress can negatively affect spermatogenesis and sperm maturation, oxygen-free radicals in physiological concentrations are also required for normal sperm function (Aitken 1995). For example, a negative correlation has been shown between the rate of fertilization in vitro and the capacity of sperm to destroy oxygen-free radicals (Yeung et al. 1996).

Antioxidants prevent or delay the oxidation of biologically relevant molecules; this is done by scavenging free radicals or by chelation (Valko et al. 2006). Oxidative stress can affect fertility through two mechanisms: first, by **damaging the sperm membrane** and second, by apoptosis and direct **alteration of sperm DNA** (Kodama et al. 1997; Lewis et al. 2013). The latter can be canceled by superimposition of normozoospermia, limitations in DNA fragmentation (DFI) can be detected by various tests. Some authors consider DFI determination as an elementary part of diagnostics.

Some vitamins and trace elements have an antioxidant effect and, like substances with a direct antioxidant effect, are part of dietary supplements. Table 39.1 shows a selection of these substances, with their corresponding antioxidant properties and natural occurrence. Further information can be found in the Cochrane Review (Smits et al. 2019). Often, these substances are part of the therapeutic desire of idiopathic infertile men who are denied causal therapy due to a lack of etiological factors.

Considering the many beneficial properties of antioxidants, dietary supplementation with these substances seems logical to improve fertility.

Of a large number of mostly uncontrolled clinical trials with diverse individual antioxidants and their combinations, a recent Cochrane Review lists 61 randomized clinical trials with **arginine, L-carnitine, coenzyme Q10, N-acetyl cysteine, carotenoids, micronutrients (calcium, magnesium, selenium, zinc), vitamins (B6, B12, E, and C), and myo-inositol**, which were studied as single substances or in combination (Smits et al. 2019). The vast majority of studies exclusively examine the effect of antioxidants on ejaculate parameters and/or DNA fragmentation, and only a few studies examine clinical pregnancy rate ($n = 12$) or live birth rate ($n = 7$). Overall, antioxidants show potential improvement in DNA fragmentation, sperm concentration, and sperm motil-

Table 39.1 Overview of antioxidants, trace elements, and other substances with antioxidant activity (from Smits et al. 2019)

	Substance	Antioxidant properties	Occurrence	References
Antioxidants	**Arginine**	Radical scavenger	Meat, dairy products, nuts	Appleton (2002)
	Coenzyme Q10	Ubiquinol (reduced form) prevents protein and DNA oxidation	Meat, fish, nuts	Littarru and Tiano (2007)
Trace elements	**Folic acid (vitamin B9)**	In synthetic form radical scavenger	Green vegetables, liver, fruits	Joshi et al. (2001)
	Zinc	Strong antioxidant effect; involved in testicular development and phys. sperm function	Meat, grains, seeds	Colagar et al. (2009)
	Vitamin E (bioactive: Tocopherol A)	Antioxidant effective in membrane damage induced by oxidation	Vegetable oil	Traber and Atkinson (2007)
	Vitamin C (ascorbic acid)	Reduces DNA damage as a radical scavenger	Fruit, vegetables	Padayatty et al. (2003)
Substances with antioxidant properties	**Myo-inositol**	Increases endogenous antioxidant enzymes	Self-synthesis possible by vitamin B5 and glucose	Condorelli et al. (2017)
	Resveratrol	Enhances antioxidant effects	Grapes, berries, wine	Branco et al. (2010)
	Vitamin B complex (B1/2/12)	Involved in homocysteine metabolism, homocysteine has prooxidant properties	Meat, beans, potatoes, bananas	Hankey and Eikelboom (1999)
	Vitamin D	Antioxidant property via activation dismutase	Sun-induced synthesis	Dorota Halicka et al. (2012)

ity in the overall Cochrane analysis, although the marked heterogeneity of the studies and high likelihood of bias allow only low to very low evidence for this statement (Smits et al. 2019).

Furthermore, in summary, the randomized trials in the Cochrane analysis show an improved clinical pregnancy rate and live birth rate of antioxidants versus placebo or no treatment, which is no longer detectable for live birth rate when the trials at high risk for bias were removed. However, because of the multitude of methodological problems in the studies involved and the overall low level of evidence, the authors consider the results of their analysis insufficient to make a recommendation for single agents or combinations of antioxidants and point to the urgent need for further large randomized trials (Smits et al. 2019).

Of the large randomized trials requested, one large study with 174 patients (Steiner et al. 2020) and one very large study with 2370 patients (Schisterman et al. 2020) have now appeared. These multicenter studies were conducted with either a combination of 5 mg folic acid/30 mg zinc or a more complex combination preparation of 500 mg vitamin C, 400 mg vitamin E, 0.20 mg selenium, 1000 mg L-carnitine, 20 mg zinc, 1000 µg folic acid, and 10 mg lycopene. Thus, in total, these two studies alone included more than three times as many patients for the live birth rate analysis as were reported in the Cochrane analysis as a whole. Neither of these two large studies showed an advantage of treatment with antioxidants over placebo in terms of sperm concentration, motility, and morphology or DNA fragmentation. Neither clinical **pregnancy rates** (Steiner et al. 2020) nor **live birth rates** (Schisterman et al. 2020) showed differences between treatment groups.

Therefore, especially considering current study results, the widespread **use of antioxidants in routine clinical practice should be clearly opposed** until large multicenter studies show otherwise.

39.2.3.1 Diets

Investigation of the effect of "healthy diets" on sperm quality is of clinical relevance, although the term "healthy diet" is a highly elastic term and often changing in content.

A recent systematic review examined observational studies with respect to the association of diet and sperm quality or fertility/fecundability (Salas-Huetos et al. 2017). Of the 35 studies included, 31 addressed the effect of specific diets on sperm parameters. Overall, the cohort appears very large with n = 12,672 participants, but the two largest studies already include nearly 9000 men studied (Jensen et al. 2013). The remaining studies split the remaining patient numbers. A cross-sectional study (2013) with approximately 700 healthy participants showed decreased sperm concentrations in men with an increased intake of saturated fatty acids (Jensen et al. 2013). Overall, an inverse association of decreased sperm parameters and intake of **Mediterranean diet** (rich in nutrients such as omega-3 fatty acids, some vitamins and antioxidants, and low in saturated fat) was detected (Salas-Huetos et al. 2017). Intake of fish, seafood, fruits and vegetables, and low-fat milk was positively associated with sperm quality (Mendiola et al. 2009; Eslamian et al. 2012).

In contrast, **high-fat diets, soy products, potatoes, cheese, coffee, and alcohol** were associated with impaired sperm parameters (Salas-Huetos et al. 2017). Whereas another cross-sectional study from Scandinavia with 8344 healthy participants showed that **moderate alcohol consumption does not negatively affect sperm** quality (Jensen et al. 2014). In terms of fecundability, this is reduced when male partners have a high intake of **alcohol, caffeine, and red meat** (Salas-Huetos et al. 2017).

These observations do not currently allow conclusions to be drawn about recommendations in clinical practice, as observational studies reflect associations but not causal relationships. Again, large prospective studies are needed to confirm these findings.

39.2.4 Herbs from Natural Medicine

The literature and evidence on the use of herbs in the treatment of male infertility are very sparse. Review articles are mostly narrative and lack objectivity or are unscientific in structure. Scientific studies/primary articles are mostly conducted on very small cohorts, with often questionable statistical significance.

Articles supporting the use of herbs, often as part of an **Ayurvedic** approach, see benefits of this treatment as including the greater accessibility of these preparations to patients, lower cost, and, underlying the concept, better holistic treatment. Overall, a stimulating effect in terms of libido, vitality, and improved ejaculation is seen through these therapeutic approaches, as well as better nourishment of (sexual) tissues. In a recent review, the following herbs are said to increase sperm count: **polygonatum verticillatum, mucuna pruriens, sesamum indicum** (Dutta and Sengupta 2018). **Studies or statistical evaluations are not listed in this regard**.

At the current time, treatment with herbs from natural medicine to improve sperm quality cannot be recommended due to lack of evidence.

39.2.5 Pentoxifylline /Theophylline

Pentoxifylline is applied as methylxanthine in peripheral circulatory disorders (e.g., intermittent claudication) and is also an inhibitor of phosphodiesterase, as is theophylline. In idiopathic infertility, Pentoxifylline has occasionally been used under the assumption of promoting blood flow to the testes (Heite 1979) without providing convincing evidence of efficacy (Wang et al. 1983; Shen et al. 1991), and few randomized trials have demonstrated improvement in ejaculate parameters compared with placebo, but pregnancy rates or live births were not recorded (Safarinejad 2011).

When used in vitro in the context of IVF treatment, neither Pentoxifyllinenor theophylline clearly demonstrated an increase in fertilization rates in asthenozoospermia, IVF failure, and sperm antibodies (review in Tournaye et al. 1995).

When used in vitro in the context of ICSI treatment with immotile or near-immotile ejaculated sperm or with minimally motile epididymal or testicular sperm, both substances show an improvement in sperm motility as well as fertility and pregnancy rates (review in Oseguera-López et al. 2019) also compared to selection of motile sperm by HOS assay (Mangoli et al. 2011).

39.2.6 Antibiotics and Anti-Inflammatory Drugs

Prior to the widespread use of antibiotics, **obstructions of the seminal ducts due to STDs** or clinical infections of the seminal ducts were the most common cause of male infertility and still are in parts of sub-Saharan Africa (Nieschlag 1993). The gold standard for treatment of clinically manifest infections of the vas deferens is antibiotic treatment depending on the result of the resistogram. If a **resistogram** is not (yet) available, the current guidelines of urologic societies recommend **fluoroquinolones** (e.g., ciprofloxacin, levofloxacin), **macrolides** (e.g., azithromycin), or **tetracyclines** (e.g., doxycycline 100 mg 1-0-1) for 10 to 14 days in first-line treatment (Bonkat et al. 2019).

As indisputable as the antibiotic therapy of symptomatic, clinically manifest infections of the vas deferens is, the antibiotic therapy of sole **leukozoospermia** and/or **nonsignificant germ detection** in asymptomatic subclinical courses is unclear. In asymptomatic leukozoospermia, the current guidelines of the European urological societies recommend an **ejaculate culture** (Jungwirth et al. 2012). However, in an attempt to validate this guideline in asymptomatic infertile patients, there were no differences in the proportion of patients with significant germ detection in patients with and without leukozoospermia, and 80% of positive germ detections were in patients without leukozoospermia (Ventimiglia et al. 2020).

Some of the few randomized trials in infertile men with asymptomatic leukozoospermia or nonsignificant germ detection show improvement in leukozoospermia and ejaculate parameters with antibiotic administration, while others fail to show any effects, as improvements were also detectable in the placebo or nontreated group (Comhaire et al. 1986; Yanushpolsky et al. 1995; Krisp et al. 2003). Therefore, due to the questionable efficacy of **antibiotics** in asymptomatic patients with leukozoospermia or nonsignificant germ detection, antibiotic treatment of these patients **cannot be recommended**, also in view of the potential side effects and development of resistance (Samplaski and Nangia 2015).

One of the main characteristics of infertile men with asymptomatic leukozoospermia or nonsignificant germ detection is **subtotal occlusion** of the seminal ducts with sometimes highly fluctuating ejaculate parameters, which cannot be explained by the abstinence period or the otherwise usual variance of ejaculate parameters. Therefore, to improve patency of the vas deferens, glucocorticoids or nonsteroidal anti-inflammatory drugs have been used in a few small studies. These mainly uncontrolled studies suggest that some positive aspect of the substances is plausible. However, the quality and number of studies are not sufficient for a reliable assessment (For an overview, see Haidl et al. 2019). Compared to treatment with antibiotics, the significantly lower potential for side effects, the rapid onset of action, and the favorable cost of treatment suggest that further evaluation, particularly of **nonsteroidal anti-inflammatory drugs**, is desirable in infertile men with asymptomatic leukozoospermia or nonsignificant germ detection.

39.2.7 Historical Deviations: Mesterolone, Pulsatile GnRH, Kallikrein, etc.

Since spermatogenesis cannot proceed normally without testosterone, therapy of idiopathic infertility with androgens has been attempted again and again, although it cannot be rationally justified, since a clinically relevant androgen deficiency has not been demonstrated in this clinical picture. Nevertheless, **testosterone esters** are repeatedly used in infertility, although they are considered **contraindicated** in existing desire for offspring (see Chap. 36).

Since **mesterolone** hardly suppresses gonadotropins and thus is not thought to have a suppressive effect on spermatogenesis, this androgen has been widely used. Finally, after years of using mesterolone in practice, WHO conducted a large multicenter, randomized, controlled, double-blind study of 246 couples, and it showed no statistically significant increase in pregnancy rate over a placebo (WHO World Health Organization 1989). Further publications followed, so that at least nine randomized, placebo-controlled double-blind studies could be evaluated in a meta-analysis. With

regard to pregnancy rates, the combined odds ratio for 1025 couples was only 1.02. In other words, 359 patients would have to be treated for an additional pregnancy to occur (Kamischke and Nieschlag 1999).

Thus, the **administration of androgens in male infertility is not justified and even contraindicated**. The fact that since 1995, despite the almost exponential increase of placebo-controlled studies in andrology, no additional studies with androgens have been performed underlines this statement.

In preliminary studies, it was hypothesized that oligoasthenoteratozoospermia with elevated FSH levels might be due to an **excessively low GnRH pulse rate**, and the term "slow-pulsing oligospermia" was coined (Wagner and Warsch 1984). In another study, lower LH with normal FSH pulsatility was reported in men with oligozoospermia compared with normal controls (Reyes-Fuentes et al. 1996). An uncontrolled study then demonstrated that such therapy did indeed bring FSH levels into the normal range, but did not demonstrate an improvement in sperm parameters or pregnancy rate (Bals-Pratsch et al. 1989). The hypothesis that the increased FSH levels were the cause and not the consequence of the spermatogenesis disorder could not be confirmed. Due to the lack of a pathophysiological concept and the lack of therapeutic success, a controlled study with this therapeutic substitute did not appear necessary.

There is no clear therapeutic concept for the use of **kallikrein**. Nevertheless, since the 1980s, kallikrein has been used in the treatment of idiopathic fertility disorders, especially to improve spermatozoa motility. In a controlled, randomized, double-blind study of 91 couples, it was demonstrated that kallikrein treatment did not result in significant improvement in seminal parameters and pregnancy rates (Keck et al. 1994). Similar negative results were reported in Japan (Yamamoto et al. 1996). A prospective, placebo-controlled, randomized study conducted in Israel with 114 patients also failed to detect any improvement in seminal parameters (Glezerman et al. 1993). These studies suggest that kallikrein, in the dosage and preparation forms chosen to date, does not show fertility chances of the patient with idiopathic infertility (Vandekerckhove et al. 1996b).

A number of other agents have been used to treat idiopathic male infertility. For example, **bromocriptine,** used successfully for hyperprolactinemia, has been tried for infertility without any positive effects being reported (Vandekerckhove et al. 1996a). Without a clear concept and without convincing data, empirically used substances also include **growth hormone and oxytocin, interferon, mast cell blockers, and antihistamines (ketotifen), angiotensin-converting enzyme (ACE) inhibitors (captopril), and zinc salts.**

Overheating of the genital organs is considered a fertility-reducing factor; **cooling of the scrotal organs** has been variously tried as a therapy. However, a convincing approach to increase pregnancy rates has not yet been developed (Jung and Schuppe 2007).

Acupuncture has also been considered for the treatment of male infertility. However, positive effects on pregnancy rates have not been reported to date (Ng et al. 2008).

39.3 Therapeutic Guideline

The previous sections of this chapter show that drug treatment of idiopathic male infertility is extremely unsatisfactory and that no drug has been clearly demonstrated to be effective. The chapter also emphasizes the **enormous importance of randomized, placebo-controlled clinical trials** in andrology (Kamischke and Nieschlag 1999). This is because only when the effectiveness of a treatment has been demonstrated by a controlled trial should it be generally applied. Until then, the principle applies:

Any therapy for male infertility must be considered experimental until its effectiveness has been demonstrated in randomized controlled trials. Therapeutic interventions without evidence of effectiveness should be used only in clinical trials.

Strict adherence to this principle requires great **discipline** on the part of the treating physicians and **consensus** among the medical profession so that therapies are not used prematurely and pregnancies that would have occurred spontaneously are not attributed to this therapy. The patient made happy by the pregnancy would not see through this placebo effect.

At present, empirical forms of therapy have taken a back seat in view of the success of **assisted fertilization procedures**. In particular, **ICSI treatment** proves beneficial in cases with marked oligoasthenoteratozoospermia and even azoospermia. However, it is precisely through assisted fertilization that previous experience has been proven once again: that intensive therapy of the woman's reproductive functions is also the best treatment of the man's infertility. This should be emphasized in the following principle:

Any therapy of male fertility disorders must be accompanied by optimization of **female reproductive functions**. This principle is also and especially valid if there is no effective therapy on the male side.

However, the lack of success of empirical therapies to date should not discourage the search for effective treatments of male infertility. For despite the successes of assisted reproduction, it remains to be said that most patients would like to conceive their children in a private atmosphere and not in a laboratory, and would therefore prefer a causal therapy for their condition. Moreover, especially in societies where the costs of assisted reproduction are not covered by the community (the insured), there is considerable demand for drugs that are reimbursable or at least cheaper than assisted reproduction. This pressure must not result in hasty prescribing, but must act as a motivation for intensified research. The treating physician has the important and time-consuming task of informing the patient and the couple and guiding them through the jungle of available drugs and procedures. In the absence of a rational therapeutic approach, intensive medical discussion among the physician and his patients remains the measure best leading to the goal, along with optimization of female reproductive functions.

Key Points

- Men with idiopathic infertility represent the largest proportion of patients with unfulfilled desire for children. Due to the lack of etiologic factors, causal therapy is not possible in these patients. Empirical therapy is often used, and here the postulate of Evidence-Based Medicine according to randomized controlled trials proves to be particularly important.
- Treatment with gonadotropins: Recent Cochrane and meta-analyses show a positive effect on pregnancy rates after treatment with gonadotropins. Due to the small number of controlled, randomized studies, no clear recommendation for FSH therapy is possible so far. Here, the pharmacogenetic approach in particular plays an important role; clearer courses of action would be derived by better selection of patients who could potentially benefit from FSH therapy.
- Recent meta-analyses on the use of antiestrogens show a positive impact of therapy on pregnancy rates and individual sperm parameters. However, again, due to the small number of studies and the different statistical methods, a clear recommendation cannot be made. Nevertheless, due to the low side effect profile and low price, antiestrogen treatment can be considered in men with low testosterone.
- Similarly, aromatase inhibitors (AIs) have an effect on testosterone concentration and can be considered in patients with low testosterone and a low T/E2 ratio.
- Antioxidants have a positive effect on DFI and individual ejaculate parameters, although there is considerable heterogeneity between studies. Few studies have looked at the effect on clinical pregnancies; here, no difference has been found between placebo and antioxidants, so their use in routine clinical practice is not recommended.
- The use of antibiotics in asymptomatic patients with leukozoospermia or nonsignificant germ detection cannot be recommended. Treatment with nonsteroidal anti-inflammatory drugs can be considered because of fewer side effects and cheaper costs.
- There is a great deal of momentum in empiric therapy for idiopathic infertility, and the coming years and findings will lead to clearer recommendations for action.
- In any case, therapy for male fertility disorders should be accompanied by optimization of female reproductive functions.

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Gynecology Relevant to Andrology

40

Ulrich A. Knuth

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Abstract

Reproductive medicine is multidisciplinary. In this context, the importance of gynecological history, somatic factors, and the influence of coital frequency and timing are presented. Algorithms for calculating the probability of pregnancy are described. Hormones influencing follicular maturation are presented. Psychological factors are taken into account. Environmental influences and noxae are discussed. Follicle maturation is described in detail with possible impairing variables. Disorders of passage in the genital tract and their diagnosis are described. Endometriosis is discussed as a frequently unrecognized cause of infertility. Immunological factors with sperm antibodies in the female organism are discussed and possible therapies are presented. An overview of physiological changes during early pregnancy, implantation, and their disorders follows. Therapeutic measures in connection with idiopathic infertility form the conclusion.

The care and the successful treatment of the infertile couple require a multidisciplinary approach, which supports and counsels the couple as an entity.

The flowchart in Fig. 40.1 enables the non-gynecologist to appreciate the main diagnostic and therapeutic steps necessary for the evaluation and treatment of female factors of a couple's infertility.

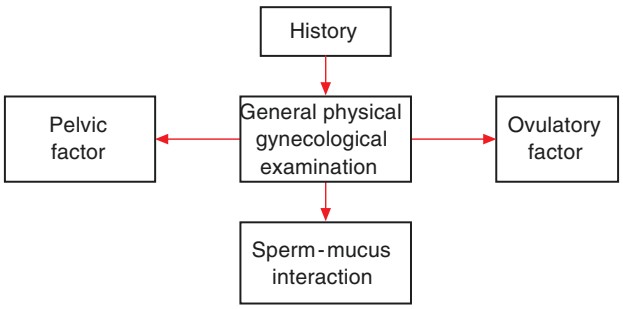


Fig. 40.1 Main diagnostic areas of female infertility workup

40.1 Medical History and Somatic Factors

40.1.1 Age

The decline in female fecundity with advancing age is well known; donor insemination studies, which allow for a constant male factor, show a decrease in female fertility with increasing female age. Figure 40.2 shows exemplary studies (Schwartz and Mayaux 1982; Yeh and Seibel 1987). In the prospectively recorded IVF cycles of the German IVF Register between 2013 and 2017, the age dependency is impressively recognizable (DIR 2020). An increase in FSH levels parallel to female age already begins at the age of 25 (Ebbiary et al. 1994).

The FSH level, in addition to inhibin B in the early follicular phase and AMH (anti-Muller hormone), combined with estradiol and progesterone, is the most important parameter for diagnosing declining ovarian function (Gülekli

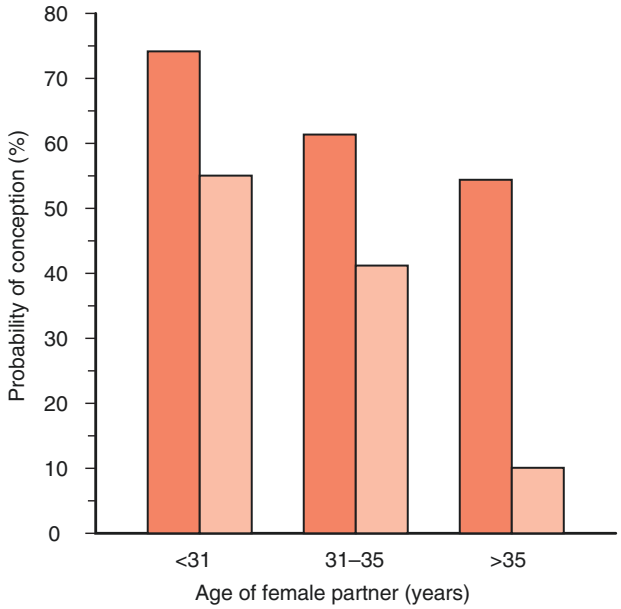


Fig. 40.2 Relationship between probability of conception and female age according to two independent studies. Black columns: redrawn from Federation CECOS, Schwartz and Mayaux (1982) reprinted with permission. Hatched columns: redrawn from Yeh and Seibel (1987), reprinted with permission

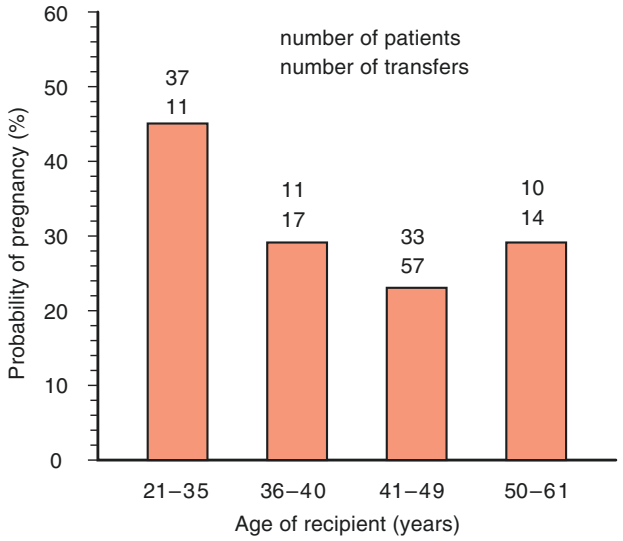


Fig. 40.3 Pregnancy rates after transfer of donated oocytes depending on recipient's age. Adapted from Flamigni et al. (1993) with permission of the publisher

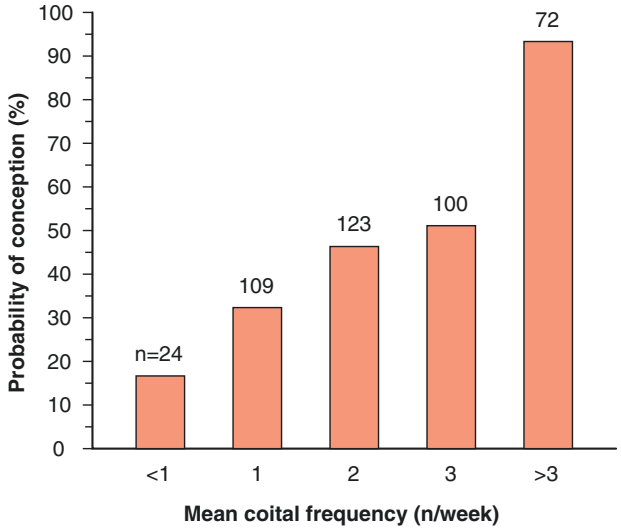


Fig. 40.4 Rate of conception depending on coital frequency per week in couples who achieved a pregnancy within 6 months of unprotected intercourse. Adapted from MacLeod and Gold (1953). The male factor in fertility and infertility: IV. Semen quality and certain other factors in relation to ease of conception. Reproduced with permission of the publisher, the American Society for Reproductive Medicine [formerly The American Fertility Society]

et al. 1999; Corson et al. 1999). Conversely, assisted reproduction studies of couples in which older women receive oocytes from younger donors show normal fertility rates (Fig. 40.3). However, even here fertility rates decline after the age of 35 years.

The older woman undeniably bears an increased risk of pathological alterations of her reproductive organs. Moreover, a subgroup of patients with decreased fertility evolves among those who do not conceive despite years of unprotected intercourse. In these cases, age is only an apparent factor for the decline in fertility. A retrospective study by structured interview after delivery showed that no change occurs in the latency rates until conception in normal fertile women between the ages of 26 and 35 years (Knuth and Mühlenstedt 1991).

40.1.2 Coital Frequency

The decline in fecundity with advancing age is seen by some authors to result solely from a decrease in coital frequency per cycle (James 1979). Other studies have shown that this factor must be assessed before forming a prognosis in the treatment of the infertile couple (Fig. 40.4).

Increased coital frequency augments the probability of fertile sperm reaching the Fallopian tubes at the optimal time. One could thus deduce that low coital frequency, perhaps only at the time of ovulation, could result in acceptable rate of conception. Such a recommendation is, however, often counterproductive. Recommendations to have intercourse on certain days or even at a certain time of day cause considerable stress, often preventing couples from having normal intercourse with intravaginal ejaculation because of erectile dysfunction (Agarwal and Haney 1994). The experienced physician will only suggest unprotected intercourse before and during the "fertile days." Recommendations as to the frequency or time of day for intercourse should be withheld. Natural conception studies have shown that a single act of intercourse timed between 6 days before and 3 days after ovulation results in pregnancy (France et al. 1992). Reevaluation of earlier publications shows that the highest rates of spontaneous conceptions occur when coitus takes place on the day prior to ovulation (Dunson et al. 1999). The use of mobile computer apps appears to increase the probability of conception by 12% to 20% per cycle (Stanford et al. 2020).

40.1.3 Length of Childlessness

The duration of barrenness is directly related to the probability of conceiving and represents an important prognostic parameter. A clinical study of 969 Dutch couples (Eimers et al. 1994) showed an 11% decline in the fertility rates with the numbers of years of unprotected intercourse. A woman who has spent 7 years unintentionally childless thus has only a 50% chance of becoming pregnant compared to a woman with 1 year of infertility. If, however, she had an earlier pregnancy, the factor increases the chances for probable conception by 74% compared with a population with primary infertility.

The original models for predicting the spontaneous fertility rate in subfertile patients have been refined further (Hunault et al. 2005) and can provide valuable help in planning the intensity of further therapy in cases of idiopathic infertility. A standardized estimate can easily be calculated using the following parameters: length of involuntary childlessness, female age, primary or secondary infertility, percentage of motile sperm, and previous care (referral by gynecologist or general practitioner). Including results of a postcoital test greatly increases the quality of prediction. The exponential function is found in the appendix of the original publication.

40.1.4 Risk of Infection

The probability that the couple's infertility is due to a tubal factor rises with the number of sexual partners in the past and is also correlated with age at first intercourse. The contraceptive form used must be taken into account, as the risk of an ascending infection when taking an oral contraceptive is reduced by the progestin effect on the mucus. A former IUD wearer will therefore be advised to check the tube passage more quickly than a woman with a long history of oral contraception or condom use by the partner.

40.1.5 Psychological Factors

Female sexual interest seems to be correlated with the menstrual phase. Increased libido in the follicular phase results in a natural rise in coital frequency during the fertile phase (Dennerstein et al. 1994). Infertility therapy can, however, deeply disturb the couple's sexuality, interrupting physiological events and resulting in functional infertility. During infertility therapy, inherent sexual dysfunction is often magnified. It can be symptomatically divided into three groups: loss of libido, orgasmic dysfunction, and inability for intravaginal intercourse (Herms 1989).

40.1.5.1 Libido Dysfunction and Orgasmic Disturbances

The primary absence of sexual desire (**alibidinia**) is extremely rare. The decrease or loss of libido is usually a secondary change. Psychological causes, such as deep-rooted fear or depression, can result in aversion reactions, in a loss of sexual interest, or even in sexual deviations. The group of organic causes includes chronic dyspareunia, endocrinological imbalance, and debilitating disease. Psychotropic drugs such as antihypertensives, tranquilizers, or sedatives can alter the patient's libido. Even the constant availability of a sexual partner can result in apathy, a loss of sexual interest, and even in sexual aversion. This, of course, results in reduced coital frequency and thus reduced fertility. Factors that cause a change in libido can also influence orgasmic capability.

40.1.5.2 Dyspareunia

In contrast to inhibited sexual desire and orgasmic dysfunction, which are usually psychological disturbances, the main factor for dyspareunia is physical discomfort. Painful intercourse, called **algopareunia**, can have organic and psychological origins, which may occur sequentially. Timed intercourse recommended during an infertility treatment program can itself be a major factor in initiating this symptom.

In some patients, **vaginismus**, an involuntary painful spasm of the pelvic floor, inhibits intercourse (de Souza et al. 2018). This is often combined with a defensive reaction of the entire body in the form of lordosis and adduction of the lower extremities. This reaction is always psychological in nature and is often combined with sexual fear or a traumatic sexual experience in the past. Care of the patient must be in the form of psychotherapy and never of surgical nature. In rare cases and necessarily in consultation with the psychologist, intravaginal insemination with a thin catheter can also be considered under these circumstances in order to break the circulus vitiosus of the ambivalence between sexuality and the desire to have children.

40.1.6 Hormones and Female Sexuality

The influence of hormonal factors on female sexuality is not as clear as it is for male sexuality. **Estrogens** are necessary for normal vaginal reaction during sexual arousal, but their influence on sexual desire and female orgasm is not as distinct. An important role in female libido is ascribed to **testosterone** . In women with androgen deficiency, loss of libido is the dominant clinical symptom (Davis 1999a). This loss can be effectively compensated by appropriate substitution of androgens (testosterone patches) or androgen precursors such as dehydroepiandrosterone (Arlt et al. 1999; Davis 1999b). However, the role of hormones in female sexuality is either strongly modified by psychosocial factors or results in a much more individualized reaction when compared to that of males (Bancroft 1993).

A critical review of androgen therapy in women recommends refraining from a diagnosis of female androgen deficiency at the present time, as the criteria for diagnosis and methods for its determination are inadequate (Wierman et al. 2014). From the clinical point of view, this position is questionable (Traish et al. 2007).

40.1.7 Stress

During the treatment of infertility, stress is often mentioned as one of the possible causes of failed conception. However, there is no clear definition of this entity, which could be used for a reproducible experimental study. Stress implies a pooling of many negative factors, which influence well-being, such as exhaustion stemming from rest or sleep deficiency, tension, and inability to find relief from real or imagined pressure. An investigation of 420 Danish couples seeking infertility treatment for the first time showed a reduction in the probability of conception from 16.5% to 12.8% in couples with a high-stress score, i.e., > 80th percentile on the General Health Questionnaire (Hjollund et al. 1999). Over and beyond such systematic studies, often anecdotal and casuistic evidence is offered to support the thesis that stress factors decrease female fertility. One example often cited is the supposedly increased pregnancy rate after adoption. A systematic study, however, shows that the conception rate of 4.3% post-adoption is equal to that of couples who do not adopt (Banks 1961). A systematic overview of the prevalence of psychogenic infertility considers 5% a realistic estimate (Wischmann 2006). A critical discussion of the correlation between stress and reproductive success finds insufficient data to make a definitive assessment (Lawson 2020). However, infertility-related distress leads to significantly higher rates of sexual dysfunction on the female side (Facchin et al. 2019). Those acutely stressful situations need not be an obstacle to pregnancy can be inferred from high pregnancy rates (5%) following rape (Holmes et al. 1996). Menstrual cycle changes, which are caused by stress, are, however, well documented and are discussed in Sect. 40.2.4.

40.1.7.1 Immunological Modulation and Stress

Recently, reciprocal relationships between stress and immunological events have been increasingly noted. Especially in females, there seem to be powerful mechanisms in which immunological factors stimulate **stress factors** and result in decreased fertility.

An increased secretion of **corticotropin-releasing hormone (CRH)**, with the resulting stimulation of ACTH, causes a rise of glucocorticoid secretion. This modulates precursors of prostaglandin synthesis, platelet-activating factor, serotonin, cytokinins, interleukin-1 and interleukin-6, and tumor necrosis factor (TNF). CRH is a part of the proopiomelanocortin system and thus regulates the secretion of α-MSH and β-endorphin, which, in combination with metencephalin, directly influences the function and activity of the immunocytes. It can be concluded that a stress situation can negatively influence implantation via autoimmune factors. This complex interaction promises to become a major area of female infertility research in the future (Negro-Vilar 1993; Chrousos 1995; Appleyard et al. 2020).

40.1.8 Environmental Factors

So-called environmental factors are often cited while searching for a cause for infertility. It is, however, almost never possible to make a clear statement pertaining to particular environmental factors and their influence on female infertility or on an individual couple's fertility status.

40.1.8.1 Definitions

Several years ago, the WHO initiated a study of environmental factors relevant to fertility (Baranski 1993). To measure the influence of potentially harmful substances, a measurable parameter is required. An often-cited parameter is the infertility rate. Using this parameter, the rate of infertility in a group of normal patients is compared to the infertility rate in a group of patients who are under the influence of a certain external environmental factor. Methodical problems arise whenever infertility is to be defined, as the condition may be subject to varying preconditions:

1. Failure to conceive after 1 year of unprotected intercourse.
2. Failure to achieve a clinically discernable pregnancy after 1 year of unprotected intercourse.
3. Failure to deliver a viable baby after 1 year of unprotected intercourse.

These three different definitions already demonstrate that methods of confirming a pregnancy alone may influence the parameter "infertility rate" and results.

Only in a few cases, e.g., as part of an IVF program, it will be possible to capture all conceptions through early hCG determinations, even if there is no clinically recognizable gravidity as a result of early abortion later on. The assessment of the birth rate may be clearer, but neglects factors that lead to early abortion or delayed conception. Therefore, the time between the initiation of unprotected sexual intercourse and the onset of pregnancy is often used as a sensitive parameter of fertility assessment. It is possible to evaluate the number of cycles that are needed until pregnancy occurs. This method offers the advantage of capturing even minor influences on the latency of conception.

Since the organism is never exposed exclusively to one potentially noxious environmental factor, the influence of possible cofactors such as nicotine, alcohol, caffeine, and other substance abuse must be considered in first instance. Certain behavioral patterns are also correlated with other socioeconomic factors, which again influence the fertility rate.

40.1.8.2 Epidemiology

Although extensive lists exist of environmental factors possibly influencing female fertility (Baranski 1993), they are

usually of little use for practical counseling of the individual infertile couple, since even the best epidemiological studies only show a slightly elevated risk for a population exposed to a potential environmental hazard as a whole. Infertility rates in women rise when they are continually exposed to toxins such as anesthetics, asbestos, textile dyes, and dry cleaning agents. The risk for infertility is increased by a factor of 2.4 in women exposed to a high level of noise (Rachootin and Olsen 1983). Even specialized databases fail to provide useful data on toxic influences on human reproductive functions (Scialli 1994). The fact that less than 1% of over 60,000 chemical substances included in lists of toxic materials have been investigated illustrates the magnitude of the problem.

Even well-controlled studies fail to give a precise picture of the influence of certain factors on fertility. A study of dental assistants who were exposed to mercury vapor showed, for example, that the fertility of women who assisted in more than 30 fillings per week only reached 63% of the fertility of those women not exposed to mercury vapor. However, the fertility rate of those women with a low exposure to mercury vapor was better than that of the nonexposed controls (Rowland et al. 1994).

40.1.8.3 Nicotine

Compared to poorly definable environmental factors, the exposure to nicotine plays a practical role, which can be usually eliminated. Although the effect of nicotine on fertility is not consistently described in the literature, a large British study clearly demonstrates a negative effect of nicotine on fertility. Female nicotine consumption resulted in a 12% rise in the conceptive latency in 11,407 persons born in 1958 and followed in Great Britain. Male smoking did not cause a negative effect on fertility after data were corrected for socioeconomic factors (Joffe and Li 1994). A prospective Danish study of 430 couples showed a reduced rate of fecundity (0.53) in women who smoked, compared with nonsmokers (95% confidence interval: 0.31–0.91). An important factor was whether the mothers of the women investigated had smoked during pregnancy (Jensen et al. 1998). It is also known that IVF results are poorer in women who smoke (Zitzmann et al. 2003) and that ovarian function also declines earlier in this population. One of the first steps in patient education and counseling during infertility treatment should thus be to **abandon nicotine consumption**.

40.1.8.4 X-Rays and Radioactivity

Although the negative effects of smoking on female fertility have been proven, most of the affected woman does not find it necessary to change their behavior. In contrast to this, the influence of X-rays and radioactivity on fertility is usually overestimated by the general population.

The serious reactor accident in Chernobyl in 1986 resulted in a dose load of 50 to 60 mSv within a radius of 30 km around the reactor. The resulting load in the European countries has been estimated at <1 mSv and is absorbed in the natural background radiation of 1–2 mSv (Boce and Linet 1994). The additional load, as far as described, did not lead to an increase in malformations. Even among the children of Japanese parents who survived the atomic bombing in 1945, there was no evidence of increased genetic abnormalities. Whether there was a restriction of fertility is not to be decided for the reasons discussed above.

The influence of high-altitude radiation during frequent flights at altitudes greater than 15 km may influence early gravidity, but restriction of fertility seems negligible (Silverman and Gendreau 2008).

Epidemiological studies and detailed analysis of radioactive exposure of females before conception show no clear effects (Committee on the Biological Effect of Ionizing Radiation 1990). A study of 627 women with thyroid carcinoma who were treated with radioactive iodine (^{131}I) showed no significant difference in the fertility rate when compared to controls (Dottorini et al. 1995). The ovarian radioactive dose in such a therapy was determined to be 1.14 ± 0.34 Gy (mean ± SEM) (Izembart et al. 1992). In this study, 12 of 50 patients developed amenorrhea and 38 showed no change in ovarian function. The effect of age on onset of menopause was, however, not clearly delineated in the study. **Total body radiation** because of neoplastic disease during childhood results in definitively higher total body doses. These cover a range between 20 and 30 Gy. Women who have survived this form of treatment and have continuing ovarian function often deliver low birthweight infants. This is explained by hypoplasia of uterine tissue and impaired vascularization (Critchley et al. 1992). Research data show a variable influence of radiation on female fertility. It cannot be deduced that a pregnancy will not be achievable in these patients. An ovarian dose of 4 Gy can cause sterility in about 30% of young women and in 100% of women over the age of 40 years (Ogilvy-Stuart and Shalet 1993). These results are underlined by data obtained from patients treated with chemotherapy and radiation in Hodgkin's disease (Bokemeyer et al. 1994).

40.1.8.5 Electromagnetic Fields

In contrast to the low number of patients who have been exposed to radioactive materials or X-rays, almost every patient seen for infertility counseling has been or is exposed to weak electromagnetic fields. Some consider so-called **electro-smog**, the causal factor for infertility (Ouellet-Hellstrom and Stewart 1993). This has not been proven for the average patient. Under experimental conditions, there is evidence of enhanced production of ROS by electromagnetic fields (Santini et al. 2020). Even in extreme situations, such as the personnel of nuclear magnetic resonance imaging departments, no change in fertility rates was apparent (Kanal

et al. 1993). The harmlessness of **ultrasonography** for the fetus will be discussed later.

40.1.9 Pertinent Medical History

40.1.9.1 Physiology of Pregnancy

An assessment of the medical history for female and male factors affecting infertility is important in infertility counseling since often causal therapeutical options become possible. Notwithstanding, consideration of diseases, which may worsen during pregnancy, is also necessary, so that the couple may be counseled to forego further therapy.

Such changes in pregnancy may be caused by adaptations in the cardiovascular system and by immunological events. Renal circulation increases 50% in early pregnancy. The circulating blood volume expands during pregnancy by between 27% and 64%. Cardiac output grows from 4.5 to 5.5 L/min, and the pulse rate also rises from 70 to about 85 beat/min at the end of pregnancy. There are no major changes in the respiratory system.

The ureters dilate during pregnancy and may also become compressed. This results in a higher infection rate and/or hydronephrosis. The motility of the gastrointestinal tract decreases. Liver function is usually not altered. Thyroidal activity is stimulated with a rise in protein-bound iodine levels. Carbohydrate metabolism usually remains constant, although a slight glycosuria is common as a result of a lower renal threshold for glucose.

40.1.9.2 Pertinent Medical Disorders

40.1.9.2.1 Pulmonary Disease

Pulmonary tuberculosis will not worsen during pregnancy. Pregnancy has a favorable influence on the course of uncomplicated **sarcoidosis** with a possible decrease in pulmonary hilum lymphomas. The course of **asthma** is also usually not altered during pregnancy.

Cardiovascular System. Manifest cardiovascular disease may deteriorate owing to the physiological changes that occur in the cardiovascular system during pregnancy. A patient with cardiac disease should thus consult a cardiologist before beginning infertility therapy. Today pregnancy is even possible following heart transplant (Morini et al. 1998).

Gastrointestinal Disease. Inflammatory bowel disease usually has no effect on fertility. In severe **Crohn's disease** or ulcerative colitis, one should, however, wait for a less active or inactive phase to plan pregnancy, if possible.

Viral hepatitis is a much more significant factor in infertility therapy. A review is found in the bulletin: The Practice Committee of the American Society for Reproductive Medicine (2006). In areas endemic to hepatitis A, women usually become immune to hepatitis A before reaching reproductive age. Children of mothers who develop **acute hepatitis** A during pregnancy show no effects of the disease and fetal transfer of the HA virus is extremely rare, and hepatitis A is not a true problem in infertility therapy. As the best protective measure, a vaccination can be carried out during pregnancy.

The situation with **hepatitis B** infection is more serious (Castillo et al. 2017). Prenatal care studies in Germany show a positive hepatitis B antigen in 0.73% to 1.73% of all women examined (Joosten and Stürner 1980). Between 20% and 30% of all HBsAg-positive mothers communicate the virus to their offspring if immunoprophylaxis is not performed. In women seropositive for HbsAg and HbeAg, the rate increases to 90%.

The importance of **hepatitis C** is more imminent than that of hepatitis B, since passive immunization does not seem to be a possibility in this infection. In northern Europe, investigations in blood donors revealed a rate of disease between 0.01% and 0.05%.

The infection persists in about 80% of all cases and leads to chronic persistent or chronic active hepatitis, with 20–30% developing into cirrhosis. Passive immunization is not currently available. Standard therapy with IFN-α and ribavirin is contraindicated during pregnancy. Intrauterine transmission of acute hepatitis C has been clearly demonstrated. Follow-up of 403 women post-delivery with hepatitis C virus antibodies and their offspring revealed infection in 13 of 403 neonates (3.2%). This applied only to infants whose mothers were virus RNA-positive. As six infants were virus RNA-positive immediately after birth, an intrauterine infection must be assumed (Resti et al. 1998). If patient history reports hepatitis C, or if a finding of such arises during a routine diagnostic step in infertility therapy, counseling must include the risk for the fetus. The potential of interferon therapy has yet to be clarified in these particular cases (Parent et al. 2018).

The risk of hepatitis C transmission to spouses of carriers is not clear (Van der Poel et al. 1994). Hepatitis B, E, F, and G are of lesser importance (see details in review cited above).

A patient with **chronic hepatitis** needs not expect a progression of the disease during pregnancy. In patients with chronic aggressive hepatitis, however, only the autoimmune form is not influenced by pregnancy. The prognosis in chronic aggressive hepatitis B is reduced. Experts are not, however, able to make general recommendations for this situation. In the case of esophageal varicosis due to liver cirrhosis, experts recommend prophylactic sclerosis prior to planned pregnancy.

When cryopreserving gametes, the possibility of cross-contamination through joint storage in liquid nitrogen must be considered. HBV and HCV can survive in liquid nitrogen, so storage in the gas phase or double containment to avoid leakage is indicated.

In contrast to the various forms of hepatitis, **ulcerative disease** is not a problem either before or during pregnancy. An initial attack rarely occurs during pregnancy. Cholecystitis is described more frequently than peptic ulcer disease. One of 1300 cholecystectomies are performed during pregnancy. There is, however, no special need for counseling concerning this subject during infertility therapy.

HIV. In contrast to hepatitis, HIV-positive women seeking pregnancy represent only a small group in Germany. When the mother is seropositive, the probability of an intrauterine infection is about 20% if a cesarean section is performed and when the mother does not nurse. If the mother is treated (e.g., with azidothymidine) during pregnancy, the risk of infant infection can be reduced to approximately 1%. Independent of possible infant infection, HIV represents a fourfold higher risk of abortion and stillborn offspring (Brocklehurst and French 1998). The same authors documented an additional albeit weak effect of pregnancy on the further development of HIV disease (French and Brocklehurst 1998). More recent investigations no longer confirm this (Tai et al. 2007). In view of this development, therapeutic possibilities must be discussed individually with the affected couple who are dependent on intensive counseling (Ethics Committee of the American Society for Reproductive Medicine 2015). A review of the recommendations of the German AIDS Society and the German Society for Gynecology and Obstetrics (Tandler-Schneider et al. 2008) was recently discussed again (Tandler-Schneider et al. 2017).

Zika virus. It is transmitted via mosquitoes. It was detected in rhesus monkeys in Uganda as early as 1947 and led to the first major human outbreak in the Yap Islands in Micronesia in 2007. A recent outbreak, starting in Brazil, spread to several countries in the Americas with acute viral symptoms in 20–50% of all affected cases. Microcephaly and CNS pathologies of newborns result from intrauterine infection during pregnancy (Coutinho et al. 2021). Since the chain of infection is predominantly mosquito–human–mosquito, the transmission rate is predominantly linked to the spread of A. aegypti.

However, since the virus has also been detected in the sperm of infected men, it can lead to sexual transmission of the disease to the partner with consequences for the fetus (Joguet et al. 2017).

If there is anamnestic evidence of a possible Zika infection due to the couple's or partner's stays abroad in countries with endemic spread, diagnostics should be considered in advance of infertility therapy for the couple, whereby laboratory analysis complicates the interpretation of serological tests due to cross-reactivity with flavivirus antibodies (Petersen et al. 2016).

COVID-19. As things stand, the oocyte appears to be completely free of possible binding sites of the COVID-19. Direct infection via the female germ cell line of the oocyte therefore seems highly unlikely. However, an infection via virus-bearing sperm, similar to Zika infection, is discussed (Aitken 2021).

Urinary tract. Preexisting diseases of the urinary tract can cause severe complications in pregnancy. In particular cases, patients should be counseled against further infertility therapy. During acute renal disease such as **pyelonephritis** or **glomerulonephritis,** pregnancy should be avoided. Treated tuberculosis of the urinary tract with negative controls is no argument against a pregnancy. A patient history of **kidney stones** or a surgically corrected congenital hydronephrosis does not present a contraindication for pregnancy. Progressive polycystic **degeneration of the kidneys** with restricted glomerular and tubular function has a poor prognosis in pregnancy and thus also in infertility therapy. **Chronic glomerulonephritis** and **diabetic glomerulonephritis** are a high risk for the development of eclampsia and intrauterine fetal demise. During counseling of patients with a nephrotic syndrome, one should more or less advise against further infertility therapy. After renal transplantation, however, patients may consider a pregnancy in consultation with a specialist 2–3 years after surgery (Kremling 1986).

Neoplasias. Malignant gynecological diseases such as **breast cancer**, **cervical cancer,** and **ovarian cancer** differ as to the effects of a pregnancy on the course of the disease (Börner 1986). In summary, a pregnancy following breast cancer will not stimulate further disease or worsen the prognosis. Patients with a history of **carcinoma in situ of the uterine cervix** should allow a period of 6–12 months to pass during which cytological controls are negative before attempting therapy for infertility. Patients with **malignant ovarian tumors**, who were treated with a conservative unilateral salpingoovariotomy, should be counseled to undertake contraceptive methods for a period of 2 years, since most recurrences appear during this period.

It is not possible to cover all of the diseases, which may be influenced by pregnancy in this chapter. Most of the other diseases are quite rare, so that even gynecological obstetrical textbooks do not suffice in counseling an individual patient. A literature search will provide helpful and up-to-date information.

40.2 Ovarian Cycle and Ovulation

40.2.1 Follicles

The mainstay of female reproductive physiology is the maturation of the oocyte in conjunction with the menstrual cycle. The evaluation of the maturing ovarian follicle, of ovulation, and of the luteal phase is therefore a central point in the workup of infertility patients.

40.2.1.1 Early Oocyte Development

The maturation of oocytes is a long process, which begins during fetal development. Oocyte maturation is completed, however, only during fertilization, which often takes place up to more than 30 years after the beginning of oocyte development. During fetal life, 6–7 million germ cells develop in the second trimester. By the time of birth, many of the germ cells have undergone atresia, and the newborn female has about 2 million ova. At this time, the primary oocytes have undergone the first part of the first meiotic division and have reached the first stage of arrest. This phase of arrest lasts until puberty, when ovulation of the oocytes begins.

40.2.1.2 Meiosis

Meiosis only continues when ovulation has taken place. One-half of the paired chromosomes are expelled in **the first polar body**. The oocyte is now haploid. This phase is called the **metaphase II** and the oocyte is **a secondary oocyte**. This is the situation at the time of ovulation. If the oocyte is not fertilized, it remains in this stage. If fertilization occurs, one-half of the chromatin is again expelled in the form of the **second polar body**. Female meiosis, thus, in contrast to spermatogenesis, begins in the early fetal period and ends at the point of fertilization in the adult female.

40.2.1.3 Follicle Development

The development of the surrounding follicle occurs in parallel with ovarian oocyte development. During the eighth week of pregnancy, the primary oocytes are surrounded by a single layer of spindle-shaped cells. These are precursors of the later granulosa and theca cells. The oocyte and the granulosa cells are surrounded by a basal membrane, which separates the **primordial follicle** from the stroma. The follicle with layered granulosa cells is called a secondary follicle. In the seventh month of pregnancy, an antrum can develop in the granulosa cell layer; the follicle is then called a **tertiary or a Graafian follicle.**

40.2.1.4 Regulatory Mechanisms

The granulosa cells begin to express **FSH receptors** during the fifth to sixth month of pregnancy. The number of receptors per cell remains constant; the absolute number of FSH receptors per follicle, however, rises with the increasing number of granulosa cells. During further development, receptors for estradiol, progesterone, testosterone and glucocorticoids can be detected. For the further development of the follicle, FSH is required. This is supported by the influence of estrogens. Thus, a **positive feedback** exists in which even minimal FSH concentrations in the follicular liquid are answered by a positive feedback. It is of note that FSH is only detectable in the follicular fluid if the ratio of estrogens to androgens is dominated by estrogens and the quotient is above 1. If androgens increase, FSH is not detectable.

In addition to estradiol and FSH, other factors influence the mitotic division of the granulosa cells. Aromatase and LH receptors are induced depending on the FSH concentration and the time phase. The aromatases convert androgens into estrogens. The development of LH receptors in the **dominating follicle** prepares it for the midcyclic LH surge and thus for ovulation. Additionally, the LH receptors are necessary for the production of progesterone, which occurs later in the corpus luteum. During follicle development, estradiol seems to act synergistically with FSH to facilitate the development of FSH receptors. Later in the cycle, estradiol stimulates LH receptor density and the activity of the LH receptors. When estrogen synthesis is blocked, none of the Graafian follicles will grow in diameter over 2.2 mm. If FSH stimulation is blocked during follicle development, FSH and LH receptor numbers decline, and granulosa cells die.

Follicle Selection. Knowledge of follicular development is important for understanding impaired follicular maturation. The preovulatory rise in estradiol initiates a negative feedback and thus a suppression of pituitary FSH secretion. Since the preovulatory follicle has a higher receptor density than the other smaller follicles, further maturation is possible, whereas the smaller follicles must degenerate to **atretic follicles**. The effect of FSH can be modulated by prolactin and sexual steroids. Estrogen, insulin, and other somatotropic factors can influence the aromatase activity and the development of LH receptors. The stimulation of aromatase, LH receptors, and prolactin receptors by FSH seems to be restricted to the membranes of the granulosa cells. Thus, a very complicated system of endocrine and paracrine regulatory mechanisms exists in which there are a multitude of steps during which the maturation of follicles can be disturbed.

Two-Cell Theory. Further LH receptors develop in the cells of the **theca interna** of the Graafian follicle. The theca interna consists of mesenchymal cells surrounding the entire follicle. Under the influence of LH, the theca interna cells change into epithelial cells, which are able to produce androgens, of which the most important is **androstenedione**. This androgen is the main precursor for follicular **estradiol** production and a significant factor for further follicle development. An excessive production of androgens, which exceeds the capacity of the aromatase to synthesize estrogens, causes a decline in the estrogen/androgen quotient and thus follicular atresia as already described. In an androgen-rich environment, the direction of conversion of androstenedione is changed even further from estrogen formation into additional androgen production. An optimal follicle can only develop if androgen production in the theca cells is followed by estrogen production from these androgens in the granulosa cells. This mechanism is called the two-cell theory of follicular estrogen production. Like granulosa cells, the theca cells can

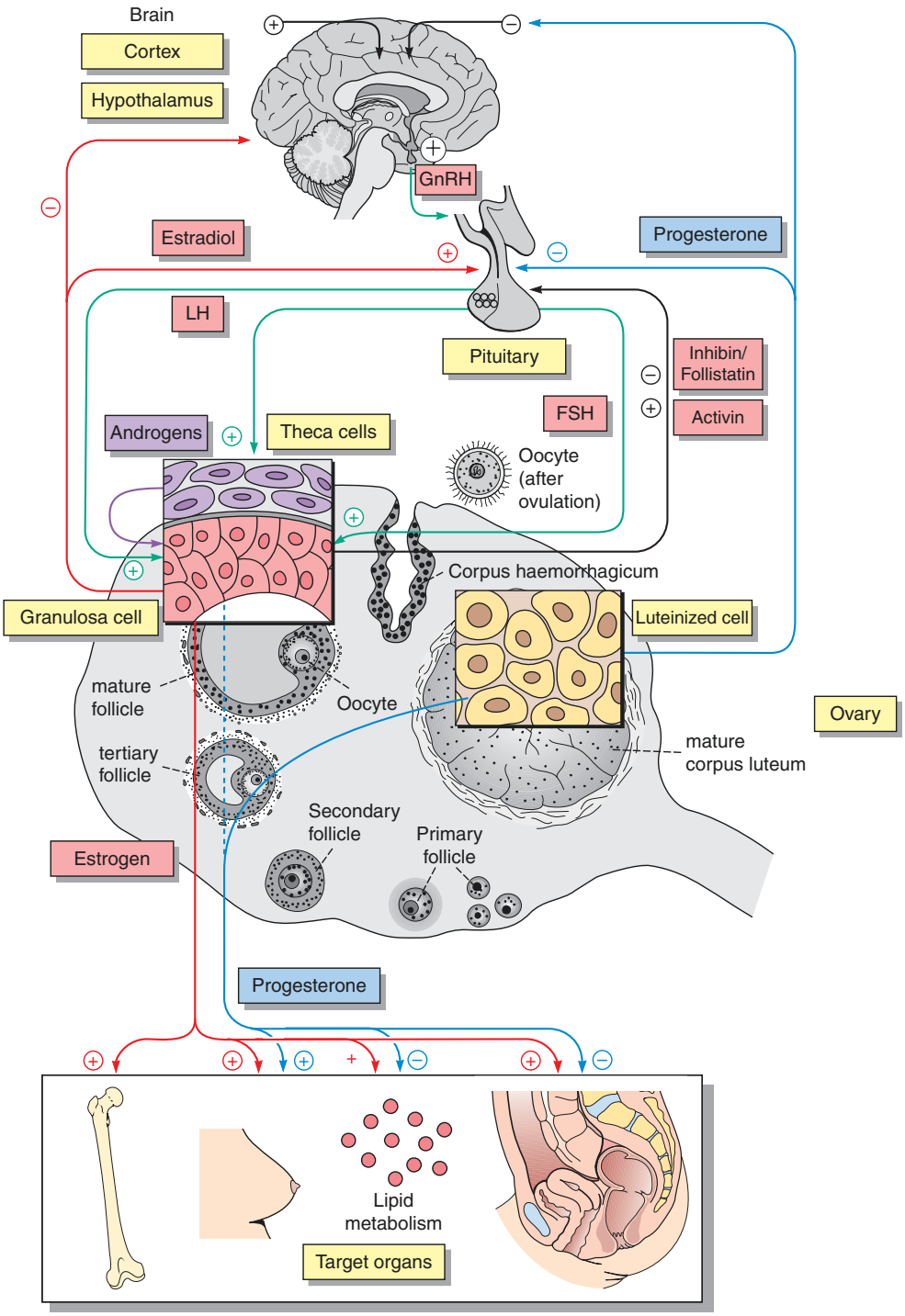


Fig. 40.5 Feedback mechanism of hypothalamic–pituitary–gonadal axis and interdependence of hormone production in the structures of theca and granulosa cell layers. The schematic drawing of the ovary shows the cyclic changes in the growing follicle (from Nieschlag et al. 1999)

also be influenced by other hormones. The regulation of the hypothalamic–pituitary–ovarian axis and the target organs is simplified in Fig. 40.5.

Paracrine Regulation of Ovarian Function. In light of recent investigations, the classic model of ovarian regulation appears inadequate. Follicle selection, growth, maturation, and ovulation seem to be regulated by complex intraovarian mechanisms in conjunction with gonadotropins and sex steroids. These local factors presumably have both paracrine and autocrine effects. In the paracrine system, locally secreted messenger substances influence neighboring target cells of the same organ, whereas autocrine regulation influences secretory cells within the organ via surface receptors.

The list of autocrine/paracrine factors is growing rapidly. Current research is concentrated on the **IGF (insulin-like growth factor**) system. Besides the liver, the ovary is the

main source of **insulin-like growth factor 1 (IGF 1). IGF 1 and IGF 2** are produced by the granulosa cell. IGF 1 increases the effect of LH and FSH and seems to be important in the coordination between theca and granulosa cell functions. FSH and LH increase the number of receptors on the granulosa cell, and this reaction is additionally intensified by estrogens. In the theca cell, IGF 1 stimulates and increases steroid formation. On the whole, IGF 1 is important for the formation and increase in FSH and LH receptors, for steroidogenesis, and for secretion of inhibin and oocyte maturation.

Along with IGF 1 receptors, the granulosa cell has receptors for insulin, which binds directly to IGF receptors. This cross-reactivity is important for many pathophysiologic events in the ovary, as insulin causes a modulation of ovarian function. The regulatory cascade is complicated by the fact that IGF molecules are bound and neutralized by IGF-binding proteins. Thus, the concentration of IGF-binding proteins directly affects cell regulation. In addition, the **TGF (transforming growth factor) β-gene** family is important. This term comprises substances such as **activin, inhibin, and "growth and differentiation factor 9,"** which modulates both stimulatory and inhibiting effects of gonadotropins. "Growth and differentiation factor 9" seems to be an oocyte-specific growth factor, which is important for development beyond the primordial follicle. Additionally, an intraovarian **interleukin-1** system seems to regulate periovulatory events reciprocally with LH (Udoff and Adashi 1999).

Besides growth processes, which are connected with an increase in cell numbers in follicle maturation and ovulation, the elimination of supernumerary cells in the ovary is a decisive factor in normal ovarian function. This programmed cell death (**apoptosis)** is becoming a major research interest (Chun and Hsueh 1999; Zhou et al. 2019). Clinically, most of the paracrine parameters, which have been discussed, cannot be quantified. To evaluate normal or pathological development of follicle maturation, one still has to rely on classical measurement of sexual steroids and gonadotropins. Figure 40.6 shows the course of the serum hormone concentrations used in routine clinical diagnosis.

Anti-Muller Hormone (AMH). In recent years, anti-Muller hormone, also known as MIS (Muller-inhibiting substance), has been identified as an important marker of ovarian function (van Rooij et al. 2002; Al-Qahtani and Groome 2006). Originally, this homodimeric glycoprotein, discovered in 1940 by Alfred Jost, was believed to be a secretory product of Sertoli cells whose function was to initiate the regression of the Mullerian ducts in the male fetus (Josso et al. 2006).

An overview of the physiology, methods of measurement, its variability in healthy women under different life and cycle phases, and resulting assessments of ovarian reserve can be found in detail in a comprehensive review (Dewailly et al.

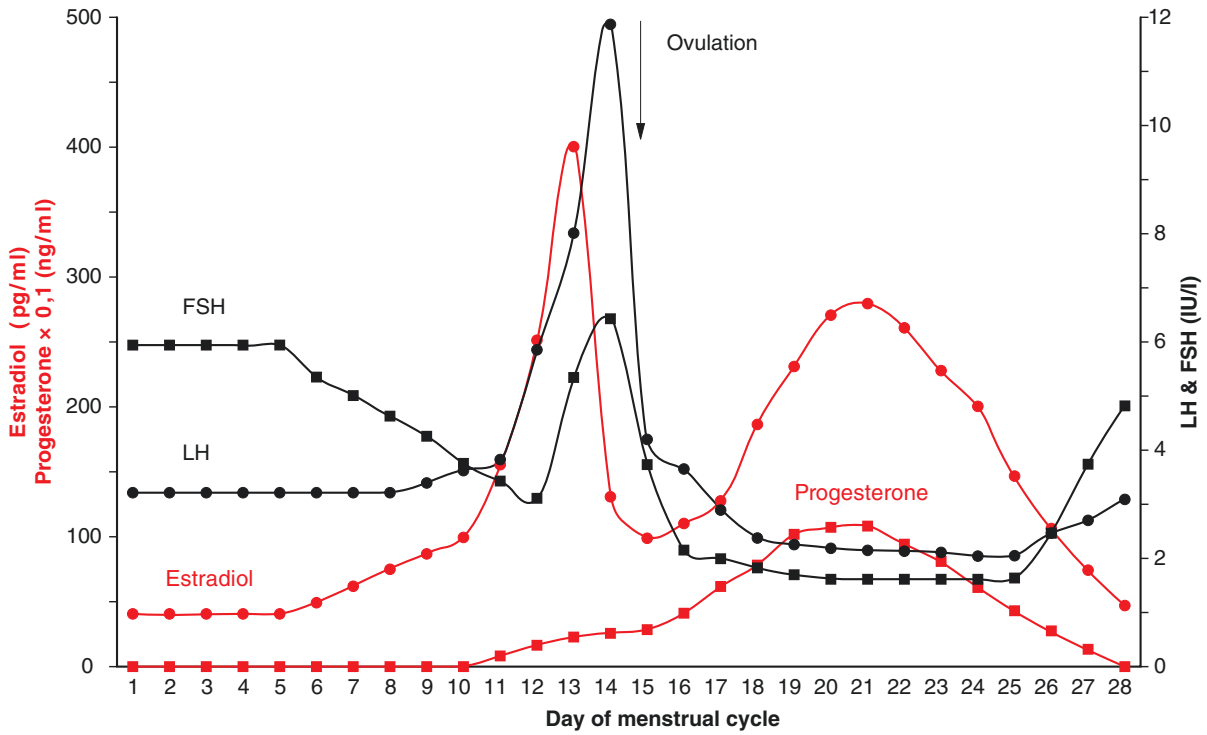


Fig. 40.6 Serum hormone concentrations during the menstrual cycle

2014). Variations over the course of age are well-documented (Cui et al. 2016).

In the female organism, **AMH** is produced by the granulosa cells and seems to be a marker for quality and quantity of follicle reserves (Visser et al. 2006). In smaller follicles, AMH inhibits the effects of FSH until follicles reach a size of 6 mm. Thereafter, intrafollicular AMH concentrations decrease, leading to selection of the **dominant follicle**, which then contains no AMH. This represents one part of the autocrine factors operative in the selection of the dominant follicle. Numerous investigations show that the basal AMH levels are the best single marker reflecting the ovarian reaction to gonadotropin treatment within the framework of IVF measures.

Its measurement can be used both to assess the onset of follicular maturation in prepubertal development and to estimate declining ovarian function perimenopausally. Since at present the determination of AMH can vary significantly depending on the measurement system, absolute values should not be compared in clinical studies when different assays are used. For the optimal use of AMH, the development of an international standard would be of great importance but is currently pending (Bungum et al. 2018).

AMH shows limited short-term variability, although the influence of prolonged treatment with oral contraceptives needs to be considered in clinical assessment. However, the influence of cycle phase is significantly less than with FSH or inhibin B determination.

There are limited data on the relationship between AMH and natural fertility during different phases of reproductive life. Predicting menopausal age based on AMH levels is only possible with great uncertainty (van Disseldorp et al. 2008). However, the hormone appears to be a useful marker for deciding on fertility protection during chemotherapy or surgical interventions on the ovaries. The risk of overstimulation is signaled by elevated AMH concentrations (Nakhuda et al. 2006), presumably by the close connections between PCOS and elevated AMH secretion in the presence of increased numbers of follicles.

40.2.2 Menstrual Cycle

40.2.2.1 Hormone Variations

The normal menstrual cycle can be divided into two phases: the follicular phase and the luteal phase. The first day of menstruation represents the first day of the cycle. The follicular phase lasts from the first day of menstruation until the preovulatory LH surge. Ovulation occurs about 36–38 h after the LH surge. As discussed above, estradiol concentrations rise during follicular development and cause a reduction in FSH during the second half of the follicular phase. Conversely, the rise of estradiol has a positive feedback effect on the production of LH and thus causes the midcyclic LH surge. In clinical practice, one can expect normal peri-ovulatory mono-follicular development with an estradiol concentration of 250 pg/mL in the serum. If during **ovulation** the ovum is extruded from the Graafian follicle, the remaining follicle develops into the **corpus luteum**, which produces progesterone, estradiol, and 17α-hydroxyprogesterone. The production of these hormones is stimulated by LH and choriogonadotropin. Progesterone secretion, combined with estradiol release, causes a negative feedback on gonadotropin secretion, so that serum levels gradually decrease at the end of the luteal phase.

LH is secreted in pulses; the interval between peaks during the follicular phase ranges from 60 to 90 min. The amplitude and the frequency rise continually as midcycle is reached. After ovulation, a lower frequency of 100 to 300 min is attained. The amplitude, however, rises in the luteal phase. The pulsatile secretion of LH is caused by the pulsatile secretion of GnRH. This discontinual release is very important for infertility therapy (see below).

40.2.2.2 Luteal Phase

Progesterone secretion after ovulation has a central thermogenic effect on the hypothalamus. Therefore, graphic representation of measurement of basal temperature (basal body temperature chart) can provide a first impression of cycle quality. A rise in morning temperature usually occurs when progesterone levels exceed 3 ng/mL. This level is, however, not representative for normal luteal function, so that even a basal temperature curve with a normal hyperthermic phase must not necessarily have an underlying normal follicular development with a normal luteal phase. On the other hand, some women do not show a rise in basal temperature after producing the thermogenic pregnan-3α-ol-20-one so that the absence of a hyperthermic phase must not necessarily imply anovulation. The basal body temperature chart (BBT chart) is thus no longer such a central diagnostic tool as it was when follicular development and ovulation could not be followed **sonographically**.

One must be especially careful not to use the BBT chart to set the time for insemination or forced intercourse, since the progesterone level described occurs 1–2 days after ovulation. The basal temperature curve should be used only in order to show variations of "important end" cycle quality and/or to time diagnostic procedures.

Clinical guidelines often describe the median cycle length as 28 days with a range of 25–30 days. The length of the luteal phase is almost always given as 14 days, although in a prospective study in a sample of women with 28-day cycles, the luteal phase varied between 7 and 19 days (Wilcox et al. 2000). With age, cycle length decreases and ovulation occurs earlier. Cycle characteristics are influenced by ethnicity, high BMI, and lifestyle factors. In contrast to such studies with a limited number of participants, data from data collection by corresponding apps offer a much larger database of the normal population. From this, information can be derived that was previously not available to the same extent (Bull et al. 2019).

40.2.2.3 Ovulation

The midpoint of the menstrual cycle is, naturally, ovulation, which must be recorded during the evaluation of the menstrual cycle (see Fig. 40.7). Next to ovulation, cyclic changes in the uterine cervix and the endometrium occur. The changes in cervical mucus are of eminent importance in the evaluation of the sperm–mucus interaction (see Fig. 40.8).

40.2.2.4 Changes in the Uterine Cervix and in Cervical Mucus Production

The cervical os is the entrance to the uterine cavity and consists of a system of folds and gaps called cervical crypts or endocervical glands. These endocervical glands have a secretory epithelium combined with ciliated epithelium in a ratio of 10:1. The secretory cells produce the cervical mucus and lie mainly in the upper part of the cervix, close to the uterus. The secretory activity of the cervical epithelium undergoes cyclic changes and thus can give easily obtainable information on the stage of the menstrual cycle. Important information is yielded by the **amount of mucus**, the **width of the external cervical** os, the ***Spinnbarkeit*** of the mucus, and the **fern-like pattern,** which develops when drying. A

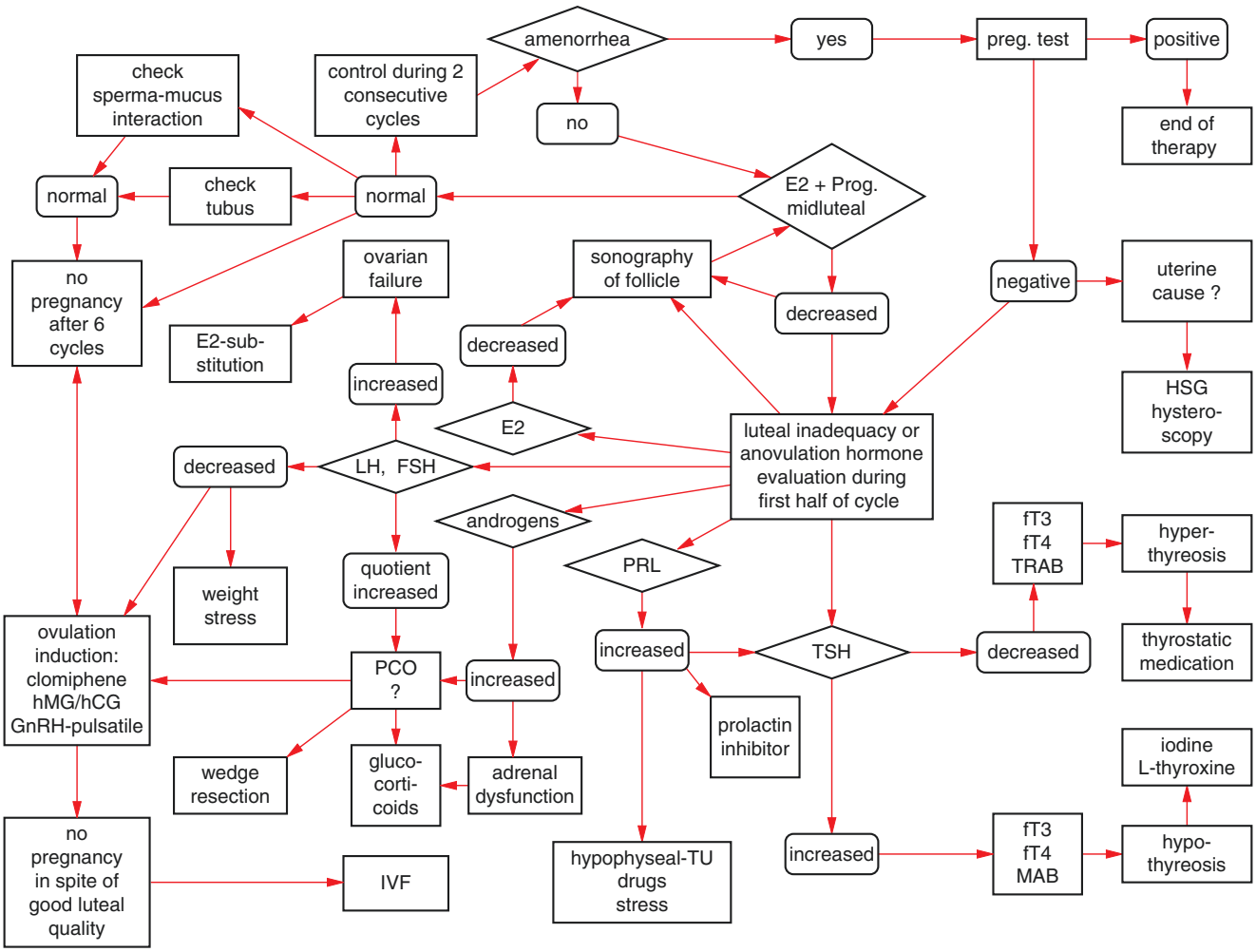


Fig. 40.7 Flow chart for the systematic diagnosis of impaired follicular growth. Only main areas are shown to facilitate readability. For more details, see text

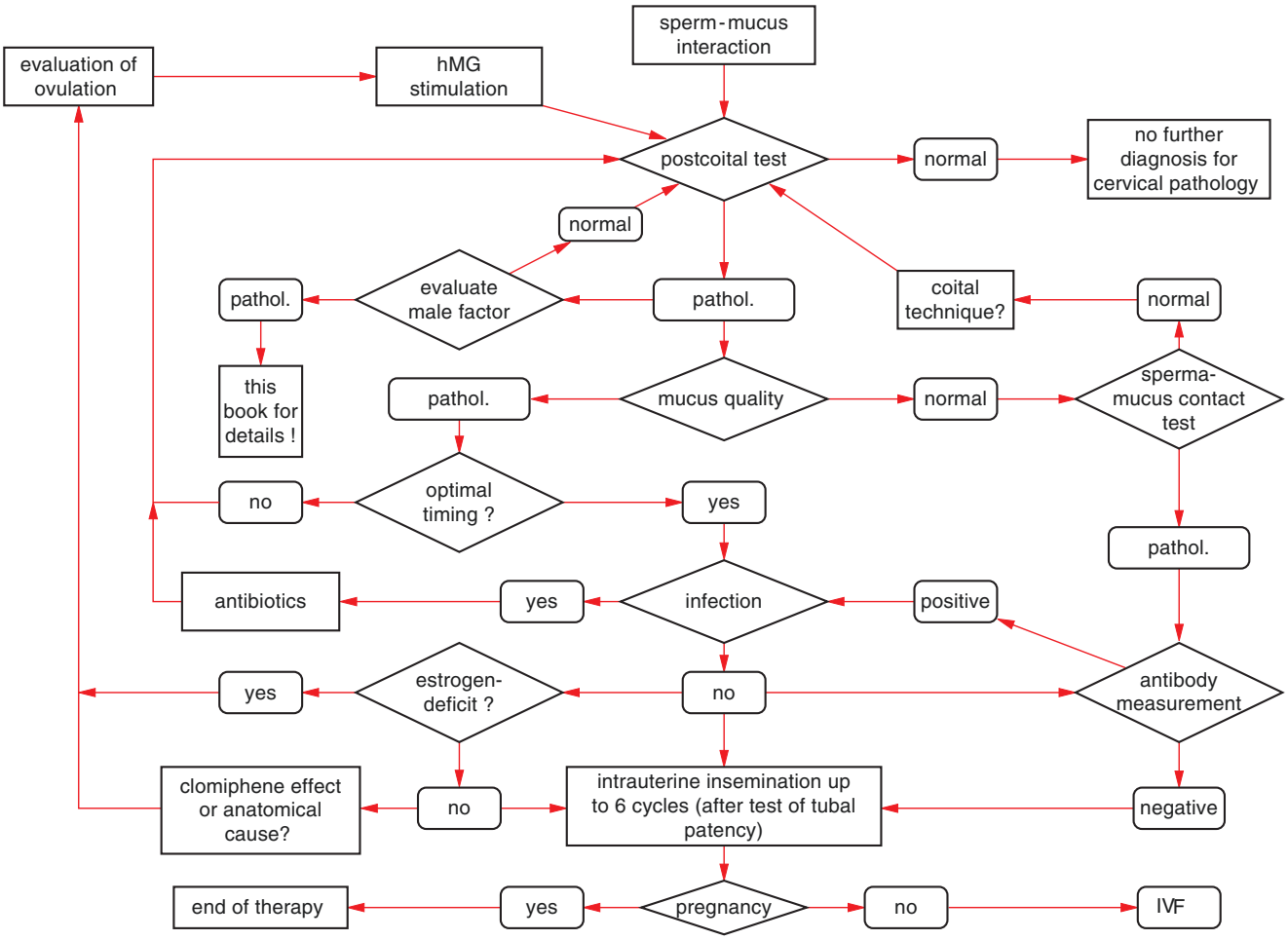


Fig. 40.8 Flow chart for the systematic evaluation of sperm–mucus interaction. Only main areas are shown to facilitate readability. For more details, see text

markedly improved prognosis for further therapy is seen when, under optimal circumstances, at least one spermatozoon per field is found postcoital at a magnification of 400× in at least six fields (Hunault et al. 2005). The amount of estrogen necessary to stimulate cervical mucus secretion varies in individual patients and can vary from cycle to cycle. Cervical secretion is influenced by cervical infection, or after surgical procedures on the cervix. In some patients, cervical mucus production can be seen several days prior to ovulation; in other cases, optimal cervix secretion occurs only a few hours before the LH surge. This must be taken into account when examining sperm–mucus interaction. The development of the cervical factor can be judged using the cervical index of Insler. The technique of cervical mucus diagnosis is described in WHO (1999).

The uterine cervix and the cervical mucus are important for the ascent of the sperm, since they protect the sperm from the acidic vaginal environment. The uterine cervix provides an energy-rich substrate for the spermatozoa, facilitates the transport of the sperm from the vagina into the uterine cavity, provides a reservoir for the sperm in the cervical crypts, and also acts as a filter, which separates normally formed sperm from those that are malformed (For a review on the cervix, see Joseph et al. 2006).

40.2.2.5 Endometrium

The cyclic changes in the endometrium, like the changes in the cervical factor, reflect cyclic ovarian function and are of eminent importance for the nidation of the fertilized oocyte. The endometrium becomes thicker during the follicular phase. Uterine glands are drawn out so that they become lengthened. This proliferation has given the first half of the menstrual cycle its name. After ovulation is complete, vascularization of the endometrium is increased. Edema develops, and the glands of the endometrium become convoluted and begin to secrete a clear fluid. Consequently, this phase of the cycle is the so-called secretory or luteal phase.

If pregnancy does not occur, the **corpus luteum** regresses; hormonal stimulation of the endometrium decreases, and it becomes thinner. This adds to the curling of the arteries and

focal necrosis occurs with local hemorrhage, which finally conflates and causes the menstrual flow. The cause of vascular necrosis is not known. Since the endometrium and the menstrual blood contain high concentrations of prostaglandins, and the infusion of prostaglandin PGF<2α> causes necrosis of the endometrium, it seems possible that the prostaglandin release causes contraction of the blood vessels.

Seventy-five percent of menstrual blood is of arterial origin. It contains necrotic endometrial cells, prostaglandins, and a relatively high proportion of fibrinolysin. This is why usually no coagulation of the menstrual blood occurs. **Menstrual blood flow** usually lasts 3–5 days. A flow of 1–8 days can still be considered normal, unless a sudden change in the regular blood flow of a patient occurs. The average woman loses around 30 mL blood per cycle. This varies between slight smearing and up to a blood loss of 80 mL. If more than 80 mL is lost, a pathological cause must be considered. After menstruation, the endometrium once again develops from the remaining cells of the basal layer.

40.2.2.6 Vaginal Epithelium

The vaginal epithelium undergoes cyclic changes under the influence of estrogen secretion as does the endometrium (for a review see Schnell 1973). Although alterations in the vaginal epithelium can indicate cycle phases, these are subject to great variation, so that no clear-cut cyclic diagnosis can be obtained through cytological analysis of the vaginal epithelium.

40.2.3 Diagnostic Evaluation of the Cycle

Essentially, evaluation of the female cycle requires the identification of optimal follicle maturation with ovulation, the development of a corpus luteum of good quality, and the physiological cyclic development of the endometrium, which is necessary for nidation of a fertilized oocyte. It is important to remember that all the tests used in the evaluation of the cycle are indirect tests, which have their limits.

40.2.3.1 Ultrasound

Although the basal body temperature chart, and the measurement of hormone concentrations in serum, urine, and saliva give indirect information about ovulation and follicle maturation, ultrasound provides the possibility of following the **maturation of the follicle** and of ascertaining **ovulation**. Sonography thus constitutes a major component in the evaluation of the female factor in infertility and is an indispensable tool.

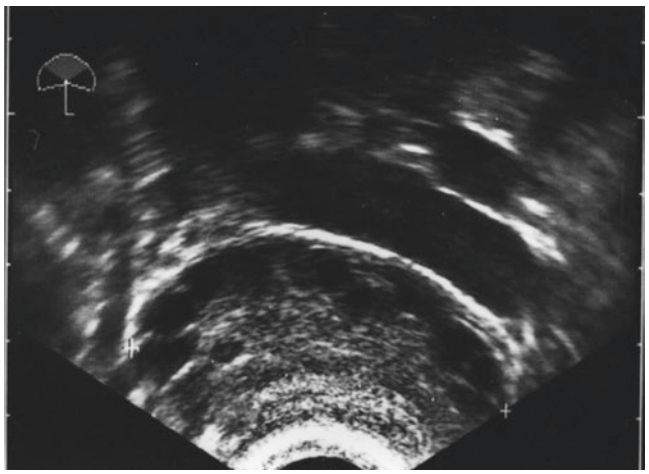


Fig. 40.9 Sonographic picture of polycystic ovary

Vaginal sonography is the methods of choice to visualize the uterus and the ovaries. As the vaginal probe lies in close proximity to pelvic structures, a high frequency of ultrasound can be used, leading to excellent resolution. In addition to classic sonography, measuring blood flow by **Doppler sonography** provides further information. Tissue damage due to energy produced by the ultrasound waves has never been shown during clinical use of this diagnostic tool. The negative effects that have been described in animal experiments require a higher output level than is used in clinical sonography.

The sonographic picture of an ovary is a rotational ellipsoid with a volume below 6 mL. Pathological changes can be seen quite readily. An important pathological picture is that of the **polycystic ovary (PCO)**, the pathophysiology of which will be discussed later. Typically, small follicles under 1 cm arranged like a string of pearls along with an ovary larger than normal with increased stroma are seen (Fig. 40.9). In addition to PCO syndrome, **endometriosis** and other **cystic changes** in the ovary can at times also be identified by ultrasound. Changes in the fallopian tube such as a sactosalpinx, in which peripheral closure of the tube causes tubal dilation by intratubal fluids, can often be diagnosed sonographically. If a **sactosalpinx** can be identified sonographically, women seeking pregnancy can benefit from salpingectomy prior to IVF therapy (Strandell and Lindhard 2002). The most important aspect of sonography in infertility therapy is, however, the evaluation of follicle growth in the normal and the stimulated cycle. Additionally, the endometrium can be evaluated and invasive procedures can be avoided.

40.2.3.2 Endometrial Evaluation

Ultrasonographically, the endometrium presents as a relatively thin and homogenous echo during the proliferative

phase. During the late proliferative phase, the thickness increases and measures about 5 mm in the anterior—posterior diameter. Edema of the stroma often causes an echodense edge of the uterine cavity, so that prior to ovulation often a multi-layered picture of the endometrium is seen. In the luteal phase, the thickness of the endometrium lies between 7 and 9 mm.

Evaluation of the Ovaries. In optimal cases, ultrasound resolution identifies ovarian structures up to 2 mm. As early as day 5 of the cycle, the dominant follicle is selected from the other primordial follicles. Between the eighth and the twelfth days, it has reached a diameter of 14 mm. During the last 4–5 days prior to ovulation, the diameter of the dominant follicle increases between 2 and 3 mm per day. The follicle thus reaches a diameter of 16–28 mm. The variable size does not allow prediction of the time of ovulation based on ultrasound alone, so that ultrasound diagnosis should always be combined with the determination of estradiol and LH levels during the preovulatory phase (Queenan et al. 1980). The prediction of the LH surge based on ultrasound is quite inaccurate and is equal to that of predicting the surge based on the day of the cycle (Buttery et al. 1983). Transvaginal ultrasonography in combination with the endocrinological methods can, however, double the fertility rate during **insemination** procedures.

After the LH surge, theca tissue becomes increasingly vascularized and the granulosa layer separates from the theca cells. This process can be visualized sonographically approximately 24 h prior to begin ovulation and is usually accompanied by a discrete rise of plasma progesterone levels. Additionally, the cumulus oophorus can be seen sonographically in about one-fifth of all follicles larger than 18 mm. If the vascular supply of the follicle, which can be ascertained by color Doppler sonography, is also taken into consideration, the quality of the egg cell can be evaluated even better and conception rates can be improved (Bhal et al. 1999).

About 25% of patients show ultrasonographically demonstrable fluid in the culdesac after ovulation. The transition from follicle to corpus luteum is seen by an indentation of the capsule and an increase in intrafollicular echo-density. Sometimes, however, the corpus luteum is a rather solid structure, which cannot readily be identified by ultrasound.

40.2.3.3 LUF Syndrome

Impaired ovulation, which is usually caused by problems of follicular maturation, is often a cause of infertility. This is frequently diagnosed by ultrasound examination, since other objective tests are often normal. A typical clinical picture is that of the syndrome of **luteinization of the unruptured follicle (LUF syndrome),** which was first discussed in 1967 (Kase et al. 1967). In this clinical entity, the hormone profiles are in a normal range and yet a rupture of the follicle does not occur. About 5% of all normal cycles are said to occur in this form. However, the significance for infertility is not yet proven since this syndrome is not one of continuous anovulation. Ultrasound examination can lead to diagnosis when, 36 h after the LH surge, the cystic follicle remains unchanged and no shrinkage can be shown. Under clomiphene stimulation, the LUF rate may increase by more than 25% (Qublan et al. 2006).

A menstrual cycle can be anovulatory even if menstruation occurs at the proper time. In anovulatory cycles, however, usually low progesterone levels are found in the luteal phase. The rise in estradiol levels in the luteal cycle is also reduced, so that the measurement of progesterone and estradiol in the second half of the menstrual cycle gives important information pertaining to ovulation. An exception is, of course, the abovementioned LUF syndrome.

In addition to the diagnostic evaluation of follicle growth and corpus luteum development, ultrasound can be used in the luteal phase to determine the quality of the endometrium. Deichert et al. (1986) compared the endometrial thickness with hormonal parameters and concluded that endometrial thickness below 10 mm is an indication for hormonal stimulation or substitution in order to optimize the implantation of the blastocyte. In a prospective cohort study comprising 1186 women seeking treatment for infertility, no correlation between thickness of the endometrium and pregnancy rate following IVF or ICSI could be found. Conversely, the pregnancy rate was found to be significantly lower in women with a thin endometrium undergoing intrauterine insemination (De Geyter et al. 2000).

40.2.3.4 Endometrial Biopsy

Because of the importance of the endometrium for implantation, an endometrial biopsy is used to assess endometrial quality. This histological procedure with dating of the endometrium in relation to the time of ovulation was long considered the gold standard for assessing the quality of luteal function and diagnosing luteal insufficiency (Noyes et al. 1975).

However, subsequent studies showed that this is not a valid diagnostic method, as both accuracy and precision are lacking (Murray et al. 2004), and no distinction can be made between fertile and infertile women (Coutifaris et al. 2004).

Therefore, endometrial biopsy is no longer recommended in the assessment of ovulation and luteal function in infertile women. The procedure should only be performed if a specific endometrial pathology (neoplasm, endometritis, and tuberculosis) is to be clarified (The Practice Committee of the American Society for Reproductive Medicine 2015).

40.2.3.5 Progesterone Levels and Evaluation of Luteal Quality

To assess luteal quality, one can resort to the measurement of progesterone and estradiol in serum. It is often critically objected that due to the pulsatile release of progesterone, this

method is afflicted with a high error rate. In contrast, a single mean progesterone value below 10 ng/mL (31.8 nmol/L) or the sum of three progesterone values <30 ng/mL (95.4 nmol/L) correlates better with the integrated progesterone concentration of the second half of the cycle than the basal temperature curve, the length of the luteal phase, or the preovulatory diameter of the follicle.

Progesterone is the primary hormone of the **corpus luteum** and of eminent importance for nidation and the maintenance of early pregnancy. A lutectomy in the seventh week of pregnancy causes miscarriage (Csapo et al. 1973). The removal of the corpus luteum during the ninth week of pregnancy, however, causes only a transitory drop in progesterone serum levels and pregnancy continues to develop. If an early decrease in progesterone concentration in the seventh week of pregnancy is compensated by substitution of this hormone, pregnancy continues to develop.

Pregnancies in women with ovarian insufficiency who become pregnant after oocyte donation can be supported by progesterone substitution during the first weeks of pregnancy. Serum levels of 20 ng/mL are sufficient. This serum level is obtained by giving 50 mg progesterone daily. Physiologically, the daily production of progesterone in the luteal phase is about 25 mg. A midluteal progesterone concentration under 3.1 ng/mL in a natural cycle signals anovulation. The median progesterone levels in conceptive cycles are around 17.8 ng/mL, although in extreme cases single conceptions also occur at lower levels around 3.8 ng/mL. In clinical practice, a midluteal progesterone level 8 days postovulation greater than 10 ng/mL demonstrates sufficient luteal function (review in Wathen et al. 1984).

The daily fluctuations of progesterone serum levels (shown in Fig. 40.10) lead one to expect that statistically about a third of all progesterone measurements will lie below 10 ng/mL, even though a normal luteal phase is present. The measurement of progesterone in combination with estradiol is, however, a clinically relevant diagnostic tool, which gives a first impression of follicular maturation when estradiol and progesterone are determined on the eighth postovulatory day. Within the abovementioned limits, it is then possible to establish whether ovulation has occurred. Several menstrual cycles must be monitored to show whether the patient has a continual restriction of her luteal quality or not. In such a case, the cause of impaired follicle maturation must be obtained before deciding on further therapy.

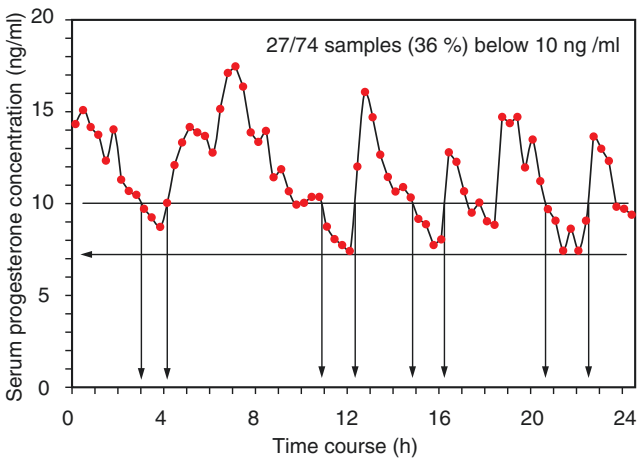


Fig. 40.10 Fluctuations in serum progesterone concentrations during a 24-h period. Adapted from McNeely and Soules (1988). The diagnosis of luteal phase deficiency: a critical review. Reproduced with permission of the publisher, the American Society for Reproductive Medicine [formerly The American Fertility Society])

40.2.4 Impairment of Follicle Maturation

The extreme form of impaired follicle maturation is that of anovulation with amenorrhea, although these cases are far less common than those in which the menstrual cycle remains unimpaired (Fig. 40.11).

40.2.4.1 Amenorrhea

Amenorrhea is a symptom that occurs in a large group of disorders, resulting in the absence of menstruation. Amenorrhea can be a result of dysfunctional endometrium with normal hormone levels or it can be due to disturbance of the hypothalamic–pituitary–gonadal axis with a normal endometrial reaction following exogenous hormone substitution. For scientific purposes, amenorrhea can be classified according to WHO criteria (Fig. 40.12).

Amenorrhea can be either primary or secondary. This classification says nothing about the cause of amenorrhea, since the same disorder can result in either primary or secondary amenorrhea. Primary amenorrhea is defined as the absence of menses by age 16. This is clinically important, since in 35–40% of the patients primary amenorrhea is a result of primary ovarian insufficiency or urogenital dysgen-

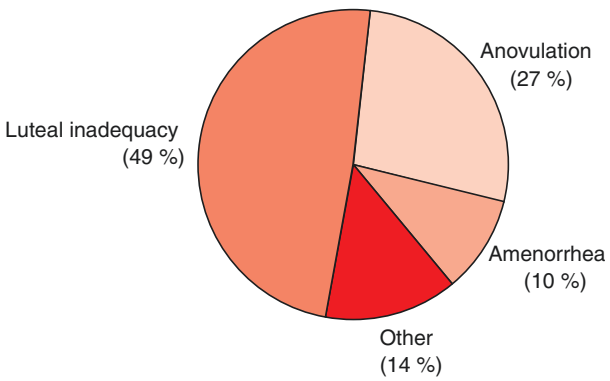


Fig. 40.11 Relative proportion of impaired follicular growth among patients of an infertility clinic according to severity of disturbance. Adapted from Bohnet (1985)

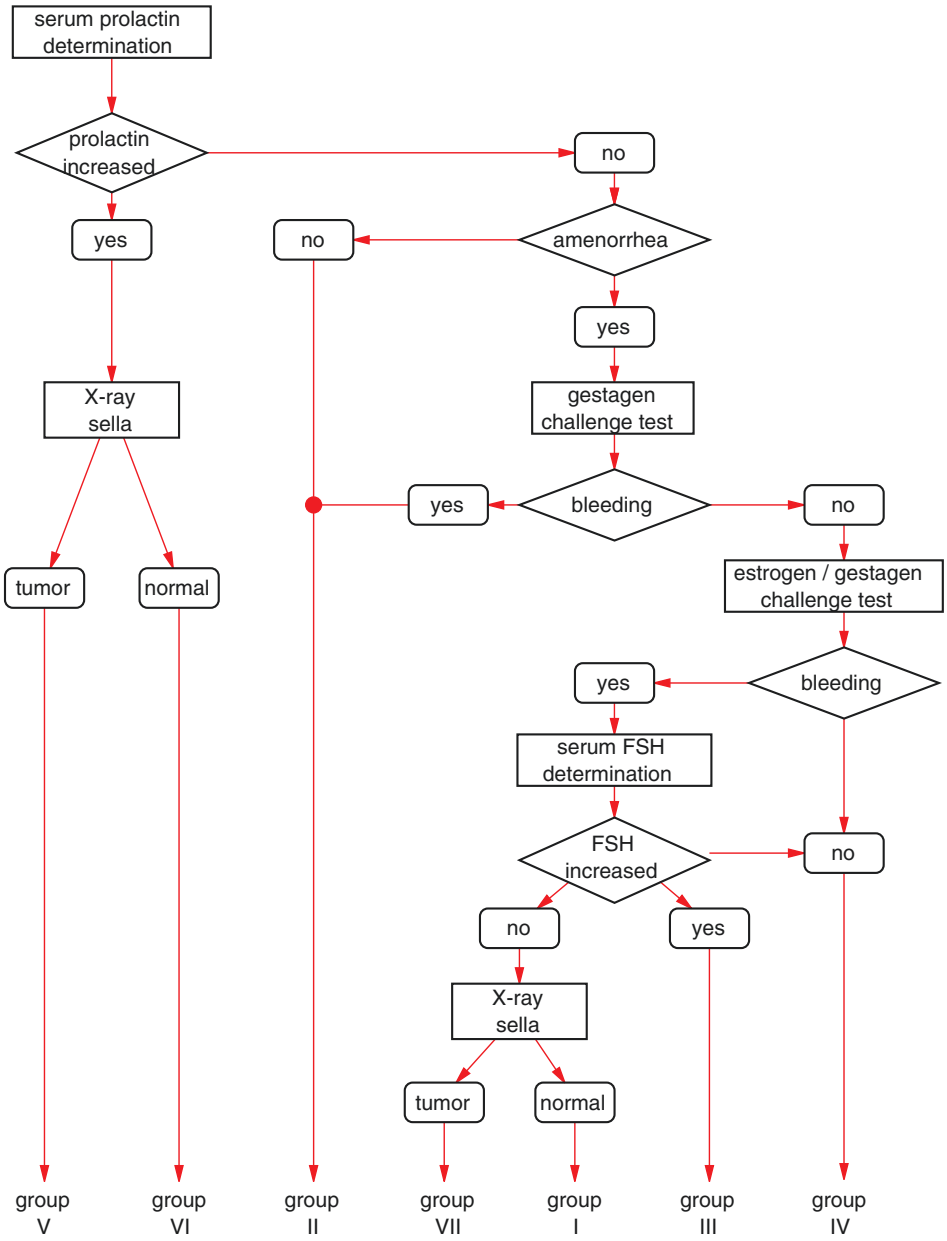


Fig. 40.12 Classification of impaired ovarian function according to WHO guidelines

esis. Secondary amenorrhea is the absence of menstruation for at least 4 months in a woman who had had at least one spontaneous menstrual cycle in her history.

40.2.4.2 Primary Amenorrhea

Patients with primary amenorrhea do not often present in infertility practice, since most of the causes are of genetic origin and have been diagnosed in puberty or in early adulthood. The primary cause of primary amenorrhea is **Turner syndrome** with the classical karyotype 45X0. In extremely rare cases, patients with Turner syndrome do have a menstrual period and develop secondary amenorrhea as a result of ovarian insufficiency.

The second most common cause of primary amenorrhea is Mullerian dysgenesis, which is characterized by the congenital failure of the Fallopian tubes, the uterus, and/or the vagina to develop. An example is **Rokitansky–Küster–Hauser syndrome**, which is a clinical entity with vaginal aplasia, a rudimentary uterus, and normal fallopian tubes. In Mullerian dysgenesis, ovarian function is intact, so that the gonadotropins and sexual steroids show normal levels. Diagnosis is obtained after clarification of the anatomy through a gynecological examination, imaging diagnostics, hysteroscopy, and possibly diagnostic laparoscopy.

Patients who are underweight in relation to height can present with primary amenorrhea. The importance of body

weight for normal development of the hypothalamic–pituitary ovarian axis is seen in the hypothesis of critical body weight, which postulates that a particular body weight must be obtained in relation to height before menstruation can begin (for review, see Knuth et al. 1977). In addition to these common causes of primary amenorrhea, a number of congenital and exogenic defects can disrupt the hypothalamic–pituitary axis, which results in a defect in hormonal regulation analogous to that which has been described in men.

40.2.4.3 Secondary Amenorrhea

Secondary amenorrhea plays a much more important role in infertility practice than primary amenorrhea described above.

A major cause of secondary amenorrhea is pregnancy. This fact has to be considered in every patient presenting with this symptom.

Even if a patient has been diagnosed with another cause of amenorrhea, it must be remembered that pregnancy can develop out of an amenorrheic phase and that menstruation need not have occurred. This is often seen in the effective therapy of hyperprolactinemic amenorrhea.

A rare cause of amenorrhea is **intrauterine synechia,** which can cause **Asherman's syndrome**, in which a destruction of the endometrium has occurred with a resulting internal adhesion of the uterine cavity. This is usually caused by a complicated dilation and curettage such as in infected abortion, or extremely vigorous curettage, but the syndrome can also occur after unspecific or tuberculosis endometriosis. Patient history is important for diagnosis. A suspicion of Asherman's syndrome can be strengthened by normal estradiol and progesterone levels in the luteal phase, or through continuing amenorrhea after hormonal stimulation. Diagnosis is obtained through hysteroscopy or, with limitations, by hysterosalpingography. Therapeutically, adhesiolysis of the uterine cavity must be followed by sequential estrogen and progesterone substitution in the form of a pseudo-pregnancy. Simultaneously, an IUD can be used in order to deter the development of new adhesions while the endometrium regenerates. Therapy is also possible through stem cell transplantation (Zhu et al. 2019).

If Asherman syndrome and a hysterectomy can be ruled out, the cause of secondary amenorrhea in the remaining cases that present in infertility practice can be divided into those with hypothalamic and pituitary defects and those with ovarian failure.

Hypothalamic amenorrhea is a diagnosis of exclusion and represents a functional defect of the secretion of gonadotropins caused by rapid weight loss, rapid weight gain, systemic diseases, extreme physical activity, and/or extreme stress situations (see Sect. 40.1.7). Hypothalamic amenorrhea is the extreme case of impaired follicle maturation, in which luteal insufficiency or anovulation with normal menstruation occurs because of the above-named causes prior to the development of secondary amenorrhea.

Hyperprolactinemia is often a cause of amenorrhea. Here, again amenorrhea is the extreme result of hyperprolactinemia; luteal insufficiencies and anovulation with normal menstruation are seen much more commonly. In hyperprolactinemia, one must think of a pituitary adenoma or **hypothyroidism** as causative factors.

An important cause of impaired follicular maturation, and thus of amenorrhea, is the **polycystic ovarian syndrome (PCO syndrome**). Primarily one thinks of an obese patient with hyperandrogenic symptoms and the typical picture in ultrasound diagnosis described above. The so-called PCO syndrome or Stein–Leventhal syndrome is, however, only an endpoint of a group of various different pathological disturbances and represents a disruption of the cyclic ovarian function with elevated androgen/estradiol levels and an unbalanced LH/FSH ratio.

In addition to these three common causes of secondary amenorrhea and impaired follicle maturation, rare causes of hypothalamic tumors and cysts and infiltrative diseases of the hypothalamus or the pituitary gland such as tuberculosis, sarcoidosis, or histiocytosis X exist. These are, however, rarities even in specialized centers.

In contrast to dysfunction of the hypothalamic–pituitary axis, which can be readily treated, the common diagnosis of primary ovarian failure is usually the endpoint of infertility workup in countries where oocyte donation is prohibited by law, since atresia of the primordial follicles results in an absolute loss of oocytes. Premature ovarian failure is characterized by the loss of ovarian function before the age of 35 years. This can be a result of chemotherapy or radiation therapy or also may have an immunological cause.

40.2.4.4 Hyperprolactinemia

A current recommendation for the diagnosis and treatment of hyperprolactinemia can be found in a Clinical Practice Guideline of the Endocrine Society (Melmed et al. 2011).

The existing relationship between disturbed reproductive function and lactation has long been known. Designation names are found in the older literature such as the Chiari–Frommel syndrome (postpartum amenorrhea with persisting lactation); Argonz–Ahumada–Castillo syndrome (galactorrhea and reduced urinary estrogen concentrations); and Albright-Forbes syndrome (amenorrhea, reduced urinary FSH concentrations, and galactorrhea). After 1972, when it first became possible to measure human prolactin, all of these syndromes were found to have a common denominator, namely that of **hyperprolactinemia**.

In humans, prolactin consists of 199 amino acids and is similar in structure to growth hormone and placental lactogen (HPL). However, the structure can be modified by glycosidic bonds, phosphorization, and different dimer formations. Accordingly, parallel measurements in different bio- and immunoassay systems occasionally show considerably different concentrations. The variance of forms ("little prolactin," "big prolactin," "big big prolactin") described can in rare cases explain typical clinical symptoms such as galactorrhea in the presence of apparently normal prolactin concentrations. The predominant form in circulation is glycosylated "little—prolactin," which is also routinely measured.

In contrast to the other hormones of the pituitary gland, prolactin is mainly regulated through a hypothalamic inhibitory factor. The main inhibitor is dopamine. In rat experiments, 70% of prolactin secretion can be inhibited through a dopamine infusion, if the androgenic dopamine synthesis has been blocked beforehand. γ-aminobutyric acid (GABA) is another inhibitory factor, which, however, is weaker in its effect than dopamine.

A number of stimulating substances have also been found, which are important for the short-term secretion of prolactin. These substances include thyrotropin-releasing hormone (TRH), vasoactive intestinal peptide (VIP), and angiotensin. Serotonin precursors cause a significant rise in prolactin concentrations. In contrast, the blockade of serotonin inhibits prolactin secretion. Endogenous opioids increase prolactin secretion by inhibiting dopamine synthesis and reducing dopamine secretion. Histamine and substance P both stimulate prolactin secretion, although the exact regulatory mechanisms are not yet known.

The differentiated regulation of prolactin secretion explains the various causes of hyperprolactinemia. A slight rise in serum prolactin concentration can be a symptom of central neurogenic dysregulation, such as the stress reaction, in the form of a functional dysregulation. Hyperprolactinemia can be due to the ingestion of a variety of drugs. **Primary hypothyroidism** can also be the cause of hyperprolactinemia. Even hormonally inactive tumors close to the pituitary gland can cause hyperprolactinemia through changes in portal circulation. Very high prolactin concentrations are usually, however, caused by prolactin-secreting tumors **(prolactinoma)**.

About one-third of all patients with secondary amenorrhea have a pituitary adenoma (for review of therapy, see Gheorghiu et al. 2020). In patients with simultaneously occurring **galactorrhea**, 50% of those women exhibit an abnormal cone view of the sella turcica. The infertility in these patients is related more closely to hyperprolactinemia than to tumor size except, of course, in extreme cases.

A prolactinoma causes a rise in hypothalamic dopamine concentration, which in turn causes a negative feedback on GnRH secretion. This causes a decrease in gonadotropin secretion, which then results in anovulation. Therapeutically, one must thus either remove the adenoma or lower the prolactin concentration by giving a specific inhibitor.

Since not all cases of hyperprolactinemia develop the typical symptoms, a check of the prolactin serum concentration belongs to the routine diagnostic workup of female factor infertility.

A blood sample is best taken during basal conditions in the early morning hours. Since this is usually not obtainable in clinical routine, one must consider the circadian rhythm and subjective situation during which the blood sample was drawn when evaluating the serum level obtained. If hyperprolactinemia is diagnosed, then the level needs to be checked in a second sample. Prolactin serum levels show great fluctuations, which can be caused by a variety of physiological stimuli such as nutrition and sleep behavior, stress, and physical activity. The intake of prolactin-stimulating drugs must also be considered.

Before beginning treatment of a slight to medium hyperprolactinemia (lower than 50 ng/mL) with a prolactin-inhibiting drug, the TSH level (less than 3 μU/L) should be obtained from the same blood sample in order to rule out thyroid dysfunction. In this case, treatment of the thyroid may be pertinent. If the prolactin concentrations exceed 50 ng/mL without known physiological stimuli, one should perform an MRI scan of the hypothalamic–pituitary area.

The probability of a pituitary adenoma is about 1:5 when prolactin serum concentrations are above 50 ng/mL. The probability rises to 1:2 if serum concentrations exceed 100 ng/mL. In cases with even higher prolactin concentrations, almost all patients have a microadenoma. When levels rise above 1000 ng/mL, a macroprolactinoma is very probable.

40.2.4.5 Pituitary Adenoma

Prolactin-secreting adenomas are the most frequent tumor of the pituitary gland. About 50% of all pituitary adenomas, which are found in male and female autopsies, fall into this group. 9–27% of autopsied humans have a prolactinoma, with the highest incidence occurring in the sixth decade of life. There is no difference in incidence between male and female patients, although clinical symptoms are seen much more commonly in women. Hyperprolactinemia can be diagnosed five times more commonly in women than in men.

When other causes for hyperprolactinemia have been ruled out, suspicion of a prolactinoma can be confirmed by an MRI scan. Microadenoma (<10 mm diameter) are distinguished from macroadenoma.

The **MRI scan** is superior to a CT scan for identifying microadenomas and is the best diagnostic test to rule out an adenoma. An ophthalmological checkup is only necessary when the adenoma is greater than 10 mm in diameter.

40.2.4.6 Empty Sella Syndrome

A radiologically abnormal sella can be seen in the **empty sella syndrome**. In this case, the diaphragma sellae is congenitally malformed. This defect causes an expansion of the subarachnoid space into the pituitary fossa, which results in a bilateral displacement of the pituitary gland causing the sella to appear empty. This syndrome is not rare. It is seen in 5% of all autopsies, 85% of which are female. The empty sella syndrome is usually a benign syndrome, whereas radiologically sometimes the misdiagnosis of a tumor occurs. In these cases, surgical intervention must be prohibited. After the diagnosis has been made, an annual check of prolactin concentrations should be performed. If a hyperprolactinemia is found, treatment with a prolactin inhibitor may be started (for review, see Speroff et al. 1994).

In the recent past, a direct inhibitory effect of elevated prolactin levels on follicular maturation has been suspected. Follicular atresia, anovulation, insufficient development of the corpus luteum, and a premature luteolysis were presumably caused solely by the pathologically elevated prolactin concentrations. These mechanisms have been established in the rat model, but it is not yet clear if the same applies in humans.

Lately, an increasing number of experts have suggested that the findings described above are caused by hypothalamic changes. Peripherally measured hyperprolactinemia appears to be secondary to a central nervous cause. Recent studies show that little-known disturbances of androgen secretion and dysfunctional regulation of steroid biosynthesis are at fault, which, according to analysis by the Androgen Excess Society, is primarily caused by excessive androgen biosynthesis, increased androgen intake, or increased androgen metabolism (Azziz et al. 2006) dysregulation caused primarily by an alteration in the pulsatile GnRH pattern. The changes in the GnRH pattern deter normal gonadotropin secretion and thus follicular maturation. Richardson et al. (1985) showed that rhesus monkeys with hypothalamic lesions treated with exogenic pulsatile GnRH showed normal postovulatory plasma progesterone concentrations independent of the prolactin serum level. A correlation between progesterone and prolactin levels in a group of women with luteal insufficiency could not be shown when compared with normal control groups (for review, see Soules 1993).

Even if it is not entirely clear whether there is a direct effect of elevated serum prolactin concentrations on follicular maturation, or if the origin lies in an alteration of gonadotropin secretion, hyperprolactinemia is undoubtedly a cause for female infertility and should thus be treated.

40.2.4.7 Surgical and Radiological Treatment of Hyperprolactinemia

In the past, before dopamine agonists were available for the treatment of hyperprolactinemia, patients with pituitary adenomas and other similar tumors were operated or received radiation therapy. Data on these patients show that transsphenoidal neurosurgery resulted in ovulatory menstrual cycles in 80% of patients with microadenomas, but only in 40% of those with macroadenomas. In 30% of the patients with microadenomas, however, a tumor reappeared. The recurrence rate of macroadenomas was around 90%. Severe side effects such as panhypopituitarism and liquor fistula occur and restrict the indication for neurosurgical treatment.

The results of radiation are inferior to those of surgery, so that this form of therapy should only be used for postoperative treatment of larger tumors, which show new growth, and do not respond to pharmacological treatment.

In the past, it was feared that a treated pituitary adenoma could expand during pregnancy. This is extremely uncommon in microadenomas (<1%) where even breastfeeding may be allowed without having to fear the stimulation of a pituitary adenoma. The risk of pituitary adenoma growth during pregnancy rises, of course, with the size of the adenoma. In the case of a macroadenoma with suprasellar infiltration, a 15–35% increase in tumor size is likely during pregnancy, so that if motherhood is desired, reduction in the tumor is recommended. In these cases, ophthalmological checkups during pregnancy at least every 3 months, combined with measurements of serum prolactin, should be performed. When the prolactinoma increases during the course of pregnancy, bromocriptine should be considered.

40.2.4.8 Pharmacological Treatment

The development of synthetic prolactin inhibitors represented an important step in the treatment of hyperprolactinemia. The introduction of bromocriptine in the 1970s allowed the direct treatment of hyperprolactinemic amenorrhea and infertility for the first time.

- **Bromocriptine** is a lysergic acid derivative with dopamine agonistic activity, which inhibits prolactin secretion by binding at the dopamine receptor. When pregnancy is desired, it is the medication of choice. Depending on the prolactin concentrations, a dose of 0.625 to 2.5 mg in the evening may be enough to normalize the prolactin level. In patients with pituitary adenomas, 10 mg or more daily may be needed.

Although this form of treatment is very effective, adverse reactions may cause poor compliance. Headaches and nausea often occur during the beginning of treatment. Dizziness is caused by orthostatic hypotension, since noradrenergic neural transmitters are interfered with. These effects can be minimized by slowly increasing the dosage. Treatment should always begin with 0.625 mg taken in the evening. The dosage may be increased every 3 days by 1.25 mg in the morning until the final dosage is reached. Vaginal application of uncoated tablets of bromocriptine can markedly reduce side effects of the drug (Ginsburg et al. 1992). Since this form of application results in a higher rate of resorption and eliminates the first-pass effect of the liver, a lower daily dosage can achieve the same effects. This is often clinically employed.

Research data show that about 80% of patients with hyperprolactinemic amenorrhea regularly menstruate as a result of treatment with bromocriptine. In 50–75% of patients with pituitary adenoma, treatment with dopamine agonists has significant influence on tumor size. Neoplasias were no longer detectable in 25–30% of these cases receiving long-term treatment. In view of this aspect, pharmacological treatment of a pituitary adenoma should be the method of choice. Transsphenoidal neurosurgery should only be considered if bromocriptine therapy does not reduce tumor size. This is also relevant in cases where prolactin levels return to normal. In this case, evidence points to a nonfunctional tumor, which causes hyperprolactinemia merely by disrupting the dopamine supply in the pituitary stalk.

If pregnancy occurs, bromocriptine treatment is usually discontinued. Three large-scale studies have shown that even with continuation of therapy, no negative consequences for the fetus have to be feared (Holmgren et al. 1986).

- A number of new prolactin inhibitors are now available. **Lisuride** has a higher activity, a longer half-life, and is better tolerated by some patients, so patients unable to continue bromocriptine therapy can switch to lisuride.
- **Metergoline** is an antiserotonergic substance, which acts via a non-dopaminergic mechanism and can be tried as an alternative product (Bohnet et al. 1986).
- A new substance is **cabergoline**, a prolactin inhibitor, which only needs to be taken 1–2 times per week and which seemed to be better tolerated than bromocriptine in first clinical trials.

Hyperprolactinemia, which is caused by thyroidal dysfunction, should be treated through adequate treatment of the thyroid gland.

40.2.4.9 Polycystic Ovarian Disease

The different entities, which are combined under the term polycystic ovarian disease, are, next to hyperprolactinemia, the most important cause of anovulatory infertility. Recommendations for the assessment and treatment of PCO syndrome can be found in an international evidence-based guideline (Teede et al. 2018). The clinical picture varies from the physiologically normal patient with an anovulatory menstrual cycle to the adipose, hirsute, and oligomenorrheic patient who was first described by Stein and Leventhal.

Depending on the investigators' point of view, three different diagnostic key elements are emphasized: hyperandrogenemia, chronic anovulation, and polycystic ovaries verified by ultrasound. Originally, the typical alterations of the ovaries, which, however, are not obligatory, led to the description of the disease. At present, two diagnostic systems define PCOS: The 1990 definition of the National Institutes of Health (NIH) requires chronic anovulation plus clinical or laboratory symptoms of hyperandrogenemia. In contrast, the criteria agreed upon in 2003 in Rotterdam specify two or more symptoms: chronic anovulation, clinical or biochemical signs of hyperandrogenemia, and/or polycystic ovaries (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004).

In the past, these typical ovarian alterations were assumed to cause PCOS; the underlying pathophysiological mechanism was misunderstood. In fact, the anatomical situation results from a hormonal dysregulation perpetuated and aggravated in a vicious circle. These changes can be caused by hypothalamic, hypophyseal, ovarian, and/or adrenal dysfunction, which are all often combined with oligo- or amenorrhea, hirsutism, and infertility. The characteristic polycystic ovary is seen when no ovulation occurs during a longer period of time. "Polycystic ovarian disease" thus is not a diagnosis, but a prototypical form of chronic hyperandrogenic anovulation.

Recent studies show that little-known disturbances of androgen secretion and dysfunctional regulation of steroid biosynthesis are at fault, which, according to analysis by the Androgen Excess Society, is primarily caused by excessive androgen biosynthesis, increased androgen intake, or increased androgen metabolism (Azziz et al. 2006).

The typical ovarian morphology with multiple small subcapsular follicles is frequent, but in no way adequate for diagnosis. Many women with no hormonal dysfunction have more than eight subcapsular follicular cysts of less than 10 mm. Epidemiological studies show that about 25% of all premenopausal women demonstrate typical signs for polycystic ovarian disease in ultrasound examination. Even women who take oral contraceptives can show the typical ultrasound picture in 14% of all cases (Clayton et al. 1992).

The prevalence of disturbances associated with anovulation is only between 5% and 10%.

Three essential characteristics are required to arrive at a correct diagnosis.

40.2.4.10 Hyperandrogenemia

Hyperandrogenemia is the predominant characteristic of a PCO syndrome and occurs in most cases. Increased androgen can be diagnosed via clinical signs, biochemical parameters, or a combination of these. **Hirsutism** is found in over 60% of all women with PCOS. Differences due to ethnicity must be taken into consideration.

Laboratory parameters are usually derived from total serum testosterone and SHBG levels, in order to objectify an increased androgen fraction. About 20–40% of all PCOS patients show no clear signs of hyperandrogenemia (Chang et al. 2005). Many testosterone assays in the routine laboratory are not able to deal with the relatively low values for women and cannot discriminate between patients with apparently normal values and those with hyperandrogenemic symptoms.

40.2.4.11 Chronic Anovulation

A diagnosis of anovulation is less arbitrary than evidence of hyperandrogenemia. When the cycle exceeds 35 days or menstruation fails to take place for more than 3 months, oligomenorrhea or amenorrhea is present. Regular cycles, however, do not exclude anovulation so that determination of progesterone in the second half of the cycle is mandatory for diagnosis. If chronic anovulation is given, prolactin, LH, or FSH must be measured additionally.

40.2.4.12 Polycystic Ovaries Confirmed by Ultrasound

In recent years, technological progress, especially in resolution of image technology, has considerably improved the measurability of ovarian alterations. Earlier standards based on abdominal measurements were adapted to the gynecological situation. The criteria for a polycystic ovary are now considered to be more than 20 follicles between 2 and 9 mm in diameter during the follicular phase or an ovarian size of more than 10 mL (Teede et al. 2018). Measuring **AMH (anti-Muller hormone)** could provide a potential marker, as its concentration closely correlates with the number of antral follicles (Pigny et al. 2006).

Increased androgen production plays an important role in the pathogenesis of PCOS. Steroid biosynthesis in the ovary and in the female adrenal cortex follows the same mechanisms as described for the male in Chap. 2. Ovarian androstenedione is the basis for both testosterone and estrogens. Unlike in males, LH and ACTH do not control androgen production by a specific inhibitory feedback mechanism, as androgens are only byproducts of estrogens and cortisone synthesis. Rather, intraovarian control of androgen production plays a critical role. In the ovary, androgens represent a "necessary evil" (Rosenfield 1999). On the one hand, they are necessary for estrogen production and accelerate growth of smaller follicles; on the other hand, when overabundant they prevent selection of the leading follicle and cause atresia. Steroidal secretory patterns of PCOS patients suggest a generalized dysregulation of androgen production, particularly affecting the level of 17-hydroxylase and 17,20 lyase activity. This dysregulation can manifest itself solely as ovarian hyperandrogenemia, but may also lead to functional hyperandrogenemia of the adrenal cortex. A combination of the two is not rare. Inherently, a PCOS can derive from pathological androgen production of the adrenal cortex.

The absence of the correct rhythmic pattern of gonadotropin and sexual steroid secretion causes persistent **anovulation**. Serum levels of testosterone, androstenedione, dehydroepiandrosterone sulfate, 17-hydroxyprogesterone, and estrone are thus elevated. The elevated production of estrogens is not due to direct ovarian secretion. The daily production of estradiol in women with polycystic ovarian disease is equal to that of normal women in the early follicular phase. The elevated serum estrogen concentration is rather due to peripheral aromatization of androstenedione to estrone in adipose tissue.

Typically, the **LH-to-FSH ratio** is greater than three in polycystic ovarian disease. 20–40% of all patients with polycystic ovaries, however, do not have this typical change in the LH-to-FSH ratio. Analysis of the LH pulse pattern in PCO patients shows a frequency identical to that of normal controls. The individual pulse amplitude, however, is elevated (12.2 ± 2.7 mU/mL) when compared to normal controls in early or midfollicular phase (6.2 ± 0.8 mU/mL) (Kazer et al. 1987). This seems to be a result of a change in GnRH pulse frequency.

An increase in the GnRH pulse amplitude with constant pulse frequency reduces peripheral FSH concentrations in the presence of normal LH patterns. This causes the typical reversal in the LH/FSH ratio. Research data thus demonstrate that the change in gonadotropin secretion often associated with polycystic ovarian disease is due to a malfunction in the frequency and amplitude modulation of GnRH production. A primary disturbance of LH secretion does not, however, seem to be the origin of PCOS.

Endogenous opiates influence hypothalamic GnRH secretion. It has been shown that endorphin metabolism can be modulated in polycystic ovarian disease when compared to controls. **β-endorphin** and **adrenocorticotropic hormone (ACTH)** stem from the same precursor called proopiomelanocortin (POMC). It is known that β-endorphin levels are elevated in situations in which ACTH production is increased. The concentrations of ACTH and cortisol are normal in PCO patients, but this does not rule out a higher rate

of metabolism of these substances. Since β-endorphin levels are elevated in stress situations, and patients with PCO show a higher rate of psychological stress reactions, it is possible that this may be one central factor in the impairment of normal regulatory mechanisms.

The influence of hyperprolactinemia in central hormone regulation was discussed above and explains the high association of hyperprolactinemia with polycystic ovarian disease.

Elevated **testosterone** concentrations reduce the measurable free sex hormone binding globulin (SHBG), so that anovulatory women with polycystic ovaries usually have a 50% reduced level of SHBG due to secondary hyperandrogenemia. Reduced SHBG concentration elevates the concentration of free estrogen, which again is positively correlated with a rise in the LH/FSH ratio. The elevated free estradiol concentration and the peripheral metabolism of androstenedione to estrogen reduce the FSH level. A residual activity of FSH, however, remains so that continued stimulation of the ovaries with ensuing follicle production occurs. The hypothalamic–pituitary axis is fully intact and is even the basis for further development of polycystic ovarian disease.

Follicular maturation in PCOS does not, however, end in ovulation. The maturation of the smaller follicles extends over several months so that typically 2–6 mm large follicle cysts are produced, which in turn give this disease its name. Since the follicles are enclosed in a hyperplastic theca layer, the follicular stroma is enlarged and constantly produces steroids under continued gonadotropin stimulation. This closes the vicious circle so that the disease is perpetuated. After the follicles die off and the layer of granulosa cells disintegrates, the thecal portion remains so that the production of androstenedione and testosterone increases, according to the two-cell theory outlined above. The increased testosterone levels further reduce SHBG with a consequent rise of free estrogens. Simultaneously, the free testosterone fraction increases, with the resulting effect on androgen-dependent tissue.

40.2.4.13 Insulin Resistance

About 40% of patients with polycystic ovarian disease show increased insulin resistance. Although obesity and age increase the probability of insulin resistance, even non-obese women and young women with PCOS may show glucose intolerance. The infusion of glucose causes an excess secretion of insulin. It is estimated that about 10% of all cases of glucose intolerance are caused by PCOS-induced insulin resistance. 15% of all later diabetes type II cases show a PCOS in their history.

Although androgens can induce a slight insulin resistance, the concentrations found in PCOS are not sufficient to explain disturbances of insulin metabolism. If androgen production is inhibited, insulin sensitivity fails to normalize. Conversely, insulin resistance increases only negligibly when androgens are given, as in female-to-male transexuals. Independent of the mechanism of elevated circulating insulin levels, binding of insulin to the IGF-I receptors in the theca cells occurs. This increases thecal androgen production by LH stimulation. The elevated circulating insulin levels thus increase androgen production in insulin resistance. Simultaneously, elevated insulin levels inhibit hepatic SHBG production and the production of IGF-binding protein I. Although there are indications that hyperandrogenemia can cause hyperinsulinemia, most experimental data suggest that disturbed insulin metabolism precedes irregular androgen metabolism (Dunaif 1999).

40.2.4.14 Obesity

Since increased body weight and abdominal adipose tissue cause hyperinsulinemia and reduced glucose tolerance, one may deduce that obesity is the major factor in polycystic ovarian disease. The typical distribution of female adipose tissue distribution in the hip area does not play such a major role. An objective method to determine the distribution of adipose tissue is the measurement of the ratio of waist-to-hip circumference. If the ratio is larger than 0.85, an android distribution of adipose tissue is probable, and hyperinsulinism should be suspected. If the ratio is below 0.75, a gynoid distribution is probable, which in turn is only rarely combined with changes in insulin metabolism.

40.2.4.15 Causes and Risk Factors

The origins of PCOS remain obscure. A combination of environmental conditions and predisposing genetic factors is presumed. According to new insights, triggering factors seem to lie less within the ovary than on the hypothalamic–hypophyseal level and in disturbed effects of insulin. Familial inheritance has been known for years and was recently confirmed by twinning studies in 79% of patients interviewed (Vink et al. 2006). A genetic association with a microsatellite marker (D19S884) on chromosome 19p13.2 is controversial.

Environmental factors in the sense of **"endocrine disruptors"** are difficult to prove in the human. In animal experiments, increased exposure to androgen during pregnancy led to a PCOS similar picture in female offspring (Abbott et al. 2005).

40.2.4.16 Diagnosis

Endocrinological investigation plays the major role in excluding the possibility of a PCOS and is mandatory even if the patient shows no physiological stigmata but is anovulatory. The typical ultrasound picture is not sufficient for diagnosis, as was formerly believed (see Fig. 40.9). Determining testosterone, androstenedione, DHEAS, estradiol, LH, FSH, and prolactin levels in the first half of the cycle may be help-

ful in deciding on a form of individual treatment. Cortisol and 17-OH-progesterone levels supplement the basic workup when adrenal involvement is suspected. This is all the more true as the latest generation of ultrasound machines with higher resolution also detects follicles smaller than 2 mm, so that recent studies raise the threshold of antral follicle count for PCO diagnosis from 12 to 19–25 per ovary.

At present, it is still impossible to give a clear threshold for AMH levels as predictive for the diagnosis of PCO syndrome. However, the correlation between AMH plasma level and follicle count is very strong, so that in the future we can expect a shift in diagnosis from ultrasound to the hormone laboratory (Dewailly et al. 2014).

40.2.4.17 Treatment

Since polycystic ovarian disease usually encompasses elevated androgen levels, a chronic elevation of estrogen levels, and an inverse LH/FSH ratio, treatment must intervene in this vicious circle in order to allow ovulation to occur. Methods of treatment are as follows:

1. Anti-estrogens (e.g., clomiphene, tamoxifen, and aromatase inhibitors)
2. Glucocorticoids (dexamethasone 0.25–0.5 mg)
3. Pulsatile GnRH treatment via a cyclic pump
4. HMG/FSH stimulation
5. Surgical reduction in ovarian stroma
6. Oral antidiabetic medication

Treatment forms 1–3 still allow feedback and regulatory mechanisms of follicular maturation to occur. In contrast, the treatment with hMG or hCG/FSH directly acts at the ovarian level and thus carries a higher risk of hyperstimulation, but even **clomiphene therapy** is associated with a 5% rate of multiple pregnancy. Surgical reduction in androgen-producing ovarian stroma should only be tried when other forms of treatment have failed.

Research data show that 63% to 95% of infertile women with polycystic ovarian disease ovulate after clomiphene treatment. Clomiphene is a weak anti-estrogen and causes a rise in gonadotropin levels. The compound is orally effective and is easily resorbed. Medication consists of enclomiphene and zuclomiphene, whereby the latter leads to strong accumulation following consecutive treatment cycles (Young et al. 1999). Treatment usually begins with 50 mg daily for 5 days, beginning between the third and seventh day of the menstrual cycle. 27–50% of the women ovulate with this low dosage. In some cases, an increase up to 150 mg daily should be considered, in which a further 26% to 29% ovulate. If this dosage does not result in ovulation, one may additionally prescribe **dexamethasone** (0.25 to 0.5 mg daily), depending on the DHEA sulfate serum concentration. If follicular maturation is seen in ultrasound and is shown by serum hormone concentrations and no ovulation occurs, ovulation can be induced by 5000 to 10,000 IU hCG i.m. If one considers that normally only 50% of all couples achieve pregnancy within 3 months and that 80% require 1 year for conception to occur, treatment should be continued for at least 6 months or cycles if ultrasound and hormone levels show an adequate luteal phase. This therapy allows for an up to 90% success rate for clomiphene treatment, if other cofactors for infertility have been ruled out.

Tamoxifen acts similarly to clomiphene (Steiner et al. 2005). The substance, which is used as an anti-estrogen as adjuvant therapy for breast cancer, is not licensed for infertility but is clearly less expensive (costs of therapy per cycle ca. 1 €) and is preferable to clomiphene for pharmacological considerations.

Letrozole is being used with increasing frequency for infertility treatments. An embryotoxic effect has been discussed, but could be excluded in a comparative study with clomiphene (Pundir et al. 2021).

Although the drug is not approved for this indication, the American College of Obstetricians & Gynecologists recommends it as the first choice for the treatment of women with anovulation in PCO syndrome.

Letrozole blocks the conversion of C-19 androgens to C-18 estrogens via inhibition of aromatase, which is an essential step in estrogen biosynthesis in ovaries and other tissues. Its use increases androgen production and decreases estrogen formation in all body tissues. If clomiphene is used to increase FSH secretion via blockade of the estrogen receptor in the hypothalamus and to increase estrogen production, letrozole is also administered with the aim of increasing FSH, although there is a reduction in estrogen production from ovaries and other tissues. Because of its special effects on androgen metabolism, this drug also interferes with follicular development and follicular atresia. The effect is very complex, as different receptors for FSH and androgens are formed depending on the degree of development and size of the follicle.

Like **anastrozole**, letrozole is a nonsteroidal aromatase inhibitor of the third generation. After a single dose, the half-life is 42 h. 2.5 mg reduces aromatase activity by more than 99%. Androgens play an important role in stimulating preantral follicles (diameter 0.2 mm) by increasing mitosis of granulosa cells and FSH receptor expression. When the follicles increase in size to 3–6 mm in diameter, the maximum of androgen receptors is reached. Thereafter, the number of androgen receptors decreases. Up to a follicle size of 7 mm, the preantral follicles produce androgens. Further growth of the follicles then goes back to the FSH increase with an increase in estrogen production. This increases the LH receptor count, which later allows ovulation to occur. From the differential effect of letrozole on very early follicular action, very early administration as early as day 1 of the cycle, as

opposed to clomiphene application, could be useful (Rose and Brown 2020). However, studies to confirm this concept are still pending, so letrozole at a dosage of 2.5–7.5 mg is currently still used similarly to clomiphene from day 3 to day 7 of the cycle. A shortening of the therapy to only one treatment day could be considered (Martin 2020).

40.2.4.18 hMG and FSH

If treatment with clomiphene does not result in ovulation or conception, the next step is treatment with gonadotropins. Patients with hyperandrogenemia have a poorer success rate than those with pure hypothalamic amenorrhea. Since patients with polycystic ovarian disease are very sensitive to hMG stimulation, this treatment form walks a fine line between the induction of ovulation and hyperstimulation with the risk of multiple pregnancy. After the introduction of purified FSH, it was hoped that the LH/FSH ratio could be corrected in order to achieve a better form of treatment. The clinical results to date do not, however, clearly show better results in the treatment with purified FSH (Bairdt and Howles 1994). One advantage of the new FSH products is that of possible subcutaneous injection. Uncontrolled studies show a higher conception rate and a lower rate of hyperstimulation.

40.2.4.19 GnRH Downregulation

Treatment with pure hMG and hCG stimulation often results in a premature LH peak with luteinization of the follicle. Some authors believe that this premature LH peak is the major cause for later miscarriage, which is often found in polycystic ovarian disease. Clinical data, however, are not clear, so that downregulation is not necessarily recommended in a treatment of polycystic ovarian disease with hMG and hCG.

40.2.4.20 Pulsatile GnRH Treatment (LutrePulse® System)

Large-scale studies in the 1980s showed that this form of treatment resulted in a relatively high pregnancy rate without the high risk of hyperstimulation. In patients resistant to clomiphene citrate, treatment with pulsatile GnRH resulted in a conception rate of 26% per cycle. The conception rate could be elevated to 38% with simultaneous downregulation followed by pulsatile GnRH treatment. The miscarriage rate of 38% was, however, also elevated (for review see Filicori 1994).

40.2.4.21 Ovarian Wedge Resection

If the abovementioned forms of treatment do not result in pregnancy, ovarian wedge resection may be considered in order to reduce androgen production in the ovarian stroma. Up to 90% of such patients show normal ovulatory cycles after surgery. About one-third of the successfully operated women, however, develop oligomenorrhea within the following year (Buttram Jr and Vaquero 1975). The conception rate is reduced to 1.8% per cycle, which may be due to adnexal adhesions after such an operation (Adashi et al. 1981). Microsurgical and endoscopic techniques seem to avoid adhesion formation by replacing wedge resection by thermocauterization, laser vaporization, and electrocoagulation. These treatments are very effective and presumably long-lasting. An advantage is that there is no increase in multiple pregnancy rate (Farquhar et al. 2007).

Oral Antidiabetic Medication. Studies were carried out with metformin and troglitazone to reduce insulin resistance. Androgen levels did indeed improve and ovulatory cycles were achieved. Systematic studies have been made especially with metformin. In Germany, this medication is not licensed for ovulation induction in PCOS. It is, however, inexpensive and seems not to be associated with fetal toxic effects so that "off-label use" is not problematic. An ovulation rate of 46% was achieved compared with placebo (24%). This corresponds approximately to the effectiveness of clomiphene (Lord et al. 2003). A direct 6-month comparative treatment with the mono-substances clomiphene versus metformin showed a significantly better pregnancy rate (22.5 vs. 7.2%; $n = 208$, $p < 0.001$) in the clomiphene group (Legro et al. 2007). A combination of both substances did not increase the pregnancy rate (Moll et al. 2006).

40.2.4.22 Reduced Body Weight and Follicular Maturation

Independent of the treatment modalities mentioned above, treatment of obese patients should be based on weight reduction. Even a relatively small weight reduction of 2–5% can achieve clear improvement of hyperandrogenemia, insulin resistance, and fertility (Moran et al. 2006) and is superior to oral antidiabetic medication.

Whereas the elevated body weight in many patients with polycystic ovarian disease propagates or even induces hormonal dysregulation, underweight patients also have a major risk factor for impaired follicular maturation and amenorrhea. A major proportion of the patients with hypothalamic amenorrhea fall into this group. This clinical entity is characterized by an inadequate pulsatile GnRH secretion. Diagnosis is one of exclusion in which other pituitary disorders must be ruled out. In addition to evident low body weight, a particularly stressful situation such as flight amenorrhea can induce hypothalamic dysregulation. Such patients show extremely low or non-measurable gonadotropin levels. Prolactin levels lie in the normal range, and the cone view of the sella shows no pathological changes. A **progesterone challenge test** with a transformation dose (such as **MPA gyn** 5 mg b.i.d. for 10 days) does not result in bleeding and thus demonstrates the absence of estrogen stimulus on the endometrium.

The most extreme form of low body weight amenorrhea is that of **anorexia nervosa**. The pure form of anorexia is

extremely rare in the infertility clinic, but minor forms may be seen more often in infertility patients.

In contrast to anorexia nervosa, which may have concurring central nervous dysregulation, **simple weight loss** may be a cause of impaired follicular maturation and may be readily overlooked. The endocrine imbalance, however, is comparable to that of anorexia. Hormone studies show low FSH and LH concentrations, elevated cortisol levels, normal prolactin, TSH, and L-thyroxine levels with low normal fT_3 and elevated reverse T_3 levels. Extreme weight loss causes the loss of sleep-associated episodic LH secretion, showing a pattern usually found in early puberty. Weight gain within 15% of ideal body weight may result in an improvement of the symptoms.

In addition to low body weight, **physical activity** may cause cyclic dysregulation. Many studies have shown menstrual dysregulation in athletes, especially in long-distance runners and ballet dancers. The percentage of amenorrhea is proportional to the weekly distance run and inversely proportional to body weight. Decreasing body weight results in a higher incidence of anovulatory cycles and a reduction in the quality of the luteal phase. The theoretic mechanism for impaired GnRH secretion is a change in estrogen metabolism, in which estradiol is converted to catechol estrogens, which seem to have anti-estrogenic properties.

Increased physical activity such as in running often results in a "**runners high**," which is thought to be the result of an increase in **endogenous opiates**. These endogenous opiates may cause an elevation of corticotropin-releasing hormone concentrations, which in turn reduce gonadotropin production. Hypothalamic GnRH production seems to be suppressed. In 49 of 66 patients with hypothalamic dysregulation of follicular maturation, treatment with 25 to 125 mg naltrexone daily resulted in a normal menstrual cycle. The pregnancy rate was almost equal to that of normal controls (Wildt et al. 1993). Alternatively, pulsatile GnRH treatment or hMG and hCG stimulation may be considered, but here again there is a risk of multiple pregnancy. First, however, body weight should be normalized.

40.2.4.23 Primary Ovarian Failure

In patients with amenorrhea primary ovarian failure must be ruled out. This can be done during the initial examination in which FSH and estradiol levels are measured. If a patient with a history of oligo-amenorrhea has elevated FSH levels and reduced estradiol concentrations, the diagnosis of primary ovarian failure can be formed. The FSH levels should lie at least two standard deviations above the mean in the follicular phase and should be confirmed by a second sample.

1% of all women have **precocious menopause** with ovarian failure beginning before the age of 35 years. The causes are usually unknown. Sometimes a chromosomal origin may be identified; other causes include autoimmune diseases, viral infections, previous chemotherapy, and/or radiation therapy.

Turner syndrome with the loss of one X chromosome is one of the most common human chromosome defects and is seen in one of 2500 live births. Typically, these patients show short stature and streak gonads. Ovarian dysfunction, however, shows extreme variations. An ultrasound study of 104 young Turner patients showed visually demonstrable ovaries in one-third of the patients (Massarano et al. 1989).

Many of these women had an incomplete deletion of the X chromosome. This explains case reports of pregnancy and live births before premature ovarian failure begins in women with X0 syndrome and partial X deletions.

Various other genetic abnormalities are associated with hypergonadotropic hypogonadism. They are, however, so rare in a normal infertility clinic that a routine genetic diagnostic check does not seem warranted.

In addition to chromosomal diseases, some genetic diseases, such as galactosemia, may cause premature ovarian failure.

Chemotherapy with antimetabolites and radiation therapy may impair ovarian function and should be identifiable in the patient's history.

It is not clear if other exogenous toxins may play a role in premature ovarian failure. Analogous to orchitis in men, it is assumed that oophoritis may occur after mumps. There are, however, only a few case reports, which do not allow a clear statement on this issue.

40.2.4.24 Autoimmune Diseases

There is some evidence that autoimmune antibodies may cause premature ovarian failure. Typical autoimmune diseases such as Hashimoto thyroiditis, Basedow's disease, Addison's disease, hyperthyroidism, juvenile diabetes, pernicious anemia, alopecia areata, vitiligo, and myasthenia gravis are associated with ovarian failure. Patients often have several autoimmune diseases simultaneously, which is known as the "polyglandular failure syndrome." The most common association is that of thyroidal disease and Addison's disease.

Circulating autoantibodies against ovarian stroma can be found in the serum of patients with primary ovarian failure. It is, however, not clear whether the antibodies are primary or secondary in nature. This is also true for cellular autoimmune disease in which lymphocyte infiltration of the ovaries can be found.

The fourth argument for an immunological cause for premature ovarian failure is the statistically significant correlation between human leukocyte antigens (HLAs) with some autoimmune diseases (for review, see Bakimer et al. 1994).

In addition to the abovementioned causes for hypergonadotropic hypogonadism, there are rare cases with defective FSH hormone receptors and others with biologically inactive gonadotropins. These are, however, not relevant in clinical routine (see review by Themmen 2005).

40.2.4.25 Treatment

After diagnosing premature ovarian failure, substitution with an estrogen and progesterone combination should be recommended. In rare cases, patients show remission and possibly may conceive (Shangold et al. 1977). A causal therapy is, however, not possible in infertility patients with hypergonadotropic hypogonadism. Oocyte and embryo donation are legally prohibited in Germany. In the USA, success rates between 22% and 50% are achieved with these procedures.

40.3 Infertility Due to Disturbances of Gamete Migration

After checking ovulatory function in female infertility, one must consider a disturbance in the passage of the oocyte and the sperm (see Fig. 40.13 for an overview) Possible alterations exist in the vagina, the cervix, the uterus, and the fallopian tubes, as well as the pelvic peritoneum.

40.3.1 Vagina and Cervix

Anatomical variations of the vagina, such as transverse and longitudinal septae, or even vaginal aplasia, are obvious. Changes in the vaginal environment may be another cause for infertility. The production of lactic acid through glycogenolysis gives the vagina a pH of 4–4.5 and thus represents a hostile environment for sperm. Physiological cervical mucus production at the time of ovulation creates the first prerequisite for passage of sperm into the genital tract.

Vaginal infections with increased discharge, a change in pH, and leukocyte transudation are detrimental to the motility and the ascent of sperm. The first clinical examination of the patient should thus include a wet mount of a vaginal smear in order to exclude the presence of pathogenic bacteria and leukocytes. A vaginal infection must also be ruled out if a pathological postcoital test has been diagnosed due to poor mucus despite good estrogenization. Bacterial, viral, and yeast infections, as well as trichomoniasis, should be consid-

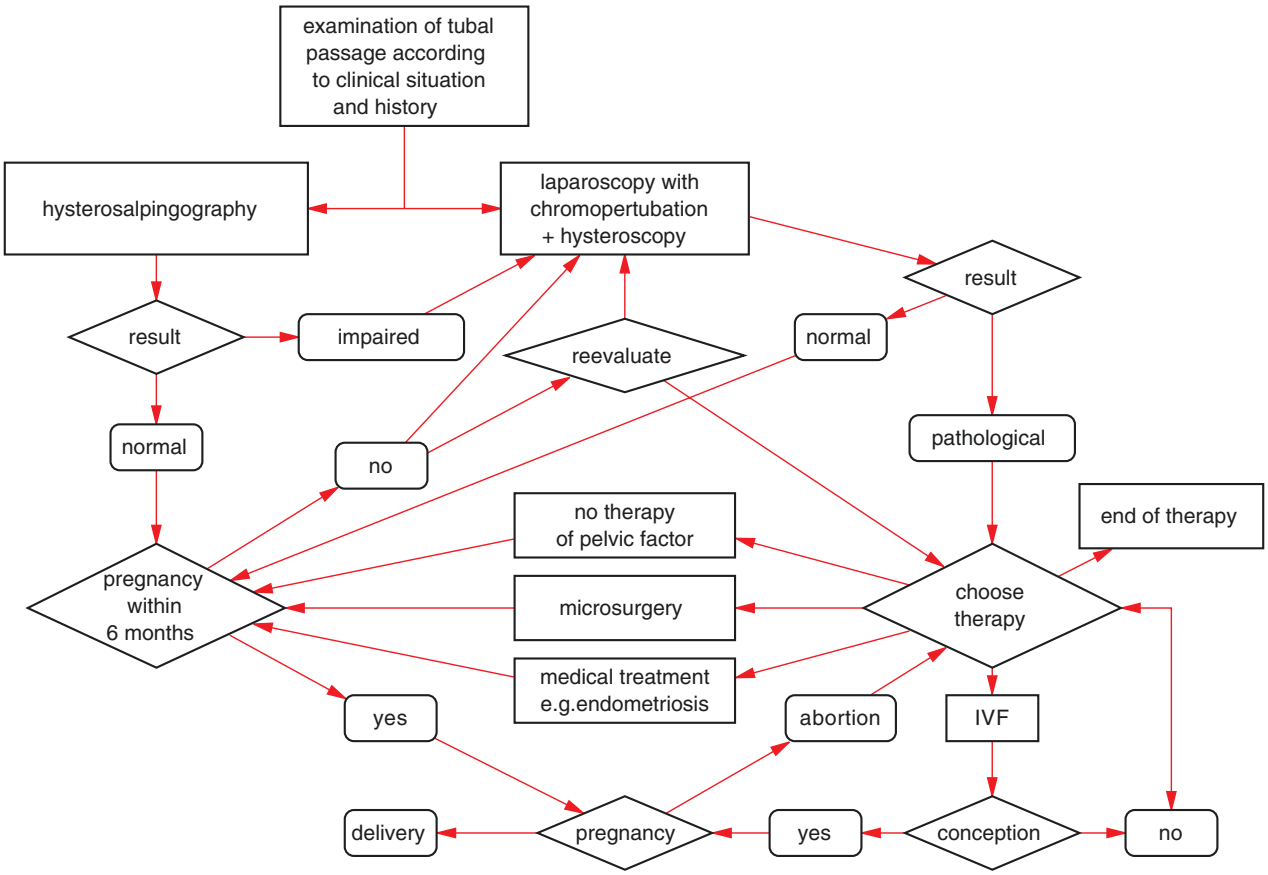


Fig. 40.13 Flow chart for the systematic **evaluation of the uterine–tubal passage.** Only main areas are shown to facilitate readability. For more details, see text

ered. The most common pathogens are gardnerella vaginalis, neisseria gonorrhea, chlamydia, mycoplasma, and ureaplasma. Viral infections may be due to herpes and cytomegaly infections. Candida albicans is the most common yeast infection.

All forms of vaginitis may include cervicitis, causing a change in cervical mucus pH, and may represent a cause of infertility. Treatment can be local or systemic depending on the pathogen isolated. Post-infectious changes in the cervix can result in a stenosis or in damage to the cervical crypts with poor mucus production. Cervical smears in infertile couples have shown a mycoplasma infection in 12%, and pathogenic aerobic bacteria in 31% of the patients examined (Eggert-Kruse et al. 1992). Cervical operations such as electrocoagulation, cryotherapy, laser vaporization, or conization may be an indication for intrauterine insemination, if cervical passage of sperm is not possible.

40.3.2 Anomalies of the Female Genital Tract

The embryonic origin of the urogenital system is complex and explains many of the congenital anomalies found. A disruption in one of the many fusion steps can result in either a partial or complete agenesis of particular ducts. Congenital anomalies often include the urinary system.

40.3.2.1 Uterus

The unilateral agenesis or aplasia of the Müllerian duct results in a **uterus unicornis**. 0.1–3% of all women have varying uterine anomalies due to disturbed Müllerian fusion. These vary from a uterus subseptate unicollis up to the double uterus, cervix, and vagina (NICE 2015). Surgical treatment should only be recommended after considering all other possible causes for infertility, since the coexistence of infertility and, for example, a septate uterus, must not be causal. A recent study of 151 women with septum resection compared to 106 women with septum but without surgery failed to demonstrate an improvement in pregnancy rate after septum resection (Rikken et al. 2020).

Acquired uterine anomalies include fibroids and adhesions. Uterine leiomyomata are found in 20–25% of all women of reproductive age. They are usually asymptomatic. These fibroids may be subserous or, more importantly, intramural, submucosal, and/or pedunculated, which may be causal for infertility. A review of 27 studies from 1982 to 1996 reported on pregnancy rates after myectomy. Nine studies were prospective (Vercellini et al. 1998); their combined pregnancy rates were 57% (95% confidence interval 48–65%). The median interval from surgery to pregnancy varied between 8 and 20 months. Unfortunately, no study compared results from surgery with non-intervention (simple waiting), so that any improvement in probability of conception following myectomy cannot be definitely ascertained.

40.3.2.2 Fallopian Tubes

Congenital defects of the fallopian tubes are very rare, especially if the remaining organs are normal. In rare cases, one-sided aplasia of the ovary and uterine anomaly may be combined with a one-sided aplasia of the fallopian tube. The cause for the rare case of missing parts of the fallopian tube is not known.

The fallopian tube can be morphologically divided into four segments:

1. The **interstitial segment** is the part of the fallopian tube, which passes through the uterine wall and is surrounded by myometrium. The endosalpinx consists of secretory and ciliated cells, which form longitudinal folds in the form of a star. The diameter is about 400 μm.
2. The **isthmic portion** of the tube consists of the proximal third of the fallopian tube. The endosalpinx here has more secretory cells than ciliated cells.
3. The **ampulla** consists of the other two-thirds of the lateral fallopian tube. The cilia beat toward the uterus and are important for the transport of the oocyte and embryo. Surgical procedures on the isthmic portion of the fallopian tubes still allow for pregnancy rates of about 80%. A resection of the ampulla, however, results in a clearly lower pregnancy rate. The major portion of ectopic pregnancies are found in the ampulla, since fertilization and early embryogenic development occur in this segment of the tube.
4. The i**nfundibulum** is fringed by the fimbriae, which have close contact with the ovary. The ostium accepts the oocyte during ovulation by means of a mechanism yet unknown. Pathological changes due to infectious diseases, adhesions, or operations result in a high infertility rate and are difficult to treat by surgery.

40.3.3 Physiology of Tubal Function

Tubal motility, secretion, and ciliary activity are the prerequisites for the complex process of sperm transport, capacitation, oocyte retrieval, fertilization, zygote development, and the transport of the oocytes and of the zygote (also see Chap. 3). The Fallopian tube shows a continuous and complex pattern of spontaneous contractions. The relationship between the contractions and gamete transport is yet unclear. In con-

trast to the gastrointestinal tract, no regular peristaltic movements occur over a greater distance. Individual segments simultaneously contract in opposing directions and short distances. Oocyte transport is thus discontinuous with movement toward and away from the uterus. Since the movement is primarily toward the uterus, the oocyte finally ends up at the endometrium of the uterine cavity.

The significance of tubal contractility for sperm transport is not yet clear. One would assume that tubal passage is the result of sperm motility. After insemination, however, sperm can be found 5 min. later in the peritoneal cavity, so that tubal contraction must play a major role in the primary transport of the sperm. A further assumption is that contractions of the uterus and the tubes during sexual intercourse play a role in rapid sperm transport. Sperm reaching the fallopian tube in this manner, however, do not seem to be important for fertilization.

Tubal ciliary movement is directed toward the uterus, so that the transport of sperm in the other direction must be due to other mechanisms. The secretory activity of the fallopian tube may play a role in spermal transport. Tubal secretion is greatest at time of ovulation, and these viscous glycoproteins seem to attenuate ciliary movement in such a manner so that the sperm are able to pass the fallopian tube on their own, as they do in the midcyclic cervical mucus. After mucus secretion decreases, ciliary movement toward the uterus is reactivated so that the fertilized oocytes can be transported in the correct direction.

Tubal mucus production is estrogen-stimulated and inhibited by progestins. A number of proteins found in the tubal secretion, which cannot be found in serum, seem to be of importance for reproductive function.

In contrast to the testis, female gametes are transported through an open system. After leaving the ovarian surface, the oocyte must pass through the peritoneal cavity in order to reach the fallopian tube. Microscopy studies in humans have shown that at the time of ovulation the fimbriae and the ostium of the tube are directly in contact with the ovary. This is due to contractions of the fallopian tube, the mesosalpinx, and the fimbriae. Interestingly, pregnancy is possible with only one ovary and a contralateral fallopian tube.

Surgical salpingostomy in a case of a fimbrial adhesion results in only a low pregnancy rate, which shows the importance of this pick-up mechanism.

Only a very small portion of the large number of sperm deposited intravaginally during intercourse reach the fallopian tube (see Fig. 40.14). Only optimally formed and motile sperm are able to pass the filtration and selection mechanisms of the female tract in order to reach the ampulla and thus the location of fertilization.

Fertilization probably occurs in the distal ampulla. The sperm seem to survive 24 to 48 h in the female genital tract, with some reports of survival up to 96 h. The oocyte can, however, only be fertilized for 24 h. The fertilized oocyte begins division in the fallopian tube. The human zygote has no nourishment reservoir nor constant cell contact during transport. The developing zygote must thus be maintained by the tubal secretions until it is implanted in the uterine cavity after about 1 week.

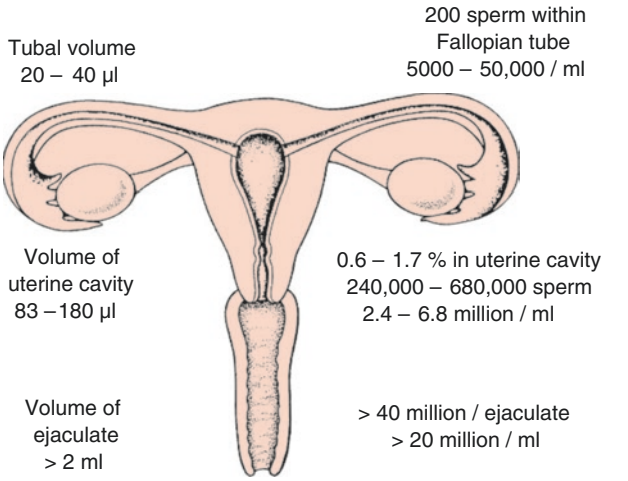


Fig. 40.14 Volume of different compartments of the female genital tract and resulting concentrations of sperm after vaginal ejaculation

40.3.4 Diseases of the Fallopian Tubes

The complex processes of tubal passage show that disturbances in this area can cause infertility. Congenital anomalies are not as important as those that are acquired, such as adhesions caused by endometriosis or infections. Chlamydia infection is of primary importance.

40.3.4.1 Salpingitis

Nineteen percent of all women with salpingitis demonstrate an infection with chlamydia (Hoyme et al. 1988). Four percent of patients who underwent laparoscopy owing to tubal infertility showed culture-positive chlamydia. Since many pelvic infections have no or only unspecific symptoms, tubal passage should be checked in all patients who do not achieve pregnancy after 6 months, despite good luteal functions.

> Especially in couples with clearly reduced male parameters, one should always consider further invasive diagnostic measures in the female patient.

The probability of tubal infertility in couples with secondary sterility rises by a factor of 7.5 with a history of venereal disease. A patient with appendectomy has a risk of 4.7 for tubal factor infertility when compared to controls. A history

of a previous extrauterine pregnancy increases the risk 21-fold, and a history of prior pelvic inflammatory disease increases the risk by a factor of 32 (Thonneau et al. 1993).

A tubal occlusion in the fimbrial part can cause a sausage-like swelling of the tube with reduction in wall structure (saktosalpinx). A bilateral salpingectomy prior to planned IVF doubles the probability of pregnancy. Whether one-sided obstruction should also be corrected cannot be concluded with certainty at the present time (Johnson et al. 2004).

40.3.5 Diagnostic Tests for Uterine and Tubal Patency

The available tests for tubal patency are as follows:

1. Insufflation of fallopian tube with CO_2
2. Hysterosalpingography
3. Chromopertubation and diagnostic laparoscopy

40.3.5.1 Tubal Insufflation

This method was first described in the 1920s and is unreliable. No clear information can be obtained whether the tubal obstruction lies on one or on both sides. Direct comparison with laparoscopy shows great discrepancy of the results. This method should not even be used as a screening procedure.

40.3.5.2 Hysterosalpingography

Hysterosalpingography (HSG) is a much more important procedure. It can be performed on an outpatient basis after giving an analgesic such as indomethacin 1–2 h prior. The examination of the tubes is performed via a fluoroscope. In rare cases, tubal spasm may lead to misdiagnosis of a tubal occlusion. Spasmolytic drugs such as 1–2 mL butylscopolamine bromide i.v. should be tried.

The ovarian radiation dose varies between 1.7 mGy for a 100 mm fluoroscopic film and 2.8 mCy for a 24 × 30 cm spot film. This procedure is usually timed 2–5 days after menstrual bleeding in order to minimize the disturbance of a possible early pregnancy.

The risk of this radiographic diagnostic procedure is that of infection, which has a probability of 1%. Identification of patients at risk has been tried through blood sampling of leukocytes, C-reactive protein, and sedimentation rates. Our own studies, however, have shown that this has a poor specificity and sensitivity (Knuth et al. 1982).

Some authors recommend prophylactic antibiotics such as 200 mg doxycycline 2 days before the procedure followed by 5 days of 100 mg. There are no uniform recommendations, and we do not use prophylactic antibiotic treatment.

During hysterosalpingography, the uterine cavity is easily delineable and septae, polyps, and submucosal fibroids are readily seen. In order to evaluate the significance of this diagnostic procedure, consequent pregnancy rates must be correlated to previous results. A normal hysterosalpingography was followed by pregnancy in 46% of cases with donor insemination. A uterine anomaly or defect with bilateral tubal patency resulted in a pregnancy rate of 34%. Normal uterine anatomy with unilateral tubal occlusion resulted in a pregnancy rate of 40% after donor insemination (Stovall et al. 1992).

The relatively high rate of conception despite pathological hysterosalpingography examination is probably due to optimal male factors during donor insemination. This must be considered if the male factor in the infertile couple is also impaired.

When comparing hysterosalpingography with laparoscopy and chromopertubation, a larger discrepancy is found. The predictions from HSG thus do not seem to be optimal. A positive correlation of the data is found in 62% of 104 patients examined (Adelusi et al. 1995). If the results of hysterosalpingography are normal, in 96% of the cases laparoscopy and chromopertubation are also normal. If the HSG results are unclear and lesions are expected, laparoscopy is only correlated in 63% of cases. Suspicious lesions in the fallopian tubes are only correlated in 54%, so that Opsahl et al. (1993) deduced that women with a normal HSG who do not conceive must undergo diagnostic laparoscopy. The interpretation of HSG can be optimized through a special catheter system, which perfuses each fallopian tube individually. In this procedure, it is possible to measure the pressure necessary to achieve patency in each individual tube. Normal tubes require a pressure of 429 ± 376 mmHg, and pathological tubes require a pressure of 957 ± 445 mmHg (Gleicher et al. 1992). A similar procedure can be performed less invasively by duplex sonography (Allahbadia 1992). Newly developed sonographic contrast substances allow the HSG to be performed entirely by ultrasound (Degenhardt et al. 1995; Maheux-Lacroix et al. 2014; Ludwin et al. 2017). This new technique may provide a simple screening procedure in the infertility clinic, although one must consider its limits in the evaluation of tubal patency.

If HSG is performed in the classical manner, an oil suspension contrast medium should be used since this results in a higher pregnancy rates compared to cases in which a hydrophilic agent is used (Fang et al. 2018). A small risk of granuloma formation following oily media must, however, be considered.

In conclusion, sonohysterosalpingography can be recommended as a screening procedure for tubal patency in women with an uneventful prior history, negative chlamydia tests, and period of non-conception less than 1 year. If these requirements are not fulfilled, laparoscopy and chromopertubation should be performed.

40.3.5.3 Hysteroscopy and Laparoscopy

The standard method for the diagnosis of pelvic conditions and tubal patency is laparoscopy combined with chromopertubation and hysteroscopy. In contrast to hysterosalpingography, endometriosis adhesions, fallopian tubal thinning, and ovarian alterations can be optimally diagnosed by an experienced surgeon.

Hysteroscopy. This procedure can be performed without general anesthesia if no consequent laparoscopy is planned. A rigid or flexible endoscope is passed through the cervical os into the uterine cavity, which, since it is usually collapsed, must be distended by a medium. High molecular dextrin, CO_2, or 5% dextrose in water may be used. A separate instrument channel may be used, through which forceps, electrocautery, or laser devices can be passed.

Laparoscopy. Laparoscopy is usually performed under general anesthesia with intubation. After subumbilical incision and insufflation of the peritoneal cavity with CO_2, the laparoscopic trocar and the laparoscope are introduced. The risks of this procedure are that of gastrointestinal or great vessel perforation. This occurs in about 0.1% of all examinations. After 1 or 2 additional incisions are made for the introduction further instruments, dye can be injected through the intrauterine cannula. Direct observation attests to tubal patency if dye spills from the ostium.

Since intra-abdominal adhesions can be associated with intratubal pathology without clear disturbance of tubal patency, new diagnostic measures have been implemented. Falloscopy is performed with extremely fine endoscopes through which the internal fallopian tube can be inspected during hysteroscopy or laparoscopy. These endoscopes have a special optical system in order to prevent damage to the fallopian mucosa (Venezia et al. 1993).

40.3.6 Treatment

Depending on the pathology of the fallopian tube, either adhesiolysis, fimbrioplasty, salpingostomy, or anastomosis may be considered. **Adhesiolysis** includes all microsurgical procedures, which are performed to regain the mobility of the fallopian tube and the ovary. **Fimbriolysis** is performed; in order to mobilize fimbriae, peritoneal defects are carefully sutured. **Fimbrioplasty** is the reconstruction of the existing fimbriae in a partially or totally occluded oviduct. In the case of total occlusion, the ostium is opened and the serosa is everted in order to form a fimbriatum. If ampullary occlusion requires the resection of a part of the tube, a **salpingoneostomy** is performed. Anastomosis can be performed at every segment of the fallopian tube.

Today, the results of microsurgical procedures must be compared to those of IVF programs. One must consider, however, that **microsurgery** may be a curative form of treatment for a patient, whereas **IVF** is only a temporary form of treatment. Pregnancy rates after surgical salpingolysis, fimbriolysis, and ovariolysis lie between 39 and 69%. In a summary statistic, a median pregnancy rate of 40% has been calculated after conventional techniques.

Post-inflammatory tubal diseases, which require fimbrioplasty and salpingostomy, however, have poorer results. A study at the University of Münster showed a pregnancy rate of only 25% in such patients (for overview, see Schneider and Karbowski 1989). When deciding on the form of treatment, the availability of an experienced surgeon must be considered. The patient should be referred to a specialized microsurgical department, if an immediate IVF procedure is not to be recommended.

40.4 Endometriosis

Although it can be argued whether hysterosalpingography or laparoscopy is the better diagnostic test to check for tubal patency, laparoscopy is absolutely necessary in order to diagnose peritoneal endometriosis.

40.4.1 Pathogenesis and Epidemiology

Endometriosis is a disorder in which normal endometrium or endometrium-like tissue is found in locations other than the uterine cavity. These ectopic tissues may be involved in pathological and normal cyclic changes in the endometrium. The true cause of endometriosis, which can cause infertility, is unknown and many theories have been discussed. To date, the accepted theory is that endometriosis occurs through implantation of individual endometrial cells and endometrium fragments, which are passed through the fallopian tubes into the peritoneal cavity in retrograde fashion during menstruation. It is assumed that these cells then implant on the peritoneal surfaces and grow into endometrial lesions. After further proliferation, these tissue particles grow to allow macroscopic visualization. A review of this frequent disease is found in Dunselman et al. (2014).

Endometriosis is found in 5–10% of all women of reproductive age. Its prevalence, however, is much higher in women with infertility problems.

40.4.2 Symptoms

The cardinal symptoms of endometriosis are pelvic pain, dysfunctional bleeding, and infertility. The pathophysiology by which endometriosis causes infertility is multifactorial. This

includes mechanical factors, toxic derivatives in the peritoneal fluid, and immunological and hormonal disturbances.

The main cause for infertility, however, lies in the adhesions found in progressive stages of endometriosis. Peritubal and periovarian adhesions inhibit the interaction between the uterine tube and the ovary, which is important at the time of ovulation. Proximal tubal occlusion is often found. Although endometriosis is often present on the ovaries, small lesions probably do not play a role in fertility. Larger endometriosis or endometriomas can invade stroma of the ovary, destroying normal tissue. These are called "chocolate cysts."

40.4.3 Pathophysiology

Physiologically, the ovaries and the tubes are surrounded by peritoneal fluid, the volume of which rises to about 20 mL at the time of ovulation. The regulation of peritoneal fluid volume is disturbed in women with endometriosis. Embryotoxic substances are also found in the peritoneal fluid of these women. After incubating sperm with the peritoneal fluid of endometriosis patients, one sees a reduction in motility in computerized analysis when compared to controls. Presumably, prostaglandins and macrophages inhibit sperm motility.

An increase in prostaglandin concentrations in the peritoneal fluid probably alters the contractility of the tubes, so that the transport of sperm, the oocyte, and the zygote are modified. Nonsteroidal antiphlogistics, however, fail to improve infertility. Normally, the peritoneal fluid contains 0.5–2 million leukocytes per mL, the major portion of which are macrophages. If endometriosis is found on the peritoneum, there is a proportional rise in macrophages. One cannot, however, discern whether these changes in the peritoneal fluid are the result or a cause of endometriosis.

Patients with endometriosis have increased levels of IgM, IgG, and IgA when compared to normal controls. An inverse correlation of the immunoglobulins with the severeness of endometriosis is, however, found. Some authors presume that endometriosis is an autoimmune disease, but this has yet to be proven.

Diagnosis of endometriosis is possible by direct visual observation or histological examination during laparoscopy or laparotomy. One must be careful to search the entire pelvic peritoneum systematically in order to find discrete endometriotic lesions. It is not enough to just perform one quick diagnostic view through the endoscope and to let the patient go home with the diagnosis of endometriosis after visualizing a few topical brown-black lesions. Often only histological probes will show the typical endometriotic alterations.

Table 40.1 Revised classification of endometriosis

	Endometriosis	<1 cm	1–3 cm	>3 cm
Peritoneum	Superficial	1	2	4
	Deep	2	4	6
Ovary right	Superficial	1	2	4
	Deep	4	16	20
Ovary left	Superficial	1	2	4
	Deep	4	16	20
Post Culdesac	**Partial**		**Complete**	
Obliteration	4		40	
	Adhesions	**<1/3**	**1/3–2/3**	**>2/3**
Ovary right	Filmy	1	2	4
	Dense	4	8	16
Ovary left	Filmy	1	2	4
	Dense	4	8	16
Tube right	Filmy	1	2	4
	Dense	4[a]	8[a]	16
Tube left	Filmy	1	2	4
	Dense	4[a]	8[a]	16

Stage I (minimal) = 1–5, stage II (mild) = 6–15, stage III (moderate) = 16–40, stage IV (severe) > 40

[a]If the fimbriated end of the fallopian tube is completely enclosed, change the point assignment to 16

40.4.4 Staging of Endometriosis

Once a diagnosis of endometriosis has been made, a staging classification must be performed (Engel et al. 2015). This is usually done with the help of the revised classification of the American Fertility Society (Table 40.1).

Since laparoscopy is an invasive test, another screening method for endometriosis has been sought for in the past. The most commonly used parameter is the tumor marker Ca 12-5, which is a membrane antigen of epithelial origin, which can be measured in the serum. Ca 12-5 levels are elevated in women with epithelial ovarian cancer and other neoplasias of the pelvis. Slight elevations are also found in women with endometriosis. The sensitivity and specificity of this parameter are, however, so low that women with slight endometriosis may have a normal level.

40.4.5 Treatment

Since the pathophysiology of the disease has not been fully clarified, there is no ideal treatment. If adhesion formation with impaired tubal function, large endometriomas, or occluded fallopian tubes can be diagnosed, the mainstay of therapy will be microsurgery, in some cases after prior drug treatment. In other cases, it is not clear whether a specific treatment of endometriosis is even necessary. The form of treatment recommended must be tailored to the individual situation of the infertile couple (Thaler et al. 2008).

Typical pharmacological treatment forms include

- Progestins
- GnRH analogs
- Aromatase inhibitors

The estrogen and progestin combination, which has been used in the past to achieve pseudo-pregnancy, is only used in rare cases (Schweppe 1995; Macer and Taylor 2012).

40.4.5.1 GnRH Analogs

GnRH analogs are a good option for inducing reversible pseudo-menopause in which a hypogonadotropic and hypoestrogenic condition equivalent to temporary castration is achieved. The principle of this form of treatment has been discussed in another chapter of this book. The mechanism in females is similar to that in males, in which downregulation results in the desired low estradiol concentration. The success of a treatment is directly proportional to the extent of estrogen reduction.

GnRH agonists probably do not have a direct effect on the ovaries. Successful treatment is due solely to estrogen deficiency. Evaluation of pregnancy rates after GnRH and danazol therapy shows similar results. Because of the hyperandrogenemic side effects of danazol therapy, it is likely that GnRH treatment will be recommended.

The main **side effect of GnRH agonist** treatment is that of profound hypoestrogenemia including flushes, vaginal dryness, reduced libido, and, at times, depression. There is no influence on plasma lipoproteins. During a 6-month treatment course, a reduction in bone mass can be reversible after conclusion of therapy.

40.4.5.2 Aromatase Inhibitors

Studies by Zeitoun and Bulun (1999) show an abnormal expression of aromatase in endometrial lesions, so that a systemic reduction in estrogen supply will be of little effect. Here, aromatase inhibitors could provide innovative therapeutic possibilities (Shippen and West Jr. 2004).

40.4.5.3 Surgical Treatment

Surgical treatment is indicated in infertile patients with extreme adhesion formation, enlarged ovaries, and pain, which is not ameliorated after pharmacological treatment, and in older patients for whom lengthy medical treatment seems inappropriate. Individualization of therapy is important, since, as in microsurgical treatment, the experience of the available surgeon has considerable significance for successful treatment.

40.4.5.4 IVF

In vitro fertilization may offer the only possibility for patients with extreme endometriosis, although results are poor in this patient group (Simon et al. 1994). Another study, however, showed pregnancy rates of 29% per transfer in endometriosis patients compared to 25% in controls (Dmowski et al. 1995).

40.5 Sperm Antibodies

If hormonal and clinical factors of female infertility have been ruled out, immunological causes are considered next.

The **mucosal immune system** represents the largest immunocompetent structure of the human body and includes the majority of endogenous antibody-producing plasma cells. Antibodies that are detected in blood or lymph samples usually belong to the IgG group, whereas antibodies found in secretions such as the cervical mucus are usually of the IgA group.

40.5.1 Pathophysiology

Although a sexually active female is exposed to a great number of potential antigens in the form of spermatozoa, the female immune system can usually not be activated in this manner. Different factors may play a role in this mechanism (Jones 1994). For example, nonspecific antibodies, which surround the spermatozoa in a protective fashion, deterring an immunological response, have been discussed (for review, see Marshburn and Kutteh 1994).

40.5.2 Antibody Testing

Sperm antibody tests usually depend on sperm agglutination and sperm immobilization by serum antibodies. The measurement of serum antibodies against spermatozoa is without clinical significance, however, since the levels are not correlated with those of genital secretions. ELISA measurements give greatly varying results, depending on which antigen is used from the wide range of spermatozoal proteins. This explains why research data are so contradictory.

The best method to determine antibodies in the female seems to be the indirect immunobead test. Donor sperm are incubated with the body fluid to be examined and then undergo multiple washings with a buffer. Antibodies against IgA, IgG, and, in some cases, IgM, which are bound on small latex particles, are added and then the number of donor spermatozoa with attached immunobeads is counted (WHO 2010).

Spermatozoal antibodies are found in about 2–3% of the general population. The possibility of causing an amplified immunological response to intrauterine insemination using a high number of spermatozoa could not be confirmed in any of several studies (Marshburn and Kutteh 1994).

Sperm antibodies in the cervical mucus are inversely correlated with the number of live sperm during the postcoital test. However, a positive correlation between circulating antibodies and pregnancy rates cannot be proven (Eggert-Kruse et al. 1989). In conclusion, the significance of female antibodies has yet to be determined. Serum antibody testing is decidedly not an integral part of routine screening of the infertile couple. If a pathological postcoital test is found despite good cervical factors, the cervical secretion should be tested for antibodies. This is also true for other cases of unexplained infertility.

40.5.3 Treatment

The detection of sperm antibodies in combination with protracted involuntary childlessness only indicates subfertility, since studies have shown that these antibodies do not absolutely impede spontaneous pregnancy. Different strategies have been recommended to optimize the probability of conception.

- The use of **condoms** for several months has been recommended in the past in order to inhibit boosting of the immunological response. Higher pregnancy rates have, however, not been achieved through this method.
- The results of **immune-suppressive treatment** with **glucocorticoids** vary. Since the significance of female antibodies is not clear, and the side effects of this treatment can be severe, it should not be recommended.
- Similarly **intrauterine insemination** does not seem to elevate pregnancy rates significantly. Clinical studies showed that after 6 months of intrauterine insemination, pregnancy rates were equal to those of patients without sperm antibodies who also had poor postcoital tests (42.4% vs. 40.5%) (Check et al. 1994). This shows that these antibodies do not play a very large role in infertility.

It is not clear if IVF outcome is influenced by female sperm antibodies. Some studies show poorer fertilization results in these cases. In a study of 2363 patients, no differences in the pregnancy rates could be found between women with or without sperm antibodies (Check et al. 1995).

40.6 Early Pregnancy Abnormalities

40.6.1 Implantation

After fertilization occurs in the ampulla of the fallopian tube, rapid cell division begins. Details of this development are described in Sect. 4.7. About 6 to 8 days after conception, the blastocyte is implanted in the uterine cavity, and hCG production can be measured as a sign of implantation. Once pregnancy has been established, the gynecologist will calculate gestational age, necessary for monitoring pregnancy, and the estimated date of birth. Using Nägele's rule, the birth date can be determined by subtracting 3 months from the date of the last menstrual period while adding 7 days to the first day of the last menstruation. This rule is based on a normal 28-day cycle, and if ovulation occurs prior to or later than the 14th day, the EDD must be corrected accordingly. Deviation from the accepted conventions of calculating gestational age can cause considerable misunderstandings and incorrect evaluation of early pregnancy.

40.6.2 Pregnancy Loss

Unfortunately, the goal of infertility treatment for the unintentionally childless couple is not yet achieved with conception or implantation, as many couples subsequently have an abortion. This proportion is considerable if one takes into account the very early proportion of miscarriages. If implantation failure, preclinical abortions, and clinical abortions are included, only 30% of all conceptions are said to result in birth (Macklon et al. 2002; ESHRE Guideline 2017).

Approximately 15% of all pregnancies end in clinically recognizable loss of fetal tissue between the fourth and 20th week of pregnancy. However, the true proportion of post-implantation abortions is likely to be closer to 50% of conceptions, as many abortions occur undetected between the second and fourth week of pregnancy and are only detectable by early hCG testing, which is not routinely done.

About 80% of spontaneous abortions occur during the first 12 weeks of pregnancy. 70% of these abortions are due to a chromosomal disorder. While younger women around 20 years of age have a miscarriage rate of 12%, the number rises to around 26% in older women at the end of their reproductive life. The probability of miscarriage increases significantly with delayed implantation (Wilcox et al. 1999). If the ultrasound shows a normal ovary, the probability of a later miscarriage decreases to about 5%. However, if there have been several miscarriages in the past, this rate increases by a factor of four to five (van Leeuwen et al. 1993).

> A single miscarriage is, as the above figures document, not a rare event. Given the frequency, a miscarriage can almost be considered a normal occurrence event in a woman's reproductive life.

The pathological occurrence of a habitual miscarriage therefore requires, according to the definition, at least three

successive miscarriages. For a review of possible causes and treatments, see Rai and Regan (2006).

Chemotherapy or radiotherapy on the male side includes the possibility of chromosomal changes, so that increased miscarriage rates are conceivable in addition to potential fetal malformations (Meistrich 1993).

40.7 Idiopathic Infertility

If all tests described above show normal results, and no causes for infertility can be found, this is called "idiopathic infertility." This must be differentiated from the so-called idiopathic infertility of the male, which is characterized by an unexplained reduction of semen quality. In 10–15% of infertile couples, no clear cause can be defined (Crosignani et al. 1993). Prognosis of this condition is correlated with the couple's age and the length of infertility (see above).

In contrast to the normal monthly fecundity rate of 25%, couples with idiopathic infertility have a conception rate of 1.5–3% per cycle. Patience and time alone will result in pregnancy in about 60% of the couples with idiopathic infertility (Verkauf 1983). As a rule, no treatment should be performed if a diagnosis cannot be made. Empirical studies, however, have shown that methods of **assisted reproduction** result in higher pregnancy rates in couples with unexplained infertility. If a couple with idiopathic infertility does not achieve a pregnancy within 3 years, stimulation with hMG in combination with intrauterine insemination may be considered. If a conception is not achieved within 4–6 months, IVF may be performed. The results of this therapy may also be diagnostic in nature. If fertilization occurs and pregnancy does not follow, another trial of conservative therapy with hMG stimulation may be justified. Following this strategy, a cumulative pregnancy rate of 40% is achieved after 6 cycles with superovulation or after 3 cycles of IVF (Simon and Laufer 1993; Romanski et al. 2020).

40.8 Prospects and Conclusion

If, despite all therapeutic efforts, delivery of a baby cannot be achieved, concluding treatment is a major step. Determining to end treatment signifies a failure for the couple and the physician and represents a psychological burden for everyone involved. In their despair, many couples may look for treatment by another physician and undergo the entire procedure a second time.

Couples must be counseled so that they can plan the rest of their lives accordingly. Psychological counseling should be an integral part not only during treatment, but also at its conclusion.

Further possibilities such as heterologous insemination or adoption must be discussed with the couple carefully and with empathy.

Key Points

- In the diagnosis of unfulfilled desire to have children, the patient is not the woman or the man but the couple as a whole!
- Female age influences the probability of conception to a great extent with a decreasing conception rate as early as after the age of 25.
- A higher frequency of coitus before ovulation increases the probability of conception.
- One day before ovulation is the optimal date for conception with intercourse in the spontaneous cycle.
- Unfulfilled desire for a child over a longer period of time can lead to a psychological stress reaction.
- Psychological strain and stress factors, on the other hand, tend not to explain a failure to conceive.
- Environmental factors, noxious substances, and electromagnetic fields are generally not clear causes of failure to conceive.
- Abstinence from nicotine and normalization of body weight are important factors for optimizing conception.
- In addition to checking the tubal passage, clarification of the cycle function is at the center of diagnostics in cases of unfulfilled desire for offspring.
- Examination of estradiol and progesterone in the middle of the luteal phase is an essential step in the evaluation of luteal function.
- If luteal quality is limited, further hormone tests, especially of prolactin and the gonadotropins, are important.
- Determination of androgens and AMH in the context of a suspected PCO round off the hormone diagnostics in addition to thyroid diagnostics.
- The measurement of AMH provides important information in cases of suspected PCO and indications of premature ovarian insufficiency.
- Gravidity must always be considered in cases of amenorrhea. A ß-hCG measurement provides clarification in suspicious cases.
- Sperm–mucus incompatibility should be ruled out by a morning postcoital test shortly before ovulation after evening intercourse.
- Vaginal sonographic examination is an indispensable part of diagnosis and therapy and, in addition to detecting anatomical disorders in the genital area, serves to monitor follicular growth and the endometrium.

- Examination of the tubal passage by sonographic tube imaging with contrast medium (sonoHSG) can take place prior to radiological imaging and possibly replace it.
- Definitive clarification of the uterine, tubal, and peritoneal conditions, however, can only be obtained by laparoscopy or hysteroscopy.
- This examination is also indicated to exclude endometriosis.
- Forms of therapy for a disorder of follicle maturation include measures to stimulate follicle maturation with clomiphene, letrozole, and hMG/hCG.
- For hyperprolactinemia, bromocriptine, lisuride, metergoline, and cabergoline may be considered.
- An adequate supply of thyroid hormones must be ensured.
- In the case of hyperandrogenemia with an adrenal component, low-dose dexamethasone administration may be useful.
- Surgical measures should be discussed in cases of endometriosis and uterine pathologies. Tubal surgery must be weighed against the possibilities of IVF treatment.
- If no cause for a failure to conceive can be found, this is referred to as idiopathic infertility.
- A single miscarriage can be considered a physiological event. If more than two consecutive abortions are complained of, genetic clarification of the couple is indicated.

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Assisted Reproduction

41

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Abstract

Assisted reproductive technology offers many possibilities for the treatment of male fertility disorders. Inseminations may be carried out, if the male partner is unable to ejaculate. However, the value of inseminations in the presence of reduced semen quality remains unproven. Instead, intracytoplasmic sperm injection (ICSI) is the first-choice treatment option in cases of

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severely reduced male fertility. ICSI can even be carried out successfully with spermatozoa directly retrieved from biopsied testicular tissue. In the complete absence of spermatogenesis, treatment has to rely on donated semen. All assisted reproductive technology is feasible only in the presence of at least one mature oocyte. Hormonal stimulation of the ovaries is often needed to enhance the likelihood of pregnancy, as several oocytes may be harvested that may be fertilized and grow to competent embryos. In the presence of several embryos, cryopreservation of embryos not only permits the elective transfer of fewer embryos per treatment trial, but also more than one pregnancy over time.

41.1 Assisted Reproduction as a Treatment of Infertility

Ideally, medicine should deal with the childlessness of each infertile couple in two subsequent steps. First, the most frequent factors interfering with natural conception should be identified through a comprehensive diagnostic work-up of both partners. Second, targeted treatment of each identified factor contributing to the infertility should be conceived and initiated. In principle, it is appropriate to first treat all potential causes contributing to the couple's infertility, as such a therapeutic strategy will be effective over a longer period of time until complete fulfillment of family planning. However, the multiple interactions of the many variables interacting between **both partners** are complex and cannot always be identified by taking the patients' histories and within the framework of a diagnostic evaluation of both partners.

In addition, neither in gynecology nor in andrology can many of the identified causes of infertility be overcome by a targeted treatment of the causes. Instead, a number of therapeutic strategies have been developed to deal with childlessness exclusively as a **symptom** without removing the cause of infertility itself. The term **medically assisted reproduction (MAR)** includes various forms of insemination, whereas **assisted reproduction technology (ART)** exclusively focuses on **in vitro fertilization** and related techniques (Zegers-Hochschild et al. 2017). The short-term efficacy of ART, which has been a disadvantage in the past, can now be compensated, as oocytes and embryos can be stored frozen without damage. In the case of an unsuccessful treatment or when the couple wishes to have more children, those oocytes or embryos can be thawed and transferred without necessarily having to go through another round of ART.

Today, the majority of infertile couples can be treated with one of the many techniques of assisted reproduction currently available. Since the introduction of **intracytoplasmic sperm injection (ICSI)** (Palermo et al. 1992), virtually all cases of **male infertility** can be potentially overcome and such relief is now being offered worldwide (de Mouzon et al. 2020). The wide availability of treatments, together with an increasing demand due to the present-day demographic trend toward delayed childbirth, has caused an impressive uprise in the number of treatments performed with ART in Europe and elsewhere. This leads to a significant proportion of newborn babies—varying between 1.5% to 6.5% in various countries—originating from ART (Fig. 41.1).

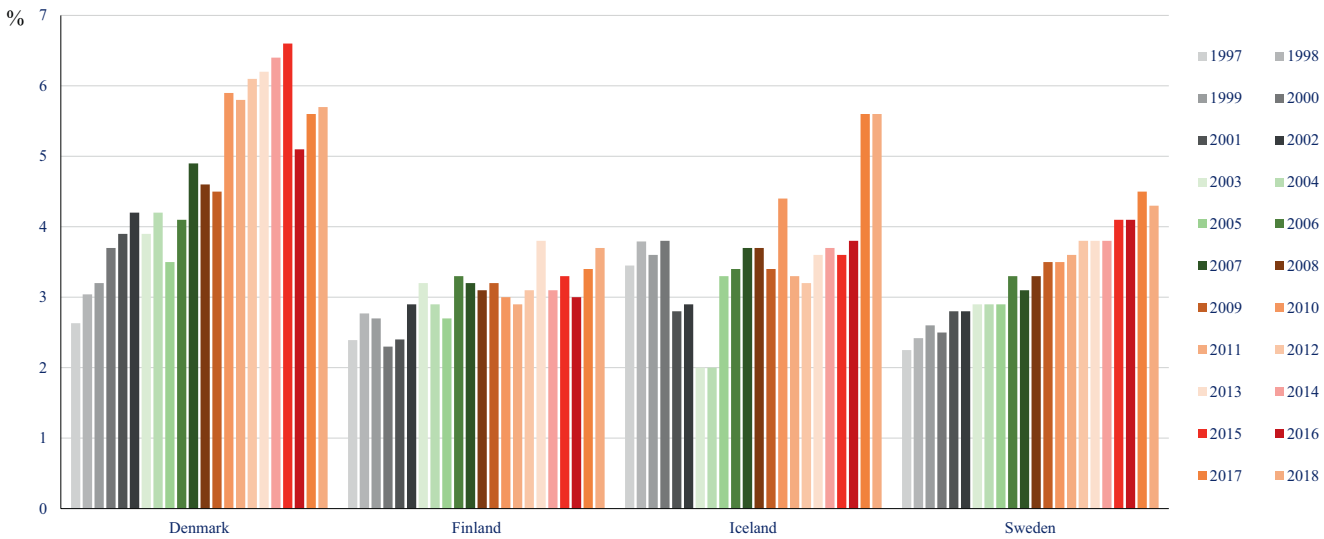


Fig. 41.1 Since 1997, the use of ART has been recorded in various European countries together with the registration of pregnancy outcomes including neonatal birth weight. These data are collected by the European IVF-Monitoring Consortium (EIM) of the European Society of Human Reproduction and Embryology (ESHRE). Since 1997 to 2018, only four European countries were able to achieve full coverage of data on birth numbers. The number of births of neonates born after ART is set in relationship to the overall births of neonates (expressed in %). Over this entire observation period, there has been a more or less progressive rise in the number of children conceived with ART

41.2 Methods of Medically Assisted Reproduction for the Treatment of Male Infertility

Medically assisted reproduction (MAR) comprises various treatment modalities, which can be differentiated by more or less stringent control over the processes of fertilization and by the degree of invasiveness. For the purpose of this chapter, the various forms of assisted fertilization are subdivided into five major groups:

- **Insemination** denotes a group of techniques in which the spermatozoa are transferred into the female partner by various methods. Insemination aims at increasing the concentration of fertile spermatozoa in the vicinity of the oocyte within the Fallopian tube around the time of ovulation. Insemination can be carried out with the semen of the husband or partner, or with donated semen.
- In **in vitro fertilization** (IVF), fertilization and early embryonic development do not take place in the fallopian tube of the female partner. Before ovulation, the oocyte is removed from the mature follicle and cultured in a specifically designed artificial environment. At the latest 6 days after oocyte retrieval, the developing embryo is replaced transcervically into the uterine cavity of the recipient, where the embryo can become implanted.
- In **intracytoplasmic sperm injection** (ICSI), a single vital spermatozoon is placed into the cytoplasm of the oocyte by penetrating it, through all its investments, with a micropipette. ICSI enables successful fertilizations not only despite severe abnormalities in motility, morphology, and capacitation process of the injected spermatozoa, but also despite functional and anatomical anomalies of the testes, the epididymis, and the efferent ducts (see also Chap. 1). ICSI is also used to fertilize thawed oocytes, because the process of cryopreservation and thawing renders the surrounding zona pellucida exceedingly hard.
- **Cryopreservation of gametes, of oocytes in the pronucleate stage, and of embryos** has become a crucial part of medically assisted reproduction. Cryostorage helps to preserve the fertility potential of individuals diagnosed with cancer facing chemotherapy or radiation, which may destroy their gonads. Cryopreservation also helps to reduce the number of embryos that need to be transferred per treatment trial. It also allows couples to have more than one child without having to go through repeated treatments or to delay pregnancy over time, when natural aging might reduce their fertility chances.
- In **semen donation,** semen of a fertile donor is used to overcome the absence of functional gametes in the husband or partner. In theory, all available techniques of assisted fertilization can be used to achieve fertilization with donated semen. However, the practice of semen donation is usually regulated by country-specific legislations, requiring maximal safety of both the recipient and the offspring through optimal diagnostic screening of the donor and through cryostorage of donated semen before use. Semen donation can be either anonymous or non-anonymous (see Chap. 50).

41.3 Likelihood of Natural Conception after Long-Term Infertility

Any treatment of infertility is effective only if its pregnancy rate exceeds the conception rate in the untreated infertile couple. The balance between the **natural conception** rate and the likelihood of a pregnancy that can be achieved with any fertility treatment may change over time, as each failed treatment is followed by lower chances in the case of another treatment trial, whereas the likelihood of natural conception remains similar during the same time interval (McLernon et al. 2014). The natural conception rate is determined by various factors, the most important being the age of the female partner (Chua et al. 2020) and duration of infertility (Léridon and Spira 1984). It is during the first three ovulatory cycles after inception of unprotected sexual intercourse that the highest conception rates per cycle are observed: 20–25%. In subsequent 6-month intervals, the success rates of naturally occurring pregnancies steadily diminish (van Eekelen et al. 2017).

Based on large data sets, a number of models have been computed to compare the likelihood of natural conception with that of ART (Collins et al. 1995; Hunault et al. 2004). These models have been validated in different populations (van der Steeg et al. 2007; Leijdekkers et al. 2018) and in different institutions (Tjon-Kon-Fat et al. 2013). The mathematics of the validation processes have been discussed in detail (Altman and Royston 2000). However, the influential parameters for the prediction of natural conception have changed over time, such as the results of postcoital testing, the assessment of progressive sperm motility, and patency testing of the fallopian tubes (Hunault et al. 2004). Instead of the **postcoital test**, one single conventional semen analysis is now considered sufficient for the prediction of the chances of natural conception (Leushuis et al. 2014). The assessment of ovarian reserve has also undergone major modifications, such as basal FSH levels now usually being replaced by antral follicle count in ultrasound or by **AMH**-serum level

testing (Bensdorp et al. 2017). Because both the diagnostic possibilities in fertility testing are constantly being improved and as the efficacy and safety of existing treatment standards may change over time, the entrance criteria for the active management of fertility treatment must be reviewed and adapted.

Today, the tremendous improvement in survival of cryopreserved embryos has resulted in the transfer of only one selected embryo per treatment trial. As a result, the outcome of ART is now assessed based on **cumulative outcome rates** instead of singe treatment trial outcome rates.

41.4 Medically Assisted Reproduction (MAR)

41.4.1 Intrauterine Insemination (IUI)

Insemination, most particularly **intravaginal** or **intracervical insemination**, is the oldest form of medically assisted reproduction. This treatment modality was originally used in infertile men unable to deposit their semen in the vagina but has also been used to overcome the cervical barrier of the uterus. The very first documented insemination was performed around 1770 by John Hunter, who treated a couple that had remained infertile for many years because of hypospadias in the husband (Ombelet and Van Robays 2015). In this form of insemination, freshly collected semen is deposited directly into the upper portion of the vagina. No preparation of the semen is needed. Other forms of insemination, such as intrauterine, direct intraperitoneal (DIPI), and intrafollicular insemination require preparation of the semen, in which the seminal plasma is separated from the spermatozoa (Fig. 41.2).

The introduction of assisted reproductive technology has helped to replace intravaginal and intracervical inseminations by **intrauterine insemination (IUI)**, because the laboratory requirements for semen preparation needed for IVF were beneficial to IUI (Kerin et al. 1984). For IUI, 0.2–0.5 mL of prepared semen, aspirated into a 1-mL syringe connected to a long fine and flexible catheter, is inserted through the cervical canal into the uterine cavity. It is deposited there without touching the fundus of the uterine cavity. IUI must optimally be carried out immediately before ovulation. With IUI, the barrier of the cervix can be overcome. This should result in higher numbers of spermatozoa that may finally reach the ampulla of the fallopian tubes, in which in vivo fertilization may take place. IUI is performed easily and requires minimal equipment, and **complications** are rarely observed. Infections after IUI, such as endometritis or pelvic inflammatory disease, are extremely rare events.

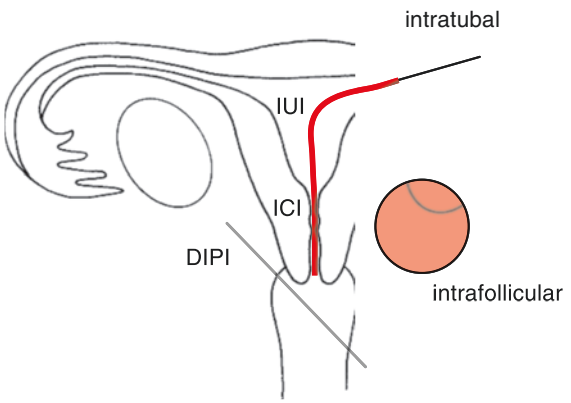


Fig. 41.2 Schematic presentation of the different modalities of insemination that have been developed over time: intracervical (ICI), intrauterine (IUI), intratubal, direct intraperitoneal (DIPI), and intrafollicular

One particular modification of intrauterine insemination involves the **perfusion** of a larger volume of a prepared sperm sample (4 mL) over a period of 4 min into the uterine cavity. Perfusion of washed spermatozoa is thought to ensure the attachment of an even larger number of spermatozoa to the mucosa of the fallopian tube (Kahn et al. 1993). The superiority of this particular method over conventional IUI has not been demonstrated (Cantineau et al. 2009).

Originally, IUI was primarily used to support infertile couples in whom sperm was not able to enter the cervix due to **ejaculatory problems**, due to stenosis of the cervical canal or because of impermeability of the cervical mucus. IUI is also commonly used in semen donation. Apart from being less invasive and costly than IVF or ICSI, the therapeutic efficacy of IUI as an isolated procedure has been questioned repeatedly. Although IUI is frequently used as a first-line therapy in both male and unexplained infertility, the evidence that substantiates the efficacy of IUI as compared with timed intercourse is lacking (Bensdorp et al. 2007; Bhattacharya et al. 2008; Cohlen et al. 2018).

Because IUI as a stand-alone procedure lacks efficacy, it is often combined with hormonal stimulation of ovarian function to increase the number of follicles that may become ovulated (Fig. 41.3). The most effective **ovarian stimulation** protocol is based on administration of exogenous gonadotropins (Cohlen et al. 1998; Guzick et al. 1999; van Rumste et al. 2008; Farquhar et al. 2018; Bordewijk et al. 2019). In contrast, clomiphene citrate has been demonstrated to be less effective (Bhattacharya et al. 2008). Registry data from the European IVF monitoring consortium have demonstrated live birth rates after IUI between 7.8% and 8.9% with partner's semen and between 12.0% and 12.4% with donated semen (De Geyter et al. 2020; European IVF-monitoring Consortium [EIM] for the European Society of Human Reproduction and Embryology [ESHRE] 2020). The disadvantage of combining IUI with some form of hormonal ovarian stimulation is the higher incidence of multiple births (van Rumste et al. 2008), although the incidence can be lowered by allowing the ovu-

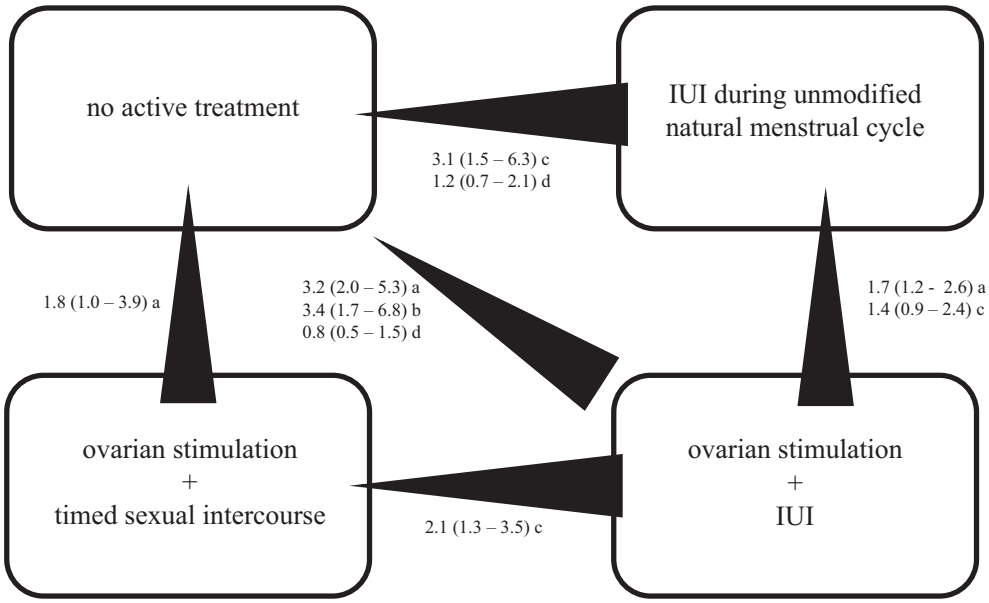


Fig. 41.3 Odds ratios with the respective 95% confidence intervals of the live birth rates after IUI, as compared with natural conception or after ovarian stimulation. Results of several prospective randomized studies ([**a**] Guzick et al. 1999; [**b**] Cohlen et al. 1998; [**c**] Farquhar et al. 2018; [**d**] Bhattacharya et al. 2008), performed in couples diagnosed with either male or unexplained infertility

lation of only one or two follicles. In the presence of more preovulatory follicles, the treatment cycle may be canceled (van Rumste et al. 2008), or supernumerary ovarian follicles may be aspirated and discarded transvaginally under ultrasound guidance without compromising the ultimate pregnancy rate (De Geyter et al. 1996, 1998).

For the estimation of the expected pregnancy rate conventional semen analysis has been shown to be unreliable (Mankus et al. 2019). The number of progressive motile spermatozoa after semen preparation may be more predictive for the outcome of IUI: The higher these numbers, the higher the chances of pregnancy. Many institutions consider less than 1 million motile spermatozoa as prognostically unfavorable in IUI (Ombelet et al. 2003; van Weert et al. 2004). Currently, there is no consensus concerning the entrance criteria for IUI. The method of semen preparation itself has no impact on the outcome of IUI (Boomsma et al. 2019).

41.4.2 In Vitro Fertilization (IVF)

Laboratory-centered and intensive treatment modalities, such as **IVF and ICSI,** and the **cryopreservation and thawing of oocytes and embryos** are ~~usually~~ designated as **"assisted reproductive techniques" (ART)** (Zegers-Hochschild et al. 2009).

During early development of IVF, the culture conditions in the laboratory, most particularly the constituents of the culture media for the embryos, were still suboptimal. In order to compensate for these deficiencies, a number of techniques were proposed to avoid or to shorten the culture of gametes and embryos (Fig. 41.4). This explains the emergence of now obsolete interventions, such as gamete intrafallopian transfer **(GIFT)**, in which, after hormonal stimulation

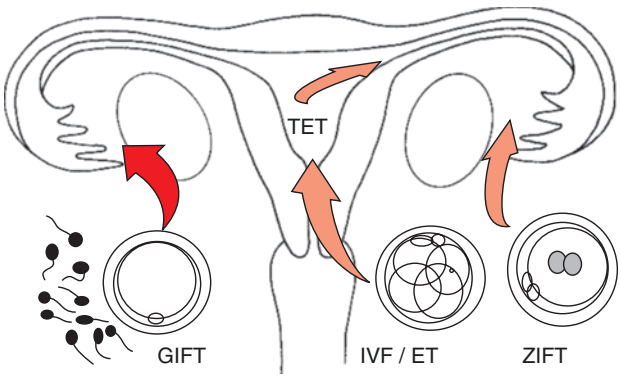


Fig. 41.4 Graphic presentation of the various organ sites, to which gametes, zygotes, or embryos can be transferred

of the ovaries, oocytes were recovered and immediately replaced together with capacitating spermatozoa into the fallopian tubes. Similar techniques were intrafallopian transfer of zygotes (**ZIFT**) and the intrafallopian transfer of embryos (so-called tubal embryo transfer, **TET**). Current ART has rendered all these former techniques obsolete.

The basic hallmark of IVF, distinguishing it from earlier procedures of medically assisted reproduction, is that the physiological process of fertilization takes place **outside the body of the patient** in an artificial environment. For this purpose, one or more oocytes are recovered from **preovulatory ovarian follicles** and subsequently co-incubated in a complex culture medium, designed to mimic the tubal fluidic milieu. The culture conditions must be strictly controlled: The temperature, the osmolarity, and the pH of the culture medium must constantly be maintained at the physiological level. Motile spermatozoa are then separated from immotile spermatozoa, non-spermatozoal cells, and seminal plasma. Subsequently, a defined number of motile sperma-

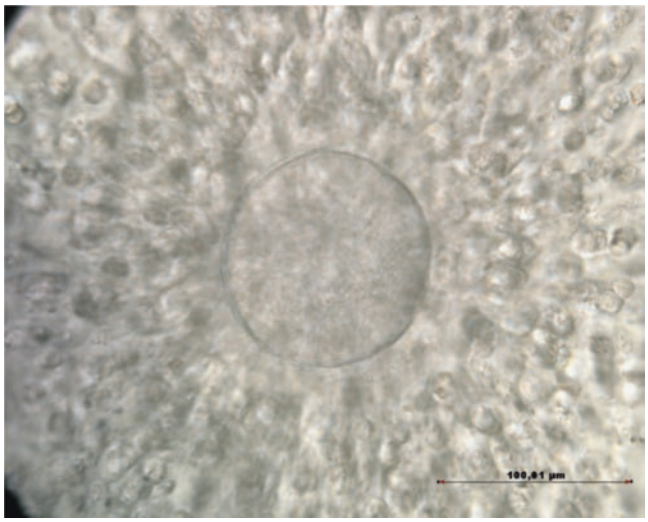


Fig. 41.5 Mature oocyte, surrounded by the corona radiata and the cumulus oophorus. During conventional IVF, prepared spermatozoa are added to the mature oocyte. Prior to ICSI, by removing the surrounding coronal and cumulus cells with the enzyme hyaluronidase and mechanically the oocyte is denuded

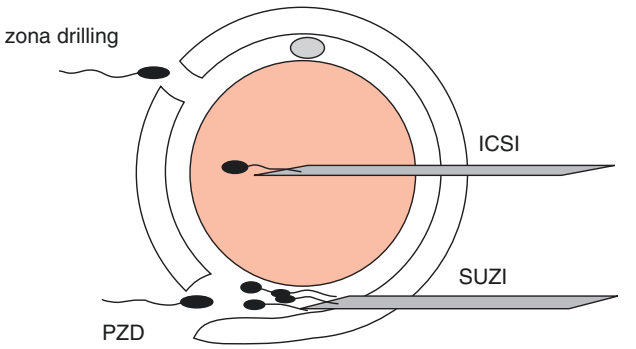


Fig. 41.6 Graphic presentation of the various forms of micro-assisted fertilization that were developed over time: zona drilling, partial zona dissection (PDZ), subzonal sperm injection (SUZI), and intracytoplasmic sperm injection (ICSI)

tozoa are then co-incubated with an oocyte (Fig. 41.5). A short coincubation period of 1 to 2 h is now recommended (Zhang et al. 2013). One day later, successful penetration of one single spermatozoon can be identified by visualizing two pronuclei in the cytoplasm of the oocyte on the heated stage of an inverted light microscope. The fusion of both pronuclei (syngamy) will occur a few hours later. After syngamy, the embryo will start to cleave. Early embryonic development takes place within the boundaries of the zona pellucida. After 4 days, the cells will start to merge, forming an **embryo** at the compaction stage. This is the **morula** stage of development. After 5 or 6 days, the embryo will start to accumulate liquid in the center of the cell mass, forming a blastocoel. At the **blastocyst stage,** the embryo will expand and ultimately hatch from the boundaries of the zona pellucida.

Embryos may either be placed into the uterine cavity for nidation in the endometrium or be cryopreserved. In principle, embryo transfer can be performed at any stage, such as the pronucleate, early cleavage, or the blastocyst.

IVF was originally introduced for the treatment of **tubal infertility** caused either by occlusion of the fallopian tubes or by impairment of their function (Steptoe and Edwards 1978). IVF is now also widely used in unexplained infertility. In cases with male infertility, ICSI is now the first choice of treatment.

Although IVF can be performed with some success in the unstimulated menstrual cycle, it has become widespread practice to carry out this treatment in combination with **hormonal stimulation of the ovaries**, so that **multiple oocytes** become available for IVF. Ovarian stimulation allows the collection of a set of mature oocytes. The improved pregnancy rate and the shorter time-to-pregnancy offered by ovarian hyperstimulation in IVF mainly stem from the identification of a set of **embryos with excellent developmental potential**, usually based on morphological characteristics. If more than one embryo with good developmental capacity is available, it may be cryopreserved for later transfer, thereby extending fertility potential over a prolonged time period, sometimes even beyond the age of reproduction.

Occasionally, fertilization fails to occur during conventional IVF. The dilemma of performing conventional IVF or, alternatively, ICSI, still largely relies on **conventional semen analysis**. Whereas the decision to perform ICSI rather than IVF is rather obvious in severe male infertility, the choice may become difficult in borderline cases. This uncertainty in the prognostic assessment of sperm function in conventional IVF motivates many institutions to exclusively perform ICSI in all cases. Globally, treatment numbers with ICSI are on the rise, with currently approximately 75% of all treatments being carried out with ICSI, at the expense of IVF (approximately 25%, De Geyter et al. 2020).

41.4.3 Intracytoplasmic Sperm Injection (ICSI)

After its initial successful use in tubal infertility, experience with conventional IVF in cases with abnormal semen quality proved much less promising. As it was thought that the main barrier for fertilization would be both the zona pellucida and the vitelline membrane of the oocyte, a number of techniques were conceived to stepwise circumvent these barriers (Fig. 41.6). First, **zona drilling** was developed to facilitate sperm penetration by boring a small hole into the zona pellucida. Later, the penetration of motile spermatozoa was enhanced further by **partial dissection of the zona pellucida** (PZD). Subsequently, one or more spermatozoa were inserted into the perivitelline space under the zona pellucida

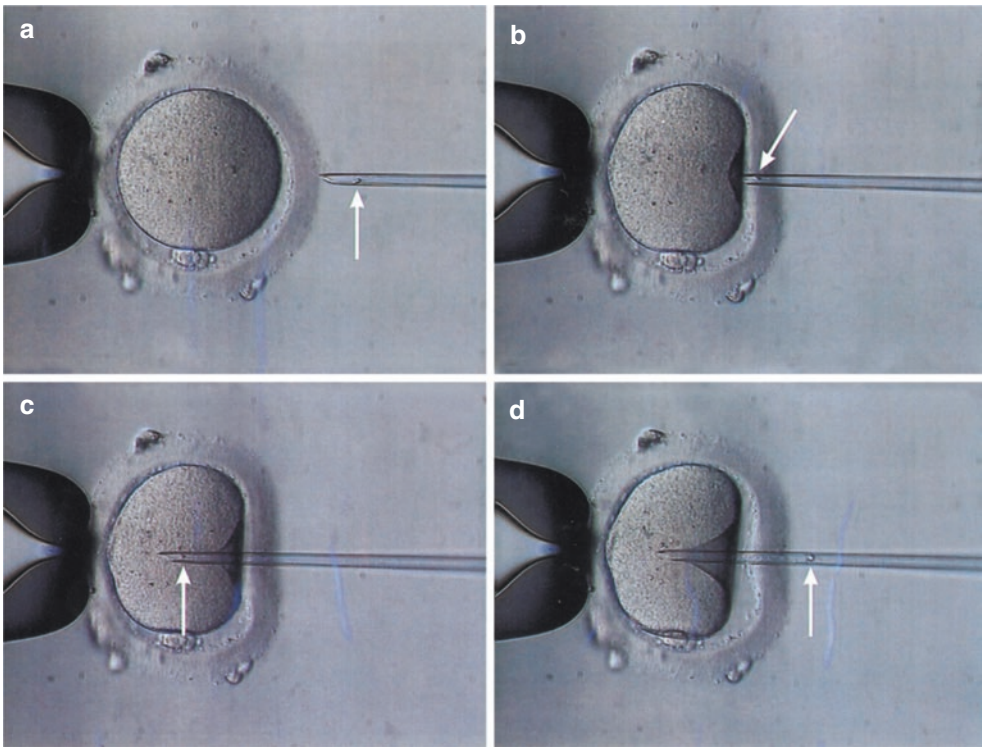


Fig. 41.7 Graphic display of the stepwise microinjection of a spermatozoon into the cytoplasm of a mature oocyte in the metaphase II stage. The **polar body** is visible at 6 h. (**a**) The immobilized spermatozoon is visible in the lumen of the micropipette (arrow). (**b**) Penetration of the micropipette through the zona pellucida. (**c**) Apposition of the micropipette through the vitelline membrane of the oocyte. (**d**) Aspiration of the sometimes very elastic vitelline membrane together with some cytoplasm into the micropipette will facilitate the rupture of the membrane before introduction of the spermatozoon into the oocyte

(SUZI). The collective experience of the different methods of micro-assisted fertilization demonstrated that only those techniques in which all fertilization steps were bypassed were the most successful. This was finally achieved with intracytoplasmic sperm injection, in which one single spermatozoon is injected through the investments of the oocyte directly into its cytoplasm.

Preclinical experience of microinjection of human spermatozoa into the cytoplasm of hamster oocytes was initially reported by Lanzendorf et al. (1988). The first report of a pregnancy with **ICSI** in an infertile couple (Palermo et al. 1992) was soon followed by the global application of this technique in routine assisted reproduction, clearly demonstrating the high therapeutic efficacy of ICSI in the treatment of couples suffering from **severe male infertility**.

In ICSI, the spermatozoon is first immobilized mechanically and then aspirated with its head toward the tip of the micropipette. Selection of the better spermatozoon may be performed based on either membrane integrity or progressive motility. Vitality of the sperm is usually identified based on motility. In single cases, in which none of the spermatozoa display motility, viable sperm may also be selected based on membrane integrity. In such a situation, the swelling test is used, in which the sperm is first transferred to a hypo-osmolar solution. The swelling spermatozoa are those that have an intact membrane and can subsequently be used in ICSI.

The oocyte is aspirated onto the tip of a holding pipette and will be held fixed in a position, in which the **polar body** is visible at its upper or lower pole (Fig. 41.7). As such, damage of the **meiotic spindle** is thought to be avoided during the microinjection, because the latter usually resides close to the polar body (Baca and Zamboni 1967; Rienzi et al. 2003). During the microinjection process, first the zona pellucida and then the vitelline membrane are penetrated with a beveled micropipette holding a single spermatozoon. To ascertain the rupture of the oocyte's membrane prior to placement of the spermatozoon into the cytoplasm and to activate the oocyte, the vitelline membrane together with some cytoplasm is aspirated into the micropipette. After withdrawal of the micropipette, the vitelline membrane closes rapidly and no trace of the process is seen within minutes after the procedure.

To facilitate capture of the sometimes rapidly moving spermatozoon into the micropipette, the prepared spermatozoa may be incubated in culture medium supplemented with the macromolecule **polyvinylpyrrolidone** (PVP). PVP will raise the viscosity of the culture medium, in which the spermatozoa are incubated, facilitating its capture for ICSI. The concentration of PVP should not exceed 5% (Ding et al. 2020).

Individual spermatozoa are immobilized by touching them with the micropipette, thereby opening their membrane. The oocyte can only be activated if cytoplasmic factors (such as the **sperm-specific phospholipase Cζ, PCLζ,** Saleh et al. 2020) are liberated from the spermatozoon's equatorial segment into the injected oocyte. After a few

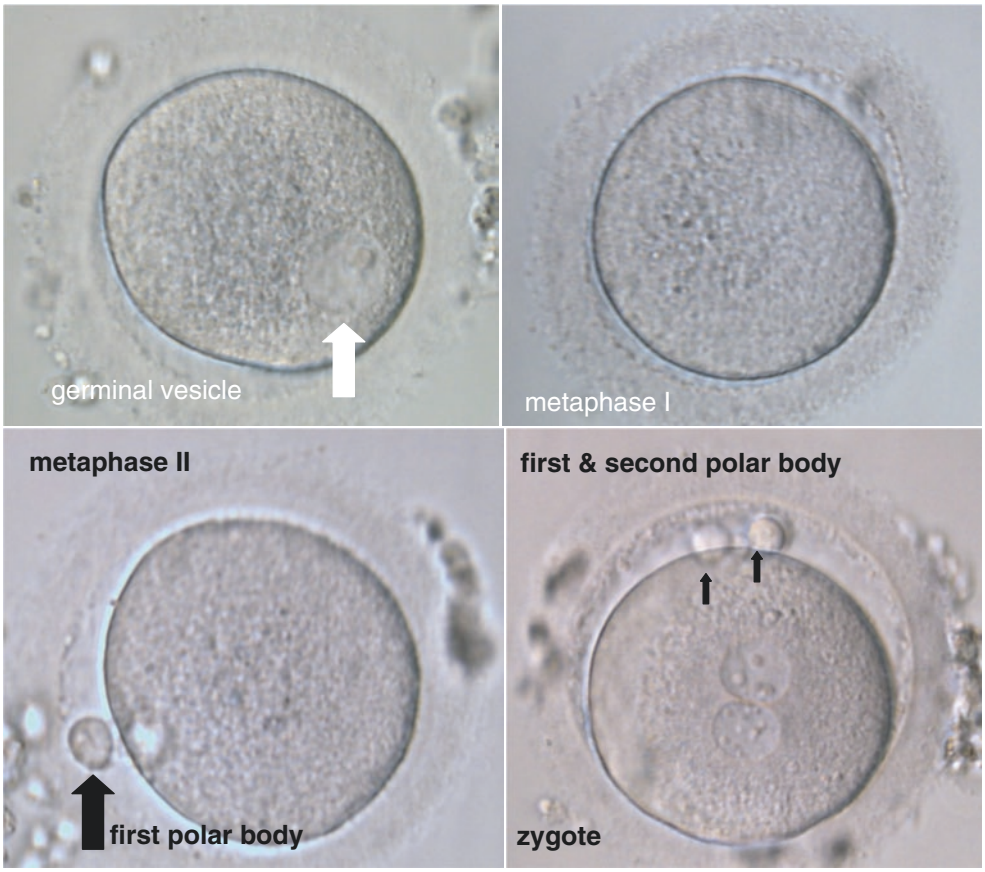


Fig. 41.8 Light microscopic images of an immature, germinal vesicle stage oocyte (surrounded by the zona pellucida), of an immature oocyte in the metaphase I stage (without polar body), of a mature oocyte in the metaphase II stage (with polar body, see arrow) and of a zygote (with two extruded polar bodies (arrows) and two pronuclei within the cytoplasm), less than 24 h after ICSI

hours, these compounds induce a pulsatile release of calcium ions from the subcortical depots of the oocytes, which lasts approximately 30 min and which is essential for oocyte activation. Indeed, failure of fertilization in ICSI seems to be caused chiefly by lack of activation of the oocyte despite successful injection of a spermatozoon into the cytoplasm (Flaherty et al. 1998).

Among the most important requirements for successful fertilization of oocytes in ICSI is the correct meiotic status of the oocyte (Fig. 41.8). Successful fertilization can only take place in the oocyte at the **metaphase II** stage, which is characterized by the presence of the first polar body in the perivitelline space. At the metaphase II stage of meiosis, the oocyte's chromosomes have become arranged at the metaphase plate at the equatorial plane of the oocyte. The chromosomes are firmly attached to the meiotic spindle. Reuptake of the meiosis is the result of the LH peak or of the administration of human chorionic gonadotropin (hCG).

Before ICSI, the oocytes have to be freed of the surrounding cells of the corona radiata and the cumulus oophorus using enzymatic digestion with hyaluronidase and mechanically as well. This denudation of the oocytes allows visual assessment of the meiotic maturity status of the oocyte. The timing between denudation of the freshly collected oocytes and their injection does not affect their developmental potential (Maggiulli et al. 2020), although a longer time interval between oocyte collection and denudation (more than 4 h) may lead to higher pregnancy rates (Carvalho et al. 2020). Normally, approximately 85% of the harvested oocytes are in the metaphase II stage.

Metaphase I oocytes are identified by light microscopy by the absence of an extruded polar body (Fig. 41.8). A significant number of oocytes, approximately 12%, may still reside in the metaphase I stage at the moment of collection. In many of the metaphase I oocytes, the first polar body will become extruded during incubation in vitro and can be used subsequently for ICSI. However, the fertilization rate of those in vitro matured oocytes is significantly reduced and both the quality of embryo development and their implantation rates have been demonstrated to be lower (De Vos et al. 1999; Shu et al. 2007). In some cases, the extension of the time interval between the administration of HCG and the collection of the oocytes from the conventional 36 to 39 to 40 h may be beneficial to reduce the number of immature oocytes (Raziel et al. 2006) (see Chap. 3).

Some of the harvested **oocytes** may be very **immature** and reside in the germinal vesicle stage (Fig. 41.8). Although these oocytes have some potential of maturation in vitro, the degree of activation and the developmental potential have

been demonstrated to be very low (Avci et al. 2020). It is therefore not recommended to use oocytes in the germinal vesicle stage for treatment purposes.

Intracytoplasmic sperm injection by far exceeds all other forms of medically assisted reproduction in the apparent absence of any correlation between sperm (dys)function and the fertilization rate and in the limited number of sperm needed for successful reproduction.

The success rate of ICSI in male infertile couples is not limited by the conventional semen characteristics such as concentration, progressive motility, or percentage normal morphology, nor by the degree of physiological maturation in the testis and the epididymis, nor by the exhibition of capacitation by the spermatozoa. The high efficacy of ICSI greatly has widely extended the spectrum of treatable abnormalities in male fertility.

ICSI has been applied successfully under highly diverse conditions with abnormal sperm function:

- Severe forms of idiopathic male infertility, such as severe **hypergonadotropic oligoasthenoteratozoospermia**, in which occasionally only few motile spermatozoa can be recovered from the ejaculate.
- Organic causes of male infertility in which the absence or loss of function of various anatomic parts of the male genital tract causes infertility: **obstructive azoospermia** with sperm recovered from the epididymis or from a testicular biopsy.
- In men suffering of severe infertility and **hypergonadotropic azoospermia,** single spermatozoa can be recovered from testicular tissue. Several biopsies from different sites in both testes are needed in order to retrieve a sufficient number of spermatozoa, which can be stored frozen, so that repetition of the testicular biopsy can be avoided.
- The presence of **anti-sperm antibodies** in male immunological infertility is an indication for ICSI. Anti-sperm antibodies may interfere with fertilization as they cover the membrane of sperm, thereby preventing attachment to the investments of the oocyte, or due to complete agglutination of all spermatozoa, or due to cytotoxicity causing premature death of all spermatozoa.
- In **necrozoospermia,** all spermatozoa in the seminal plasma have undergone apoptosis due to degenerative processes, such as chronic infection of the afferent ducts. In necrozoospermia, the spermatozoa have not only lost their motility, but also their vitality. In such cases, ICSI can be carried out successfully with spermatozoa retrieved upstream, i.e., through testicular biopsy.

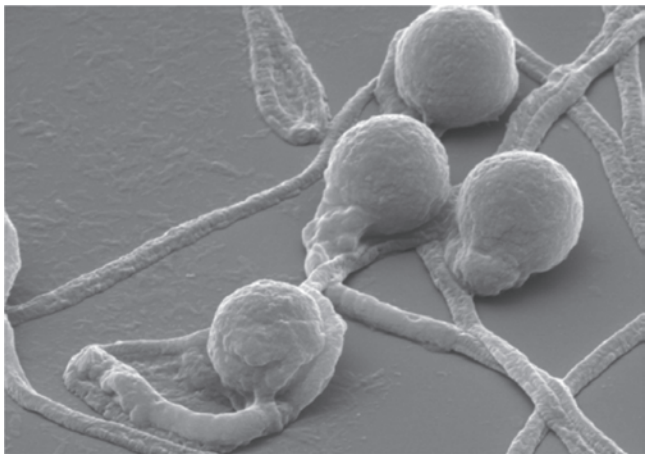


Fig. 41.9 Electrone microscopic image of globozoospermia

- Rare and sporadic forms of male infertility, which are probably caused by mutations, such as **globozoospermia**, in which sperm lacks the acrosome (Fig. 41.9).
- Structural lack or deficiency of dynein structure or other components of the sperm tail causing total sperm immotility (Fig. 41.10), such as in the Kartagener syndrome.
- The ICSI can be performed successfully with **frozen-thawed spermatozoa** from tumor patients in whom sperm quality was often already impaired in the original ejaculate and who could not be treated successfully with earlier treatment modalities.
- ICSI can also be performed more or less successfully with **round spermatids (round spermatid injection, ROSI**), which are collected in the testicular tissue through biopsy (Yamanaka et al. 1997). Round spermatid are haploid precursors of mature spermatozoa that, during spermatogenesis in the seminiferous tubules, have not yet undergone full condensation of the nuclear chromatin nor elongation of the sperm tail. Despite low fertilization and pregnancy rates, ROSI is still being applied in some institutions. Although no negative impact on the condition of infants has been demonstrated (Tanaka et al. 2018), ROSI must still be considered experimental.

ICSI is increasingly being used in other, non-male, causes of infertility (De Geyter et al. 2020), such unexplained infertility or after **insufficient fertilization** in conventional IVF. However, the likelihood that all inseminated oocytes remain unfertilized does not primarily depend on the fertilization technique itself, but rather on the number of available oocytes. When fewer than five oocytes are available, the probability of complete lack of fertilization doubles with each oocyte that is not harvested (Krog et al. 2015). The risk that none of the collected oocytes is fertilized does not differ in IVF or in ICSI (Supramaniam et al. 2020).

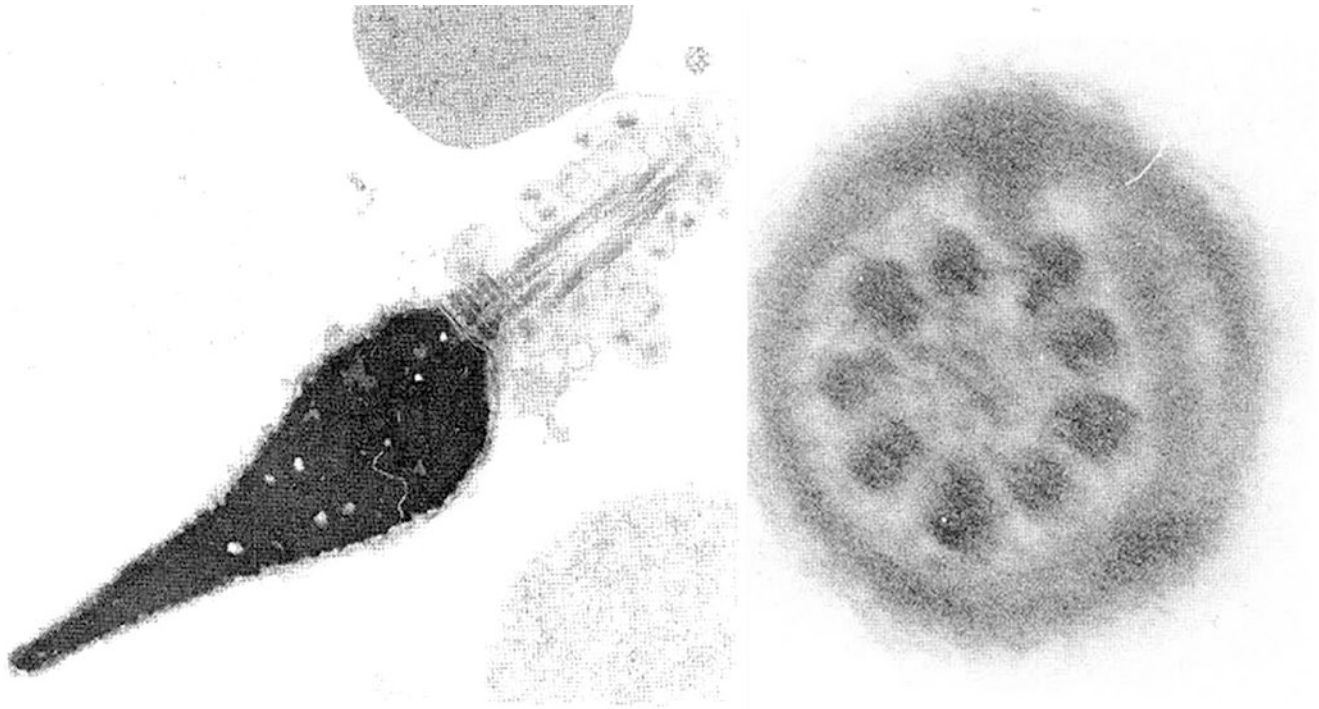


Fig. 41.10 Structural lack or deficiency of dynein structure or other components of the sperm tail causing total sperm immotility

In male infertility, the cause of infertility often remains unexplained. However, some of the male infertile patients scheduled for ICSI do present with hitherto unidentified genetic abnormalities (Meschede et al. 1998, 2000). In singleton pregnancies, congenital abnormalities are more often diagnosed after ICSI than after IVF (Davies et al. 2012). De novo chromosomal abnormalities are detected 3.2% more often after ICSI as compared to natural conception in couples not previously suffering of infertility (Belva et al. 2020). Because of these concerns, the decision to embark on ICSI should still depend on practical grounds, such as the results of conventional semen analysis and of semen preparation. However, exact **cutoff limits to decide in favor of IVF or ICSI** have not been elaborated so far.

41.4.4 Semen Donation

For the purpose of semen donation, spermatozoa of another person, i.e., fertile donor, are collected, analyzed, prepared, and used instead of those of the infertile partner/husband. The donated spermatozoa can be used for insemination or, alternatively, in ICSI. Nowadays, semen donation is used much less frequently, as ICSI can now be performed successfully in most cases of male infertility. Semen donation is used in those cases in which no spermatozoa can be retrieved, neither from the semen, nor from testicular biopsies. Semen donation may also be applied in men who underwent chemotherapy or radiation for malignant disease, in whom semen was not stored frozen. Semen donation is increasingly being used in homosexual lesbian couples (van den Akker 2006).

The selection of appropriate **semen donors** depends on several conditions, which must be fulfilled and which are similar as those when donating an organ for transplantation purposes. Complete history of the candidates for semen donation must be taken, including the family history. They must also undergo a thorough physical examination, which includes phenotypic characteristics (Chap. 50), such as hair and eye color. A number of laboratory tests must be carried out, including blood group typing and a peripheral blood count (to exclude hemoglobinopathies). The most common recessive disorder in Caucasian populations is cystic fibrosis and the carrier status must be excluded. In addition to conventional semen analysis, the chromosomal complement must be determined. Finally, transmittable diseases, such as hepatitis B, hepatitis C, and HIV, must be ruled out.

Current safety requirements mandate that **donated semen must be cryopreserved** before use. Cryostorage of semen consistently interferes with the vitality of the sperm. The degree of progressive motility, especially after thawing, is crucial for the ensuing pregnancy rate (Yogev et al. 2012) and that correlates with the duration of abstinence and sperm velocity (Jiang et al. 2017).

Pregnancy rates after semen donation reach 9% to 12% per insemination (De Geyter et al. 2020; European IVF-monitoring Consortium [EIM] for the European Society of

Human Reproduction and Embryology [ESHRE] 2020). When used in the framework of IVF or ICSI, much higher pregnancy rates are observed.

In most countries, many aspects of semen donation are strictly regulated by law. Usually, the number of children per semen donor is limited, but these numbers may vary. In France, up to five children may be conceived by one semen donor, in Switzerland eight, and in Belgium, the United Kingdom and Austria 10, and in the Netherlands up to 25. There is no rational justification for any of those limitations.

The use of donated semen may be anonymous or non-anonymous. The right of the child to have the chance to trace back the identity of the semen donor is increasingly being recognized. The infertile couples should be informed about the long-term consequences of using donated semen. Accidental disclosure of the secrecy of the origin of the semen may have serious and irreversible consequences for the relationship between the child and his parents. Current global and low-threshold access to ancestry databases has the potential of breaking anonymity (Pennings 2019). Therefore, it is of utmost importance that the legal rights of children resulting from semen donation but also those of the former donors are clearly defined.

41.5 Collection and Preparation of Sperm

41.5.1 Collection of Semen

Usually, semen for assisted fertilization **is collected by masturbation** after a **period of abstinence** of 2–7 days, as recommended by the World Health Organization (Cooper et al. 2010). After longer duration of abstinence, the total number of progressive spermatozoa and the volume of the ejaculate tends to rise, as does the **DNA fragmentation rate** in the sperm nuclei (Agarwal et al. 2016). Shortening the duration of abstinence improves the condition of the DNA. Shorter duration of abstinence (from 1 h to 1 day) may have beneficial effects on the incidence of aneuploidy in embryos (Scarselli et al. 2019) and on the pregnancy rate in ICSI (Borges Jr et al. 2019).

Sometimes, owing to an **anatomic or emotional blockade,** semen cannot be collected by masturbation at the moment of oocyte collection. In those cases, semen may be stored frozen prior to the treatment and be thawed. This preventive measure may reduce the emotional burden on the couples prior to oocyte collection. In patients suffering from **anejaculation** caused by paralysis in the segments ThXI-LII, ejaculation can also be induced by **vibration** or by **electroejaculation**. Although these procedures are effective in 90% of the patients, viable spermatozoa may also be collected through a testicular biopsy.

In patients suffering from **retrograde ejaculation,** three different methods can be used for sperm collection for assisted fertilization.

- First, **antegrade ejaculation** may be achieved by the administration of specific drugs, such as **alpha-adrenergic** substances (phenylpropanolamine, oxedrine) or **anticholinergics** (brompheniramine) and antihistaminic medication (such imipramine, chlorpheniramine, and phenylpropanolamine). **Imipramine**, a tricyclic antidepressant, has been most widely used for this purpose (Kamischke and Nieschlag 2002).
- An alternative strategy consists of collecting the freshly ejaculated sperm from the bladder through a catheter. Because of the acidity and the high osmolarity of the urine, adequate preparation of the patient is necessary to protect the sperm before and after ejaculation: Before ejaculation, the bladder should be emptied and rinsed with 10 mL of the culture medium.
- A third method consists of the aspiration of spermatozoa from testicular tissue.

In some cases with severely agglutinating **male immunological infertility** or in patients with very **viscous semen**, special care must be taken for appropriate collection of the semen sample. Sperm preparation can be facilitated by collecting the semen into 2 mL of equilibrated culture medium in order to reduce not only the **agglutination** of sperm, but also to limit the binding of antibodies to the sperm surface during liquefaction. In some cases, dissociation of agglutinated sperm can also be achieved by the addition of **proteolytic enzymes**, e.g., trypsin. These enzymes can be added to the sperm suspension in an albumin-free culture medium for up to 20 min without damage (Pattinson et al. 1990). The medium containing the enzyme should be washed away before insemination to prevent any interference of the proteolytic enzyme with the interaction between the gametes.

In some forms of **azoospermia,** spermatozoa are collected from the epididymis through microsurgical incision of the epididymal tubule and aspiration into a capillary (**"microsurgical epididymal sperm aspiration," MESA**) (Silber et al. 1995). Alternatively, spermatozoa may also be aspirated through percutaneous puncture of the epididymis (PESA, Tsirigotis et al. 1995) or through extraction of spermatozoa from testicular biopsies (TESE, Tournaye 1999). Although within the frame of a retrospective analysis higher live birth rates were obtained with epididymal spermatozoa (MESA) than with testicular spermatozoa (van Wely et al. 2015), both techniques for sperm retrieval are considered equally effective. Because of the shorter duration of the surgical procedure, TESE has become the preferred method. For practical reasons, several testicular tissue samples are

extracted from both testes and cryopreserved prior to ovarian hyperstimulation for ICSI (Oates et al. 1996; Salzbrunn et al. 1996).

Sometimes, ICSI can be performed successfully with very few spermatozoa retrieved from the testicular tissue. As in testicular azoospermia residual spermatogenesis is very often limited to a few focal sites only, three or four biopsies are usually carried out in each of testes in order to retrieve a sufficient number of viable spermatozoa. The likelihood of not finding any mature spermatozoa for ICSI varies between 40% and 50%. In difficult cases, it may be necessary to open the tunica albuginea of each testis to identify microscopically those seminiferous tubules that may still harbor spermatogenesis (Okada et al. 2002). So far, no prospective randomized studies have been carried out comparing both methods of sperm retrieval from testicular tissue.

In 0.7% of all biopsies, **testicular cancer or carcinoma in situ** was detected (Schulze et al. 1999).

In necrozoospermia, all spermatozoa in the semen are dead and cannot be used for ICSI. Cell death is differentiated from 100% asthenozoospermia using the hypoosmolar swelling test (HOS). True necrozoospermia is the result of an apoptosis-inducing event in the epididymis and viable spermatozoa may then be collected surgically upstream, e.g., from the testicular tissue (Tournaye et al. 1996; Negri et al. 2014).

41.5.2 Basics of Sperm Preparation

Before the actual processing of the semen sample can be started, the semen must be liquefied by simple incubation at room temperature for 30 min.

A subsequent washing procedure of the semen is required, because **seminal plasma** can be toxic both to the sperm itself and to the oocytes, even at low concentration (Kanwar et al. 1979). Furthermore, bacteria may be present in the semen, which, after insemination into the genital tract of the female partner or after insemination in vitro, may cause damage. Seminal plasma also contains high concentrations of prostaglandins, which, after introduction into the uterine cavity, may induce paroxysmal and painful contractions of the myometrium. Furthermore, white blood cells may be admixed to the seminal plasma and these produce large amounts of oxygen radicals, which may damage the fluidic membrane of capacitating spermatozoa (Aitken and Clarkson 1988). The preparation of the semen not only separates the spermatozoa from the surrounding seminal plasma, but the proportion of highly motile and normally formed spermatozoa is raised, thereby triggering the process of capacitation through the removal of blocking factors from the surface of the ejaculated spermatozoa.

41.5.3 The Swim-Up Method

Swim-up remains the most widely used method for sperm preparation in medically assisted reproduction (originally described by Lopata et al. 1976). It is based on the principle that the most motile and capable spermatozoa can swim vigorously upward against gravity into a layer of culture medium positioned on top of the sperm pellet (Fig. 41.11). After 60 to 90 min of incubation, a fraction of highly motile sperm free of seminal plasma and leucocytes is separated. The significance of swim-up for sperm preparation is further enhanced

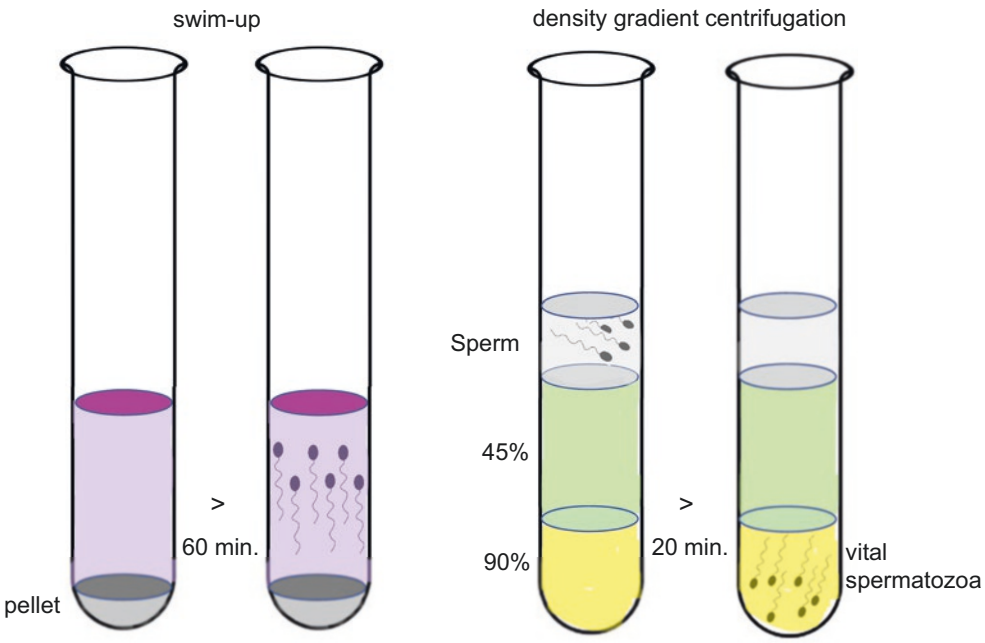


Fig. 41.11 Graphic presentation of the swim-up method and of density gradient centrifugation

by the finding that those properties needed for the penetration of motile sperm into the supernatant are the same required for the **penetration into cervical mucus** and for the **actual fertilization of oocytes** (De Geyter et al. 1988). Thus, the concentration of sperm after swim-up is also correlated with the results of assisted fertilization. In contrast to earlier reports, no selection of Y-chromosome bearing spermatozoa can be achieved with the swim-up procedure (Han et al. 1993).

The main disadvantage of the swim-up method is the low recovery rate of motile spermatozoa, especially in cases with severe abnormalities of the original semen sample, and by the lengthy time interval needed for sperm preparation. Furthermore, the centrifugation of the semen diluted with culture medium and the subsequent concentration of capacitating spermatozoa and oxygen radical secreting leukocytes into a densely packaged pellet potentially damage both the membrane and the DNA of the spermatozoa (Aitken and Clarkson 1988).

41.5.4 Density Gradient Centrifugation

In density gradient centrifugation, motile and normally formed spermatozoa are separated not only by centrifugal force, but also by differences in cell density. The density of living spermatozoa differs from that of degenerated or dead spermatozoa or of admixed cells, such as leukocytes and skin cells. To achieve a separation with density gradient centrifugation, the washed sperm suspension is layered onto a column comprising of two or more layers of a colloid suspension with different density and is subsequently centrifuged. The colloidal solution contains **particles made of silicone** with diameters between 15 and 30 nm. These particles are coated with biologically inert **polyvinylpyrrolidone** (in Percoll) or with a polymer made of **saccharose and epichlorohydrin** (in Ficoll). For separation of the spermatozoa, discontinuous density gradients with two colloidal solutions are predominantly used (e.g., 45% and 90%). The enriched fraction containing motile spermatozoa can easily be identified as a discrete turbid band at the bottom of the tube. Colloidal suspensions are available commercially and can be used for many purposes. However, as some of commercially available suspensions may be contaminated with endotoxins (Henkel and Schill 2003), only products quality-certified for use in reproductive medicine should be used.

In the presence of very low sperm concentrations, the recovery of viable spermatozoa may be insufficient both with the swim-up method and with regular density gradient centrifugation. In such a situation, the **mini-Percoll method** was developed (Ord et al. 1990). With that technology, the colloidal suspension is performed in a volume of only 0.9 mL. The mini-Percoll method is particularly suitable for the preparation of cryopreserved semen, as in semen donation.

Although density gradient centrifugation results in higher numbers of motile spermatozoa as compared with swim-up (Ricci et al. 2009), similar pregnancy rates are achieved with either method (Boomsma et al. 2019). Compared with the swim-up method (at least 60 min), sperm preparation with density gradient centrifugation (20 min) is less time-consuming. Density gradient centrifugation is not associated with any enrichment of Y-chromosome-carrying spermatozoa (Vidal et al. 1993; Lin et al. 1998). Density gradient is not suitable for raising the number of spermatozoa with non-fragmented, intact DNA (Muratori et al. 2016).

41.5.5 Removal of Infectious Viral Particles from the Semen

In men presenting with fertility problems, accompanying chronic **viral infections**, such as **HIV, hepatitis B, hepatitis C, and Zika**, may be diagnosed as well. These viral particles may contaminate the semen and constitute a risk for the partner, for the staff and for the safety in the laboratory. All measures and precautions about how to deal with infected material should be described in detail in the quality management system of the andrology and embryology laboratories. Both density gradient centrifugation and swim-up have the potential to effectively separate the spermatozoa from those viral particles.

During an acute infection with the Zika virus, the amount of viral load in the semen may be very variable and not in all cases can the viral contamination be completely removed (Joguet et al. 2017).

In contrast to HIV, hepatitis C, and Zika, some parts of the DNA of the hepatitis B-viral genome may enter the cytoplasm of sperm and be carried over to the oocyte and the embryo (Zhong et al. 2017, 2018). The infertile couple should be informed that in the presence of a paternal hepatitis B infection the virus may be transmitted vertically to the offspring, even if the virus can be eliminated completely from the prepared semen sample.

41.5.6 Sorting of Spermatozoa Based on Predefined Characteristics

Recently, more experimental work is being carried out to promote the sorting of fertilizing spermatozoa based on defined biological properties.

41.5.6.1 Selection of Sperm with Flow Cytometry and Sorting (FACS) Based on the Sex Chromosomes

With FACS, spermatozoa can be sorted based on the higher desoxyribonucleic acid (DNA) content of spermatozoa car-

rying the X-chromosome and be separated from those carrying the Y-chromosome. To this purpose, the nuclei of spermatozoa are stained with the fluorescent dye Hoechst 3342, which reversibly binds to the DNA allowing the distinction of spermatozoa based on the quantity of DNA in their genome. Spermatozoa with an X-chromosome contain approximately 3% more DNA than spermatozoa carrying a Y-chromosome.

With flow cytometry, sufficient quantities of motile spermatozoa can be selected rapidly enough to be used in assisted fertilization in families with an X-chromosome-linked genetic disease (Cran and Johnson 1996; De Geyter et al. 2013; Karabinus et al. 2014). This method of sperm selection can also be used for family balancing. The enrichment of spermatozoa carrying the X-chromosome should exceed 85%. The enrichment of spermatozoa carrying the Y-chromosome is less efficient, as the sorting process cannot differentiate between the small Y-chromosome and sperm with fragmented DNA. The risk of alterations in the DNA structure induced by the fluorescent dyes has been excluded in extensive animal research (Karabinus 2009).

41.5.6.2 Selection of Spermatozoa Through Binding to Hyaluronidase

During their migration toward the oocyte, spermatozoa must penetrate the matrix of the cumulus oophorus, which consists of **hyaluronic acid**. To that purpose, the enzyme hyaluronidase is located at the surface of the membrane surrounding the sperm head and dissolves the hyaluronic acid-containing matrix as the sperm migrates toward the oocyte. The capacity of the sperm head in binding hyaluronidase has been used to separate viable sperm for ICSI. Although initial studies have suggested lower aneuploidy rates in embryos arising from selected spermatozoa based on their binding to hyaluronidase (Jakab et al. 2005; Mokánszki et al. 2012), prospective randomized clinical trials have not been able to replicate these findings (Parmegiani et al. 2012; Worrilow et al. 2013; Majumdar and Majumdar 2013; Miller et al. 2019, for a comprehensive summary: Lepine et al. 2019).

41.5.6.3 Sorting Based on the Morphology of Spermatozoa

In ICSI, a single spermatozoon is selected from a larger population and captured in a micropipette. This process is performed on the stage of an inverted microscope at 100× magnification. At this magnification, details of sperm morphology cannot be estimated appropriately. The sperm nucleus often contains vacuoles, which have been linked to chromosomal abnormalities. Raising the magnification level to 6000× allows the appreciation of abnormalities in the organelles of moving spermatozoa. At this magnification intact normally formed spermatozoa can now be selected for ICSI (intact morphology sperm injection, **IMSI**, Bartoov et al. 2001). This can only be achieved with an interference-contrast microscope equipped with digital image magnification. Unfortunately, prospective randomized clinical trials have not demonstrated improved outcome rates of ICSI with IMSI (Teixeira et al. 2020; Duran-Retamal et al. 2020).

41.5.6.4 Selection of Normal Spermatozoa Based on Exclusion of Spermatozoa with Fragmented DNA

During conventional semen preparation one or two centrifugation steps are needed. Centrifugation of sperm may induce damage through exposure to oxygen radicals (Aitken and Clarkson 1988) or due to shearing forces (Zhu 2018).

In order to avoid multiple centrifugation steps and aiming at a more physiological approach in separating functional spermatozoa, the principles of microfluidics have been applied to the preparation of sperm for ART. In microfluidics, small numbers of spermatozoa can be separated by capillary forces in the fine channels that constitute the microfluidic system. Spermatozoa may either swim against capillary forces active in the microfluidic channels (as in diffusion processes) or against currents, pumped into the microfluidic channels. All microfluidic systems consist of an array of fine channels that allow the separation of small numbers of spermatozoa with specific characteristics. These small numbers of spermatozoa require the use of ICSI.

A number of additional properties have been applied to microfluidic systems. The membrane of spermatozoa is coated with sialic acid, which is negatively charged. Under electrophoresis, the spermatozoa are filtered through a membrane with pores that permit the passage of negatively charged, functional spermatozoa (Ainsworth et al. 2005; Fleming et al. 2008). Chemical attractants, such as progesterone and acetylcholine, have been added to a microfluidic system for the separation of spermatozoa with optimal characteristics (chemotaxis: Zhang et al. 2015; Ko et al. 2018). Temperature gradients have also been used in microfluidic systems (thermotaxis: Bahat et al. 2003; Pérez-Cerezales et al. 2018). To date, the performance of all these systems in daily practice has not yet been evaluated.

FACS is also based on the alignment of spermatozoa in a microfluidic channel. One or more specific dyes may be added to the spermatozoa and spermatozoa can be sorted based on combination of characteristics according to the absorbance of the light signal. Although the intracellular uptake of the dye YO-PRO-1 by capacitating spermatozoa has been shown to correlate with the degree of DNA fragmentation (Ribeiro et al. 2013), the ongoing pregnancy rate could not be improved significantly by sorting spermatozoa with FACS based on exclusion of YO-PRO-1 (De Geyter et al. 2019). Sorting of fertilizing spermatozoa can also be achieved by using magnetic bead sorting (MACS). Semen contains a sizeable subpopulation of damaged spermatozoa

undergoing apoptosis. Apoptosis or programmed cell death entails damage both to the cell's integrity and to its DNA. Using magnetic beads loaded with Annexin V, a phospholipid-binding protein with high affinity for transmembrane surface markers, which become externalized during apoptosis, damaged spermatozoa destined to undergo cell death can be sorted and removed from the sample to be used for assisted fertilization (Grunewald et al. 2009). However, the clinical value of MACS for the exclusion of apoptotic spermatozoa based on Annexin V remains unclear (Lepine et al. 2019).

41.5.7 Chemical Treatment of Spermatozoa in Culture

Next to mechanical separation of spermatozoa with either the swim-up methodology or with density gradient centrifugation, chemical enhancement of **sperm function** has also been applied in assisted reproduction. A number of substances have been used to stimulate the motility of spermatozoa, such as 2-deoxyadenosine (Aitken et al. 1986), pentoxifylline (Yovich et al. 1988), theophylline (Ebner et al. 2011), and caffeine (Imoedemhe et al. 1992). Pentoxifylline, theophylline, and caffeine act as phosphodiesterase inhibitors, thereby raising the intracellular level of cAMP within the cytoplasm of the incubated spermatozoa. Both pentoxifylline and theophylline have been used most frequently to induce the motility of sperm in TESE testicular biopsies. The effect of these compounds on the oocytes or on the embryos remains unclear.

41.6 Ovarian Follicular Development, Ovarian Stimulation, and Ovulation Induction

41.6.1 Monitoring of Ovarian Follicular Development and Ovarian Stimulation for Insemination

The therapeutic efficacy of **insemination** results not only from an increased number of motile spermatozoa in the lumen of the fallopian tube (Hansen et al. 2020), but also from the exact **timing of ovulation**, with which **insemination must be optimally synchronized**. Ideally, intrauterine insemination must be carried out 24 to 40 h after ovulation induction with hCG (Cohlen et al. 2018; Hansen et al. 2020). In cases with unexplained infertility or male infertility, higher pregnancy and live birth rates have been observed after ovulation induction with **gonadotropins** as compared to pretreatment with **clomiphene citrate** or **letrozole** (Cohlen et al. 1998; Guzick et al. 1999; Berker et al. 2011; Hansen et al. 2020). Within the frame of a meta-analysis, the expected pregnancy rate can be doubled through the presence of two dominant follicles induced by exogenous gonadotropins (Van Rumste et al. 2008). The higher efficacy of this treatment modality entails a higher incidence of multiple gestations.

Semen quality, however, is more decisive for the final outcome of intrauterine insemination than ovulation induction itself. If semen quality is severely reduced, expectant management has been shown to be more effective than one or more inseminations (Cohlen et al. 1998). In the presence of slightly reduced semen quality, the parameters "normal morphology" and "total number of motile spermatozoa" are most predictive for the outcome of insemination (Ombelet et al. 2014): Whereas normal morphology rates should exceed 4% to 5%, the total number of progressively motile spermatozoa should exceed at least 1 million in some studies (Ombelet et al. 2014) to 5 million in others (Tomlinson et al. 2013). In unexplained infertility cases, the live birth rate after insemination is reported to be significantly reduced if fewer than 15 million motile spermatozoa are present in the seminal plasma (Hansen et al. 2020).

The most frequent complication of gonadotropin stimulation in insemination is the higher incidence of **multiple pregnancies**. The risk of multiple pregnancy in IUI can be reduced significantly by consistently using low doses of gonadotropins. Any insemination should be canceled in the presence of more than three follicles with a diameter of at least 14 mm (Van Rumste et al. 2008). Cancelation of cycles can be avoided by the aspiration of supernumerary follicles immediately before IUI (De Geyter et al. 1996, 1998).

Gonadotropin stimulation of the ovaries may also be complicated by the **ovarian hyperstimulation syndrome (OHSS)**, which is a potentially life-threatening complication. The severe form of the ovarian hyperstimulation syndrome is rare in patients treated with insemination, compared with treatment cycles for IVF or ICSI, because of the generally lower doses of gonadotropins used in the former group.

41.6.2 Ovarian Stimulation for IVF and ICSI

The first successful treatment with IVF was performed in **a natural, unstimulated menstrual cycle** (Steptoe and Edwards 1978). Although IVF and ICSI can be performed with some degree of success in the untreated, natural menstrual cycle or with minimal ovarian stimulation, the reported pregnancy rates are generally low, approximately 10% to 15% per treatment cycle (Von Wolff 2019). It has been a long-standing experience that the pregnancy rates in IVF and ICSI can be raised significantly by the presence of higher numbers of available oocytes (Sunkara et al. 2011;

De Geyter et al. 2015; Polyzos et al. 2018). Many different treatment protocols for the stimulation of ovarian follicular development were developed over time and were correlated with the results of assisted fertilization. **Ovarian hyperstimulation protocols** combine several pharmaceutical preparations that must be administered at different time points during the process. The effects of those medications must be monitored with repeated ultrasound examinations and using sequential endocrine analyses. The various sequences of the process can be divided into the following steps:

41.6.2.1 Planning the Treatment

In order to regulate the number of treatments per week and to prevent work overload in clinical embryology, many institutions initiate actual treatment with ART with **hormonal preparation**. Oral contraceptives should not be used for this, as they have been demonstrated to have a negative impact on the expected pregnancy rate (Griesinger et al. 2008, 2015). Instead, gonadotropin-static progestogens are often given (such as norethisterone acetate) or estrogens (such as estradiol valerate) or a GnRH agonist (Farquhar et al. 2017). All these treatments induce luteolysis, while inhibiting early follicular recruitment and inducing menstruation. None of these preparatory treatments have an impact on the final outcome of ART (ESHRE Guideline Group on Ovarian Stimulation 2020).

41.6.2.2 Ovarian Hyperstimulation with Exogenous Gonadotropin Preparations

An increasing number of pharmaceutical gonadotropin preparations are now commercially available with which ovarian follicular function can be stimulated. These preparations differ in biological source, in purity, in the hormonal mixture, and in duration of activity. Despite these differences, no differences in the clinically relevant outcome parameters among the various preparations have been described. There is, however, important variability in the handling of the substances (mixing with solvent, how to administrate, syringe or pen system) and, of course, in the pricing.

The daily administration of the exogenous gonadotropins must be monitored. This is usually carried out with repeated vaginal **ultrasound examinations**, during which both the number and the diameter of the main growing follicles are measured. In addition, the thickness of the proliferating endometrium is measured (distance between the anterior and posterior lamina basalis). Frequently, the repeated ultrasound examinations are accompanied by serial measurements of the **estradiol and progesterone concentrations** in the serum or plasma. During ongoing follicular growth, the serum levels of estradiol first progressively rise, later undergo a sudden upsurge. In contrast, the serum levels of progesterone remain low throughout follicular growth but start to rise at the end of follicular development.

Without additional preventive measures, **ovulation** will occur prematurely in a significant number of patients treated with gonadotropins due to a premature upsurge of the circulating LH levels secreted by the anterior part of the pituitary. Premature ovulation must be distinguished from the preovulatory rise of the circulating progesterone levels, which typically are FSH-dependent (De Geyter et al. 2002). Premature ovulation is effectively prevented by downregulating the endogenous gonadotropin secretion in the pituitary with a long-acting **GnRH agonist**, which desensitize the pituitary after an initial release of the endogenous gonadotropin reserve stored in the pituitary (the so-called flare-up effect) (Fig. 41.12). Premature ovulation can also be prevented with **GnRH antagonists**, which immediately inhibit the secretion of endogenous gonadotropins. Several combinatory protocols with gonadotropins and GnRH agonists have been used over time.

The **"long protocol"** involves downregulation with **long-acting GnRH agonists** well before ovarian hyperstimulation is initiated. The GnRH agonist is applied either during the preceding menstrual cycle, either during the luteal phase, or at the very early beginning of the menstrual cycle or during intake of a birth control pill.

In the **"short protocol,"** the treatment with the GnRH agonist is initiated together with ovarian hyperstimulation, thereby using the flare-up effect on the endogenous gonadotropin secretion for ovarian hyperstimulation. In the short protocol, the GnRH agonist is given until the end of the follicular phase.

In contrast to GnRH agonists, the **GnRH antagonist** has an immediate inhibitory effect on the secretion of endogenous gonadotropins and can therefore be given during ongoing ovarian hyperstimulation (Fig. 41.13). The GnRH antagonist is usually started at 12 mm follicular diameter until ovulation induction (Diedrich et al. 1994).

41.6.2.3 Ovulation Induction

After ovulation induction with exogenous gonadotropins, ovulation must be induced, because under those conditions the preovulatory LH peak not only may take place prematurely, but the signal is often blunted. Still, the preovulatory signal based on LH or LH activity must be applied in order to disrupt the intercellular connections between the granulosa cells and the oocyte, which in turn will result in a sudden decrease in the cAMP levels in the enclosed oocyte (Gilchrist et al. 2016). That event will induce the resumption of meiosis from the prophase I stage, in which the oocytes have been residing since early fetal development, to the metaphase II stage 36 to 37 h later. Only in that stage may the oocyte be fertilized.

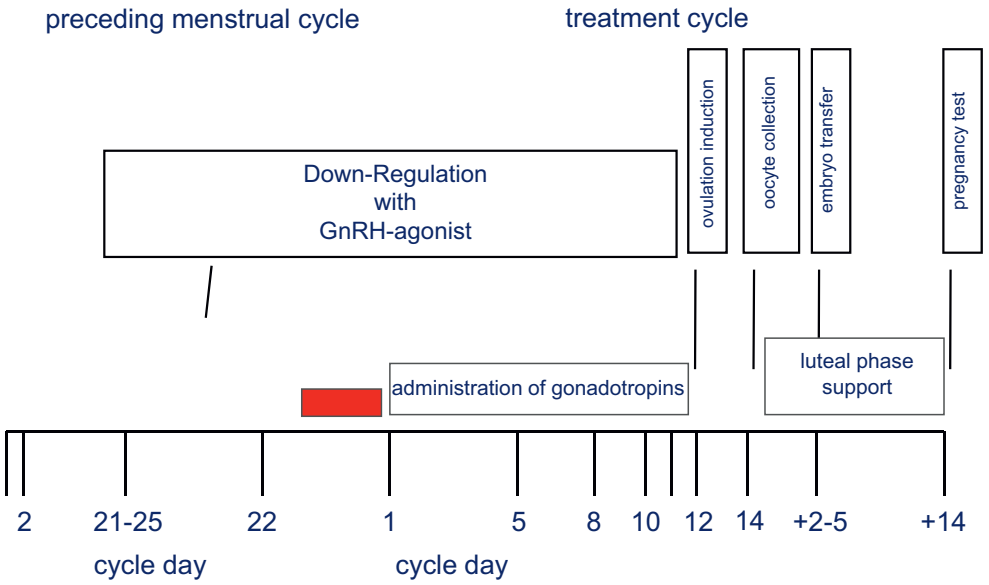


Fig. 41.12 Schematic presentation of the long GnRH agonistic protocol. Premature ovulation is effectively prevented by the administration of a long-acting GnRH agonist during the luteal phase of the preceding menstrual cycle. It induces luteolysis and thereby also menstruation (red). Ovarian stimulation with exogenous gonadotropins is initiated after the onset of menstruation and usually lasts approximately 12 days. In a GnRH agonistic protocol, ovulation can only be induced with hCG. Oocytes are harvested exactly 37 h later. From then onwards, the luteal phase needs to be supported with progesterone and hCG. Embryo transfer can be carried out 2 to 5 days after oocyte collection. The pregnancy test is performed at the earliest 14 days after oocyte collection

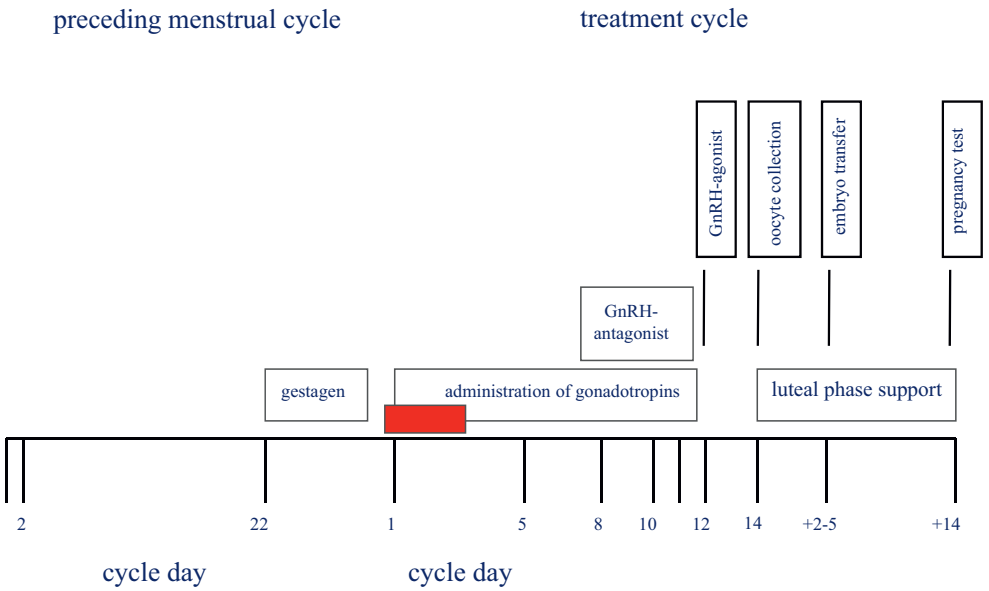


Fig. 41.13 Schematic presentation of the treatment protocol of IVF or ICSI based on prevention of the premature LH surge using a GnRH antagonist. Ovarian stimulation with exogenous gonadotropins is initiated after the onset of menstruation and usually lasts approximately 12 days. In most institutions, the administration of the GnRH antagonist is started when the diameter of the dominant follicle reaches 12 mm and is continued until ovulation induction. In a protocol based on a GnRH antagonist, ovulation may be triggered either with a GnRH agonist (which acts as an antidote to the GnRH antagonist) or with hCG. Oocyte collection must be done exactly 36 h after ovulation induction. From then onwards, the luteal phase must be supported with progesterone and hCG. Embryo transfer can be carried out 2–5 days after oocyte collection. The pregnancy test is performed at the earliest 14 days after oocyte collection

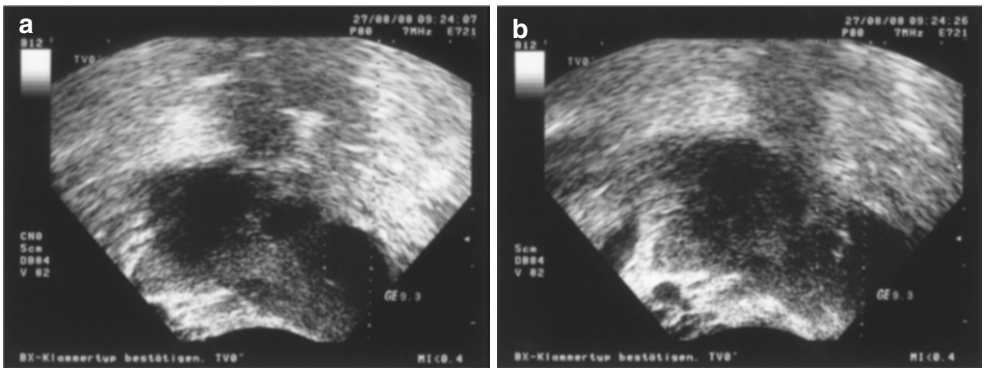


Fig. 41.14 In ultrasound-guided transvaginal aspiration of ovarian follicles, one of the prevailing dominant follicles is first aimed at (**a**). The guidance line on the screen helps in positioning the probe with the hollow needle. Then the hollow needle is inserted through the vaginal wall and through the peritoneal lining into the antrum of the follicle (**b**). Normally, only few millimeters separate the ultrasound probe from the ovary. The tip of the needle is beveled such that its reflexion can be seen on the screen (arrow). Then, the ovarian follicular content is gently aspirated with a continuous pump at −180 mmHg. Usually, the cumulus oophorus–oocyte complex can be identified in the aspirate

Ovulation is induced either with **hCG** (which substitutes the binding of **LH** to the LH-receptor) or with a **GnRH agonist**, which triggers the sudden release of both LH and FSH in the pituitary. The latter form during the GnRH antagonist protocol, in which the gonadotropin-producing cells in the pituitary are *not* desensitized. Avoidance of administration of hCG through ovulation induction with a GnRH agonist bears the advantage of significantly reducing the risk of the ovarian hyperstimulation syndrome (Santos-Ribeiro et al. 2020). Triggering the LH surge with a **GnRH agonist** cannot be done in cases with abnormal hypothalamic–pituitary function, such as in hypogonadotropic ovarian failure (WHO I, often characterized by a BMI <19 kg/m^2), in hyperprolactinemia or after treatment with a long-acting GnRH agonist in the 3 months preceding the actual treatment.

41.6.3 Oocyte Collection

Approximately 38 h after ovulation, induction natural ovulation might take place. Immediately after ovulation, oocytes enter into the lumen of the fallopian tubes, from which they cannot be retrieved. In order to collect the oocytes for IVF or ICSI, they must be recovered from the antrum of the fully matured ovarian follicles, approximately 36 to 37 h after ovulation induction.

Originally, laparoscopy was one of the basic techniques for oocyte collection, originally contributing to the first successful introduction of IVF during the 1970s (Steptoe and Edwards 1978), because it gave the physician easier and less traumatic access to the ovaries than laparotomy. Instead of the earlier laparoscopic method, oocytes are now almost universally collected by **ultrasound-guided transvaginal puncture of the follicles**, which can be performed easily as an outpatient procedure with less general anesthetics (Fig. 41.14). The ovaries are positioned just beneath the vaginal wall and can easily be reached with a hollow needle, which can be inserted into one follicle after another. The content of each follicle can be aspirated under controlled conditions, and each aspirate may be examined for the presence of an oocyte. Ultrasound-guided transvaginal access to preovulatory follicles has contributed to the widespread, low-threshold availability of ART as an outpatient procedure.

The **complication rate** of this procedure is very low. Vaginal or intra-abdominal bleedings may rarely occur (<0.2%). Infections may occur after incidental puncture of endometrial cysts or other intestinal structures, such as the appendix (<0.1%, De Geyter et al. 2020). Aspiration of endometrioma must be avoided, as they may become infected (Yaron et al. 1994; Younis et al. 1997).

41.6.4 Embryo Transfer

The embryos resulting from successful IVF are placed in the uterine cavity of the patient for later implantation (Fig. 41.15). **Uterine placement of embryos** is a procedure causing no or little pain and is nowadays performed within a few minutes as part of a routine gynecological investigation. Despite the apparent ease of the procedure, the exact technique is crucial for the success of the entire treatment (see a complete list of all details: Schoolcraft 2016).

After visualization of the cervix with a speculum, the anterior portion of the cervix is clamped with a forceps. Under slight traction, a catheter is introduced into the cervical canal. Then, the embryo is aspirated, along with a small drop of cultured medium (approximately 10 μL), into a fine and soft catheter. This catheter is then inserted carefully through the previously positioned broader catheter into the

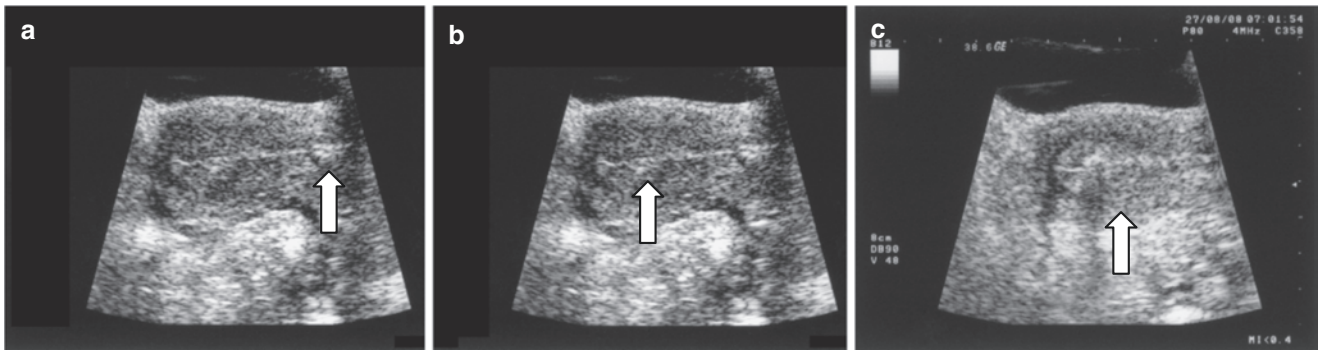


Fig. 41.15 Vaginosonographic image of the intrauterine transfer of an embryo. Usually, the embryo transfer is performed under ultrasound guidance. To that purpose, the transfer is performed with full bladder, which allows visualization of the uterus during the procedure. (**a**) The tip of the mandarin is visible in the cervix. (**b**) The tip of the catheter is visible in the uterine cavity. (**c**) The embryo is transferred into the uterine cavity together with two air bubbles

uterine cavity (about 6 cm depth). Not only the low volume of fluid introduced along with the embryos is important, but also carrying out the procedure in an absolutely atraumatic fashion. After the transfer, the presence of embryos adhering to the catheter should be checked under the microscope. The transabdominal ultrasound-guided insertion of the catheter into the uterine cavity leads to higher pregnancy rates (Abou-Setta et al. 2007; Cozzolino et al. 2018). After a few minutes, the patient is allowed to stand up and to leave the unit.

The optimal technique of embryo transfer is one of the most important factors contributing to consistently high pregnancy rates in IVF and ICSI.

The **complication rate** of intrauterine embryo transfer is very low. In rare cases, endometritis may occur. The replaced embryos may slip into the fallopian tube, causing **ectopic** pregnancies, especially in patients with widened tubal diameters (**sactosalpinx**). The surgical removal of unilateral or bilateral hydrosalpinges visible in ultrasound, prior to assisted reproduction (Strandell et al. 1999; Johnson et al. 2010), has considerably contributed to reduce the risk of ectopic pregnancies after IVF or ICSI.

Occasionally, stenosis of the cervical canal may constitute an insurmountable obstacle to the atraumatic replacement of an embryo. Intrauterine adhesions, which may be result of one or more surgical interventions, may interfere with the normal proliferation of the endometrium and even cause accumulation of liquid within the uterine cavity (serometra). Endometrial polyps are also a common finding, which may not always be readily visible in ultrasound. Higher ongoing pregnancy rates have been observed when **hysteroscopy** is performed prior to the first IVF treatment to exclude all endometrial pathology (Di Spiezio et al. 2016; Cao et al. 2018; Mao et al. 2019). Currently, evidence that live birth rates are improved by systematic hysteroscopic screening before IVF is still lacking.

Embryo transfer may potentially be carried out at any stage of early embryonic development, including the **pronucleate stage** (day 1 after oocyte collection) until the late **blastocyst stage** (day 6, Fig. 41.16). Due to the natural selection process during early embryonic development, the likelihood of implantation is higher if the most appropriate embryo, usually selected based on morphological aspects, is transferred at the blastocyst stage. The better the developmental capacity of the available embryos, the fewer embryos must be transferred to achieve an acceptable pregnancy rate leading to the concept of **elective single embryo transfer** (eSET). The systematic use of eSET together with the cryopreservation of embryos in the blastocyst stage has been demonstrated the most effective method to reduce the incidence of **multiple gestation** without compromising the overall success rate in ART (De Geyter et al. 2020).

The ovarian hyperstimulation syndrome can also be prevented effectively by not replacing the embryo during the stimulated cycle. Instead, all available embryos are now stored frozen until the endocrine environment has normalized again. This protocol is known under the label "freeze all" or "freeze only" (Stormlund et al. 2020; Santos-Ribeiro et al. 2020).

41.6.4.1 Luteal Phase Support

In a menstrual cycle hyperstimulated with exogenous gonadotropins, the negative feedback exerted by the elevated serum levels of both estrogens and progesterone on the pituitary inevitably reduces the output of LH during the **luteal phase,** resulting in luteal phase insufficiency. This undesired consequence of ovarian hyperstimulation necessitates support of the luteal phase with **exogenous progesterone**, with **hCG,** or with both. When ovulation was induced with hCG, the latter will sustain the luteal phase during the first few days after oocyte collection due to its longer half-life. When,

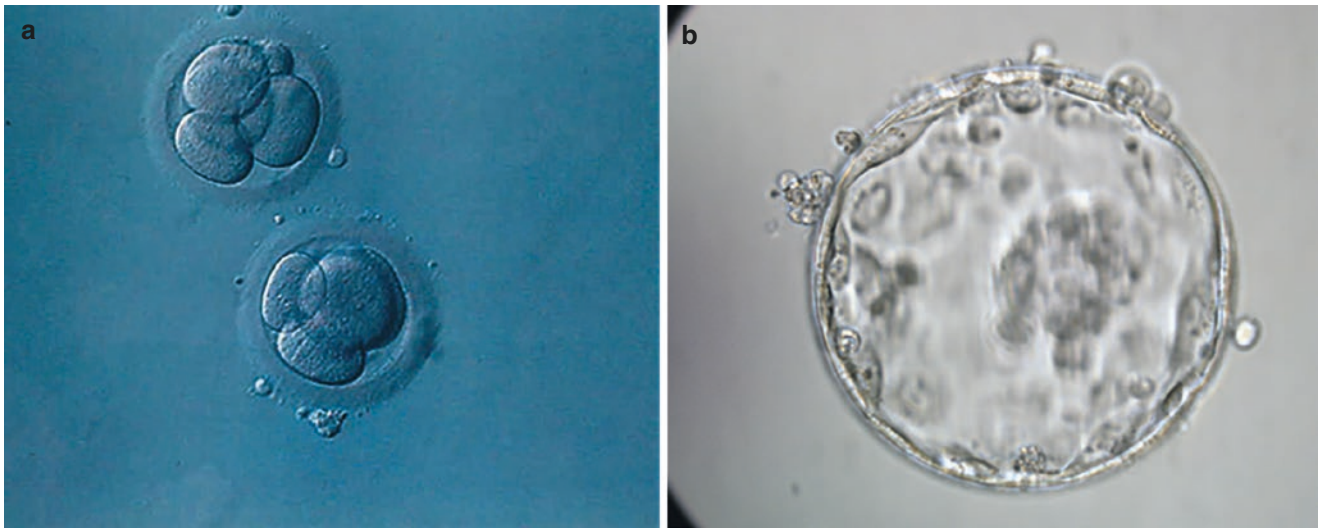


Fig. 41.16 Embryo transfer can be performed, either on day 2 or 3 after oocyte collection, in the cleavage stage (**a**), or on day 5, in the blastocyst stage (**b**)

however, ovulation was triggered with a GnRH agonist, luteal phase support must be initiated immediately after oocyte collection (Humaidan et al. 2013).

Although luteal phase support with hCG is more effective than with progesterone, hCG is associated with more side effects and a significantly higher risk of the ovarian hyperstimulation syndrome.

For luteal phase support, a number of progesterone preparations are available, such as the natural progesterone, which can be given either intramuscularly, subcutaneously, or transvaginally. During transvaginal administration, progesterone is selectively taken up in the uterus, so that the circulating progesterone levels in the serum may remain low (Cicinelli and De Ziegler 1999). Dydrogesterone may also even be given orally. The additional administration of estrogens has been demonstrated not to be effective (Scheffer et al. 2019; Jing et al. 2019).

In the event of a pregnancy, luteal phase support must not be continued (Nyboe Andersen et al. 2002).

41.6.5 Cryopreservation of Oocytes in the Pronucleate Stage and of Embryos

If one or more oocytes are successfully fertilized with IVF or ICSI, they can be cryopreserved in the pronucleate stage prior to completion of the fertilization process or in various stages of early preimplantation embryonic development. When pregnancy does not occur in the stimulated treatment cycle with fresh oocytes, the cryopreserved material may be thawed and replaced in a subsequent menstrual cycle, thereby avoiding repeated ovarian stimulation and oocyte collection. Eventually, cryopreservation of oocytes in the pronucleate stage or of embryos allows couples to have a second or even a third pregnancy at a time when another treatment with ovarian hyperstimulation might not be feasible anymore.

Legislation may limit the number of cryopreserved embryos per couple. The **German Embryo Protection Act** stipulates that not more oocytes per woman may be fertilized as can be transferred within one treatment cycle (see also Chap. 50). The **Swiss Legislation on Medically Assisted Procreation** limits the maximal number of embryos per couples at 12. Both legislations allow the cryopreservation of oocytes in the pronucleate stage, as these are considered as not yet fertilized. **Cryopreservation of oocytes in the pronucleate stage** must be carried out prior to the approximation of both pronuclei, about 20 h after in vitro insemination in IVF or after the ICSI procedure.

Due to much higher success rates, ultrarapid freezing (**vitrification**) has now replaced earlier slow freezing protocols (Cobo and Diaz 2011; Cobo et al. 2012; Rienzi et al. 2017). Vitrification defines an ultrarapid freezing method (less 1/10,000 s), in which no crystallization takes place, thereby reducing the risk of damage to the biological material. For vitrification the biological material is first dehydrated with cryoprotectants and then plunged into liquid nitrogen at −196 °C. The frozen samples have the transparency of glass, hence the term "vitrification."

Prior to thawing, the endometrium must be proliferated and be receptive for nidation of the embryo. Transfer of thawed embryos can be carried out in the **natural menstrual cycle**, but for timing purposes ovulation is usually **induced with hCG**. Thawed embryos may also be transferred in a

so-called "**artificial cycle,**" in which the endometrium is proliferated with exogenous estrogens and then made receptive with exogenous progesterone. In that case, the ovaries do not contain any luteal body. Thawing and transfer of embryos can be better organized and scheduled with the artificial cycle protocol. However, if a pregnancy is achieved during an artificial cycle, the administration of both estrogen and progesterone must be continued until the tenth week of pregnancy, until the placenta has developed. Similar results are achieved with either method (Greco et al. 2016; Groenewoud et al. 2018), but recent findings indicate that pregnancies achieved in the artificial cycle are more often complicated by hypertensive disorders and pre-eclampsia (Pereira et al. 2021).

The importance of cryopreservation of embryos in ART is rising. Both in Australia and New Zealand and in the United States, more pregnancies are now achieved after thawing of embryos than in freshly stimulated treatment cycles (De Geyter et al. 2020). Cryopreservation of oocyte and embryos are valuable adjuncts in eSET to prevent multiple pregnancies and for the prevention of the ovarian hyperstimulation syndrome.

41.7 Complications of Medically Assisted Reproduction

The widespread use of assisted reproduction for the alleviation of infertility, the rising age of the infertile partners and the broadening of conditions that can be treated with assisted reproduction have all raised concerns about **short-term and long-term health risks**, not only to the treated women but also to the offspring. As infertility itself is known to lead to a higher incidence of complications during pregnancy and delivery, appropriate conclusions can only be drawn from pregnancies achieved in previously infertile couples conceiving naturally. Dissection of the risks of the various therapeutic components of assisted reproduction from those resulting from infertility has been difficult and is still ongoing. Minor differences in outcome and risk can only be identified through the collection and combination of large data sets, as in nationwide registries. There are many examples of the potential detrimental action of assisted reproduction on both the mother and the offspring. Prolonged and repeated hormonal treatments may impact the health status of the treated patients by inducing cancer. Repeated stimulatory treatments of the follicles may provoke ovarian cancer. During the first trimester of pregnancy after ovarian hyperstimulation, the multiple luteal bodies continue to produce high levels of sexual steroids, which may influence the conceptus.

41.7.1 Short-Term Complications of Assisted Reproduction

A number of procedures during ART are to some extent invasive, such as the collection of the oocytes and embryo transfer, but the complication rates of those procedures are generally quite low**. Intraperitoneal hemorrhage** after oocyte collection is a rare event (<0.2%), as are **infections** (<0.1%, De Geyter et al. 2020; Blumenauer et al. 2020).

The ovarian hyperstimulation syndrome (OHSS) is a much more common complication of all forms of medical stimulation of ovarian function, affecting up to 2% of all treatments with assisted reproduction, particularly IVF and ICSI (Delvigne and Rozenberg 2002). The more severe form of OHSS (grade III, WHO) occurs in approximately 0.3% of all treatments with IVF and ICSI (Blumenauer et al. 2020). The presence of **hCG** in the patient's blood circulation is essential for the development of OHSS, which usually develops about 12 days after ovulation induction, occasionally earlier. HCG enhances the production and secretion of the vascular endothelial growth factor (VEGF) in the corpora lutea of the stimulated ovaries (Neulen et al. 1995). In recent years, the incidence of OHSS has dropped due to the frequent avoidance of the use of hCG in infertile patients at risk of OHSS and of the freeze-all strategy, in which all embryos are cryopreserved until later (current reported incidence of OHSS in Europe: 0.5%, De Geyter et al. 2020).

OHSS is characterized by a massive and rapid swelling of the abdomen due to an enlargement of the ovaries accompanied by **ascites**. The massive shift of fluid from the vascular space into the peritoneal cavity often leads to **hemoconcentration** (as measured by the hematocrit), thereby causing general distress (with high leukocyte numbers in the peripheral blood count) and a risk of intravascular blood clotting. Hypovolemia may even cause anuria. OHSS is characterized by low sodium, high potassium, and low albumin levels in the serum. Women with an otherwise subclinical diaphragmatic hernia may also develop pleural effusion, which is characterized by dyspnea and frequent coughing. Hemoconcentration increases the risk of thrombovascular events, including pulmonary embolism. Thrombotic events in OHSS occur predominantly in the upper part of the body, sometimes several weeks after the acute phase of OHSS. Increased pressure in the peritoneal cavity also impairs perfusion of the kidneys leading to oligo- or anuria. Renal insufficiency ranks among the most life-threatening conditions in OHSS.

OHSS is the most dangerous complication of ART. Approximately 50% of all **maternal deaths** in ART are caused by OHSS (Braat et al. 2010). Not only should all patients be informed about this potential complica-

tion, but also all individual risk of kidney disease and thromboembolic processes should be enquired before treatment.

In OHSS, many of the events can be reduced through a number of interventions. OHSS may present with various degrees of severity. Although **mild forms** can be treated without hospitalization, the **severe forms** must be treated in the hospital. The most effective intervention to alleviate the patient with OHSS is the transabdominal or transvaginal aspiration of **ascites**. Pleurocentesis is beneficial in the presence of a hydrothorax, which is mostly unilateral. Severe OHSS must also be treated through the transfusion of adapted sodium solutions (thereby avoiding administration of potassium) and intravenous albumin together with anticoagulant treatment to prevent thromboembolism. The latter must be continued until the twelfth week of gestation, as thromboembolism may occur with delay. In the presence of anuria or oliguria, renal excretion may be stimulated with medication, but this must be done while administrating sufficient amounts of liquid and proteins into the vascular space in order to maintain the oncotic pressure.

Ovarian hyperstimulation leads to a more or less pronounced enlargement of the ovaries, particularly during the luteal phase and early pregnancy. After the ninth week of gestation, the size of the ovaries will gradually normalize. Particularly in slender women treated with ICSI because of male infertility and in whom intraperitoneal adhesions are rarely encountered, these enlarged ovaries may undergo **torsion**. In ovarian torsion blood circulation from and to, the organs may become compromised leading to the organ's infarction. It occurs in 0.1 to 0.3% of all cases and is observed more often in women in early pregnancy or with multiple pregnancies (Källén et al. 2005).

41.7.2 Complications During Pregnancy

For many years, several embryos were transferred per treatment trial in order to raise the likelihood of pregnancy. Because the implantation potential of embryos is different among individual couples, this strategy resulted in a high incidence of multiple gestations and, together with rising treatment numbers, in an **epidemic of multiple births**. Not only births of twins were common, but also those of triplets and other high-order pregnancies. All multiple births are linked to a much higher risk of prematurity often with long-term health consequences for the affected children.

With time, many aspects of assisted reproductive technology were gradually optimized, not the least the culture conditions for the embryos. As a result, the developmental capacity of the embryos was gradually improved, so that fewer embryos are now needed for one healthy pregnancy. Meanwhile, the replacement of only one selected embryo has become standard practice in many institutions.

Not only dizygotic twin pregnancies are more common after ART, but also monozygotic twin pregnancies. Although the exact cause of this has not yet been completely elucidated, hardening of the zona pellucida during embryo culture may be responsible, most particularly in blastocyst culture (Hviid et al. 2018).

Not only multiple pregnancies but also singleton pregnancies after ART are characterized by a higher incidence of complications such as **prematurity** (Pinborg et al. 2013) and must be considered as pregnancies at risk that need to be monitored with care (De Geyter 1994). Placentation abnormalities occur more frequently in ART, including placenta previa (Vermey et al. 2019). The incidence of preeclampsia is 1.7 to 2.0 more common after ART than after natural conception (Almasi-Hashiani et al. 2019; Omani-Samani et al. 2020). As compared to neonatal birth weight of children born after natural conception, the neonatal birth weight of children born after embryo transfer in a stimulated cycle is significantly lower (150 g) (De Geyter et al. 2006). In contrast, children born after a thawing cycle have a 1.4 times higher risk of being born with a neonatal birth weight > 4500 g (Pinborg et al. 2014).

41.7.3 Long-Term Complications for the Mother

Hormonal stimulation of ovarian function in ART, especially if repeated many times, and the endocrine changes resulting from these interventions may potentially influence the incidence of various endocrine-dependent malignant diseases, such as breast cancer, ovarian cancer, and others. Any difference in the observed incidence must be differentiated from known correlations between the failure to conceive or delayed conception and the occurrence of **malignant disease** (such as ovarian cancer or breast cancer). In addition, conditions causing infertility have been linked to a higher risk of malignancy, such as the association of endometriosis with endometrioid ovarian cancer (2.3-fold, Kvaskoff et al. 2021) and the association of polycystic ovary disease with endometrial cancer (1.9-fold, Gottschau et al. 2015).

In order to detect any correlation between hormonal stimulation of the ovaries and the occurrence of malignant disease, registry data of ART and of cancer were linked together. As a result, no consistent connection between previous hormonal treatment of the ovaries in ART and the incidence of the following malignant diseases was detected: breast cancer, ovarian cancer, endometrial cancer, and cervical cancer (Venn et al. 2001; Dor et al. 2002; Siristatidis et al. 2013; Sergentanis et al. 2014; van den Belt-Dusebout et al. 2016).

41.7.4 Pediatric Aspects of Assisted Reproduction

From its early beginnings, the introduction of ART for the treatment of male infertility has raised much concern about the safety of the techniques with respect to the risk of malformations in the offspring. It is crucial to distinguish between any abnormality potentially arising from the technology itself, from abnormalities that may arise accidentally or that result from pre-existing infertility issues in the couple. Similar consideration concerning the incidence of neonatal malformations results from the condition of infertility, which itself pertains to a higher health risk to the offspring, and from the current trend to delay the age of childbearing.

Results so far have demonstrated that, apart from those cases in which male infertility has a definite genetic background, ICSI is not associated with a major risk of malformation in the offspring. Despite hitherto comforting findings, close observation of the outcome of assisted reproduction, of ICSI in particular, has revealed some risks:

- In children born after ICSI, a higher risk of de novo **chromosomal abnormalities** in the offspring, particularly of the sex chromosomes, but also in the autosomes has been observed (Bonduelle et al. 2002; Woldringh et al. 2010; Belva et al. 2020). The risk of chromosomal abnormalities in the offspring has been shown to be higher in the presence of very few spermatozoa.
- The **perinatal condition** of the children conceived with assisted reproduction is similar to that of children conceived naturally, especially in singleton deliveries (Henningsen et al. 2015; Luke 2017; Henningsen et al. 2018). The current trend of replacing fewer embryos per treatment and the lower incidence of multiple deliveries has contributed significantly to improve the health status of newborn children after ART.
- The **cardiovascular condition** of children after ART is currently being examined in greater detail. Among adolescent children conceived with ART, the elasticity of the large blood vessels has been demonstrated to be reduced, suggesting higher cardiovascular morbidity during adulthood (Scherrer et al. 2012; Meister et al. 2018). Similar changes in the blood vessels were observed in animal research (Vrooman and Bartolomei 2017). In animal experiments, the extent of cardiovascular abnormalities was correlated with the duration of embryo culture in vitro (Aljahdali et al. 2020). Some of these vascular changes may be caused by suboptimal culture conditions. The mild hypertension detected in adolescent children that underwent ART decades ago could not be confirmed anymore in children of similar age treated more recently (Guo et al. 2017) or in young adults (Halliday et al. 2019). Further research with larger sample sizes is needed to corroborate or refute these findings.

Key Points

- Intrauterine insemination with prepared semen is an effective treatment in cases in which the partner is not able to have sexual intercourse, but not in men with abnormal semen quality.
- Semen donation is carried out with semen that has been cryopreserved before use.
- Intracytoplasmic sperm injection can be performed successfully in a large variety of male infertility forms and is the most effective treatment in couples suffering from infertility caused by abnormal semen quality.
- Whereas conventional semen preparation methods, such as swim-up and density gradient centrifugation are still the most commonly used, novel forms of semen preparation are targeted at improving predefined properties of the spermatozoa.
- The efficacy of any form of medically assisted reproduction very much depends on the hormonal treatment of the female partner.
- Cryopreservation of semen, of oocytes, and of embryos can now be used to disconnect timing of certain procedures. Segmentation of assisted reproduction helps to prevent complication and to preserve fertility potential over prolonged time periods.
- Whereas the incidence of short-term complications has been drastically reduced in recent times through the use of cryopreservation and by avoiding the use of human chorionic gonadotropin for ovulation induction, the future long-term health condition of the children conceived with assisted reproduction remains a matter of concern.

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Cryopreservation of Human Sperm and Testicular Germ Cell Tissue for Fertility Reserve

42

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Abstract

Cryopreservation of human sperm can be offered as fertility reserve to adolescents and adults in oncological and non-oncological diseases, as a preventive measure before vasectomy and securing sperm availability in ART procedures. The methods of cryopreservation differ with regard to fresh ejaculate and surgically (epididymal or testicular) retrieved tissue. Current cryopreservation methods allow sperm to survive for at least 30 years, probably indefinitely. Cryopreserved sperm can be used as part of ICSI therapy with pregnancy and birth rates comparable to fresh sperm. Since 2012, cryopreservation of immature testicular tissue has been available to cryopreserve spermatogonial stem cells for pre- and early pubertal boys in Germany. This currently experimental procedure is on the cusp of clinical application, and intensive research will hopefully increase the chances of refertilization for affected individuals in coming years.

42.1 Cryopreservation of Sperm and Testicular Tissue for Fertility Protection and Fertility Reserve

The following chapter provides an overview of the current state of the art, applicability, and limitations of human sperm cryopreservation. When informing the patient(s) and planning treatment, the physician should include long-term concerns of a patient's quality of life, taking into account his or her fertility. This has a special significance in oncological diseases and, since 2017, is also required in the corresponding AWMF S2k guideline (AWMF 2017). Sperm cryopreservation is an important part of the work of many andrology and reproductive medicine laboratories, especially in collaboration with reproductive medicine centers or practices. In addition, since 2012 the experimental procedure for cryopreservation of spermatogonial stem cells in boys with still immature testicular tissue has been in place in Germany and will also be presented in this chapter.

Cryopreservation of sperm from ejaculate or also from testicular tissue (in the presence of azoospermia) represents a preventive, adjunctive therapeutic measure to preserve the ability to reproduce in oncological patients and in non-oncological patients whose fertility is threatened by the loss of their gonads or reproductive functions. Since July 2021, the cost of cryopreservation of sperm from the ejaculate or testis for medical indications, as outlined in the Cryopreservation Guideline (Kryo-GL) of the Federal Joint Committee, is to be paid by health insurances in Germany. Indications outside of this guideline continue to be a self-pay benefit for patients.

42.1.1 Historical Development of Sperm Cryopreservation from Ejaculate and Testicular Tissue

The observation that human sperm can retain their motility after freezing and thawing was first made by Lazzaro Spallanzani in 1776. In the mid-nineteenth century, the idea of establishing a sperm bank for cattle breeding emerged. Almost at the same time, consideration was given to freezing the ejaculates of soldiers to preserve the possibility of fertilizing their wives should they become victims of warfare. However, serious efforts at cryopreservation technology were not made until the first half of the twentieth century (Trottmann et al. 2007).

Sweeping improvements in cryopreservation technology were achieved with the introduction of the first cryoprotectant, glycerol, into the freezing process to protect spermatozoa. Thus, spermatozoa could be stored on dry ice at −79 °C (Polge et al. 1949). Later, liquid nitrogen (−196 °C) was used, after which the first successful fertilizations with cryopreserved sperm were reported. Since then, thousands of pregnancies and births have been achieved worldwide using cryopreserved sperm (Leibo et al. 2002; Ferrari et al. 2016). With the introduction of intracytoplasmic sperm injection (ICSI), cryopreservation was extended to single spermatozoa and testicular and epididymal extracted spermatozoa obtained by surgical procedures [open multilocular or microsurgical testicular

sperm extraction using testicular biopsies (TESE, mTESE), and fine needle biopsy or (microsurgical) aspiration from the epididymis (MESA)]. After thawing, the cryopreserved sperm are promisingly usable for intracytoplasmic sperm injection (ICSI), now considered the standard in medical-assisted reproduction (MAR) (Huang et al. 2022; Corona et al. 2019; Esteves et al. 2020).

42.1.2 Emergence of the "Andropotect®" Network: Cryopreservation of Immature Germ Cell Tissue (Spermatogonial Stem Cells)

In contrast to the standardized and established cryopreservation of sperm as a fertility reserve in adolescents and adults, the presence of spermatogonial stem cells in immature testicular tissues offers a new, still experimental option for creating a fertility reserve in the pre- and early pubertal boy. Target cells of such a therapy are the **spermatogonia**, which are located on the inner side of the basal lamina of the germinal epithelium (see Chap. 11 "Testicular histology"). Through regular divisions, the stem cells ensure their self-preservation while producing differentiating sperm precursors. This spermatogonial stem cell system is established in the fetus during differentiation of the testis and remains active throughout life, so that in principle an option for stem cell-based fertility reserve exists at any time and does not depend on the developmental state of the male (Table 42.1).

If puberty has not yet begun or is only in its early stages, preservation of spermatogonial stem cells, which are in principle available throughout life, should be considered. As puberty progresses, focal islets with intact spermatogenesis are present in the germinal epithelium at a very early stage, so that both spermatogonial stem cells and occasionally already mature spermatozoa may be present during bioptic sampling of testicular tissue. The chances of success for cryopreservation of spermatozoa from testicular tissue in adolescence are difficult to estimate when sexual maturity is still lacking. In these cases, the combination of testicular tissue sampling for cryopreservation of stem cells with cryopreservation of (isolated) spermatozoa can be attempted (Sect. 42.4).

The following table summarizes the situation (Table 42.1).

The understanding of spermatogonial stem cells has expanded tremendously due to intensive research and relevant methodological breakthroughs (Sharma et al. 2019). Stem cells do not form a homogeneous population. Rather, spermatogonia exist as slowly expanding, randomly splitting clones capable of transdifferentiation. Like other stem cells and cells in the germline, spermatogonia are characterized by high and dynamic plasticity (Sharma et al. 2019), so there is no reason to isolate and preserve a defined subpopulation of stem cells. In particular, in immature and recovering testicular tissues, their density is sufficiently high that it is possible to leave stem cells in the tissue composite and preserve them as small tissue fragments. Here, sufficient quantities of stem cells should be present in the biopsy-derived tissues to

Table 42.1 Development-dependent range of methods for creating a fertility reserve

Developmental status	Infancy	Childhood	Puberty	Adult
Spermatogonial stem cells	Present, leading stem cell population	Present, leading stem cell population	Present, decreasing proportion of total germ cell population	Present, very low proportion of total germ cell population
Spermatogenesis	Inactive	Inactive	Onset	Active
Mature spermatozoa	None	None	Depending on the degree of maturity	Present
Cryopreservation of spermatozoa	Impossible	Impossible	Possible	Possible
Cryopreservation of testicular tissue for sperm collection	Impossible	Impossible	Eventually possible	Clinically established method
Cryopreservation of testicular tissue for spermatogonial stem cells	Possible	Possible	Possible	Difficult and not sufficiently studied

use them prospectively for the stem cell-based approaches for sperm derivation. As with other stem cell systems (e.g., bone marrow), it is expected that few stem cells have the potential to fully induce spermatogenesis from even a few progenitor cells through expansion and differentiation, provided that conditions can be created to resume tissue function. This has already been demonstrated for many species in animal studies, e.g., for germ cell transplantation or engraftment of immature testicular tissues (Tran et al. 2022).

Against this current background of experimental development, for pre- and early pubertal patients in whom spermatogenesis has not yet been initiated, cryopreservation of immature testicular tissue and the spermatogonial stem cells it contains is the only way to establish a fertility reserve. This approach is still considered experimental, as the protocols for differentiation of spermatogonial stem cells into sperm are still under development. In order to provide cryopreservation of immature testicular tissue to affected patients throughout Germany, and to further develop approaches for sperm retrieval from these tissues, the Andropotect® network, based at CeRA in Münster, was founded in 2012 (Schlatt and Kliesch 2012). Members of this network are pediatric oncology centers, pediatric transplantation centers, and transgender outpatient clinics, which offer this fertility-securing measure to affected children and their parents in cooperation with the CeRA Münster. The corresponding ethical votes and delimitation of responsibility agreements (VAV) and regular sample transfers have already been established with five large clinics in Germany. Special transport boxes are available for the transport of tissue biopsies to CeRA, which must be carried out in accordance with the tissue medicalization law. Thus, transfer of the tissues into culture medium and at ideally 8 °C (not above 10 °C) is possible on the same day. Immediately after receipt of the samples, they are then processed as under the required sterile conditions. One of the three tissue samples collected will be fixed for histological processing to test for the presence and differentiation of stem cells and will be used for research. Two samples will be cryopreserved with DMSO-based cryomedia according to current protocols for immature testicular tissue and will be stored for patient use.

Internationally, the "Nordfertil" network (Nordic Centre for Fertility Preservation) was established in the Nordic countries in 2013 and the Oncofertility Consortium was established in the USA as early as 2006. Within the framework of research networks also in Europe ("GROWSPERM," 2014 to 2019), intensive work was carried out on the further development of the methodology to achieve a refertilization of the affected person from testicular stem cells by means of in vitro or in vivo procedures at a later point in time. A breakthrough was published from Pittsburgh in 2019: In the nonhuman primate, for the first time it was demonstrated that prepubertally harvested testicular tissue leads to the maturation of seminiferous tubules and mature spermatozoa after scrotal transplantation, resulting in pregnancy and subsequent birth of a monkey baby ("Grady," graft-derived baby) following ICSI (Fayomi et al. 2019) (see Sect. 42.6.2).

42.1.3 Guidelines and Legal Framework for Sperm and Germ Cell Tissue Cryopreservation

The cryopreservation of sperm and testicular tissue is—unlike the processing of ejaculates—not subject to regular quality control according to Rili-BÄK, but to control according to AMG and the "Guideline for the collection and transfer of human germ cells and germ cell tissue in the context of assisted reproduction" of the German Medical Association as amended (RL-Assistierte Reproduktion, Bundesärztekammer 2022). However, the "WHO Manual for the Examination and Processing of Human Semen Samples" in its most recent edition is also the standard for the preparation for and performance of cryopreservation of sperm from the ejaculate and testicular tissue (WHO 2021).

Cryopreservation and the storage of sperm are complex and make high demands on the medical and medical–technical laboratory staff. In Germany, the legal requirement for the cryopreservation of sperm is the authorization according to AMG §20b and for the processing and use the permission according to §20c AMG. A current HIV and hepatitis test must also be available, also at a facility that has permission according to §20b AMG or cooperates with it by contract. A contract with the patient for cryopreservation and storage of the samples is also required, as is appropriate liability insurance for the laboratory responsible.

The first German-language S2k guideline for fertility protection in patients with oncological diseases has existed since 2017 and includes all established indications for cryopreservation of gametes of both sexes and is due for revision in 2023. This provides a good guideline for all involved medical disciplines for consultation.

The "**Guideline for the cryopreservation of egg or sperm cells or germ cell tissue as well as corresponding medical measures due to germ cell damaging therapy" (Kryo-RL) of the Joint Federal Committee (G-BA)** (Bundesanzeiger BAnz 19.2.2021, last revised 2022) became effective as of July 1, 2021. It regulates the reimbursement of costs for cryopreservation and storage of germ cells (from puberty) for medical indications in men up to the age of 50 and in women (from menarche) up to the age of 40. The medical indications are not limited to oncological diseases, but refer to planned treatments that, according to the state of scientific knowledge, **may be harmful to germ cells**: These include surgical removal of gonads, radiation therapy with expected damage to the gonads, and the use of potentially

fertility-damaging drugs. Congenital disorders of testicular function with progressive deterioration, such as patients with Klinefelter syndrome, adrenogenital syndrome (AGS), or cystinosis, do not yet fall under this indication. The scope of services of the Kryo-RL covers the medical measures required for cryopreservation: preparation, collection, processing, transport, freezing, storage, and subsequent thawing of **egg or sperm cells and germ cell tissue**, such as laboratory tests (HIV, hepatitis serology), measures in connection with the collection, examination, and processing **of sperm cells in males from puberty onwards including semen analysis,** and, if necessary**, testicular sperm extraction (TESE)**, and the selection of the appropriate **cryopreservation procedure** including the associated (subsequent) measures in accordance with the regulations of the RL-Assisted Reproduction of the German Medical Association. The service providers must be licensed in accordance with §20b or §20c of the German Medicines Act (AMG), and in the case of treatment of male insured persons, they must have additional training in andrology.

42.2 Indications for Cryopreservation of Sperm and Testicular Tissue

The indications for cryopreservation of sperm from ejaculate or testicular tissue vary depending on the patient groups involved. Most men who opt for sperm cryopreservation are patients diagnosed with malignancy, in whom the curative strategy for their disease is still unclear, and who are undergoing potentially fertility-damaging therapy (Table 42.2). Cryopreservation should be offered to adolescents and adults prior to potential gonadal damage (AWMF Guideline 2017) so that sperm cryopreservation can also be performed prior to initiation of therapy. Cryopreservation can be performed promptly (within 24–48 h), so that a delay in therapy is not expected in most cases. In case of azoospermia (or aspermia), surgical sperm retrieval should be offered immediately.

Table 42.2 Frequency of diagnoses of oncological and non-oncological diseases at the time of presentation for cryopreservation (using the example of patients of the Center of Reproductive Medicine and Andrology of the University of Münster), as of December 31, 2022

Diagnoses	Number of patients (n = 3527) (%)
Testicular tumor	2071 (58.7)
Hodgkin/non-Hodgkin lymphoma	451 (12.8)
Leukemias	264 (7.4)
Bone tumors	189 (5.3)
Rectal and colon carcinomas	75 (2.2)
Malignant melanoma	43 (1.2)
Transwomen	82 (2.3)
Paraplegia	81 (2.2.)
Other	271 (7.79)

In this situation, it must be decided in principle whether the time delay associated with surgery is acceptable for the planned therapy and whether the underlying disease permits surgery of the testis.

In countries where the use of cryopreserved semen samples is also allowed posthumously, cryopreservation is also recommended to men in the context of a dangerous activity, e.g., military service. There is also the basic possibility for every man to create a fertility reserve, such as before a sterilization vasectomy or in the context of fertility treatment due to an unfulfilled desire for children or without a medical indication ("social freezing") (Gromoll et al. 2015; WHO 2021).

42.2.1 Oncological Diseases and Gonadotoxic Therapies in Adults and Adolescents

The indication for sperm and testicular tissue cryopreservation extends to all systemic therapies that have potential gonadotoxic effects and to all local therapies that may affect gonadal function directly or through their control mechanisms, in both oncologic and non-oncologic diseases. In addition, the medical need for fertility protection also exists for surgical procedures that affect seminal deposition (ejaculation and/or erection) in the long term.

Sperm cryopreservation should be offered not only to **adults** but also even to **adolescents**. Adolescent boys show comparable ejaculate parameters before and after performing cryopreservation compared to adult patients regardless of the underlying disease (Kliesch et al. 1996; Kamischke et al. 2004). Since the documentation of cryopreservation in the CeRA's **Androbase©** database, a total of 3527 (1987–2022) cryopreserved ejaculates have been recorded. The proportion of patients under 18 years of age is 7.5%.

The adolescent oncology patients in our patient population showed age-appropriate normal or near-normal testicular volume independent of sperm concentration. An age-appropriate normal testicular volume in the **low-to-subnormal adult range** can be considered a positive predictor of successful spermatogenesis and subsequent cryopreservation (Kamischke et al. 2004). Although many chemotherapy protocols used in adolescents have produced fewer side effects, prognosis regarding toxicity to the gonads and corresponding long-term effects on fertility remains difficult. It depends on the type of combination therapy and the need for multimodality treatments. Up to 16% of patients exposed to chemotherapy protocols without alkylating agents (e.g., adriamycin, vincristine, and methotrexate) in adolescence showed persistent azoospermia in adulthood. After cisplatin-containing chemotherapies, azoospermia is observed in a mean of 37% of cured adult patients. When using alkylating agents (e.g., cyclophosphamide or procarba-

zine) in the adolescent, adult infertility must be expected in 68% of patients (AWMF 2017). Modern therapeutic regimens aim to reduce toxicity, but this cannot exclude permanent damage. However, recovery of spermatogenesis can be observed even after long time intervals after completion of therapy and testicular volume is also an important indicator here (Wilhelmsson et al. 2014).

Generally, the oncological patients are informed about chemotherapy and receive an offer to perform cryopreservation. This is already required in the currently available guideline on fertility preservation in oncological diseases for all age groups (AWMF 2017). However, a recent meta-analysis shows that the education rate and also the acceptance of the offer still vary considerably (Valipour et al. 2019). There is still a need for education and improvement of the educational structure in this regard. By implementing SOPs for education and information on fertility protection issues into the procedures of the tumor centers now established at all major centers, an improved education rate can be achieved.

Chemotherapy and/or radiation, as well as surgical procedures, can have a negative impact on the male reproductive system with sometimes irreversible effects depending on the therapeutic measures, the drugs and their dose. For this reason, fertility protection is now a firmly integrated component of the first "S3 Guideline on the Diagnosis, Therapy and Follow-up of Germ Cell Tumors" (AWMF 2019).

Malignancies may occur at a time when the patient's family planning either has not yet begun or is not yet completed, and reproductive function is considered to be of high importance in terms of quality of life. A previous evaluation showed that of 1132 adult oncology patients (mean age 28 years) who presented to our center for cryopreservation of their semen between 1989 and 2008, only 24% were married; only 10% had fathered at least one child before the onset of the disease.

The extent of fertility reduction due to chemotherapy and/or radiotherapy cannot be predicted with certainty, and the possibility of recovery of testicular function depends on the type of therapy, dose, and individual sensitivity and baseline situation regarding the patient's testicular function (Kliesch et al. 1996; Palmieri et al. 1996; Meistrich 2013; Sander 2013; AWMF 2017) (Table 42.3).

To date, it has not been possible to effectively protect testicular function from the gonadotoxic influence of such treatment (Kamischke et al. 2003). Retrograde ejaculations after retroperitoneal lymphadenectomy can be treated in a drug therapy trial. In contrast, for impotentia generandi after radical surgery (prostatectomy, cystoprostatectomy, rectal exenteration), TESE is the only therapeutic option (Kamischke and Nieschlag 1999; AWMF 2017).

In patients with testicular tumors or other malignancies (e.g., Hodgkin's lymphoma, leukemia), limitations in testicular function are known, although the mechanisms leading to impairment by the underlying disease are not clear (Hansen et al. 1991; Viviani et al. 1991). In our own patient population, 63% of testicular tumor patients, 43% of patients with lymphatic or leukemic diseases, and 45% of men with various solid tumor diseases show limitations of ejaculate parameters in the sense of oligozoospermia even before cryopreservation (Fig. 42.1) (modified after Kamischke et al. 2004). At diagnosis, azoospermia is much more frequent (14%) in patients with testicular tumors than in lymphoma patients (van der Kaaij et al. 2009) (see Table 42.4). In view of these facts, cryopreservation prior to the start of therapy is urgently recommended, as then surgical removal of the diseased testis can be combined with surgical sperm retrieval, especially in testicular tumor patients.

In almost every case, solid urogenital tumors (prostate, bladder, and penile carcinoma) lead to irreversible infertility shortly after surgical therapy. Despite patients' older age at disease, the desire for fertility protective measures may exist and should be included in counseling. In 495 patients with prostate cancer disease, at a median age of 62 years, the desire for fertility protection was thus present in one in five men, particularly among those without children (21%) (Salonia et al. 2013).

Patients with osteosarcomas, Ewing sarcomas, and soft tissue sarcomas are at higher risk for posttherapy permanent infertility as are men with anal and rectal cancers who are at high risk for permanent infertility. Unfortunately, too often this is still not considered in surgical education and counseling fails to be offered.

Table 42.3 Results of ejaculate values at a mean of 7 years after the end of therapy in patients with oncological and non-oncological diseases and gonad-damaging therapy who created a cryodepot with sperm before the start of therapy (according to Sander 2013)

	Normozoospermia (%)	Oligozoospermia (%)	Cryptozoospermia (%)	Azoospermia (%)
	25.2	42.6	6.4	25.8
Germ cell tumors	26.8	50.5	4.0	18.8
Lymphomas	32.0	27.2	8.0	32.8
Leukemias	10	47.5	5.0	37.5
Sarcomas	3.1	34.4	18.8	43.8
Non-oncologic diseases	41.7	33.3	8.3	16.7

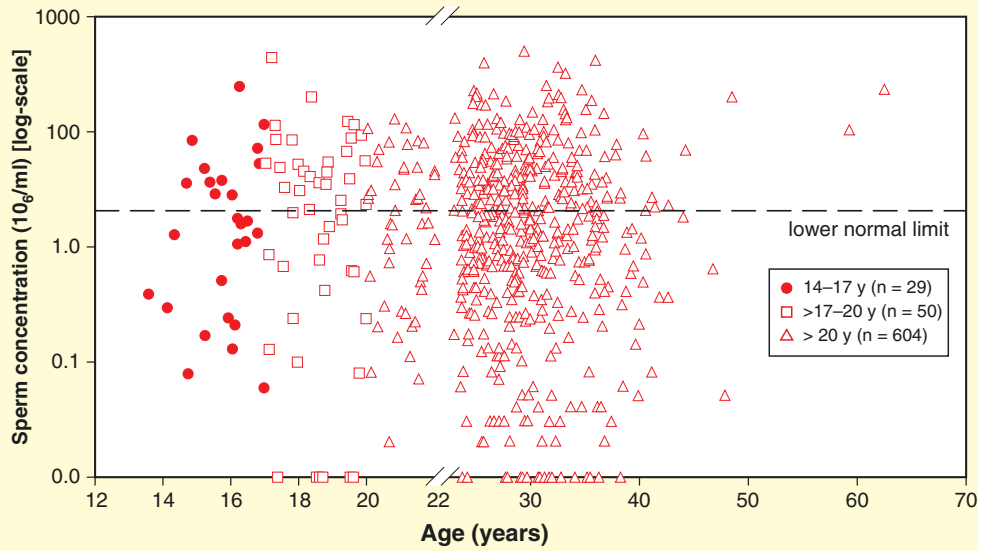


Fig. 42.1 Age and sperm concentrations in semen of 683 patients with malignancies at the time of cryopreservation. Note logarithmic scale. The lower normal limit for sperm concentration is 20 million spermatozoa/ml semen (broken line) [updated and modified from: Kamischke et al. 2004]

Table 42.4 Semen parameters in testicular tumor patients of our own collective (Kliesch 2012) compared to data of the Lymphoma Study group (van der Kaaij et al. 2009) (modified according to Kliesch 2012)

Ejaculate parameters (according to WHO)	Testicular tumor ($n = 563$), (Kliesch 2012) (%)	Hodgkin's lymphoma ($n = 474$) (van der Kaaij et al. 2009) (%)
Normozoospermia	25	41
Oligozoospermia	56	56
Azoospermia	14	3
(Aspermia)	4	Not examined

Of course, all patients with hematologic neoplasms (leukemias, Hodgkin's and non-Hodgkin's lymphomas, and follicular lymphomas) should also be informed about the risk of infertility and be offered cryopreservation.

Information about options for fertility-preserving measures is required for all age groups. Sperm cryopreservation as a preventive measure with regard to family planning must be taken into account, especially for younger patients, as it affects the quality of life after the underlying disease has been overcome. It should also play a role in the education of young patients in adolescence and their parents before a potentially gonadally damaging therapy is carried out.

42.2.2 Oncological and Non-Oncological Diseases in Children

Although spermatogonial proliferation activity does not increase until puberty, prepubertal testes are sensitive to gonadotoxic exposure (Jahnukainen et al. 2011). In this context, spermatogonia are highly sensitive regardless of the type of noxious agent, whereas somatic cells in the testis show much higher resistance. While testicular hormonal function is only disrupted at very high doses, both irradiation and chemical noxious agents lead to a dose-dependent depletion of spermatogonia even at low exposures. Because germ cells constitute only a very small volume fraction of the immature testis, this effect is not evident in immature testes by the reduction of testis size. However, the potential for efficient future spermatogenesis depends directly on the survival of the spermatogonia population. In particular, it is relevant that at least a few stem cells are preserved in as many testicular tubules as possible, because although they can achieve recolonization and induction of one tubule at a time by clonal expansion and migration, colonization of completely germ cell-free seminiferous tubules is not possible. Therefore, an "all-or-nothing" phenotype is often observed in cross sections of the testis after intensive chemo- or radiotherapies, as seminiferous tubules with remaining stem cells show complete spermatogenesis, while other testicular tubules do not contain germ cells (Fig. 42.2).

For this reason, no linear correlation exists between the dose and the recovery of spermatogenesis, as the critical factor is not the number of spermatogonia per se, but the presence of stem cells in as many seminiferous tubules as possible. In addition to the degree of recovery, the stem cell-based system of spermatogenesis also affects the kinetics of a recovery. Even very few surviving stem cells can achieve efficient recolonization long after depletion. Depletion of active spermatogonia following noxious agents occurs acutely. Subsequent activation of dormant stem cells occurs. Their survival and transition into active progenitors also depend on the dose of exposure. Although the mechanisms of selection of healthy stem cell clones are poorly understood, stringent checkpoints appear to be responsible for

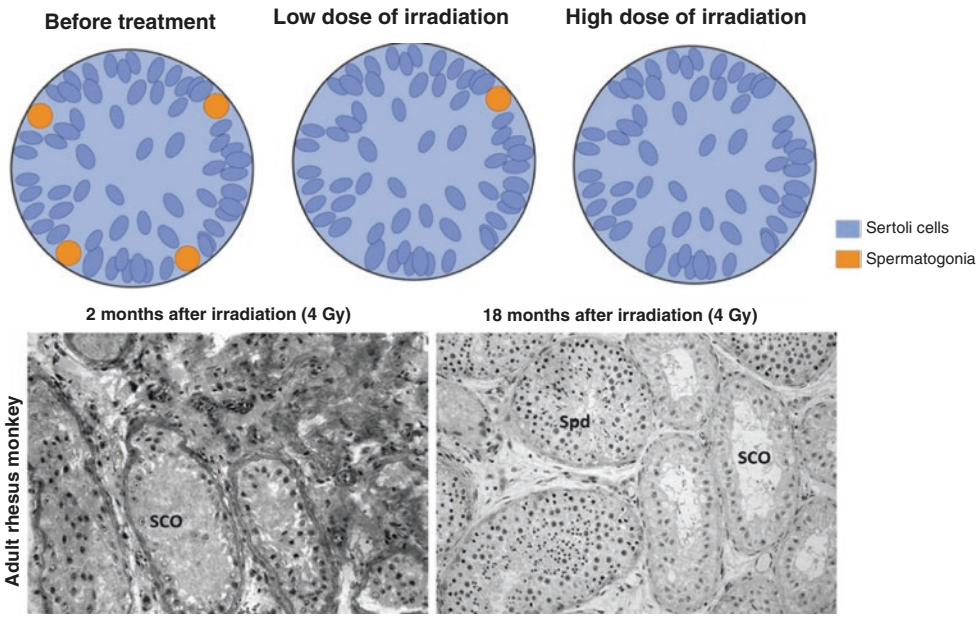


Fig. 42.2 Effect of low and high irradiation on the number of spermatogonia (orange cells at the basement membrane). Recovery of spermatogenesis in the tissue of an adult rhesus monkey in individual seminiferous tubules based on the remaining spermatogonia and germ cell-free (SCO, Sertoli cell only) tubules after a complete loss of spermatogonia (modified after Heckmann et al. 2018, image material CeRA, UKM)

ensuring that no severely damaged germ cells survive. These mechanisms appear so reliable that paternity as a result of spontaneous recovery of spermatogenesis after chemotherapy or radiotherapy is not considered particularly risky. Only the recommended waiting period before conception of a child is controversial.

Carcinogenic agents show different degrees of damage to spermatogenesis in childhood. In a list of the "International Late Effects of Childhood Cancer Guideline Harmonization Group," alkylating reagents in particular are named as problematic, and among them cyclophosphamide, busulfan, mechlorethamine, and procarbazine are particularly noteworthy, especially due to their widespread high-dose and additive combined use. Thus, conditioning therapies prior to bone marrow transplantation are particularly problematic (Skinner et al. 2017). However, a defined dose for the occurrence of azoo- or oligozoospermia cannot be named and appears to vary on a patient-specific basis (Green et al. 2014; Skinner et al. 2017). An assessment of recovery after exposure can only be collected by quantifying spermatogonia in biopsy-derived specimens. To this end, numerous studies have been performed in recent years and scores have been described that allow estimation of spermatogonial stem cell reserve (Heckmann et al. 2018; Stukenborg et al. 2018; Valli-Pulaski et al. 2019). If samples are collected prior to exposure, it is possible to determine the number of stem cells in the collected and cryopreserved tissue samples and thus assess the suitability for prospective use of the tissues for sperm retrieval.

In addition to chemical noxious agents, **radiation** plays an important role in tumor therapy. If testes are in the radiation field, spermatogonia are damaged and acutely eliminated similar to chemoexposure. Depending on the radiation dose, inactive stem cells may also be depleted at a later time. As with chemical noxious agents, there is high variability and no universally applicable doses that result in azoo- or oligozoospermia. Differentiating spermatogonia are highly sensitive and respond with depletion to doses as low as 0.1 Gy. However, at low radiation doses, there is only a short-term depletion and subsequent complete re-initiation of spermatogenesis (Rowley et al. 1974), but long-term damage can occur at doses as low as 2 Gy and is very common at doses of 6 Gy and above, leading to long-term infertility (Rowley et al. 1974; Centola et al. 1994).

Assessment of the risk for permanent damage to fertility is difficult because of the high variability and individual dispersion. Recommendations for the use of invasive stem cell-based fertility interventions therefore vary worldwide. For the most part, removal of immature testicular tissue is only advised when a significant risk of future infertility due to high levels of radiation and chemoexposure can be assumed (Wyns et al. 2011; Picton et al. 2015; Goossens et al. 2020). Preexisting conditions or previous treatments are also factored into the decision. An ever-increasing group of patients are affected by gonadotoxic exposure even in the presence of non-malignant or gene-induced diseases, as well as loss of stem cells for other reasons (e.g., sickle cell anemia, thalassemia, Fanconi anemia, and immunodeficiency in variants of the FOXP3 gene).

42.2.3 Cryopreservation in Congenital Diseases with Gonadal Damage

Congenital diseases may be associated with infertility as a major or concomitant symptom. Some examples will be focused on here, for which new knowledge has been gained in recent years.

Klinefelter syndrome is the most common numerical chromosomal aberration (47,XXY), which is associated with azoospermia after puberty in >95%. Patients with Klinefelter syndrome represent approximately 15% of patients with non-obstructive azoospermia. The cause of azoospermia is apoptosis of spermatogonia carrying a supernumerary X chromosome. Focal residual spermatogenesis, particularly in adolescents or young adults with Klinefelter syndrome, originates in intact spermatogonia. With the onset of puberty, a marked FSH increase indicates testicular dysfunction. Adolescents who still have good endocrine Leydig cell function have a much better chance of still having residual spermatogenesis and may have sperm in the ejaculate in up to 7% of cases, which can be cryopreserved depending on quality (Rohayem et al. 2016). In the 15- to 25-year-old age group, microsurgical testicular sperm extraction to create a fertility reserve can be successfully performed in approximately 55% of patients with intact Leydig cell function and still normal testosterone serum levels. With increasing age, Leydig cell function also decreases profoundly and with it the chance for successful sperm retrieval (Rohayem et al. 2015). Information about fertility-preserving measures in patients with Klinefelter syndrome should be provided early, before the onset of puberty, to avoid missing the best time for this measure (Zitzmann et al. 2021).

Congenital adrenogenital syndrome (AGS) is variable in its clinical expression and its course is highly dependent on patient compliance with regular medication regimens. Inadequate steroid medication often results in testicular adrenal residual tumors (TARTs), which are easily mistaken clinically for testicular tumors if the patient is unaware of AGS. In addition to the risk of unnecessary testicular surgery, depending on the severity of the TART, deterioration of testicular function and even azoospermia may result. Fertility reserve with cryosperm may also be useful in these cases.

The rare clinical picture of **cystinosis**, a lysosomal storage disease, leads to a disturbance of Leydig and Sertoli cell function and subsequently to testicular dysfunction. The course of the disease cannot always be predicted with certainty; most patients decompensate in the fourth decade of life. Spontaneous paternity has not been reported in the literature. In these cases, sperm cryopreservation represents a simple and safe fertility preservation measure. A small cohort study demonstrated that TESE can also be successfully performed in the case of azoospermia in these patients (Rohayem et al. 2021a, 2021b).

Disorders of sexual differentiation (DSD), which can present a wide spectrum of gonadal damage, are also clinical pictures in which sperm cryopreservation can be discussed and offered depending on the severity and course of the condition.

42.2.4 Cryopreservation in Spinal Cord Lesions

Patients with a spinal cord syndrome, in whom loss of ejaculatory ability is observed in up to 95% of cases, represent a patient group that has received little attention overall. Electrovibration or rectal electrostimulation can be used to induce ejaculation, and assisted fertilization can be performed with the semen obtained (Kamischke and Nieschlag 1999; Nassau and Ramasamy 2021). Electrovibration, which is quite simple, can be successful in some cases and can also be used in the home setting. However, electrostimulated ejaculation is significantly more complex and definitely also associated with (surgical) risks or potential side effects. Furthermore, the sometimes considerable interdisciplinary and logistical effort for the attending physicians and the patient is not unproblematic. Both procedures represent a possibility of cryopreservation of the ejaculate obtained by stimulation for a later medically assisted fertilization procedure (Nassau and Ramasamy 2021).

42.2.5 Cryopreservation in the Context of Infertility Diagnosis and Therapy

Men with an unfulfilled desire to have children may cryopreserve their sperm for various reasons for subsequent therapy of their partner by intrauterine insemination (IUI), in vitro fertilization (IVF), or intracytoplasmic sperm injection (ICSI). Possible reasons may include severe oligoasthenoteratozoospermia, only intermittent presence of scattered motile sperm in the ejaculate (cryptozoospermia), or only partially successful treatment of existing infertility, such as surgical intervention for obstruction of the vas deferens or gonadotropin treatment for hypothalamic–pituitary hypogonadism. In special circumstances, such as assisted ejaculation for anejaculation, sperm can be processed from urine for retrograde ejaculation or obtained from the genital tract by surgical means (mTESE or TESE). Sperm cryopreservation is also an option in rare cases when patients are unable to provide ejaculate or be present on the day of oocyte aspiration as part of IVF or ICSI. The current “minimum requirement” for cryosperm samples is the presence of less vital sperm from testis, epididymis, or ejaculate.

Cryopreservation of surgically obtained sperm for further use in assisted fertilization methods has gained considerable importance in recent years. In combination with cryopreservation, testicular sperm extraction and, in individual cases, epididymal sperm retrieval have become routine procedures in centers specialized in reproductive medicine and urology; today, these procedures are used in cases of azoospermia (microsurgically reversible, irreversible), obstructive, or pri-

marily testicular (= non-obstructive). The current preferred technique is microsurgical TESE to detect focal islands of residual spermatogenesis intraoperatively (Salonia et al. 2022; Corona et al. 2019; Esteves et al. 2020). Predictive parameters are intensively discussed, and in addition to FSH, testicular volume, and underlying disease, genetic causes of infertility will play a greater role in the future (Busch et al. 2019; Wyrwoll et al. 2023). The German IVF Register shows a stable share of just under 5% TESE-ICSI cycles in the total number of ICSI cycles over recent years (DIR 2022). In our Center, the proportion is 20% to 25% of treatments due to specialization (Nordhoff 2014).

42.2.6 Fertility Protection in Transpersons (Transwomen)

The literature on fertility protective and reproductive interventions in individuals with gender incongruence has increased rapidly in recent years in light of the fact that at least half of transpeople want to start a family and desire genetic parenting (Auer et al. 2018). Hormone therapy for puberty blocking or gender reassignment suppresses spermatogenesis in transwomen. If orchiectomy is performed, this results in irreversible loss of fertility (Schneider et al. 2019). Current guidelines, some of which are under development, take this into account and request counseling patients with gender incongruence about fertility preservation options. The *World Professional Association for Transgender Health* (WPATH) first mentioned in its *Standards of Care* (2001) that reproductive health issues should be discussed for individuals diagnosed with gender incongruence before sex reassignment hormone therapy begins (Meyer et al. 2002). With this in mind, the option of cryopreservation of sperm from the ejaculate or germ cell tissue should be offered to these patients prior to more extensive measures (hormone therapy, surgical removal of the gonads).

42.2.7 Cryopreservation Before Vasectomy or After Vasovasostomy (VV) (or Vasotubulostomy (VT))

Cryopreservation as a procreative reserve in patients undergoing vasectomy for family planning reasons may be helpful to those affected, as no reversible male hormonal contraceptive comparable to female hormonal contraception is available in clinical use. Vasectomy as an invasive and often irreversible (see Chap. 20) method of contraception requires the affected patient or couple to make a definitive decision about their family planning. Clinical experience shows that changed life circumstances (separation of spouses and establishment of a new relationship with renewed desire for children, loss of a child or partner) may well give rise to a desire for reversibility and an attempt at microsurgical VV or VT. For this group of patients, the frozen semen depot could open up the possibility of fulfilling the desire for a child with the help of assisted fertilization procedures if the refertilization operation is unsuccessful. After successful VV or VT, there is a risk that the patency of the draining seminal ducts may not remain permanent and a new occlusion may occur. In this case, the patient can also be offered the option of cryopreservation of intraoperatively collected sperm or fresh ejaculate after successful refertilization. Seminal secretions obtained intraoperatively during VV should not be cryopreserved with the exception of epididymal aspirates containing motile sperm in the setting of VT, which can be used for assisted fertilization procedures that may be performed at a later date if refertilization is unsuccessful. Alternatively, microsurgical refertilization surgery can be combined with testicular sperm extraction (TESE). However, these measures certainly need to be critically discussed due to the resulting costs for the patient, especially in view of the small number of patients who resort to the cryopreserved semen depot after successful refertilization (Am Soc Reprod Med 2008; Kolettis et al. 2005).

In our own patient population, 48% of 73 patients with an active desire to conceive became pregnant after vasovasostomy over a 5-year period. Sixteen patients conceived spontaneously, and 19 patients underwent ICSI treatment with ejaculated sperm ($n = 15$) or testicular sperm ($n = 4$) during the course (Spiliopoulos 2014). A recent study showed that abortion rates were significantly higher after TESE-ICSI following vasectomy than after spontaneous conception following successful refertilization surgery (Uvin et al. 2018).

42.2.8 Cryopreservation for Nonmedical Indication ("Social Freezing")

In principle, every man is free to freeze his sperm in order to build up a fertility reserve for himself. Opinions differ as to whether "social freezing" makes sense. However, in light of the significant increase in the age of men in couples seeking help for childbearing to over 38 years and the existing evidence that paternal age may be associated with risks to offspring, the considerations should be taken seriously (Gromoll et al. 2015; Pennings et al. 2021).

42.2.9 Sperm Donation

Sperm donation is mentioned here only for completeness, as cryopreservation of gametes is a prerequisite. Healthy, potentially fertile donors are eligible for sperm donation. These men can be recruited by clinics or sperm banks and

provide sperm samples as anonymous donors or as donors known to the recipient couple. The use of donor gametes is not permitted in all countries. Where this procedure is possible, there are no uniform rules for donor selection. Legislation varies widely. For example, in some countries donor anonymity is mandatory; in others, children conceived with donor sperm may learn the identity of the donor after reaching the age of majority. The use of donor sperm for heterologous assisted fertilization depends on the respective legislation (Dondorp et al. 2014; ESHRE 2022).

Donor sperm can also be used in Germany in the form of IUI, IVF, or ICSI to treat the woman who has an infertile partner in whom no usable vital sperm are detectable for ICSI therapy. In addition, donor sperm can be used in cases of repeated treatment failure or excessive treatment costs, to protect against the transmission of hereditary diseases, or to prevent fetal hemolytic anemia in cases of blood group incompatibility. In addition, donor sperm can be used in patients with recurrent miscarriages. In addition, donor sperm can lead to successful induction of pregnancy in lesbian women of childbearing potential and a child can be carried to term.

42.3 Sperm Analysis, Processing, and Cryopreservation

Cryopreservation of a semen sample is always preceded by ejaculate analysis according to the currently valid criteria of the WHO. The basic spermiogram includes, as a minimum requirement, the determination of sperm count and concentration, sperm motility and morphology, and analysis of sperm vitality. According to WHO recommendations, the frozen quantity should be sufficient for 10 or more ICSI treatments. This is sufficient with the standard sets for ejaculate sperm cryopreservation in most cases with normal ejaculate quality. However, in cases of severely impaired ejaculate quality, it may be necessary or advisable to fill more cryosets—provided that the underlying disease and the time window until the start of therapy for the respective disease allow this.

There are several cryopreservation protocols for ejaculate sperm and commercially available cryoprotectants.

Intracellular ice crystals lead to intracellular damage and cell death during the thawing process, whereas extracellular ice crystal formation increases extracellular tension and induces cell membrane damage by shrinking cell volume. To perform cryopreservation, the ejaculate must be diluted with a cryoprotectant. The usual commercially available cryoprotectants are glycerol-based compositions. The ejaculate diluted in this way is then placed in prefabricated mini-straws (0.25–0.3 mL). These special straws ("straws") are labeled with an identification number and are sealed at the ends. The "straws" are then placed in prefabricated cryocassettes, and these are sealed.

Nowadays, cooling and freezing of the ejaculate are usually performed with programmable freezers. These control the supply of liquid nitrogen vapor to the freezing chamber and electronically document the freezing steps. The cryopreservation process is gradual, and the ejaculate is cooled down to −170 °C. The material is then transferred to appropriately monitored storage tanks for storage in the vapor phase of liquid nitrogen at −180/−190° for permanent storage. In the vapor phase for storage, special attention must be paid to the temperature of the samples, which must never reach above −130 °C in order not to damage the sperm. Currently, sperm vitrification procedures are also being tested, which may offer an advantage, especially when the sperm count is low (Berkovitz et al. 2018; Endo et al. 2022).

Before using the cryopreserved samples, they must be thawed and separated from the cryoprotectant. After thawing at room temperature, the contents are processed for assisted fertilization treatment. The cryoprotectant must be washed out by dilution in small volume steps.

For quality control of cryopreservation and a check of the suitability of the sample for later assisted reproduction, it is useful, but unfortunately not the rule, to reserve a small aliquot of the semen sample separately that is then thawed again after freezing on the same or the following day to check the sample quality (especially vitality). Especially in case of cryptozoospermia, this is an important aspect, because, in case of complete loss of vital sperm by cryopreservation, supplementary ejaculates should be cryopreserved or a TESE should be performed if necessary, in order to really have a usable fertility reserve available for the patient after completion of the planned (gonadotoxic) therapy.

Basically, the preparation and cryopreservation of epididymal and testicular sperm follow the same principles as described, except that the samples are additionally mixed with a culture medium and a cryoprotectant and the TESE samples are cryopreserved in minitubes.

A special feature of the testicular samples is their preferential storage in the tissue composite. Only after thawing does mechanical tissue disruption and enzymatic tissue digestion occur to release the testicular sperm. Various processing methods are used here, which are not standardized and depend on the experience of the individual laboratory.

Another special feature of testicular samples, depending on the severity of testicular atrophy, is the assessment of whether there will be sufficient material in the tissue for ICSI therapy. Here, a special, individualized screening of all collected samples has been developed in our center, which allows a reliable prediction for the presence of usable testicular sperm (Kappes 2023).

Once semen or tissue samples are frozen, transport is possible using commercially available transport containers cooled with liquid nitrogen. During evaporation, appropriately low temperatures can be maintained for several days to several weeks depending on the size of the container. Compliance with local, national, and international regulations must be observed when transporting liquid nitrogen. Appropriate UN display codes must be used and demarcation agreements signed between the dispensing and receiving laboratories to ensure responsibilities and regulatory compliance.

42.4 Analysis, Processing, and Cryopreservation of Spermatogonial Stem Cells (Androprotect®)

The protocols for the preparation and cryopreservation of mature spermatozoa and spermatogonia differ very substantially. For example, glycerol-based sperm-freezing media are completely unsuitable for preserving testicular tissue with stem cells. Therefore, especially in peripubertal patients, it is important to carefully analyze which procedure should be used or whether a multi-pronged approach should possibly be taken. If no sperm are present in the ejaculate, a two-pronged approach should be taken depending on pubertal development: A part of the testicular tissue samples is processed with the glycerol-based cryoprotocols already described (see above), so that sperm can be obtained from the supernatant or from the tissue. Another piece of tissue is frozen with DMSO-based cryomedia in order to create a stem cell reserve from them.

Freezing of immature testicular tissue with spermatogonial stem cells is fundamentally different from protocols for cryopreservation of sperm from testicular tissue or ejaculate. International standardization of protocols for cryopreservation of immature testicular tissue is one of the current clinical challenges in the field of fertility protection (Goossens et al. 2020). According to a survey by the ESHRE *Special Interest Groups "Andrology"* and "*Fertility Preservation,"* slow freezing protocols are used by all 24 specialized institutions offering cryopreservation of immature testicular tissues. After a usually unilateral biopsy, the immature testicular tissue is transported in transport medium at 4 to 8 °C to avoid warm ischemia and to ensure the fastest possible processing in the laboratory. Transport times up to 72 h are possible at 4 °C in DMEM/F12 medium (Faes and Goossens 2017). Tissues are cut into small fragments of approximately 0.5 mm in diameter. To date, there is no defined and standardized freezing protocol. However, there is a recommendation for slow and controlled freezing in dimethyl sulfoxide (DMSO)-based cryomedia including induced seeding. DMSO appears to be superior to other cryomedia such as 1,2-propanediol and glycerol and is recommended in most publications (Wyns et al. 2011; Baert et al. 2013; Poels et al. 2013; Pietzak et al. 2015). Sucrose is often additionally added to the cryomedium as a non-penetrating substance (Wyns et al. 2011; Baert et al. 2013; Poels et al. 2013). Vitrification of testicular tissue fragments has not yet provided evidence of improved tissue quality after freezing and appears to be more of a disadvantage due to the size of the tissues.

Thawing of cryopreserved specimens should be rapid to prevent ice crystal formation. Transferring the cryotubes to a 35 °C water bath is suitable for this purpose. Immediately after thawing, the tubes should be filled with cold DMSO-free medium in several steps to avoid osmotic effects, and then the fragments should be freed from residual cryoprotectant by rinsing them several times in medium.

It is not possible at this time to conclusively evaluate which of the cryoprotectants is best suited to preserve spermatogonial functionality. In contrast, cryopreservation and subsequent use of sperm in assisted reproductive treatments are a validated clinical approach. Accordingly, a two-pronged approach should be adopted in boys whose testes are potentially already producing sperm, as described above. In these cases, part of the testicular tissue should be frozen in glycerol for sperm retrieval and another part should be processed and cryopreserved with DMSO for spermatogonial retrieval.

42.5 Safety and Quality Requirements and Risk Assessment in the Context of Cryopreservation of Human Germ and Spermatogonial Stem Cells

42.5.1 Safety and Quality Control Measures

Recommendations for risk management are also provided in the WHO Laboratory Manual (WHO 2021): The basic requirement for handling cryogenic material is an alarm system for the detection of liquid nitrogen and low atmospheric oxygen.

It is imperative to ensure unambiguous identification of samples. A secure coding system for labeling cryotubes and vials is essential, and the appropriate codes are noted in all laboratory data sheets and computer databases related to the patient. If aliquots of cryopreserved sperm were used, the number of samples remaining will be appropriately documented in the database. Ideally, an MTA should process only one sample at a time.

42.5.2 Handling of (Potentially) Infectious Specimens

The emergence of acquired immunodeficiency syndrome (AIDS) HIV (human immunodeficiency virus) in the early

1980s necessitated the introduction of a quarantine period and more extensive testing of donors prior to cryopreservation to reduce the risk of transmission of the infectious disease. HIV-positive men on retroviral therapy can freeze their semen for IUI, IVF, or ICSI if their viral load is undetectable, in order to induce conception with reduced risk of HIV transmission to the partner (Gilling-Smith et al. 2005). Equal protective measures (quarantine and laboratory diagnostics) are used for patients with hepatitis disease. Whereas in hepatitis B, with a viral load <1000 copies, ICSI therapy can be performed with minor protective precautions with a vaccinated partner, this is not possible without an increased risk in hepatitis C. Here, there is an increased risk of transmission to the partner and thus also to the unborn child. Zika viruses have attracted further attention in recent years. Detectably infectious specimens must be stored in separate storage tanks. To reduce the potential risk of cross-contamination with infectious agents during storage between specimens (e.g., transmission of HIV and hepatitis B or C through cryopreservation containers), preference is given to storage in the vapor phase of liquid nitrogen and care is taken to securely seal cryostraws. Reported cases of cross-contamination are not found in the literature.

42.5.3 Risk of Tumor Cell Contamination of Spermatogonial Stem Cells

A risk in autografting and in transplantation of tissues and cells from testes of patients with malignant diseases is the possible transfer of tumor cells. The large lymphoid spaces in the interstitium of the testis provide an opportunity for tumor cells to infiltrate and colonize. This risk is particularly relevant to patients with nonsolid tumor diseases such as leukemias or metastatic bone tumors. Some animal studies demonstrate such a risk for both the presence of tumor cells in the testis and their potential to re-induce disease by transfer (Jahnukainen et al. 2001). Although initial approaches to purification of cell suspensions using cellular surface markers have been tested (Hermann et al. 2011), there are currently no reliable protocols to eliminate this risk by depletion of tumor cells or selective purification for highly specific transfer of spermatogonia. The risk cannot be conclusively assessed at this time, so this should be considered when counseling these patient groups.

42.6 Use and Quality of Cryopreserved and Stored Spermatozoa and Germ Cell Tissue

42.6.1 Use of Cryopreserved Sperm Cells from Ejaculate and Germ Cell Tissue

For the success of a later, medically assisted fertilization treatment, the restriction of sperm concentration during the creation of a sperm cryodepot does not play the decisive role, but rather sperm motility (Palermo et al. 1992). Sperm motility is sensitively affected by the cryopreservation process (Fig. 42.3). While the mean progressive sperm motility in oncological patients is about 40%, a deterioration of progressive motility after trial thawing of an aliquot immediately

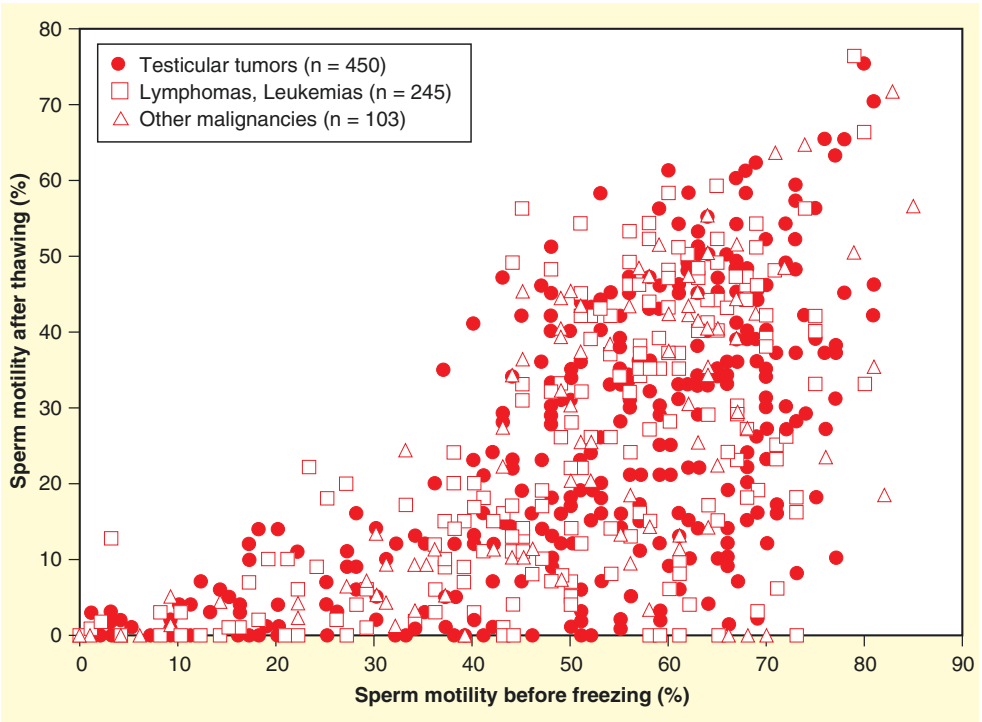


Fig. 42.3 Sperm motility prior to (abscissa) and after (ordinate) freezing and thawing of semen from patients with different oncological diseases at the time of cryopreservation of semen. The lower normal limit for the pre-freeze progressive sperm motility is 50%

after the freezing procedure is shown to still be a mean 21% regardless of the underlying disease or the age of the patients (Keck and Nieschlag 1993; Kliesch et al. 1996). The higher the sperm motility before freezing, the better the thaw motility tends to be. Since motility is considered one of the crucial parameters for the chances of success of insemination or in vitro fertilization treatment (De Geyter et al. 1992), this loss of sperm motility due to the cryopreservation process explains the frequently unsuccessful insemination or in vitro fertilization attempts. In contrast, for microinjection treatment (ISCI), the proportion of motile sperm is less relevant. In this procedure, motility is more of an indicator of the viability of a sperm that is available for selection. When using immotile sperm in the context of ICSI therapy, nowadays there are possibilities for sperm vitality testing, e.g., with modern laser technology (Nordhoff 2014).

The problem is fundamentally similar when using testicular samples: Testicular sperm are immotile to a high percentage and cryopreservation and re-thawing of the tissue can further reduce motile testicular sperm. In these cases, additional options such as laser testing of sperm in combination with TESE-ICSI therapy have also been shown to result in better fertilization and pregnancy rates (Nordhoff 2014).

There is limited information on whether long-term storage of cryopreserved semen samples frozen using modern cryopreservation technology leads to a decrease in sperm quality.

A small risk arises from long-term storage due to possible deterioration of semen quality after thawing, although the data here are inconclusive. Huang et al. (2019) described a sperm survival rate for storage of 0–5 years of 85.7% on average. After storage of 6–10 years and of 11–15 years, this dropped significantly to 82.1% and to 73.9%, respectively. However, the success rates of IVF therapies were independent of storage duration. However, this study was conducted in healthy sperm donors rather than patients. Furthermore, it remains unclear whether or not the expected decrease in semen quality due to the freezing and thawing process was already included in this analysis. From the clinical experience of one of our tertiary centers to date, there has been no significant decline due to permanent storage of cryopreserved semen samples in oncologic and non-oncologic patients, even after >14 years of permanent storage. In addition, children have been born conceived with sperm cryopreserved for longer than 28 years (Clarke et al. 2006; Feldschuh et al. 2005).

42.6.2 Experimental Use of Immature Germ Cell Tissue

Unlike cryopreservation of mature sperm, preservation of germline stem cells requires the use of procedures to generate and mature gametes before fertilization can occur. While there are already clinically applicable protocols for cryopreservation, all procedures for generating sperm from stem cells must still be considered experimental.

The experimental approaches by which spermatozoa can be derived from undifferentiated spermatogonia use cells or tissue fragments as starting material. The isolation and culture of spermatogonial stem cells for subsequent germ cell transplantation represent the only approach that could lead to actual refertilization of patients.

In animal models, this approach was already successfully applied in the 1990s. For this purpose, spermatogonia from one mouse (donor) were transplanted into the germ cell-free testis of another mouse (recipient). After successful transplantation, the stem cells populated the basement membrane of the seminiferous tubules and differentiated to fertile spermatozoa (Brinster 2007). However, xenograft transplantation of human spermatogonia into mouse seminiferous tubules resulted only in nesting of spermatogonia at the basement membrane, but not in differentiation of these germ cells (Sadri-Ardekani et al. 2009). Accordingly, it seems unlikely that the xenograft germ cell transplantation approach can be translated into clinical application.

In contrast, the studies on autologous germ cell transplantation in the primate model (Macaque) yielded very promising results. For example, autologous germ cell transplantation was shown to support and improve recovering spermatogenesis after radiation (Schlatt et al. 2002a; Hermann et al. 2012). For this approach to have perspective application in the clinic, it is necessary to sufficiently propagate spermatogonia in vitro obtained from immature testicular biopsies. For mouse spermatogonia, the protocols for isolation and long-term culture are already established (Kanatsu-Shinohara et al. 2005). While protocols are available for isolation and culture of human spermatogonia for a few days (Kossack et al. 2013), propagation of these cells in vitro has not been successful to date. Another aspect relevant to individual patient groups is that the cell suspension to be transplanted can be screened for the presence of tumor cells. Protocols to remove tumor cells by flow cytometry are under development (Hermann et al. 2011). Thus, the risk of cancer recurrence in patients with nonsolid tumor disease could be minimized.

In contrast to this cell-based approach, the use of tissue fragments has the advantage that the spermatogonial stem cells remain in their stem cell niche in the testicular tissue composite and thus the structural and secretory function of the surrounding niche can be largely maintained. Transplantation of immature testicular tissue fragments has also been advanced in recent years as an approach to differentiate spermatogonia into spermatozoa. Ectopic transplantation of immature testicular tissue from pigs (donor) to mice (recipient) resulted in complete germ cell differentiation and spermatogenesis in the transplanted tissue fragments (Honaramooz et al. 2002). Xenotransplantation of

immature mouse, hamster, bovine, and primate tissues to mice was also successful and resulted in complete germ cell differentiation (Schlatt et al. 2002b; Rathi et al. 2005, 2008; Liu et al. 2016). These studies suggested that xenotransplantation might also be an approach to obtain human sperm, but germ cells in immature human testicular tissues after xenotransplantation differentiated only to spermatocytes (Schlatt et al. 2003; Yu et al. 2006). Because the mouse environment does not appear to support full human spermatogenesis, **autologous transplantation of tissue fragments** was developed. This approach was successful in the primate model specifically in macaques (Jahnukainen et al. 2012) and marmoset monkeys (Luetjens et al. 2008), where only single orthotopic grafts showed complete germ cell differentiation. Meanwhile, the use of larger tissue fragments for autologous transplantation, as well as a longer retention of tissue fragments after transplantation, has resulted in a significantly higher yield of tissue fragments with complete spermatogenesis in the macaque model (Fayomi et al. 2019). In addition, this study has provided evidence of the functionality of sperm generated via autologous transplantation, as the first graft-derived baby ("**Grady**") was conceived via ICSI treatment. Thanks to this success, the autologous tissue transplantation approach can be considered the one most likely to be considered for transfer to the clinic (Neuhaus and Schlatt 2019).

In the in vitro spermatogenesis approach, all germ cell differentiation based on spermatogonial stem cells takes place in the culture dish. To allow diffusion of the medium into the tissue, small tissue fragments also form the starting material here. The advantage that the intercellular structure of the immature testicular tissue is preserved is offset by the challenge of adequately supplying the tissue. To supply the tissue fragments, they are placed on membranes or agarose blocks at the interphase of medium and air. This approach has been successful in the mouse model and the functionality of the retrieved sperm has been demonstrated using ROSI and ICSI (Sato et al. 2011). Germ cell differentiation to round spermatids has also been successful in the rat model (Reda et al. 2016). However, these protocols were not successful with either testicular tissues in the primate model or human tissues. This may be due to the different duration of spermatogenesis. While the differentiation of spermatogonia into spermatozoa in mice spans approximately 39 days, these differentiation processes take nearly twice as long in humans (approximately 72 days; Adler 1996). It is therefore likely that the tissues cannot be adequately supplied by diffusion of the medium into the tissues. Recent approaches therefore use micro-fluidics systems that provide a continuous and controllable supply to individual seminiferous tubules (Sharma et al. 2020).

Other approaches to derive sperm from stem cells include 2D, 3D, and on-chip systems for in vitro procedures, which are currently being intensively researched.

Stem cell-based sperm generation procedures can also be performed independently of the presence of cryopreserved testicular tissue if patient-specific induced pluripotent stem cells are used as the starting material for germ cell generation. The derivation of undifferentiated germ cells has already been shown experimentally for human cells and is based on sequentially induced differentiation steps under defined culture conditions. These cells can be eventually used instead of the germ cells obtained from immature testicular tissue described above. With these procedures, which are still purely experimental, it is possible in principle to obtain sperm from somatic cells (Saitou and Hayashi 2021).

42.6.3 Quality Assessment of Immature Germ Cell Tissue

For an assured quality assessment of immature germ cell tissues, functional tests could provide information on whether the spermatogonia present in the tissues have the potential to form fertile sperm. As respective protocols are still under development, the functionality of human spermatogonia can currently only be evaluated using a standardized morphological assessment. Therefore, for quality assessment of immature testicular tissues before cryopreservation, a number of spermatogonia are analyzed as the main parameter. It is of note that the number of spermatogonia is subject to age-related dynamics: In the first 3 years of life, their number decreases, followed by a gradual increase in the number of spermatogonia until the age of 6–7 years. Then, until the 11th year of life, the number of spermatogonia remains relatively constant, to increase significantly in the following years (Masliukaite et al. 2016). To achieve comparability of results between studies, the results of quantification studies are reported either as the number of spermatogonia per round cross section of a seminiferous tubule or per tissue area. The reliable identification of immature germ cells is secured by immunohistochemical markers (immunohistochemical stains, IHC). Besides the general germ cell marker DDX4/VASA (Stukenborg et al. 2018), MAGEA4 is also used as a marker for undifferentiated germ cells (Heckmann et al. 2018) and also marker combinations such as DDX4/UTF1 (Valli-Pulaski et al. 2019). Regarding the number of spermatogonia, it must be emphasized that the patient cohorts and therefore the testicular tissues in international fertility preservation programs are very heterogeneous. While the number of spermatogonia is within the reference range in some patients, other patients have significantly limited spermatogonia numbers (Heckmann et al. 2018). These data highlight the need to analyze the testicular tissues for each patient so that an individualized assessment can be made regarding the germ cell potential of the respective cryodepots. In this context, the patient groups of boys with sickle cell disease should be highlighted: First studies report that the spermatogonia

pool is reduced (Stukenborg et al. 2018; Van Saen et al. 2015; Portela et al. 2020). In this regard, the influence of hydroxyurea treatment is the subject of recent studies, but has so far remained without evidence of an additionally adverse effect on the spermatogonia population (Gille et al. 2021; Benninghoven-Frey et al. 2022). Moreover, histological evaluations of oncological patients who have already received a biopsy prior to testicular tissue collection indicate that treatment with alkylating, but not non-alkylating, agents leads to a reduction in spermatogonial number (Stukenborg et al. 2018). However, since the aforementioned analysis only considers the quantitative assessment of spermatogonia, no statement can be made regarding the quality and functionality or, if applicable, damage to the cells. Therefore, testicular tissue sampling before starting gonadotoxic treatment must be the goal.

42.6.3.1 Histological Evaluation of Immature Testicular Tissues

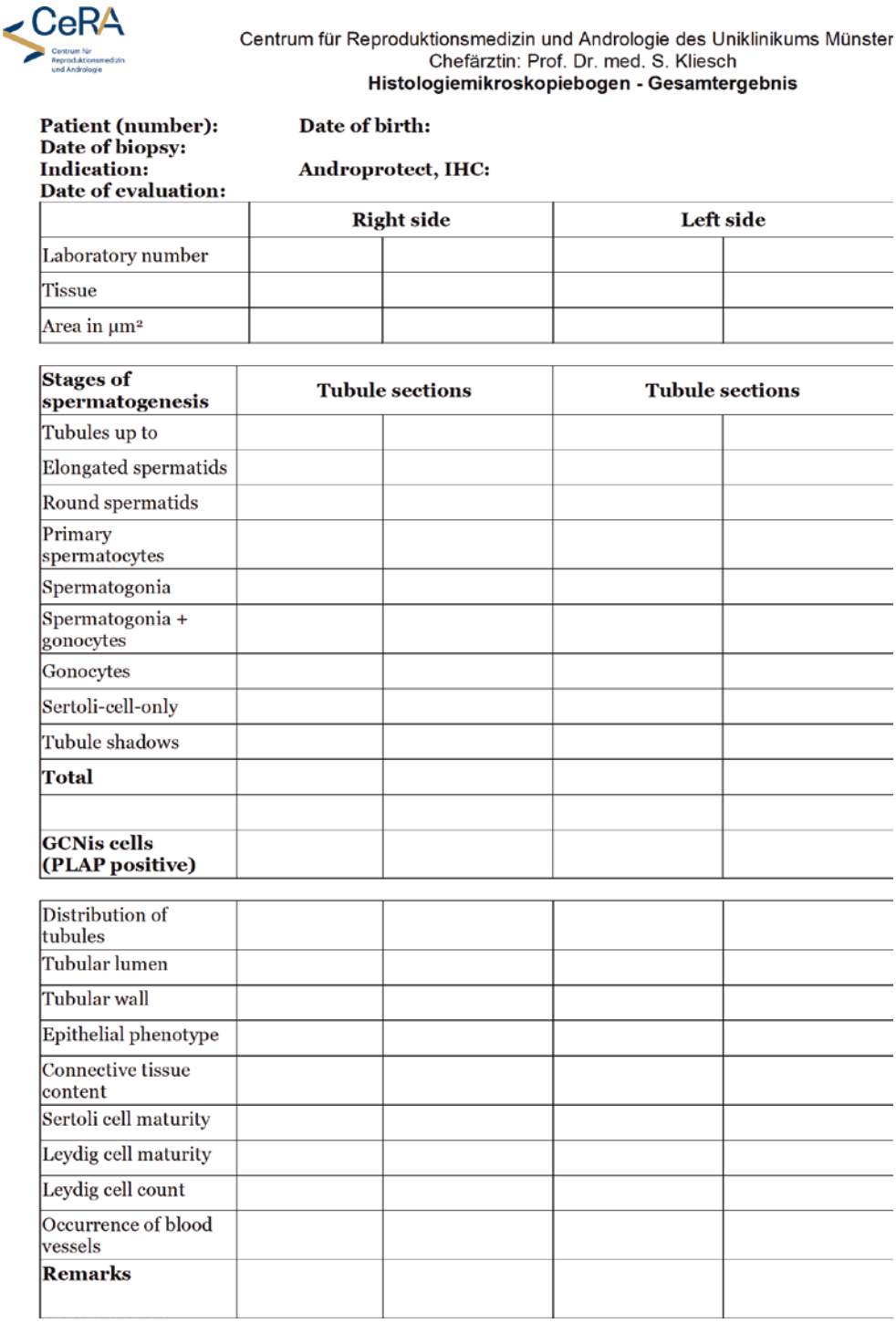
Tissue processing and histological analysis are based on protocols established for adult tissues. The immature tissue fragments are fixed in Bouin's solution and embedded in paraffin. Immunohistochemical detection of placental alkaline phosphatase (PlAP) is used to assess the presence of GCNIS cells, the progenitor cells of seminomas and nonseminomas (see Chap. 11 Histology) (Beckstead 1983). For further histological evaluation, two independent (distance >15 µm) tissue sections per tissue biopsy are stained with a germ cell-specific marker such as MAGEA4 (Heckmann et al. 2018) to reliably identify the undifferentiated germ cells, some of which are only focally present. In addition to the categories of germ cell differentiation considered for the adult tissue samples, the number of tubules containing spermatogonia and gonocytes or exclusively the undifferentiated gonocytes is also noted for the immature tissues. In addition, histologic evaluation also includes cytologic changes such as multinucleated spermatogonia. The findings are recorded in a documentation sheet developed for the Androprotect® specimens (see Fig. 42.4), similar to the documentation of adult testicular histology in the context of a TESE (see Chap. 11).

42.6.3.2 The Androprotect® Score

Spermatogonia form the basis for all protocols currently being developed to obtain sperm from immature testicular tissues. The Androprotect® Score therefore determines the percentage of tubules that contain spermatogonia. Each tubule of the respective tissue section is analyzed according to the data sheet shown in Fig. 42.4 and the number of tubules containing spermatogonia is determined. The score of 10 means that 100% of the tubules contain spermatogonia, a score of 1 accordingly means that 10% of the tubules contain spermatogonia (Fig. 42.5).

The degree of differentiation and the integrity of the testicular tissues are further mapped via the following categories. With regard to the integrity of the testicular tissues, the distribution of tubules in the tissue section, the percentage of connective tissue, and the thickness of the tubule wall are assessed. Moreover, the number of Leydig cells and blood vessels in the tissue section is recorded.

With regard to the degree of differentiation, the formation of a tubular lumen and the epithelial phenotype are assessed. A distinction is made whether the seminiferous tubules are already organized as tubules or whether they are entirely or partially present as aggregates of Sertoli cells. Finally, the maturity of Sertoli and Leydig cells is assessed (Heckmann et al. 2018; Funke et al. 2021).



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Histologiemikroskopiebogen - Gesamtergebnis

Patient (number): **Date of birth:**
Date of biopsy:
Indication: **Androprotect, IHC:**
Date of evaluation:

	Right side		**Left side**	
Laboratory number				
Tissue				
Area in µm²				

Stages of spermatogenesis	**Tubule sections**		**Tubule sections**	
Tubules up to				
Elongated spermatids				
Round spermatids				
Primary spermatocytes				
Spermatogonia				
Spermatogonia + gonocytes				
Gonocytes				
Sertoli-cell-only				
Tubule shadows				
Total				
GCNis cells (PLAP positive)				

Distribution of tubules				
Tubular lumen				
Tubular wall				
Epithelial phenotype				
Connective tissue content				
Sertoli cell maturity				
Leydig cell maturity				
Leydig cell count				
Occurrence of blood vessels				
Remarks				

Fig. 42.4 Evaluation sheet for immature testicular tissue of the Center of Reproductive Medicine and Andrology, UK Münster (CeRA UKM, Androprotect®)

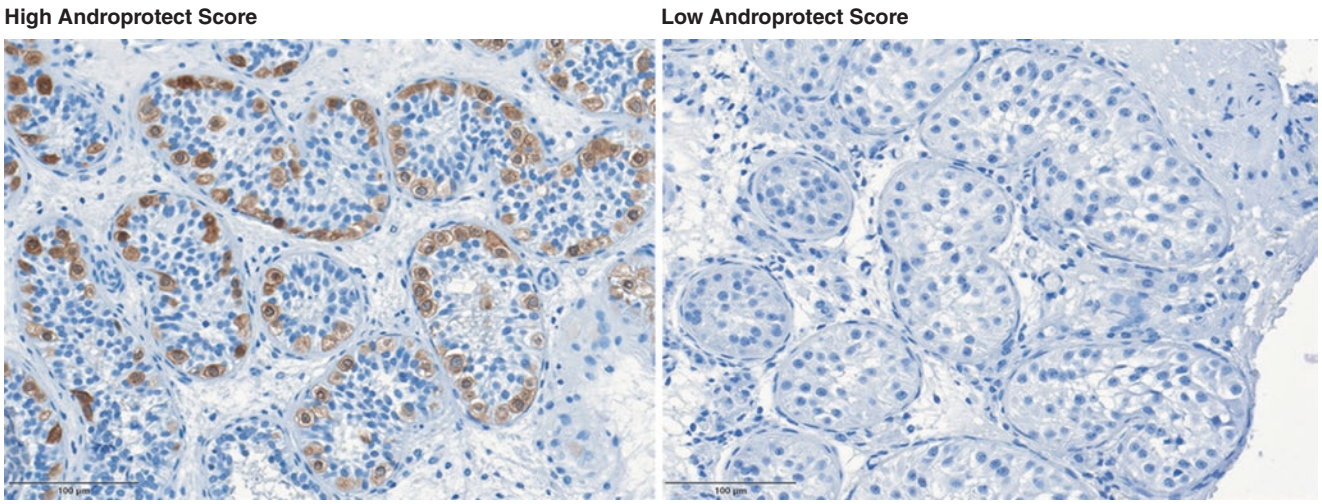


Fig. 42.5 Left immature testicular tissue from a patient with a high Androprotect® score based on the presence of spermatogonia in the vast majority of seminiferous tubules and an Androprotect® score of 0 based on the absence of spermatogonia (image material CeRA UKM, Androprotect®)

42.7 Prospects, Limitations, and Risks of Cryopreservation

42.7.1 Prospects for Success and Risks for Later Paternity

There are no risks associated with the preventive therapy option of sperm cryopreservation for the patient. Cryopreserved sperm can be used for ICSI treatment with comparable chances of success as freshly obtained sperm. There is also no risk of transmission of tumor cells when cryopreserved sperm are used in fertility treatment. This also applies to testicular-derived sperm. Especially in patients with a germ cell tumor of the testis, testicular sperm are frequently obtained from regions adjacent to the testicular tumor. Again, there is no increased risk from the therapeutic procedure per se, either for the use of these sperm or for the offspring.

Differences in pregnancy and birth rates exist depending on ejaculate quality. Cryptozoosperm ejaculates, like TESE-ICSI treatments, show somewhat lower success rates depending on sperm quality. In the current German IVF Register Yearbook (DIR 2022), clinical pregnancy rates are 23.1 and birth rates after TESE-ICSI are 17.1% based on all TESE-ICSI treatments. When ICSI is performed with ejaculated sperm, clinical pregnancy and birth rates are 26% and 18.8%, respectively, based on all ICSI treatments. However, the cumulative birth rate can be improved to over 40% by the fourth treatment cycle.

Financing of ICSI therapy in Germany is provided by health insurance companies up to the age of 40 for the woman and 50 for the man at around 50% for three treatment cycles. There are some federal states and also health insurance companies that still grant co-payments after the third treatment cycle. With the aforementioned age limit, storage at health insurance costs also ends with corresponding medical indication for cryopreservation (Gemeinsamer Bundesausschuss 2021, 2022).

42.7.2 Genetic Risk for Offspring

With regard to subsequent offspring, there is no evidence that the use of cryopreserved semen samples from oncologically or non-oncologically diseased patients is associated with an increased risk of malformation in the offspring. However, there are risks associated with the method of ICSI therapy per se, which—according to current knowledge due to problems inherent in the couples—is associated with a 1.3-fold higher risk of malformation of the offspring (von Wolff and Haaf 2020). For this reason, genetic counseling prior to initiation of ICSI therapy is recommended for all couples and, since 2018, has been required accordingly in the guideline of the German Medical Association on "Collection and Transfer of Human Germ Cells in the Context of Assisted Reproduction" (Bundesärztekammer 2022) and the AWMF S2k guideline "Diagnostics and Therapy Prior to Assisted Reproductive Medicine Treatment" (AWMF 2017).

Hereditary components of a disease, such as the familial increased testicular tumor risk for male offspring of a testicular tumor patient or, for example, genetic aspects of leukemia disease, should be considered in the consultation (Al-Jebari et al. 2018).

Older cytogenetic studies performed on spermatozoa from patients after prior chemotherapy or radiotherapy show a significantly increased proportion of structural abnormali-

ties in the patients studied compared to a control population (Genescà et al. 1990; Rousseaux et al. 1993). Recent studies have shown for polychemotherapy (cisplatin, etoposide, and bleomycin, BEP) in germ cell tumor patients that sperm aneupoidy rates return to pretherapeutic levels 24 months after the end of chemotherapy. For radiotherapy, an increased existing aneuploidy rate was shown over a longer period of time (Rives et al. 2017). Temporarily increased aneuploidy rates due to both disease and therapy have also been demonstrated for lymphoma patients (Martinez et al. 2017). For these data obtained by in vitro experiments, there are no clinical studies indicating a resulting increased rate of malformation of the children. For the clinical aspect of potential genetic risk, there are national registry studies, particularly in testicular tumor patients, that show no evidence of increased genetic risk for congenital malformations in children of fathers treated for germ cell tumors (Al-Jebari et al. 2019). Furthermore, recent studies of offspring of oncology patients, including in particular cured pediatric cancer patients in the European Childhood Cancer Survivor Cohort, have failed to show increased risks for malformations, tumor disease, or increased abortion rates compared with healthy fathers and mothers (Borgmann-Staudt et al. 2022).

Permanent storage of cryopreserved sperm does not lead to a reduction in sperm quality. There is no risk of transmission of tumor cells during fertility treatment by cryopreserved sperm. Study data from offspring of oncology patients allow the conclusion that there is no increased genetic and malformation risk associated with either the underlying disease or with artificial insemination using cryopreserved sperm in oncology patients.

42.7.3 Experimental Use of Spermatogonial Stem Cells and Their Prospects for Clinical Application

Experimental development of many fertility preservation strategies using stem cell-based strategies is far ahead of clinical application. Animal studies have already produced offspring for many species after tissue grafting and germ cell transplantation (Sharma et al. 2019). The principle of application has also been confirmed for these two approaches in primates, making successful application in humans highly likely.

Similarly, it has already been shown in mice that generation of offspring using pluripotent cell-derived germ cells is possible. So why has a transfer of almost all procedures to clinical application failed to materialize? A decisive factor is probably the direct influence on and manipulation of the genetic integrity of the germline, since all procedures have at least the potential risk of a germline effect.

In particular, the derivation of gametes from pluripotent cells and the cultivation of spermatogonial stem cells and their expansion in vitro appear to be problematic. Outside the germinal epithelium, these processes take place without the inherent control mechanisms. Whether checkpoints are anchored in the somatic environment and how selection of germ cell clones occurs are largely unexplored issues. Without understanding these mechanisms, it is almost impossible to assess potential risks from altering the checkpoint mechanisms. Whether xenologically produced sperm or artificially produced gametes will ever be used for human ART appears to be an open question until these processes have been researched and valid knowledge has been gained.

On the other hand, based on the available data, it seems appropriate and indicated to use autologous applications of fertility preservation measures. Harvesting of immature testicular tissue is not problematic and the data situation suggests a clinical–experimental application of, e.g., autografting of immature testicular tissue. After experimental confirmation in nonhuman primates, the first birth of a child conceived with sperm from cryopreserved and transplanted testicular tissue is conceivable in the near future.

42.7.4 Psychological Aspects

Counseling a patient about the possibility of cryopreservation occurs at a time when the patient's physical and psychological integrity is sensitively disturbed. In view of the acutely stressful situation for the patient (confrontation with diagnosis and therapy planning), it sometimes seems difficult for physician and patient alike to give cryopreservation as a procreation reserves the importance it deserves in the context of long-term quality of life. Sperm storage prior to potentially gonadotoxic therapy is also of psychological importance, as it can give the patient hope for future paternity. Particularly in adolescent patients, this requires a high level of commitment from all involved and a willingness to transcend taboos that still exist today and address the issue of sexuality. However, cryopreservation can contribute to the personal stabilization of the patient in this area of life in the acutely stressful situation.

Due to the improvement in survival and cure rates, the effects of long-term toxicity and late effects of therapeutic interventions are becoming increasingly important. Particularly in adolescents with oncologic disease, European network studies of fertility as late toxicity have led to the development of European recommendations for recording these long-term toxicities (Skinner et al. 2017). Education on fertility protection, recording the consequences of therapeutic procedures on fertility, and training on how to deal with

the problems are the subject of current studies (PanCareLIFE; Skinner et al. 2017). Appropriate intervention studies have resulted in better information and thus better management of fertility issues (Borgmann-Staudt et al. 2019). This development contributes to relief in the difficult situation for adolescents and their parents who are confronted with a malignant disease.

Key Points

- Cryopreservation of sperm from the ejaculate or testicular tissue of the adolescent and adult is established and safe procedure to preserve fertility.
- Indications span oncologic and non-oncologic conditions and "social freezing" and can be offered to adolescents and adults.
- Crucial to the successful use of cryopreserved sperm is their viability, which is reduced by 40–50% during the freezing and thawing process.
- The requirements in terms of risk minimization, safety, and quality control for the andrological and reproductive cryolaboratory in the processing and preparation of gametes are high.
- Permanent storage of sperm is possible almost indefinitely; the decisive factor is the stable storage temperature, nowadays in the vapor phase of liquid nitrogen, which must be constantly below −130 °C.
- With current cryopreservation methods, sperm can survive for at least 30 years, probably indefinitely.
- Since July 2021, cryopreservation and permanent storage have been a service reimbursed by health insurance companies in Germany for appropriate medical indications according to the Kryo-RL.
- Cryopreserved sperm can be used in the context of ICSI therapy with pregnancy and birth rates comparable to results from fresh sperm. Since 2012, there has been the possibility in Germany within the Androprotect® network to also provide pre- and early pubertal boys with a potential fertility reserve by means of cryopreservation of spermatogonial stem cells after removal of immature testicular tissue. This experimental procedure is being intensively researched and is on the threshold of clinical testing.

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Part IX

Sexual Health

Sexual Medicine and Andrology

43

Klaus M. Beier and Frank-Michael Köhn

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Abstract

Sexual medicine issues play a role in medical practice in all disorders and diseases that affect sexual functions, sexual and/or partner experience and behavior, and gender identity. Sexual disorders can also be consequences of other illnesses and/or their treatment and can manifest as the result of sexual trauma. In any case, a basic understanding of human sexuality is beneficial for clinical work, distinguished into three different dimensions (desire, reproduction, and attachment), all closely intertwined. This basic understanding also permeates the principles of sexual medical diagnostics and therapy, the couple dimension being of essential importance. As a result, an integrative approach in sexual medicine, taking somatic and psychosocial factors into account, makes it possible to deal with couple-related problems in the same way as somatic therapy options.

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43.1 Sexual Medicine in Clinical Practice

Sexual medicine is concerned with the assessment, recognition, treatment, prevention, and rehabilitation of disorders and diseases of sexuality. These can affect sexual functions, sexual and/or partnership experience, and behavior (also as a result of diseases and/or their treatment), as well as gender identity and/or be associated with sexual traumatization.

With regard to the etiology, diagnosis, and treatment of these disorders, sexual medicine takes into account the findings and procedures of medical and psychological and social science disciplines, with **special emphasis on the couple dimension** (Vogt et al. 1995; Beier et al. 2021). In this context, sexual medicine adopts the findings of behavioral research and developmental psychology, according to which mammals, especially primates and above all humans, are **relational beings programmed for bonding,** whose chances of survival depend on the fulfillment of their basic existential needs for acceptance and belonging; these can be realized particularly intensively in the physical closeness of (intimate) relationships—with resulting feelings of security and safety. Sexual medicine interventions are based on this basic understanding (Sect. 43.5).

There is considerable overlap between sexual medicine and andrology in some areas. The definition of andrology, according to the current qualification regulations of the German Medical Association, includes the "prevention, detection, conservative treatment and rehabilitation" of "partnership disorders" and "erectile dysfunction, including libido, ejaculation and cohabitation disorders."

The andrological workup of patients and couples thus offers a suitable opportunity to detect concomitant relevant disorders in the context of sexual medicine beyond the foremost shown symptom (e.g., **involuntary childlessness**; Chap. 45). It would therefore be desirable for physicians working in the field of andrology to have **well-founded competence in sexual medicine** or at least be part of a network with the opportunity of referring couples to trained sexual physicians. Consequently, sexual medicine has been integrated as postgraduate training for physicians since 2007 by the Berlin Medical Association and the qualification can be acquired by colleagues from various specialist fields (including dermatology and sexually transmitted diseases, internal medicine and general medicine, psychosomatic medicine, and psychotherapy or urology and gynecology). By providing theoretical knowledge and practical skills, this continuing training qualifies physicians for the diagnostics and therapy of all sexual disorders and gender dysphoria symptoms (Sect. 43.3). In 2018, the German Medical Association integrated sexual medicine in their catalog of postgraduate training for physicians as well. One requirement of the regulation is having "specialist recognition in an area of direct patient care" and additionally "80 hours in courses of further training in basic psychosomatic care or additional further training in psychotherapy or additional further training in psychoanalysis" and additionally "120 hours of courses of further training (…) in sexual medicine" as well as additionally "120 hours of case seminars under supervision" (case seminars can be replaced by 6 months of further training under supervision at further training institutions).

Critical examination of anthropological, biomedical, psychological, and sociocultural aspects of gender is essential, since sexual medicine is designed to be **interdisciplinary** and integrates knowledge from other medical disciplines (in particular general medicine, gynecology, urology, andrology, endocrinology, neurology, psychiatry, psychosomatics, and psychotherapy) and associated human sciences (in particular biology, psychology, sociology, etc.) (Fig. 43.1).

As to be expected, the circle of those affected is wide: the diabetic patient who suffers from sexual dysfunction as a result of his chronic disease (erection problems in men, arousal and orgasm problems in women), the hypertensive patient whose medication has a negative effect on sexual response, and the patient with depression who loses his sexual appetence (and can develop additional arousal and orgasm problems due to antidepressants), and the young man with fear of sexual failure, the couple whose unresolved conflicts or power struggles are expressed in sexual symptoms, or the woman who has pain during coitus after menopause.

Considering the increasingly important field of disorders concerning sexual reproduction (including the often massive effects of an unfulfilled desire for having (own) children on sexual and partnership relationships), the spectrum of sexual disorders (Sect. 43.3; Chap. 45) and the associated challenges for medicine become obvious.

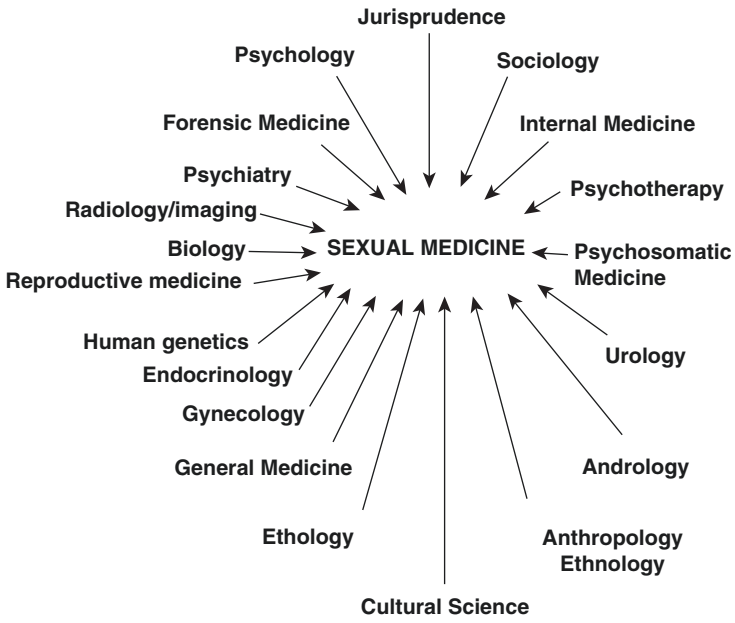


Fig. 43.1 Interdisciplinary references of sexual medicine

43.2 Basic Understanding of Human Sexuality

Sexuality defines a one-sided definitional approach. As a **biologically**, **psychologically, and socially determined dimension** of human experience, in its individual form it depends on biological factors and on biographic development. In contrast to earlier dualistic views, the somatic, psychological, and social factors involved are always active at the same time. Therefore, instead of an "either-or" approach (e.g., either body or psyche), an "as-well-as" approach should be taken. This needs to be emphasized because everyday medical practice still disregards the "and-simultaneous" view: Foremost, somatic reasons are looked for in finding the cause of a disease. Only when somatic causes are not found, a psychological problem is considered, overlooking the fact that both levels are always intertwined.

This biopsychosocial view is neither a cliché nor a fashionable attitude. It has been convincingly demonstrated by scientists in the fields of psychoneuroendocrinology and psychoneuroimmunology, whose research results additionally emphasize the importance of attachment and relationships for human development and for physical and mental health (Schedlowski and Tewes 1996; Eisenberger and Cole 2012).

From a functional perspective, it is appropriate to speak of a multifunctionality of sexuality, whose individual aspects are closely interrelated and can be conceptually distinguished as follows:

The **dimension of desire** encompasses sexuality in all conceivable ways of experiencing and increasing desire by sexual stimulation.

The **dimension of reproduction** stands for the significance of sexuality in reproduction.

The **dimension of attachment** emphasizes the importance of sexuality for the fulfillment of biopsychosocial fundamental needs for acceptance, closeness, warmth, and security by sexual communication in relationships (Beier and Loewit 2004; Beier et al. 2021).

> Comparing the various dimensions of sexuality, the reproductive dimension is phylogenetically the oldest.

In humans, for females, reproduction is limited to the period of fertile capacity from puberty to menopause and is also dependent on biographical choices; i.e., it is optional. Recent availability of reliable **contraceptive methods**, on the one hand, and the development of reproductive medicine, on the other hand, have made it possible—to a large extent—**to extricate the dimension of reproduction from the dimension of attachment and from the dimension of desire.**

> The dimension of attachment is established later in evolutionary development of the human species, but for mankind today it is without doubt an integral and essential part of sexuality.

Its great importance results from the fact that humans are **relational beings**: Their basic needs for acceptance, safety,

trust, security, and closeness, which exist from the beginning of life, can only be met in relationships (Bowlby 1969, 1980; Brisch 1999). This happens in infancy through physical and emotional experiences of **being accepted**, for example, by mothers holding their infant protectively while breastfeeding. Through this parental attention, the ability to fulfill basic psychosocial needs through skin contact is experienced and learned by the infant and at the same time neurally preordained, as generally happens in learning processes of elementary skills (Rüegg 2003).

The feelings conveyed through interaction and body language determine human development from birth on and remain a core feature of **relationship formation**. They do not initially rely on genitalia, but nevertheless enable deep satisfaction, which is obtained through skin and eye contact, and through sensory impressions in general. They are therefore the first "sexual" dimensions of experience, which is later merely extended by the implementation of genital—sexual communication.

By means of imaging techniques, it was shown (Bartels and Zeki 2004) that the same brain regions are deactivated when mothers are presented with pictures of their children or their partners (compared to distant acquaintances as control subjects). Possibly, the functions for fear and rejection, which are functionally–anatomically located in the amygdala, are deactivated at the same time. This means that any apprehension concerning the partner is "turned off" in order to enable close interaction, presumably the origin of the traditional proverb "love is blind."

Another study by Zeki and Romaya (2010) demonstrates that this occurs independently of sexual orientation; i.e., same-sex-oriented individuals respond to the beloved partner in the same way. In a study by Acevedo et al. (2012), neural correlates of longer-term romantic relationships were again investigated, suggesting very similar conclusions.

This would also support assumptions by earlier authors—who did not yet have access to imaging techniques—such as Balint's (1965) consideration of the "primordial forms of love" as the core and basis of adult intimacy, or Montagu's (1987) view that adult sexual interaction is "in some respects a reproduction of the tender love between mother and child."

Neurobiological findings increasingly show the extent to which the brain is a system attuned to interpersonal relationships and dependent on attachment, with the **neuropeptide oxytocin**—according to experimental findings—playing a prominent role (Meyer-Lindenberg et al. 2011); this is also likely to be a relevant variable for the psychobiological stress response or reduction in couple relationships (Ditzen et al. 2009).

The data of the meta-analysis by Holt-Lunstad et al. (2010), in which approx. 150 studies with 300,000 subjects were examined with regard to protective factors for mortality in chronic diseases, corroborate these observations. According to this, the quality of social relationships is the factor with the strongest effect—this exceeding, for example, that of cessation of nicotine abuse. In this context, neurobiological mechanisms between social ties and physical health (e.g., influence on the cardiovascular system and on the immunological system) are self-evident (Eisenberger and Cole 2012).

The dimension of desire provides sexuality with a quality that sets it apart from other human experiences through the unique sensual experience of sexual arousal and orgasm. Sexual pleasure establishes the motivational quality of sexuality and represents, as it were the drive and reward of sexual behavior. The dimension of desire can play an important role in subjective experience, in autoeroticism, and in the experience of erotic attraction, passion, and ecstasy. It is nevertheless difficult to consider in isolation because it is closely related to the other functions.

Nevertheless, data from neuroscientific research justify the assumption that, in addition to basal, midline cerebral areas and higher areas (such as the limbic system) play a decisive role in triggering the actual, physiologically detectable sexual reaction. In this context, the **area preoptica of the hypothalamus** seems to be of central importance; during sexual activity, an increase in activity in this nucleus, which in turn is anatomically connected to other nuclei in the hypothalamus, can be demonstrated in animal experiments. The activity of the area preoptica can be modulated by steroid hormones; in single-cell derivations, both male and female animals show selective activation in sexually promising or coitus-related situations (Pfaff 1999). In humans, this core area is apparently also wired in such a way that, for example, nasally ingested **androgen or estrogen metabolites** are recognized as signals. In this context, activation processes are dependent on **sexual orientation** (and not on gender): Androgen metabolites activate core areas of the hypothalamus of females oriented toward males and of males oriented toward males, whereas in males oriented toward females this is caused by estrogen metabolites (Savic et al. 2005). The dependence of central sexual activation patterns on sexual orientation (rather than gender) was shown for visual stimuli by Ponseti et al. (2006).

On aspects of motivation, emotion, and autonomic nervous system, Stoléru et al. (2012) described the interplay of excitatory and inhibitory brain areas, i.e., the activation and the inhibition of sexual responses; for the inhibitory processes, they cited the medial parts of the orbitofrontal cortex, as well as the caudate nucleus and the caudate anterior cingulate cortex. It is particularly important to note that an interplay of cortical and subcortical structures (especially the putamen and claustrum) appears to be relevant for information processing (Poeppl et al. 2016), which highlights the

complexity of central nervous processing of sexual stimuli, which is further complemented by processes of peripheral organization.

43.3 The Spectrum of Sexual Disorders

The classifications proposed in the clinical classification systems **ICD-10** (WHO 1993) or **ICD-11** (WHO 2018) and **DSM-5** (APA 2013) are purely descriptive and arbitrary schemes that, on closer examination, do not do justice to the complexity of human sexuality. Even the differentiation of the three dimensions of sexuality, such as required from a sexological perspective, makes attempts at classification implausible. Sexual dysfunction can affect not only the dimension of desire but also the attachment dimension to such an extent that the actual cause of suffering is not so much the disturbed sexual function as the resulting irritated relationship satisfaction.

> The chronic lack of feelings of security generated by repeated frustration of basic psychosocial needs increases the likelihood of developing psychological and physical disorders, according to current knowledge. It also makes it more difficult to overcome and cope with existing illnesses (Egle et al. 1997).

In practice, the symptoms presented by patients are mostly referred to as "psycho-vegetative disorders or complaints," "depressive mood states," "anxiety and/or nervous restlessness," or "nervous anxiety, tension, and restlessness states," or also as "emotionally induced restlessness states." It can therefore be assumed that in very many areas of medicine patients present with various disturbance patterns in which—again for various individual reasons—functioning and thus emotionally stabilizing social/intimate ties are not or no longer sufficiently available. This also includes chronically ill or elderly people, who experience psychosocial destabilization due to reduced contact opportunities.

This explains why very different symptoms can dominate the clinical picture, so that different medical disciplines come into contact with affected patients: the orthopedist for muscle tension, the general practitioner for vegetative symptoms, the psychiatrist for intrapsychic tension or depression, or the andrologist for unfulfilled desire to have children. Any other sexual dysfunction may also be only one of many possible symptoms (apart from disease-related sexual disorders, e.g., condition after paraplegic lesions). The special feature of therapy options in sexual medicine (Sect. 43.5) is that only these address the roots—the frustrated basic needs. This happens with an intensity hardly otherwise achievable in that the (re-)fulfillment of basic needs is achieved through physical acceptance in intimacy with the partner, also having healing effects on other areas of life or symptoms (Fig. 43.2).

Accordingly, if back pain is successfully treated by an orthopedist, nothing changes at the level of basic needs; possible frustration may remain and can consequently be the cause for a recurrence or for other symptoms.

A first overview of the spectrum of sexual disorders—still listed under ICD-10 and DSM-IV—was presented by Ahlers et al. (2005). Here, due to revisions of the classification systems, assessment of the disorder patterns (up to deletion of the same) and the chosen terminology are in flux, which is shown by the ICD-11, published in 2018.

Taking into account these limitations for a systematic recordability, the **clinically significant sexual disorders** of humans are briefly characterized in the following and their (partly, however, limited) coding options according to the internationally valid classification systems **(ICD-10 and DSM-5)** are presented, partly also with reference to innovations of the ICD-11 available since 2018:

1. Disorders of sexual function.
2. Disorders of sexual development.
   (a) Disorders of sexual maturation.
   (b) Disorders of sexual orientation.
   (c) Disorders of sexual identity.
   (d) Disorders of sexual relationship.
3. Disorder of gender identity (gender dysphoria).
4. Disorders of sexual preference (paraphilic disorders).
5. Disorders of sexual behavior (dissexuality).
6. Disorders of sexual reproduction.

43.3.1 Disorders of Sexual Function

> Sexual response can be divided into phases of appetence, arousal, orgasm, and relaxation, and each of these phases can be disturbed as such (Masters and Johnson 1966).

All sexual disorders, such as psychological and behavioral disorders, have biological and psychological and sociological aspects, so that only an integral view of these aspects offers an accurate description and thus promises effective treatment. Since sexual dysfunctions do not affect the person with the respective symptoms alone, but always affect the **well-being of the respective partner**, in principle treatment should include both partners. In addition, all functional

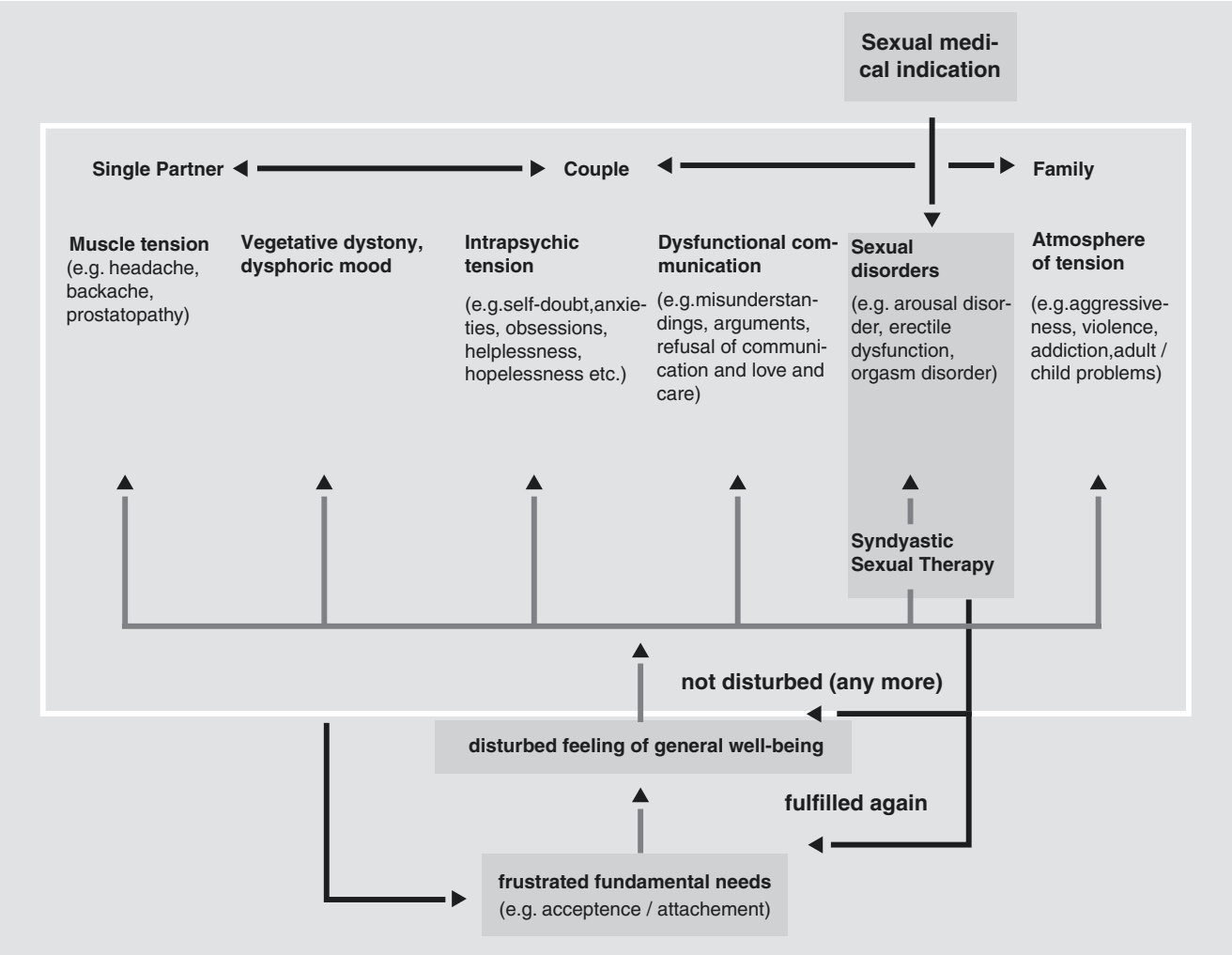


Fig. 43.2 Relationship between basic psychosocial needs and various symptoms. It is shown that—although syndyastic sex therapy (see Sect. 43.5.2) is applied in only one of the many possible levels of disturbance—if the (re-)fulfillment of basic needs is achieved, this can have a healing effect on all other symptoms as well

disorders can occur independently of other disorders or diseases, as well as presenting as a consequence of other diseases and their treatment.

Both ICD-10 and DSM-5 allow sufficient coding of sexual dysfunctions, whereby the criteriology in the DSM-5 is more differentiated and as a disorder criterion "suffering in a clinically significant way" must be connected with sexual function restrictions. Subtypes are also provided in the DSM-5 to indicate the onset of difficulties.

For example, the specification "lifelong" refers to a sexual problem that has been present since the first sexual experiences; "acquired," on the other hand, applies to those sexual disorders that have developed after a period of "relatively normal sexual functioning." "Generalized" refers to sexual difficulties that are not limited to specific types of stimulation, situations, or partners, and "situational" refers precisely to those that exhibit this characteristic (and usually occur in a partnership situation).

In ICD-11, the specifications ("lifelong," "acquired," "generalized," "situational") were also considered and even included in the coding to be assigned.

The **DSM-5** lists the following five factors that may be of relevance to the development and/or treatment of the disorder:

1. Partner (his/her sexual problems, if any, their health status).
2. Relationship (communication problems, different sexual desires, and needs).
3. Individual vulnerability such as low self-esteem and negative body image, but also childhood trauma, comorbid mental disorders, or different stressors.

4. Cultural and/or religious influences (especially regarding attitudes toward sexuality and partnership with usually gender-related differences).
5. Medical conditions, for example, due to other illnesses and/or their treatment.

Accordingly, assigning a diagnosis of sexual dysfunction requires excluding problems that can be explained by medical disease factors (e.g., postoperative pelvic nerve damage), by substance use (e.g., alcohol or medication), or by other serious stressors (such as within the partnership or post-traumatic genesis).

In a representative sample of 18- to 59-year-old US Americans, Laumann et al. (1999) found that approx. 15% of the test persons had "lacked interest in sex" or "trouble in maintaining or achieving an erection" and 31% had orgasm disturbances in the sense of ejaculatio praecox. In an international comparison, there were some similarities, but partly also significant intercultural variations, proving the biopsychosocial basis of such disorders (Laumann et al. 2005).

The "prevalence estimates of sexual dysfunctions" in the survey "Health and Sexuality in Germany—GeSiD," a Germany-wide survey of 4955 men and women, conducted from 2018 to 2019 on the basis of a population registration office sample, was based on the new ICD-11 guidelines. Among men, "reduced desire for sex" was found in 14.7%, "erection problems" in 13.6%, "problems achieving orgasm" in 9.7%, and "premature ejaculation" in 11.7% in the 12 months before the survey. 27.1% of women experienced "reduced desire for sex," 23.4% "reduced response to sexual stimuli," 23.1% "problems achieving orgasm," and 10.9% "tension or pain during sex" in the 12 months before the survey. 45.7% of women and 33.4% of men had experienced at least one of the problems mentioned (Briken et al. 2020).

Various studies have also been able to demonstrate the negative effects of sexual dysfunction on partnership and quality of life in general (Rosen et al. 2004; Chevret et al. 2004; Fisher et al. 2005; Abraham et al. 2008). This is also in accordance with further findings from the survey "Health and Sexuality in Germany—GeSiD," according to which a "mediocre or poor state of health, a chronic illness or disability perceived as restricting sexuality, and the joint occurrence of several specific illnesses" are associated with lower sexual activity and lower sexual satisfaction, with the latter in particular correlating with the relationship situation (Dekker et al. 2020).

43.3.1.1 Disorders of Sexual Desire

Sexual appetence disorders are an **increasing problem** among men seeking sexual health treatment, they themselves often citing erectile dysfunction as the reason for their presentation. The causes are often unacknowledged **subdepressive states of exhaustion** (with and without substance abuse), **partnership disharmonies,** and—very often**—sexual preference disorders** (Sect. 43.3.4). Organic causes (**testosterone deficit, hyperprolactinemia**, and **drug side effects**) are differentially diagnostically significant, but are occasionally overemphasized in somatomedical literature. For example, studies of improvement in **libido** in hypogonadal men with testosterone replacement therapy have shown significant improvement in libido in up to approximately 50% of treated men; however, a similar positive effect was also detectable in approximately 20% of hypogonadal men treated with placebo (Stephens-Shields et al. 2019).

43.3.1.2 Disorders of Sexual Arousal

The **prevalence of erectile dysfunction** is well studied. The Massachusetts Male Aging Study (MMAS) identified minimal erectile failure in 17% of 40- to 70-year-old men surveyed, moderate failure in 25%, and complete failure in as many as 10% (Feldman et al. 1994). Braun et al. (2000) found erectile dysfunction in 19.2% of their 4489 respondents over 30 years of age, although the authors were able to show that by no means all subjects reporting erectile dysfunction were dissatisfied with their sex lives; here, as well as in the frequency of the dysfunction, they found a clear age effect. The same applies to the results of the Berlin Men's Study (Schäfer et al. 2003; Englert et al. 2007), whereby the authors strongly advocate a differentiation between "erectile dysfunction" and "erectile disorder" and only the latter would be considered a disorder worthy of illness status due to the suffering of the affected person that can be proven for the diagnosis. An important aspect of these studies was the high coinciding with general medical conditions (especially diabetes, heart disease, and high blood pressure). It is often misunderstood that the occurrence of erectile dysfunction beyond the age of 40 may be a first indicator of chronic ischemic heart disease (Chew et al. 2008; Görge et al. 2003).

The 12-month prevalence of "erectile problems" in the survey "Health and Sexuality in Germany—GeSiD" related to age groups was as follows: 18–25: 6.5%; 26–35: 6.8%; 36–45: 10.2%; 46–55: 9.2%; 56–65: 20.8%; and 66–75: 33.7% and correspondingly significantly lower when the criterion "severe impairment" was included: 18–25: 2.7%; 26–35: 4.9%; 36–45: 5.0%; 46–55: 5.4%; 56–65: 13.9%; and 66–75: 17.7%. The **lifetime prevalence** across all age groups was 11.4% for "erectile problems" with severe impairment (Briken et al. 2020). Thus, all the data available today suggest that erectile dysfunction is very common in both the general population and clinical settings and is, indeed, a significant health problem.

As with **libido dysfunction**, there is no justification for the common view among patients and some physicians that testosterone deficiency is the sole cause of erectile dysfunc-

tion. When groups with and without abnormal scores for Androgen Deficiency in the Aging Male (ADAM) and International Index of Erectile Function-5 (IIEF-5) are compared, no significant differences in their serum testosterone levels are seen (Lee et al. 2020).

43.3.1.3 Disorders of Orgasm

Male **orgasmic disorders** include two symptom patterns: premature orgasm (also referred to as "**premature ejaculation**" or "ejaculatio praecox") and inhibited or absent orgasm (synonyms: "**delayed ejaculation"** and "ejaculatio retardata ").

While premature orgasm has been the focus of numerous research efforts and there is an orally administered drug (dapoxetine) approved for this indication in addition to locally anesthetizing external agents, the opposite is the case with delayed orgasm. Here, there is the impression that this disorder is only taken up very cautiously by clinicians, which may be related to the supposed poor prognosis for therapy.

It is also true here that the change in perspective from function to relationship is the valid key to improvement of the symptomatology.

Premature orgasm, defined as persistent occurrence before or immediately after penetration, with the subject having almost no control over it and the sensation of orgasm remaining unsatisfactory, is the **most common sexual disorder** in men. Approximately 20% to 25% of adult men surveyed in modern industrialized countries experience premature orgasm with distress (Mathers et al. 2007; Porst et al. 2007). However, two problems are encountered in reporting valid **prevalence figures**: On the one hand, to a great extent, normal ejaculation/orgasm duration is assessed subjectively and is subject to great interindividual and also cultural variations (Althof 2006; Montorsi 2005); on the other hand, it becomes apparent right here that "functional impairment" and clinically relevant disorder are not congruent (see above).

For Germany, a lifetime prevalence of 7.6% for "premature ejaculation" (with "severe impairment") has been calculated in a population-based sample of 18- to 75-year-old men in the survey "Health and Sexuality in Germany—GeSiD" (Briken et al. 2020). The 12-month prevalence is highest among the youngest: 16.3% in the 18–25 age group (when the "severe impairment" criterion is included: 6.2%) and only 6.9% among the oldest (3.3% with "severe impairment"). Obviously, in some cases, persistence of the problem (i.e., the lifelong type) must be assumed.

The causes of this sexual dysfunction are still not clear; a multifactorial genesis can be assumed, in which penile hypersensitivity, exaggerated ejaculatory reflex, hyperexcitability, endocrine causes, genetic disposition, and dysfunctions of the 5-hydroxytryptamine (5-HT) receptor are discussed in addition to psychogenic factors (El-Hamd et al. 2019).

Compared with premature orgasm, **male orgasm inhibition** is a **less common disorder**. "Problems reaching an orgasm" associated with distress were 4.6% over lifetime among men (18–75 years old) included in the German GeSiD survey (Briken et al. 2020). Clinically, the lack of a sexological approach is particularly glaring here, as both psychotherapy and somatotherapy "gnash their teeth" over the disorder, but this is likely to be related to the fact that there are only limited modifiable conditions to be taken into account here, which might result, for example, from particularities of the male sexual preference structure, but may also be linked to individual techniques of masturbation, which require specific stimulation, variable only to a limited extent. This makes it all the more necessary to focus on the couple—combined with the willingness—similar to sexual preference particularities (see Sect. 43.3.4)—to accept the limits of changeability and not to see them as an exclusion criterion for sexual and partnership relationship satisfaction, but to look for couple-related solutions on which both partners work together (Beier et al. 2021).

43.3.1.4 Sexual Dysfunctions Due to Illness and/or Treatment

Practically all clinical disciplines in medicine care for patients in whom a disease and/or its treatment can lead to the development of a sexual disorder. Of particular importance in terms of sexual medicine are cardiovascular diseases, with heart failure, coronary heart disease, myocardial infarction, and hypertension, as well as metabolic diseases such as diabetes mellitus, severe general diseases, especially cancer, diseases of the musculoskeletal system (e.g., arthritis), neurological diseases such as multiple sclerosis or Parkinson's disease), but also neurologically related disabilities, psychiatric diseases such as anxiety disorders or depression, but also mental retardation, and finally the gynecological and urogenital diseases.

Independent coding of disease and/or treatment-related sexual disorders according to ICD-10 is only possible in a cumbersome way, since the underlying disease must be coded separately. In the DSM-5, coding of the main disorder is again required; in the case of multiple sclerosis, this is coded and then the sexual dysfunction, if necessary, via an additional coding. Coding of surgical sequelae is not possible according to either ICD-10 or DSM-5. In ICD-11, separate categories are provided, which would have to be coded additionally if a medical disease factor, an accident, or treatment consequences (e.g., radiation) are seen in connection with the sexual dysfunction.

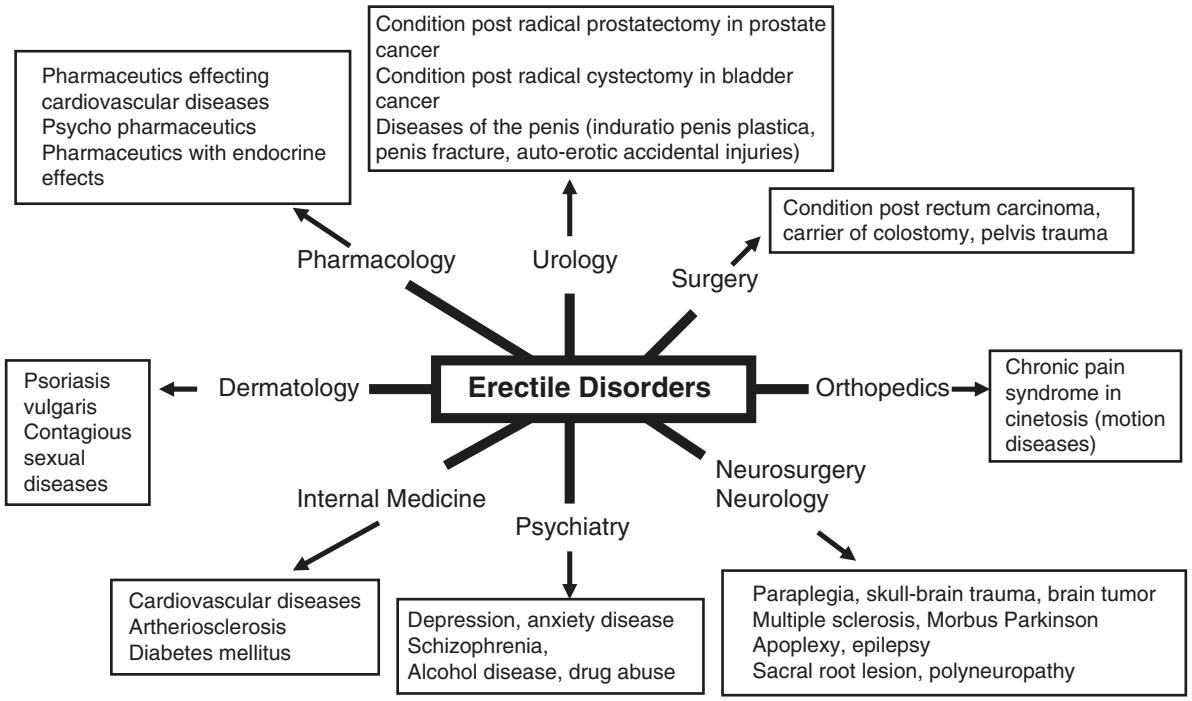


Fig. 43.3 Erectile dysfunction due to disease and/or treatment (after Rösing et al. 2009)

The multitude of possible disease- or treatment-related reasons for the development of erectile dysfunction is shown in Fig. 43.3.

43.3.2 Disorders of Sexual Development

This indication area includes disorders that occur in the context of somatosexual, psychosexual, and sociosexual development over the **entire life span** and impair the affected person's ability to **interact sexually**, up to and including the impossibility of sexual contact. These disorders often lead to the secondary development of other psychological and behavioral disorders in the affected persons, which are then cited as the reason for presentation rather than the causal problem itself. Presumably, most sexual development disorders themselves remain unconsidered and are treated solely on the (secondary) symptom level, among other reasons because the affected persons themselves are usually unable to discern that their difficulties actually or also lie in the area of their sexual development.

This is all the more true if the problem is embedded in an overall **developmental delay** (retardation; including physical and mental developmental disorders) or if the affected persons are retarded as a whole. Retardation does not change the need for sexual and partner contact.

According to the internationally valid classification systems, sexual development disorders can only be coded to a limited extent or in residual categories, but are nevertheless of great clinical relevance for sexual medicine.

43.3.2.1 Disorder of Sexual Maturation

The category "disorder of sexual maturation" (ICD-10: F66.2 and 66.9, respectively) primarily includes psycho- and sociosexual effects of delayed or absent physical sexual maturity (e.g., "**pubertas tarda"**) (Chap. 14). In many cases, this causes irritations in the formation of gender and sexual identity and subsequently also leads to developmental delays at the psycho/sociosexual level, as a result of which those affected lag behind in their sexual development and have difficulties in establishing sexual relationships with age-appropriate partners.

A disturbance of sexual maturation becomes particularly serious when sexual assaults on children occur because an adult is unable to engage age-appropriate sexual partners due to somato-, psycho-, or sociosexual retardation and therefore targets children as a substitute (Beier et al. 2021).

43.3.2.2 Disorders of Sexual Orientation

Sexual orientation to the male and/or female sex is an axis of the human sexual preference structure, which, quite independently of its respective expression (same sex, bisexual, or opposite sex), is not to be regarded as a disease or disorder, but as one variation of human sexuality (Sect. 43.4).

Nevertheless, a problematic or pathological "sexual orientation disorder" (ICD-10: F66.1) can occur, which is due

to conflictual processing and insufficient integration of the given preferred orientation, causing the person to suffer.

The most frequent manifestation of the sexual orientation disorder consists in an ego-estranged same-sex orientation (so-called **ego-dystonic homosexuality**), not least against the sociocultural background of a sexually opposite-sex-oriented majority of the population, people either fear being homosexually oriented or cannot accept a realistically perceived **homosexuality**, let alone integrate it into their own sexual identity (see below). As a result, there are attempts at denial or repression, but these usually have a short half-life and often ultimately lead to a categorical rejection of one's own sexual orientation, with the resulting desire to change it.

43.3.2.3 Disorders of Sexual Identity

Sexual identity disorder (ICD-10: F66.8) is understood as an insecurity about one's own masculinity or femininity—not about one's own gender identity (see Sect. 43.3.3).

If **gender identity** is expressed in the question, "**Am I a man or a woman?", sexual identity** is expressed in the question, "**Am I properly, or sufficiently masculine or feminine?"** Thus, for those concerned, it is a question of whether they can perceive and accept themselves as a "real man" or a "real woman" also and particularly in terms of sexual attractiveness.

Whereas with regard to gender identity, the question of sexual attractiveness does not play a primary role, sexual identity is about the possibility of being able to integrate one's own gender-related characteristics into a sexual self-concept and to translate them congruently into gender-typical sexual behavior (Diamond 2002).

Ideas and needs that (supposedly) deviate from the **stereotypical gender roles** are particularly relevant in this context, especially if they impress as unchangeable components of the sexual preference structure, but may not be adequately perceived as such.

The establishment of sustainable, continuous sexual relationships can be difficult, especially for men, because self-doubt and fear of failure regarding one's own sexual sufficiency and "potency" are too strong and thus too anxiety provoking. This manifestation can be described as "**sociosexual self-uncertainty,**" which goes far beyond the extent of general, i.e., surmountable "shyness toward the opposite sex" and can be a reason why affected persons remain unattached to partners for long periods of time, contrary to their wishes.

43.3.2.4 Disorders of Sexual Relationship

Disorder of Sexual Relationship (ICD-10: F66.9) occurs when a person suffers from being unable to initiate or maintain sexual relationships over a long period of time.

The resulting distress is often a negative influence on general and health-related quality of life. The reasons for these problems can lie in the spectrum of other sexual disorders and in psychological and behavioral disorders that make it difficult or impossible to initiate and/or maintain sexual relationships. A disturbance of the sexual relationship is thus almost always secondary; this means that the impossible or disturbed sexual relationship is usually a sociosexual expression of another, predominantly causal problem, which can regularly be found in a non-integrated attachment dimension of sexuality.

If, for example, the dimension of desire is separated from the dimension of attachment, this can manifest itself in an overemphasis of the desire dimension in relation to the attachment dimension, which is more frequently found in men. In their sexual experience, they are so fixated on pleasure and its satisfaction that they experience it as not being linked to a partner relationship. The partners then often react with sexual withdrawal because they feel "not meant," "used," or sometimes even "abused" by their partners in the context of sexual interactions.

This disengagement also occurs with an exclusively **pharmaceutical symptomatic treatment** of erectile dysfunction (e.g., PDE-5 inhibitors) without **sex therapy embedding** and without any involvement of partners and can unintentionally intensify a sexual relationship disorder. One reason why many men use erection-enhancing drugs only for short periods of time—in addition to the costs—is that their partners signal that their actual needs for a relationship, closeness, and security are not being taken into account, neglected or even disregarded when their husbands focus on their erections.

Even if the dimension of reproduction is unilaterally overemphasized, this can lead to a disturbance, for example, if one partner strives for sexual contact exclusively or mainly for reproduction, but the other partner does not share the desire for children and as a result, for example, is reluctant to have sexual intercourse. It is not uncommon for such a form of sexual relationship disorder to express itself by the absence of sexual reactions or in sexual dysfunction.

43.3.3 Disorders of Gender Identity (Gender Dysphoria/Gender Incongruence)

This widening field of different symptomatologies includes insecurities, irritations, and misperceptions about one's own **gender**. The inner feeling of belonging to the gender opposite to one's own assigned gender, i.e., having to live in the "wrong" body, with an increasing desire to change this state, is characteristic.

Within this group of disorders, there are different gradations and manifestations, which can have different backgrounds and must be treated differently. This is why these complaints have been summarized in the ICD-10 under the generic term "**gender identity disorders,**" while the DSM-5 speaks of "**gender dysphoria**" and the ICD-11 of "**gender incongruence.**"

The most severe and irreversible form of gender identity disorder is called **transsexuality** or **transsexual gender dysphoria**. In this (rarer) case, there is a biographically lasting, irreversible, or final disintegration of one's own gender corporeality ("feeling of living in the wrong body"). This is a progressive diagnosis, which requires careful differential diagnostic clarification—especially in connection with the indication for body-changing measures, which have irreversible consequences and should therefore only be considered if the diagnosis is (as far as possible) confirmed. In this context, the procedure of **opposite-sex hormone treatment** can be regarded as largely standardized, and with regard to **surgical techniques of gender reassignment**, the aim is for surgical measures to be part of an interdisciplinary treatment concept. Thus, a cooperation of several surgical disciplines is useful for this purpose (cf. in detail on gender-transforming treatment in Bosinski et al. 2021).

According to the assessment guideline **"Gender Reassignment Measures in Transsexualism (ICD-10, F64.0)"** published in August 2020 as a guideline of the central association of health insurance funds in the German health care system (MDS 2020), psychiatric or psychotherapeutic treatment must be undergone before the costs for body-modifying treatments are covered by health insurance funds. "Everyday experiences" in the desired gender role "in as many areas of life as possible" and the exclusion of psychiatric differential diagnoses are still called for.

From the point of view of medical ethics, it should be pointed out above all that **irreversible body-changing measures, too rapidly initiated** can lead to harm for those affected, who at the time of initiation of these measures may have been completely convinced that this is the right thing for them, but at a later date regret this step and would prefer to reverse it. These requests for reversion should prompt the initiation of body-modifying measures not before diagnosis is completed, as is also evident from the current S3-guidelines of the German Association of the Scientific Medical Societies concerning **"Gender incongruence, gender dysphoria and trans-health"** (AWMF 2018).

In the case of **non-transsexual expressions** of gender dysphoria, body-modifying measures (hormones, surgery) are not indicated; rather, the focus is on psychotherapy accompanying the search for identity (Bosinski et al. 2021).

43.3.4 Disorders of Sexual Preference (Paraphilic Disorders)

"Disorders of Sexual Preference" (term used in ICD-10, i.e., the previous diagnostic manual of the World Health Organization) or **"paraphilic disorders"** (term used in DSM-5, i.e., the diagnostic manual of the American Psychiatric Association and in the ICD-11, i.e. the current version of the diagnostic WHO-manual, which is not yet implemented in many countries, including Germany) are disorders in which the persons concerned suffer from sexual impulses that deviate from the norm. Accordingly, persons who exhibit deviant sexual tendencies but do not suffer from them are not considered disturbed, ill, or in need of treatment as long as they do not harm or endanger others or themselves through their deviant sexual needs. This prevents overly extensive pathologization. However, since such an additional condition does not permit recording of a preference particularity that would not be associated with suffering, the DSM-5 provides for a distinction to be made between "**paraphilia"** and "**paraphilic disorder."**

The following lists peculiarities of the sexual preference structure, which can be seen in each person in terms of three axes (Sect. 43.4):

(a) With regard to the preferred gender of the sexual partner (male and/or female).
(b) With regard to the preferred (physical developmental) age of the sexual partner (children, pubescents, adolescents, adults, old people).
(c) With regard to the preferred mode of sexual activity with and without sexual partner(s) (type, object, mode, etc.).

It should be emphasized that a person's sexual preference structure in its individual characteristics (from norm-conforming to paraphilic) manifests itself for the first time in adolescence and, according to current scientific knowledge,

Table 43.1 Overview of sexual preference/paraphilic disorders according to ICD-10/DSM-5/ICD-11

ICD-10	Sexual preference disorders	DSM-5	Paraphilic disorders	ICD-11	Paraphilic disorders
F65.0	Fetishism	302.81	Fetishist disorder	6D36	Paraphilic disorder involving autosexual behavior or consenting persons
F65.1	Fetishistic transvestitism	302.3	Fetishist transvestic disorder		
F65.2	Exhibitionism	302.4	Exhibitionistic disorder	6D30	Exhibitionistic disorder
F65.3	Voyeurism	302.82	Voyeuristic disorder	6D31	Voyeuristic disorder
F65.4	Pedophilia	302.2	Pedophilic disorder	6D32	Pedophilic disorder
F65.5	Sadomasochism	302.83	Sexual masochistic disorder	6D36	Paraphilic disorder involving autosexual behavior or consenting persons
		302.84	Sexual sadistic disorder	6D33	Sexual sadistic disorder involving coercion
		302.89	Frotteuristic disorder	6D34	Frotteuristic disorder
F65.6	Multiple disorders of sexual preference				
F65.8	Other sexual preference disorders	302.89	Other paraphilic disorder specified	6D35	Other paraphilic disorder involving non-consenting persons
F65.9	Unspecified sexual preference disorders	302.9	Unspecified paraphilic disorder	6D3Z	Unspecified paraphilic disorder

cannot then be categorically changed for the rest of a lifetime, i.e., an orientation toward the male sex cannot be transformed into an orientation toward the female sex, a fetishist inclination cannot be extinguished, etc. (Seto 2012; Spitzer 2012; Grundmann et al. 2016). Moreover, a paraphilic responsiveness may characterize the entire preference structure (so-called exclusive type), or constitute only a part of it (so-called non-exclusive type), so that norm-conforming parts exist in addition to the paraphilic pattern.

Table 43.1 provides an overview of the sexual preference disorders according to ICD-10 (WHO 1993) and the paraphilic disorders according to DSM-5 (APA 2013), as well as the innovations in ICD-11 (WHO 2018), where the terminology of DSM-5 has now been adopted, but fewer paraphilic disorders are explicitly covered, namely, only exhibitionist disorder, voyeuristic disorder, pedophilic disorder (with specification exclusively to prepubescent children, no longer also to early pubescent children as in ICD-10), frotteuristic disorder, and sexual sadistic disorder involving coercion. In particular, fetishism or fetishist disorder and sadomasochism or sadomasochistic disorder are no longer explicitly included, but they characterize a large and also clinically significant part of sexual preference particularities that will have to be coded in residual categories in ICD-11 in the future. For example, there are the "other paraphilic disorders involving non-consenting persons," which include disorders that can be classified as sadistic, or the category "paraphilic disorder involving autosexual behavior," which includes fetishist tendencies if they are associated with suffering.

According to the first epidemiological data (Långström and Zucker 2005; Ahlers et al. 2011), the prevalence of paraphilic tendencies is higher than previously assumed.

For the German-speaking world, this is evident from the results of a subsample of the "Berlin Male Study," which initially investigated a representative sample of 6000 men aged between 40 and 79 years with regard to the presence of erectile dysfunction and the associated effects on quality of life, health, and partnership. In this first part of the survey, 1915 men participated (Schäfer et al. 2003) who were then invited to take part in a detailed sexological questionnaire survey including their (then also examined) female partners. The resulting sample consisted of 373 men, 63 of whom were single and 310 of whom were partners (in addition, it was also possible to personally survey 108 female partners). The data suggest the possible prevalence of paraphilic tendencies in the general population, because the most frequent sexual arousal patterns were queried with regard to their occurrence in sexual fantasies, in masturbation (as fantasy content), and in actual sexual behavior (Ahlers et al. 2011).

57.6% of the men surveyed identified one of these arousal patterns as part of their fantasy world, 46.9% used them to increase arousal during masturbation, and 43.9% lived them out on the behavioral level. Even if a transfer of these figures to the general population seems inadmissible due to the obligatory, almost unavoidable selection effects, an idea of the presumed prevalence can be gained, which also makes the extent and variety of corresponding offers of the pornographic industry more comprehensible (Table 43.2)

Among women (specifically here: the partners included in the study), the proportion of those who are aware of impulses attributable to paraphilias as part of their sexual preference structure is also remarkably high, although significantly lower than among men (this ratio is exactly reversed in the case of sexual reproduction disorders, which occur more frequently in women than in men; Chap. 45). 27.8% of the women interviewed acknowledged paraphilic impulses as part of their fantasy world, 23.1% used them to increase arousal during masturbation, and just as many lived them out

Table 43.2 Prevalence of paraphilia-associated arousal patterns at different levels of experience in men between 40 and 79 years of age (no clinical use population; results of the "Berlin Men Study II")

	Levels of experience					
	Sexual fantasies		Accompanying fantasies during masturbation		Sexual behavior	
Arousal patterns	*n*	%	*n*	%	*n*	%
Nonhuman objects (e.g., cloth or shoes)	110	29.5	97	26.0	90	24.1
Wearing women's clothing	18	4.8	21	5.6	10	2.7
Being humiliated	58	15.5	50	13.4	45	12.1
Torturing other persons	80	21.4	73	19.6	57	15.3
Secret observation of intimate situations	128	34.3	90	24.1	66	17.7
Genital presentation to strangers	13	3.5	12	3.2	8	2.1
Touching strangers in public	49	13.1	26	7.0	24	6.4
Children's bodies	35	9.4	22	5.9	17	3.8
Other	23	6.2	23	6.2	17	4.6
Sexual responsiveness to at least one arousal pattern/stimuli	215	57.6	175	46.9	163	43.7
As responsiveness to different arousal patterns was surveyed on a 5-point scale with the gradations: none–little–moderate–strong–very strong. All responses from "little to strong" were scored as responsiveness to a sexual arousal pattern						

Table 43.3 Frequency of different sexual arousal patterns among female partners of participants in the Berlin Men Study (Part 2; $n = 108$), with multiple responses

	Levels of experience (%)		
Arousal patterns	Sexual fantasies	Accompanying fantasies during masturbation	Sexual behavior
Nonhuman objects (e.g., cloth or shoes)	13.9	13.0	12.0
Being humiliated	17.6	15.7	16.7
Torturing other people	7.4	5.6	6.5
Secretly observing intimate situations	9.3	9.3	0.9
Presenting genitals to strangers	1.9	1.9	0.9
Touching strangers in public	2.8	2.8	2.8
Children's bodies	1.9	2.8	0.9
Other	2.8	1.9	0.0
Sexual responsiveness to at least one arousal pattern	**27.8**	**23.1**	**23.1**

on the behavioral level. This also shows that considerably fewer women feel that paraphilic content—if it occurs—is inadequate for them and want to refrain from acting it out (Table 43.3).

However, it can be assumed that most "deviant" impulses only become a disorder worthy of illness definition when they are isolated and generalized.

This is also taken into account in the DSM-5 (APA 2013) in that a diagnosis can only be made if the person in question suffers from the paraphilic tendency or if this has led to restrictions in important social or occupational areas of functioning or, in the case of paraphilias associated with potential danger to others (e.g., a pedophilic disorder), if the person in question has acted in accordance with these impulses (irrespective of any suffering pressure that may be present).

Against this background, however, it is noteworthy that a significant proportion (almost one-third) of the men included in the BMS II feel that paraphilia-associated arousal patterns are inadequate for them and refrain from acting out on them—even if they are not associated with a danger to others (e.g., fetishist tendencies). Nevertheless, a likewise considerable proportion of respondents exhibit a sexual assault potential (e.g., exhibitionism, voyeurism, frotteurism) or has already committed sexual assault (e.g., pedosexual acts).

43.3.5 Disorders of Sexual Behavior (Dissexuality)

All sexual behaviors in which **the well-being and sexual self-determination of other people are impaired or harmed** and which can be prosecuted for this reason are subsumed under this heading. Overall, this group of disorders—regardless of their criminal relevance or prosecutability—refers to all sexual assaults (whether psychological or physical) against sexual self-determination, subsumed under the term dissexuality (Beier 1995).

Sexual behavior disorders also include attempts or performance of sexual acts in front of, on, or with children (so-called "**pedosexual acts,**" in criminal law: "**sexual abuse of children**") or adolescents or other persons who cannot consent to the sexual acts.

On the one hand, sexual behavior disorders can be due to the acting out of corresponding paraphilias; i.e., paraphilic impulse patterns can represent a causal background for disturbed sexual behavioral expressions and are then to be understood as acts of inclination. On the other hand, there are also dissexual behaviors that do not represent the acting out of a paraphilic impulse pattern, but can be traced back to another background problem (e.g., a personality disorder, a reduction in intelligence, and an addiction problem). Such sexual assaults are then to be understood as **substitute acts** for actually desired sexual interactions with partners, which for various reasons cannot be achieved in a socially adequate way. This means that in the process of sexual assessment, disorders of sexual preference and disorders of sexual behavior must be precisely differentiated and not confused or even equated.

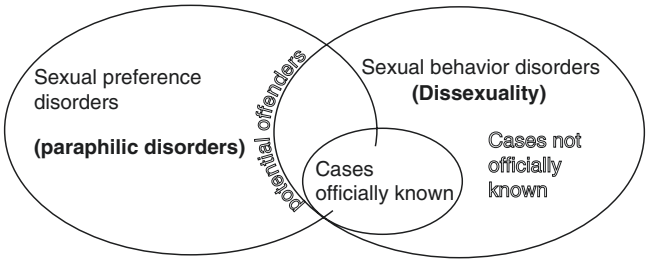


Fig. 43.4 Spectrum of sexual preference and behavior disorders

Figure 43.4 shows schematically that in the whole spectrum of paraphilias the largest part is not associated with sexual assault, i.e., dissexuality. Conversely, in most cases dissexuality does not go back to a paraphilia. Simplified, a large number of men with sexual preference disorders ("paraphilic disorders") are not dissexual, and most men with sexual behavior disorders (dissexuality) do not have paraphilic disorders.

According to the internationally valid classification systems, sexual behavior disorders can only be recorded via residual categories—namely "Other abnormal habits and disorders of impulse control" (ICD-10: F63.8; here it is generally explained: "The action is preceded by tension, which is followed by a feeling of relief during the course of the action") and as "Other specified impulse control disorder" (DSM-5: 312.89). From this, however, the decisive characteristic of dissexual acts—the violation of the integrity and individuality of another person (caused by them)—is not sufficiently clear. In addition, the possibility of indicating an overlap with "disorders of sexual preference" is missing.

From an epidemiological point of view, it should also be noted that only a small proportion of dissexual acts are committed and accordingly prosecuted, i.e., known to the legal authorities (so-called "Hellfeld" in German, literally "bright field"), whereas most of them are not known to the justice system (so-called "Dunkelfeld" in German, literally "dark field")—although they play a major role in clinical practice in sexual medicine.

43.3.6 Disorders of Sexual Reproduction

These disorders are characterized by psychological and psychophysiological **impairments of reproduction** in its different phases (conception, pregnancy, birth, and childcare/childrearing); they cause significant suffering and/or interpersonal difficulties.

Unfortunately, they cannot be adequately coded in the international classification systems (ICD-10 and DSM-5) and are not included in ICD-11. From a clinical point of view, however, they are distinguishable (Beier et al. 2006):

Preconceptional Disorders: For example, unfulfilled desire to have children; imagined pregnancy—in this case, the psychological and psychophysiological conversion processes (cessation of menstruation, weight gain, fetal movements) associated with a pregnancy that does not actually exist but is longed for are experienced by the affected woman as given and presented to the environment accordingly.

Prenatal Disorders: For example, unperceived pregnancy—in this case, the psychological and psychophysiological processes of change (cessation of menstruation, weight gain, fetal movement) associated with a pregnancy that is already established are not perceived by the affected woman herself or are hidden from the perception of others.

Postnatal Disorders: For example, postpartum depression, peculiarities in parenting behavior such as child abuse, but also treatment of the child as a "self-object" (Beier 1995, 2000).

In these disorders, the reproductive dimension of sexuality is psychologically and/or psychophysiological impaired in its different phases such as procreation, conception, pregnancy, birth, and child care/raising leading to symptom patterns from which the affected persons suffer to a considerable extent and/or which endanger their social integration.

In addition, disorders of sexual reproduction are regularly associated with disorders of sexual relationships (see above), which can also open up therapeutically useful aspects for therapeutic procedures (Sect. 43.5; Chap. 45).

43.4 Principles of Diagnosis in Sexual Medicine

The clarification of sexual and/or partner relationship dissatisfaction takes place through an **adequate conversation about and exploration** of sexual disorders and competent evaluation of physical findings and laboratory results.

The therapist provides a setting for the [allowed] openly talking about sexuality as an integral part of human life. This is one of the most important foundations of sexual medicine and itself has a therapeutic effect. It has long been documented that patients wait for appropriate signals (Vincent 1964; Buddeberg 1996; Zettl and Hartlapp 1997; Fröhlich 1998). For example, when prescribing new medication, physicians can give such a signal by commenting: ("If something should change in your sexual experience as a result of the disease or the treatment, or if problems should arise, we would be happy to talk about it and look for possible solutions"). It should be noted here that the treatment provider cannot retreat to the role of expert, but is always confronted with subjective attributions of meaning—the patient's and his or her own—and therefore cannot avoid personal involvement.

43.4.1 Exploration of a Sexual Disorder

The implementation of goal-oriented treatment is based on **comprehensive information** about concrete sexual experience and behavior of a patient/couple; this must make it possible (in the case of an existing partnership) to assess the level of sexual functioning and sexual preference structure of both partners, because otherwise therapeutic steps will remain ineffective or could even be harmful (e.g., if a preference disorder remains unrecognized as the reason for a functional disorder).

In the case of couples, it must be decided whether the sexual history should be taken in individual sessions with each partner or whether it should be taken immediately in the presence of both partners.

Individual anamnesis has the advantage that—especially in the case of unfamiliar topics such as masturbation and related fantasies, as well as paraphilic tendencies and previous relationships—the partners can communicate in a more unbiased manner and reveal things that they might not say in front of each other. However, this can turn the therapist into a "keeper of secrets," which can complicate further work with the couple.

On the other hand, the crucial thing is not for the therapist to learn as much as possible about each partner; rather, it is essentially about information that is relevant to the couple and, consequently, can be addressed in couple treatment.

Against this background, the sexual anamnesis taken directly in conversation with **the couple** provides great advantages, because all information is processed together from the onset and can show or increase the degree of openness and trust between the partners.

It is important to know what type of disorder (e.g., indirect or direct sexual dysfunction; sexual relationship dysfunction) is involved and under what circumstances or conditions (e.g., primary or secondary; generalized or situational) it occurs.

- What is the partner's attitude toward the complained-of disorder?
- Who takes the initiative in sexual activity?
- What are the differences, and how and where do they express themselves?
- What preferences or dislikes exist and how are they dealt with?

For the systematic exploration of sexual experience and behavior, attention to certain aspects has proven helpful. This has been continuously refined by clinical work at the Berlin Institute for Sexual Medicine of the Charité-Hospital in order to make it particularly useful for schooling, training, and further education, which is reflected in summarizing anamnestic tools (Ahlers et al. 2004). These also serve to record feelings of pleasure as one of the three dimensions of sexuality, which in this sense is understood as multifunctional, depending on the individually different sexual preference structure, which can be described on three axes:

1. Gender of the preferred partner.
2. Physical developmental age of the preferred partner.
3. Type and manner of interactions/practices with the preferred partner, which can be explored on three different levels that are equally interrelated (levels of fantasy, behavior, and self-concept) (Table 43.4).

The effects of illness, treatment, or old age on sexual experience and behavior can also only be recorded by asking specific questions, whereby the necessity of including the partner already results from the fact that sexuality is designed to form a "we" and partner-related intimacy must take into account the wishes and ideas of the partner if it is to succeed.

This is also shown by an interview study by Kleinplatz and Ménard (2007), in which men and women (older than 65 years and in long-term partnerships) considered characteristics such as authenticity, intense emotional connection, communication, and feeling accepted to be the hallmarks of "great sex." Sexual functioning is not necessarily a prerequi-

Table 43.4 Sexual experience and behavior: exploratory aids (modified after Ahlers et al. 2004)

Three axes	– the **gender** (of a desired partner): the opposite or the same sex (or both)
The sexual preference structure of humans is configured on three essential axes, which are interrelated and should all be explored (from norm-conforming to paraphilic)	– the **age** (of a desired partner): children, adolescents, adults, old people
	– the **mode** (of a desired partner or object or interaction): type, object, practice, etc.
Three levels	– of **sexual fantasies**
Sexual experience and behavior should be explored at three different levels, which are interrelated and all should be explored	– of the concrete **sexual behavior**
	– of the **sexual self-concept**
Three forms	– **masturbation**: Self-stimulation and self-gratification
The concrete sexual behavior, in turn, should be included in the three forms to be explored, all of which should be inquired about	– **extragenital sexual interaction**: e.g., stroking, cuddling, kissing
	– **genital stimulation**: manual, oral, or other stimulation, e.g., petting, including sexual intercourse (insertion of penis or penile surrogate into vagina or anus)

site for this; on the other hand, sexual functioning alone is also not sufficient to provide sexually fulfilling experiences.

Therefore, from the perspective of sexual medicine, the following questions are of particular diagnostic interest:

- What is communication like within the relationship in general and specifically in the sexual relationship?
- Can one's own feelings, needs, and wishes be communicated, and does this happen?
- Are boundaries respected?
- Are there self-reinforcement mechanisms, "vicious circles," self-fulfilling prophecies, and how do these effect partnership communication?
- Can misunderstandings due to misinterpretations of the partner's behavior play any part?

43.4.2 Exploration of the Three Dimensions of Sexuality

Of central importance—diagnostically and therapeutically—is the recording of patients'/couples' attributions of meaning to the three dimensions (desire, reproduction, and attachment.

As a rule, these are questions that initiate an examination of the **patient's own sexuality and partnership** previously unknown to them, so this already having therapeutic effects. Ultimately, a picture is needed of what sexuality means to the patient/couple ("What does sleeping with your partner mean to you?"), which already leads many patients to realize that some things are not as self-evident as they had assumed ("I/we have never thought about that before" is frequently given as an answer to the above question).

43.4.2.1 The Dimension of Attachment

Is there (and how is) a connection between sexuality and partnership and how is it perceived? Which contents or values are indispensable within the relationship? To what extent does the fulfillment of basic human needs succeed in the couple relationship? How far is sexuality understood as a body language communication possibility that can communicate and realize these basic needs at the same time, so that tenderness, cuddling, or coitus can be experienced as "mimic and gestures" of couple intimacy? So, for example, is the physical closeness and acceptance of the partner achieved in coitus also an expression and realization of psychosocial or partnership closeness and acceptance? Is this communicative view conscious, is it implicitly lived, or is it neither factual nor mental?

43.4.2.2 The Dimension of Reproduction

What is the significance of the reproductive capacity, and what is the significance of child(ren) in the relationship? Do the partners' views and attitudes differ in this regard? Do problems exist that result in, for example, an exaggerated desire to have children?

43.4.2.3 The Dimension of Desire

What importance, what status, and what place within the three dimensions are given to genital–sexual pleasure? (How) has the significance of desire changed over the course of the relationship? Are there disturbing discrepancies between the partners? Furthermore, is there awareness of the complexity of experiencing lust?

43.4.2.4 Individual and Partner-Related Interaction of These Three Dimensions

Can imbalances be identified in the individual partners or within the couple that could be related to the sexual disorder at hand? This includes discrepancies between fantasized and actually experienced sexuality and the predominance of one dimension at the expense of the other or completely different distributions between the partners.

43.4.3 Medical History and Somatic Findings

As far as possible, all significant illnesses requiring medical treatment should be recorded, especially those that could be related to a sexual disorder. This includes any urologic, gynecologic, andrologic, reproductive, or psychosomatic conditions and interventions, as well as medication and/or substance ab(use) or dependence. Information should also be obtained on previous pregnancies and, if applicable, existing parenthood. Furthermore, all treatment attempts concerning the sexual disorder, as well as previous psychotherapies must be mentioned and taken into account.

The biopsychosocial history includes somatic findings. It concerns sexual functions and general bodily functions. An overview of the required physical diagnostics in male sexual dysfunctions is given in Table 43.5 (after Rösing et al. 2009).

Table 43.5 Organ diagnostics in male sexual dysfunction (according to Rösing et al. 2009)

Body-related diagnostics	Type of diagnostics	For exclusion	Indication
Clinical examination	Inspection, palpation, pulse, blood pressure, (stress) ECG	Urogenital, neurological and cardiovascular diseases	Basic examination depending on risk factors (e.g., age, obesity)
Laboratory	Blood sugar	Diabetes mellitus	Basic examination
	Lipids	Metabolism disorder	Basic examination
	Testosterone	Hypogonadism	If necessary, in case of appetence or erectile dysfunction (ED) depending on other hypogonadism symptoms
	Prolactin	Prolactinoma	
Imaging	Duplex sonography with intracavernosal pharmacotesting	Cavernosal insufficiency	If necessary in ED for expert or scientific questions
	Neurophysiology (e.g., corpus cavernosum EMG)	Neurogenic deficit (e.g., consequences of an accident)	If necessary in ED for expert or scientific questions
	Penile angiography	Pelvic vessel occlusion	Only if revascularization surgery is planned

43.5 Principles of Therapy in Sexual Medicine

The biopsychosocial causation of sexual disorders described also requires a corresponding therapeutic approach, i.e., the combination of methods of "**talking medicine**" with those of **somatic medicinal interventions** (Beier et al. 2021).

Using the example of care for erectile dysfunction after radical prostatectomy for prostate cancer in Germany, Herkommer et al. (2006) were able to show that when only medicinal or mechanical therapeutic agents were used over the long term, patient satisfaction with treatment was significantly lower than was estimated by the treating urologists. Patient responses were also significantly discrepant with their practitioners' assessment of the choice of therapeutic agents. Surveys on the importance of partnership, non-genital sexuality (exchanging expressions of affection), and genital sexuality (sexual intercourse) in prostate cancer patients and their partners showed that before and after radical prostatectomy only the importance of genital sexuality decreased in both sexes. Partnership and the importance of physical affection (exchanging expressions of affection) remained as important as before (Rösing and Berberich 2004). The higher value of fulfilling psychosocial familiarity, closeness, and security compared to striving for sexual erotic satisfaction has also been demonstrated in other studies (Deneke 1999).

Interventions in sexual medicine are therefore fundamentally based on the consideration of the biopsychosocial references of sexuality and on the systematic inclusion of the couple and communication aspects. Modifications of the usual concept of the patient also mean a changed understanding of therapy.

The usual **therapist–patient relationship** expands into a **therapist–couple relationship**, especially in the case of sexual dysfunctions. Support for the individual patient expands to encompass both partners and their relationship, as long as this is the therapeutic mandate of the couple to the therapist. In the sense of Paracelsus' words, "The patient is the doctor and the doctor is his assistant" the actual, therapeutically effective work lies with the couple; the "assistant" accompanies them—making suggestions, clarifying, supporting, confronting, possibly also as an interpreter between different personalities and genders. He should enable the patient or the couple to gain new insights, especially new experiences, which are suited to improve the **quality of life**. Thus, the therapeutic goal is focused on relationship and sexuality.

43.5.1 Sexual Counseling

Here, too, the main task is to make the patient/couple aware of the attachment dimension of sexuality and to use this therapeutically. Many patients/couples are not aware that genital/coital sexuality is also one of many ways of fulfilling desires for validity, recognition, satisfaction, closeness, security, etc. that are sought in a partnership.

Counseling in sexual medicine will then be adapted to the specific needs of the patient/couple, whereby the following focal points can play a role either **alone or in combination:**

- **Passing on knowledge** (where deficits are obvious) about anatomical, physiological, or psychological processes of sexual reactions, which also includes the correction of misconceptions in the sense of sexual myths (e.g., supposed harmful effects of masturbation)—if necessary, in

Table 43.6 Comparison of treatment options for sexual disorders (modified after Beier and Loewit 2004; Rösing et al. 2009; Beier et al. 2021)

Treatment	Somatotherapy	Psychotherapy	Classical sex therapy	Syndyastic sex therapy
Focus on sexuality	Sexual function	Inner-psychic and couple conflicts	Sexuality and sexual functions	Basic psychosocial needs and their meaning, multidimensional concept of pleasure
Target group	Patient	Patient (couple)	Couple	Couple
Goal	Restoration of disturbed function	Strengthening of social and sexual competence	Strengthening of social and sexual competence, restoration of disturbed function	(re-)fulfillment of basic needs/ improvement of relationship, sexual behavior as their embodiment
Methods	Medications, mechanical aids, surgical interventions	Behavioral–therapeutic analytical therapy methods, couples therapy	Body-centered "exercises" to restore the ability to experience sexuality (sensate focus)	"Live" body language—also sexual—communication, collecting "self-ordered" new experiences, use of somatotherapy if necessary
Therapy success	With restored function	Depending on agreed therapy goals	At the end of the exercise steps	At each stage of "practicing" in relationship/sexual communication

both partners, explaining the different dimensions of sexuality (Sect. 43.4).
- **Clarification of mutual ideas and expectations** regarding sexuality and partnership.
- **Teaching communicative strategies**, if it has been shown diagnostically that general communication difficulties could already be decisive for the development or maintenance of the dysfunction.

In the case of underlying diseases, this also includes concrete information:

- For example, concerning the time of resumption of sexual contact after surgery (generally about 6 weeks) or after completion of radiation therapy (about 4 weeks).
- On the use of a lubricant if, for example, the vaginal epithelium has been altered by radiation or chemotherapy or during menopause.
- If necessary, about the use of aids (e.g., erection ring, vacuum pump, oral–medicinal or invasive–medicinal options)—but not before discrepancies between the partners regarding the interpretation of the various dimensions of sexuality have been worked through.

43.5.2 Sex Therapy

If sexuality is subjectively experienced in a predominantly negative way (pathogenic attribution of meaning), this can be ground for the development and maintenance of sexual disorders, which can put considerable strain on the partnership. In this case, the (first time or renewed) awareness of the importance of the three interrelated dimensions of sexuality for the couple can be combined with a targeted evaluation and discussion of new physical experiences in the context of sexological treatment. This can lead to a revitalization of intimacy in the partnership, because joint sexual experiences may change the previous experience of intimacy.

However, the general therapeutic goal here is to make the basic human needs, which are oriented toward comprehensive acceptance, fulfillable again for both partners through their sexual relationship.

The method of **syndyastic sex therapy** developed by Beier and Loewit (2004) (the newly introduced term "syndyastic" is borrowed from the Greek word "syndyastikós"—"disposed to be together as a couple or to be in a relationship of two or a couple," which Aristotle used for the first time in his Nicomachean Ethics) accordingly places the basic psychosocial needs in the focus of therapy. Thus, it differs fundamentally from all other forms of treatment (Table 43.6).

> It is not primarily sexual function that is to be restored, but the therapy goal is rather to expand the understanding of sexuality (in particular by increasing the ability of perceiving the desire dimension), thereby enabling new experiences of (sexual) bodily communication and improving (sexual) relationship satisfaction overall (the use of effective medication or aids is not a contradiction here, but rather a helpful addition at the appropriate time; Sect. 43.5.3).

To this end, new—self-arranged—intimate experiences by the couple also occur in the course of therapy, which make possible a new assignment of meaning to sexuality and thus an expanded context of meaningfulness in general. However, these are not "exercises," since one cannot enter into relationships "by way of exercise," but real, holistic, and consciously experienced relationship experiences, i.e., "practicing relationship."

Syndyastic sex therapy sees and uses sexuality in the treatment of sexual and partnership disorders primarily as the "most potent" vehicle for fulfilling basic biopsychosocial needs for affection, closeness, and security, i.e., ultimately for appreciation, acceptance, and belonging. The fulfillment of these basic needs is of vital importance for all human beings. Both non-sexual and sexual intimacy can make them possible in the most intense ways, which is why improving intimate communication becomes the focus of therapeutic efforts.

In an established partnership, the **syndyastic** focus is most effectively achieved by authentic involvement of the partner(s), whereby the relationship itself, i.e., the couple itself becomes the focus. This is based on the fact that these basic needs can be fulfilled in the most intensive way via sexually pleasurable physical acceptance and intimate attention. However, this is basically an activation of already existing experience potentials. Something is merely reactivated and nothing is "added," which at the same time shows the limits of the treatment method.

Consequently, the focus shifts from a disturbed sexual function to the disturbed fulfillment of basic needs, i.e., to the quality of the relationship and thus from the symptom-bearing individual to the couple.

Finally, it should be emphasized that syndyastic sex therapy is not tied to particular special therapeutical skills, nor to specific psychotherapeutic schools. It merely calls for a **basic psychosomatic understanding** and the willingness to abandon the position of "superior expert" and to actually concentrate on the syndyastic focus—acknowledging the fact that improvement in the fulfillment of basic psychosocial needs will have an impact on all other areas of life.

43.5.3 Integration of Somatic Therapy Options

Inclusion of somatic options within sex therapy is consistent with the biopsychosocial nature of sexual dysfunction. It often necessitates less invasive somatic interventions, but could short-cut sex therapy and improve compliance and prognosis of all treatment approaches—but, unfortunately, is rarely used in practice.

As with any therapy, a combined approach bears potential for both problems and opportunities; these must be carefully weighed up in each individual case. The **use of somatic options** may involve the following **problems**:

- The patient/couple may misinterpret the use of a medication as an uncomplicated "quick fix."
- Cripple the couple's ability to heal themselves.
- May reduce motivation to deal with personal or partnership problems.

This is in contrast to the fact that a combined approach

Improves the effectiveness and prognosis of treatment for many patients.

Conveys to the patient that the therapist also takes him/her seriously in his/her own perspective, which is often fixated on somatic causes.

Facilitates the establishment of an initially working relationship.

In the sense of taking the patient seriously, takes him from "where he stands" and in this way can open up an approach, also taking the psychosocial side into account.

> In the practice of sexual medicine, especially with male patients, the task usually consists in making the patients, most of whom are convinced of a physical cause of their problems, aware of psychological or couple-related aspects, and convince them of a biopsychosocial concept of sexuality.

This can only be done (or done much better) if the sex therapist is well informed about the advantages and disadvantages of medical treatment options (which, according to the definition given here, are part of sex therapy) and discusses them with the patient and signals his willingness to try out certain methods—if the examination findings suggest it makes sense and the patient agrees. If he can convey to the patient that it is not a question of "withholding" certain somatic options such as self-injections or oral medication from him, but rather of jointly exploring their possibilities and limits, especially with regard to the couple relationship, it is often possible to build up a sustainable working alliance that also enables psychological and partnership problems to be dealt with.

43.5.4 Treatment of Disorders of Sexual Preference and Behavior

Due to the manifestation of paraphilic impulse patterns in adolescence and their stability over the rest of the life span, it is necessary for those affected to "come to terms" with these inner experiences and, in the course of developing their sexual identity, consequently be more or less confronted with their own self-doubt to a greater or lesser extent. This, in the end, results from their concern that their partner might not accept the way they truly are (i.e., not love them anymore if their sexual fantasy content were out in the open—even if they have no intention of putting their fantasies into practice). This insecurity is so substantially affected by relationship experience ("Can I find true acceptance from the other person just the way I am?") that a partnership is difficult to

establish or to maintain. On the one hand, there might be anxiety about the course of a paraphilic inclination ("Will it pass, will it stay the same, or might it even grow stronger?"), while, on the other hand, a long hide-and-seek situation is established, i.e., shielding the paraphilic pattern from the perception of the partner, and this can lead to an irreparable loss of confidence when the inclination is revealed through other circumstances (e.g., discovering significant internet activities is increasingly common). However, if a relationship does exist and both partners have an authentic interest in a common perspective, couple-oriented interventions can improve satisfaction and contentment within the relationship. Five factors are crucial to planning **treatment options** (Beier 2010):

1. **The Proportion of the Paraphilic Pattern According to the Sexual Preference Structure in General**: It makes a big difference whether the paraphilic experience characterizes the entire sexual preference structure (exclusive type) or whether it exists in addition to the non-paraphilic pattern, possibly being able to integrate it on a partnership level (non-exclusive type).
2. **Additional Occurrence of Sexual Dysfunction**: Any sexual dysfunction can be paraphilia-related—especially if the affected person does not want to burden the sexual interaction with the paraphilic stimulus and as a result is insecure in intimate contact and is worried that an occurrence of paraphilic fantasy content could distance him from the actually desired closeness with a partner (e.g., in the case of a gynephilic orientation). This is always a starting point for therapeutic work, whereby the disclosure and the explanation of the connection with the paraphilic experience for both partners are already an important step in the treatment process.
3. **Value of the Paraphilic Stimulus in Inner Experience**. In its significance, the paraphilic stimulus (e.g., a fetish in case of a fetishist inclination) can sometimes substitute or overweigh attachment. This is because experiencing the stimulus is not only sexually arousing, but is also a psycho-emotionally stabilizing component, comparable to the attachment to another person. This not only puts the partnership at risk, but also limits the success of therapeutic interventions, especially when the bond to the paraphilic stimulus has reached the same (or an even greater) relevance for the intrinsic value as the attachment to a real partner.
4. **Ability of Self-Restriction**. A limiting factor in therapeutic work is not only the significance of the paraphilic stimulus in terms of self-estimation, but also—and therefore by no means congruent—is the ability for self-restriction within the relationship. An important motivational factor in this context is a sense of responsibility for any existing (or planned) children of their own.
5. **Being a Potential Danger to Others or to Oneself** Through the Paraphilic Stimulus. In the case of self-endangerment (e.g., a severe masochistic tendency) and endangerment of others (e.g., exhibitionism, frotteurism, pedophilia, and hebephilia), acquiring behavioral control (including controlling the habit of consuming child sexual abuse images) is the main goal.

Points 1–4 in particular make it understandable why syndyastic focusing (Sect. 43.5.2) can also be a useful therapeutic strategy in disorders of sexual preference or paraphilia—namely if both partners are authentically interested in an improvement of their sexual and partnership satisfaction and if this can be combined with the necessary self-restriction ability within the relationship. This, however, is only the case when the paraphilic stimulus is not regarded as more significant than the attachment itself. At the same time, this shows that in the diagnosis of paraphilias the exploration of the three dimensions of sexuality (attachment, desire, reproduction; Sect. 43.4.2) should be an indispensable element, because this makes it possible to assess the choice of therapeutic options and the associated developmental opportunities.

Beyond that, **drugs for the de-dynamization** of sexual impulses have become indispensable in the therapy of dissexuality and paraphilias as additional treatment options. They offer promising chances for the psychosocial stabilization of affected persons and at the same time reduce the dangers for potential victims, namely when the factor mentioned under 5 is relevant. Clinical experience also shows that for quite a few patients admission disclosure of their sexual preference and/or behavioral disorders becomes possible only after they have learned about drug treatment options and their effectiveness.

The main drugs available for pharmacotherapy are **selective serotonin reuptake inhibitors (SSRIs), medication for androgen deprivation therapy such as cyproterone acetate-type androgen receptor** blockers (CPAs), or gonadotropin-releasing hormone analogs (GnRH **analogs),** which can reduce sexual impulses and fantasies to varying degrees. **Opiate antagonists** (e.g., naltrexone) are sometimes an option.

Recently, increased attention has been focused on GnRH analogs, which are used extensively in the treatment of prostate cancer and are thought to be highly effective in terms of reducing sexual impulses. More recently, the GnRH antagonist degarelix was shown to be effective in a randomized controlled design in 52 men seeking treatment for pedophilic sexual preference (Landgren et al. 2020).

Prescribing **impulse-attenuating medications** requires a trusting relationship with the patient, not least because feedback on the intended effects is crucial and therefore reliable information needed. Monitoring the use of antiandrogenic

drugs includes checking blood lipid and blood glucose parameters, bone metabolism parameters, sex hormones and related parameters, and, in the case of CPA therapy, liver values (Berner et al. 2007).

The use of medication should always be linked to an overall treatment plan in which self-acceptance of the paraphilic inclination or its integration into the self-concept would be promoted, and an improvement in self-regulation strategies (via changed attitudes toward sexuality), the ability of taking on perspectives, and successful management of emotions, stress, and conflicts in relationships would also be targeted (Beier 2021).

Key Points

- Sexual medicine is holistically oriented through its biopsychosocial concept and being linked to sexual science through its interdisciplinary approach.
- The three dimensions of human sexuality—desire, attachment, and reproduction—form the basis for describing sexual health and sexual disorders.
- Disorders of sexual function and/or sexual relationship often result from unfulfilled basic biopsychosocial needs. This is related to a biological programming for attachment that is set in all mammals, having a stress-reducing effect within the relationship with a familiar other person and thus provides enormous health-preserving potential.
- Epidemiological studies leave little doubt that sexual dysfunction is widespread. It can be concluded that sexual dysfunction is one of the most common medical conditions of our time.
- It is foreseeable that due to the age demographic change and due to the increase in chronic diseases, the (secondary) sexual disorders due to disease and/or their treatment will continue to increase.
- On the basis of a structured sexual anamnesis, the various sexual disorders can be represented beyond the criteria of the internationally valid classification systems (ICD and DSM), also with regard to the individual and couple-related interplay of the three dimensions of sexuality.
- Sexual medicine treatment principles focus on the (re)fulfillment of basic biopsychosocial needs, taking into account general communication between partners and sexual communication.
- This shifts the focus from disturbed sexual function to relationship satisfaction, which can resolve the functional impairment.

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Involuntary Childlessness from the Perspective of Sexual Medicine

44

Klaus M. Beier, Julia Bartley, and Frank-Michael Köhn

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Abstract

The article deals with the different aspects of human sexuality and develops an approach allowing biopsychosocial diagnosis and treatment of couples afflicted by involuntary childlessness. Here biomedical and psychosocial ways of thinking have to be combined with a view toward the couple.

The possible effects of assisted reproduction on the sexual and partnership experience of the couple must be taken into account. Accordingly, key dimensions of sexuality (attachment, reproduction, and desire) and their relationship to each other, are also discussed. This includes the motives for wanting to have children as well as an understanding of sexuality not only as a means of procreation, but also as a form of body language communication that expresses the quality of the relationship (fulfillment or frustration of basic needs). Not only the potential parents but also the desired child/children depend on this—in sum—mutual "love." In the context, reproduction as a site for conflict processing (especially for women) is also discussed. Finally, possibilities and limits of couple-related interventions are presented on the basis of an expanded concept of sexuality.

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44.1 Couple-Related Effects of Involuntary Childlessness

The World Health Organization (WHO) recognizes involuntary childlessness as a disease and estimates that it affects 48 million couples and 186 million individuals worldwide (WHO 2020). For many couples, involuntary childlessness represents a severe crisis of life and meaning which they try to overcome with the help of reproductive medicine. In this context, the diagnostic and therapeutic concepts offered by

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professional helpers in the health care system are of considerable importance, especially since these have long been attributed to the mechanisms of high-performance medicine, which may always result in an isolated view of the services offered and thus, some distance away from the view of the "whole person." This alienation process may be multiplied insofar as (predominantly) not single individuals but a couple becomes the reference point of technical as well as psychosocial interventions.

Besides traditional treatment methods of reproductive medicine such as ovulation induction and inseminations, modern techniques such as in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI) including cryotransfer, sperm extraction from the testis (TESE) or microsurgical sperm aspiration from the epididymis (MESA) as well as gamete donation have become routine. Despite some therapeutic approaches to male infertility (e.g., stimulation therapy with hCG/rFSH for hypogonadotropic hypogonadism or ligation of a varicocele), in the majority of cases when there is an andrologic indication, invasive procedures are performed on the female partner rather than on her male partner as the causative agent.

In Germany, a total of 110,786 treatment cycles were performed in 2019, according to data from the German IVF Registry (DIR) (data from 131 of 137 member centers). The pregnancy rate per embryo transfer after fresh cycles in 2018 was 32.2% and the birthrate per embryo transfer was 23.5%. There were 21,385 children born from treatments in 2018. The proportion of multiples had fallen below 20% for the first time (in 2017 still 78% singletons vs. 22% multiples).

The proportion of cycles with thawed cells is increasing steadily and the resulting pregnancy rate, which is now 29% per transfer, is thus increasingly approaching the pregnancy rate after fresh cycles. About 261 treatments occurred in the absence of a male partner and 345 treatments occurred in lesbian couples (DIR Registry 2019).

The guideline 016-003 of the German Association of the Scientific Medical Societies (AWMF; n.d.), "Psychosomatically oriented diagnosis and therapy for fertility disorders," was updated in 2019 to include the following new recommendation:

> "Since involuntary childlessness usually burdens both partners of a couple, a psychosomatically oriented counseling service should explicitly address the couple with an unfulfilled desire to have children."

"The existing counseling services should be expanded to also address the male partner and secure availability to couples with a migration background as well as all social milieus (…). These counseling services should be offered in a destigmatizing and proactive manner as well as being available at a low threshold (financially and logistically). Psychosomatically oriented counseling for the couple should also proactively address lifestyle matters and behavioral factors that are known to have a fertility-limiting effect."

This is preceded by the following "statement": "Psychopathological abnormalities are not more frequent in women and men with an unfulfilled desire to have children; not even when no organic cause for the childlessness could be determined. A behavioral—and thus potentially psychosocially (co-)caused—fertility disorder is found in 5% to a maximum of 10% of all couples."

> "For many couples, experiencing fertility dysfunction as well as the psychological effects of reproductive medical treatment, represent a considerable emotional burden. In this context, women and men experience themselves as equally burdened."

The German Society for Infertility Counseling—Beratungsnetzwerk Kinderwunsch Deutschland (BKiD) e.V.—evaluated a total of 1719 first-time **infertility counseling sessions** conducted between June and December 2019 (Thorn 2020). According to this, heterosexual couples make up the largest proportion of those seeking advice, at around 71%. About 16% of counseling sessions were attended by single women, 12% by lesbian couples, and a total of four counseling sessions (0.2%) by gay couples. The most frequent concern of heterosexual couples is counseling in the context of treatment with their own gametes. After all, almost half (about 45%) of this group sought counseling to discuss sperm donation. Counseling in lesbian couples was often (74%) related to enquiries concerning sperm donation possibilities abroad. The proportion of single women was similar at approximately 72%. Two gay couples were counseled in the context of surrogacy.

This inventory was essentially about the various forms of assisted reproduction for which counseling is sought. A survey of the effects on sexual and partnership relationship satisfaction in the respective counseling fields would be necessary, since it can be assumed that many couples do not have their desire for a child fulfilled through assisted reproduction.

44.2 Importance of the Dimension of Reproduction

From a functional perspective, it is appropriate to speak of a multifunctionality of sexuality, whose individual aspects are closely interrelated and can be conceptually distinguished as follows:

– The **dimension of desire** includes the possibilities of gaining pleasure through sexual experience.

- The **dimension of reproduction** includes the importance of sexuality for reproduction.
- The **dimension of attachment** emphasizes the importance of sexuality for the satisfaction of basic biopsychosocial needs for acceptance, closeness, safety, and security through sexual communication in relationships (Beier and Loewit 2004; Beier et al. 2021).

These **three dimensions of sexuality** are experienced as having different significance depending on the phase of life (in fantasy and reality): the **reproduction dimension** with its beginning in puberty (menarche, ejaculatory) can range between excessive and no significance with the gender difference that men are in principle capable of reproduction until old age, whereas the reproductive capacity in women fades out many years before the climacteric. The **attachment dimension** already experiences its individual form in infancy (in the sense of the not yet genitally centered preforms of infantile sexuality) and reaches a high level very early, which is determining for later life, but often remains unreflected. The dimension of pleasure begins with the appearance of bodily pleasurable sensations probably even during intrauterine existence, in any case very early in childhood (so-called infant onanism, etc.) and maintains its importance from sexual maturity onwards for the rest of life.

Of the three dimensions of sexuality, the **dimension of reproduction** is phylogenetically the oldest in higher organisms.

At the level of unicellular organisms, so-called parasexual processes such as conjugation, a type of mating in which genetic material is exchanged between two cells, serve not for reproduction but for genetic recombination, which provides the raw material for evolution. Reproduction is asexual. Only more highly developed multicellular organisms have combined genetic recombination (during germ cell maturation) and reproduction into the type of (sexual) reproduction we are familiar with. In humans, it is limited for females to the period of reproductive capacity between puberty and menopause, and its realization also depends on biographical choices, i.e., it is optional. The availability of reliable contraceptive methods on the one hand and advances in reproductive medicine on the other have made it possible to largely decouple the reproduction dimension of sexuality from its other two dimensions, desire and attachment.

The **dimension of desire** gives sexuality a quality that sets it apart from other human experiences through the unique sensual experience of sexual arousal and orgasm. Sexual pleasure establishes the motivational quality of sexuality and represents, as it were, the drive and reward of sexual behavior. The dimension of desire can be quite prominent in subjective experience, autoeroticism, and the experience of erotic attraction, passion, and ecstasy. In the historically dualistic Western world, which is hostile to the body and to lust, it has always been caught between demonization and glorification. It can be lived separately from procreation and relationships, in isolation, but it is difficult to view it in isolation because it is closely linked to the other functions and is influenced by numerous factors outside and inside sexuality. Relevant media, porn and sex industry, etc. propagate such a one-sided predominance of the dimension of desire that the other dimensions of sexuality fade into the background. At the same time, however, listlessness is currently the most widespread sexual dysfunction (Sect. 43.2).

The **dimension of attachment** emerges later in the phylogenetic development of sexuality, only after the reptilian stage in birds and mammals, especially primates.

So this social or bonding function of sexuality—which many are not aware of—is not a new acquisition by humans. In the animal kingdom, sexuality had received a social, pair, and group cohesion-promoting meaning in the sense of a "meaning and function change" (Wickler 1969, 1971; Wickler and Seibt 1984). In humans—who differ most from their prehuman primate relatives in their linguistic and cultural capacity—the bonding dimension of sexuality becomes a communication function in a specific way: bonding and relationship arise through communication; communication and relationship are interchangeable terms. Because of the "impossibility not to communicate" (Watzlawick et al. 1969) or "not to relate," the social bonding function of sexuality is an obligatory function, not subject to arbitrary availability, and relevant throughout life. At the same time, it highlights the specifically human elements of sexuality. For contemporary man, the attachment dimension undoubtedly constitutes an integral and essential part of his sexuality. Its far-reaching significance results from the fact that human beings are relational beings.

Therefore Beier and Loewit (2004) have tried to emphasize this conceptually by speaking of the **syndyastic** dimension of sexuality and thus expressing its importance for the satisfaction of basic biopsychosocial needs for acceptance, closeness, security, and safety through sexual communication in relationships which must be explored separately from the desire and reproduction dimensions in order to determine syndyastic deprivation of patients or the syndyastic functioning level of relationships. The term "syndyastic" goes back to Aristotle and is derived from the Greek word *syndyastikós* ("disposed to the commonality of two or to the relationship of two or couple"). In his Nicomachean Ethics, Aristotle explains the fact of "becoming familiar with one another" (*synoikeioûsthai*) in the sense of "belonging"; from this he distinguishes the relationship with a "significant other" in

the sense of the couple relationship (*syndyastikós)*, in which familiarity and belonging can develop particularly intensively (Krapinger 2001).

44.2.1 Biological Influencing Factors

The three dimensions of sexuality—reproduction, desire, and attachment—are neurobiologically very closely linked.

There is a close interplay of neuronal processing and neuroendocrine regulation involving sex steroids, cerebral amines, and neuropeptides (Beier et al. 2021).

Different disciplines of medicine deal with the subaspects of this process, such as reproductive medicine with the hormonal aspects, psychiatry, and neurology with questions of cerebral organization, and (neuro-)urology and urogynecology with neuromuscular organization and peripheral neuronal connections.

The **hypothalamus** can be considered as the control center for sexual experience and behavior. It is an endocrine and neuronal regulatory organ that integrates autonomic and endocrine functions as well as visceral and somatosensory afferents and influences behavior. With respect to sexuality, it has both organizational and coordinating functions, the latter with partly promoting and partly inhibiting influences. In many areas, there are neurons that contain both testosterone and estrogen or corresponding receptors in varying densities. It is possible that the former have more efferent, the latter more afferent functions, but this difference is not yet well established.

In addition, a variety of neurotransmitters and neuromodulators are active in neurons: e.g., the following neurotransmitters/modulators, in addition to hypophysiotropic-releasing hormones, were detectable in a single nucleus close to the ventricle, which overall regulates more metabolic functions: somatostatin, VIP, neurotensin, substance P, cholecystokinin, vasopressin, oxytocin, enkephalin, the two angiotensins, and other neuropeptides (Beier and Pauls 2021).

Important regulatory tasks of the hypothalamus related to sexuality involve:

1. The formation and secretion of hypothalamic-releasing hormones, which in turn are subject to feedback mechanisms that are influenced by both hormones and neuronal transmitters.
2. A reduction of hypothalamic dopaminergic neurotransmission causes an increase of prolactin in the adenohypophysis, i.e., is linked to reproductive functions.
3. Oxytocin release from hypothalamic nuclei, triggered by sensory afferents (breast and vaginal stimulation), but also neurochemically (e.g., by sex hormones or catecholamines) and acts on the one hand neuromuscularly efferent (orgasm, contractions, lactation) and on the other hand (by intracerebral mechanisms) on behavioral dispositions (the latter is also controlled by sex steroids) (Ishak et al. 2011; Borrow and Cameron 2012).

From an evolutionary biological perspective alone, sex-related differences in these central processes are not surprising, e.g., to the extent that women activate emotionally processing structures, such as the anterior cingulate gyrus, the left insular region, or the orbitofrontal cortex, more strongly in midcycle than during menstruation (e.g. Dreher et al. 2007). When environmental factors or partner aptitude are thoroughly considered, the higher burden of reproduction on women compared to men becomes understandable. Therefore, gender-related considerations of the neurobiology of the "desire system," "the attachment system" as well as the "reproductive system" are useful.

Prolactin, in particular, highlights this interplay because there are prolactin receptors throughout the body and its release in the adenohypophysis does not appear to be directly controlled by a hypothalamic-releasing hormone. Several influencing factors are known to promote release, including most strongly nipple sucking (Grattan 2015). This promotes interaction with the infant, which in turn promotes the release of **oxytocin**, which can result in a deepening of the bond—in addition to maintaining the mother's milk production. A significant influencing factor on prolactin secretion is **dopamine**, which inhibits secretion; conversely, when dopamine levels are low, prolactin is secreted in increased amounts. This knowledge is used (off-label) to promote breastfeeding by using the dopamine antagonist **domperidone** in mothers with breastfeeding problems (Grzeskowiak and Amir 2014).

The link with the dimension of desire is again evident in the postorgasmic increase in prolactin in men and (more so) in women (Krüger et al. 2003). While these effects are not yet well understood, they suggest that (in both sexes) a switch to reproductive functions is likely to occur as a result. Moreover, hyperprolactinemia (also in both sexes) is accompanied by an attenuation of sexual desire and clinically should initiate the workup of a prolactinoma—and again is treated pharmacologically with dopamine agonists. What this also reveals, however, are the functional connections between the dimensions of sexuality, which must be understood as the foundations of sexual health (Beier et al. 2021).

44.2.2 Psychosocial Influencing Factors

In addition to gender-typical biological differences with regard to the reproduction dimension of sexuality, it must also be considered that **psychological and social influences** can have a different impact on the sexes and in this respect

could affect not only the desire to have children itself, but also a possible conflictual internal processing of the same—especially in the "case of disturbance."

Some insight into the frequency of problems in the area of sexual reproduction is provided by the second part of the Berlin Men's Study, in which 108 female partners were also included (Beier et al. 2005; Sect. 43.3.4) and—like their male counterparts—were interviewed in detail on the topic of reproduction. This showed that a total of 42.6% of the women had experienced at least one of the following five characteristics of sexual reproduction themselves and had processed it as problematic: abortion, miscarriage, unfulfilled desire to have a child, imagined or unperceived pregnancy.

Moreover, even imagined (12%) or unperceived pregnancy (4.6%) was reported surprisingly frequently. More than 15% had even cited stressful experiences in at least two areas (Tables 44.1 and 44.2).

The corresponding figures for men are much lower: 23.5% had experienced at least one of the five above-mentioned characteristics of sexual reproduction with their partners and had processed it as problematic. It is also noticeable that—in contrast to the women themselves—imagined pregnancy (2%) or unperceived pregnancy (2%) was again rather rarely stated. If one considers this high prevalence of conflicts in the area of sexual reproduction among women in relation to the figures among men, then this corresponds to the reverse situation in the case of paraphilic patterns (which occur much more frequently among men (Sect. 43.3.4). The specific and typical differences of male and female sexuality and respective disorders should therefore be considered separately, since it can be assumed that these result in different symptoms in women and men.

In Fig. 44.1, these correlations are shown graphically by linking the frequency differences in disorders of sexual preference (more frequent in men) and disorders of sexual reproduction (more frequent in women) with gender-typical differences, which concern biological as well as psychological and sociological levels. It is noticeable that disorders of sexual preference are perceived more strongly socially than disorders of sexual reproduction.

44.2.3 Conflict Processing Via Reproduction

From a psychodynamic point of view, it would be plausible to "think up" an inner-psychic coping strategy mediated via sexuality in women more with the functions of the female

Table 44.1 Frequency of various problems with sexual reproduction among female partners of participants in the Berlin male study (Part 2; $n = 108$); with multiple responses

	N	%
Terminated pregnancy	16	14.8
Miscarriage	17	15.7
Unfulfilled desire for a child	18	16.7
Imagined pregnancy	13	12.0
Unperceived pregnancy	5	4.6
Total (without multiple answers)	46	42.6

Table 44.2 Frequency of various sexual reproduction problems among partnered participants in the Berlin men's study (part 2; $n = 307$); with multiple responses

	N	%
Terminated pregnancy	25	8.1
Miscarriage	27	8.8
Unfulfilled desire to have a child	33	10.7
Imagined pregnancy	6	2.0
Unperceived pregnancy	6	2.9
Total (without multiple answers)	73	23.8

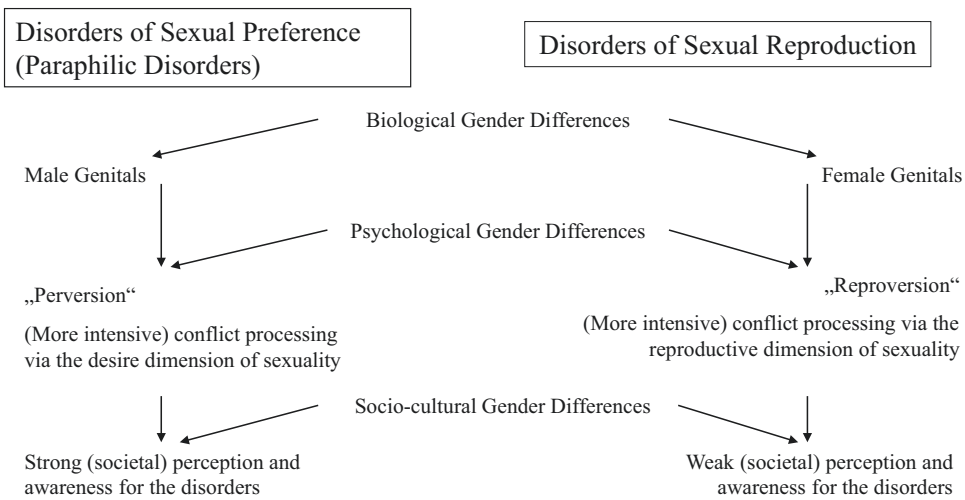


Fig. 44.1 Sexual disorders and gender differences

sexual organs—i.e., the reproductive domain. Then one would theoretically be able to assume that there is a "female analogy" to male perversion, that is, that a comparable specific mode of **conflict processing** can be assumed, which in principle follows the same mechanism, but has a different subject in each case: in the male more strongly linked to the desire dimension of sexuality (and the external genitals) and in the female more strongly linked to the reproduction dimension (and the internal genitals).

Following this line of thought, Beier has proposed speaking of **reproversion** in linguistic analogy to the conflict-processing mode of **perversion**, meaning an inner-psychic coping strategy that is mediated by the reproductive aspects of sexuality and is primarily linked to the reproduction dimension of sexuality (Beier 1994). "Plugging" in the sense of Morgenthaler (1987) as denial of libidinous sexuality would primarily concern the reproduction dimension of sexuality in women and not (as preferred in men) the dimension of desire. The precise term for this processing mode would therefore not be "perverse" but "reproverse." Thus, if—for whatever reasons—in the psychic development, the self-system in a woman shows (considerable) structural deficiencies, then the associated fears of a fragile female identity can be successfully warded off by forcing reproductive impulses (in analogy to forcing genital pleasure impulses in men).

Perverted men and analogously reproverted women try to close a gap in the psychic structure by intensifying normal (pleasurable-genital or reproductive) aspects of experience. In this way, the conflicts are identified and fixed in reproduction—the rest of the person is no longer burdened with them.

According to this, its mechanism is identical to that in the case of perversion, which, however, is given a different thematic form due to the biological preconditions in women. The shift of conflicts into the reproductive aspects of sexuality can be differentiated, at least theoretically, in the same way under clinical guiding aspects as the forcing of genital pleasure impulses as a conflict processing mechanism in men: here, too, the intensity of the symptomatology and its significance in the personality structure can be very different.

As an example, the **desire for bearing a child in a dissolving partnership** (where the separating tendencies outweigh the unifying tendencies) would be an example of a reproverse impulse that occurs uniquely and is tied to a current conflict. In contrast, in women with very many (sometimes more than 10) abortions, the conflictual desire to have children "counteracted" by the "decision to have an abortion" rather suggests a recurring pattern of conflict resolution in the sense of a neurotic compulsion to repeat. Finally, there are highly intense reproverse symptom formations as stable orientations, which express themselves in the overvalued dominant relationship to the child: The child becomes the mother's "**self-object"** and is "used" by her for the fulfillment of her own needs, which can go as far as giving way to destructive impulses if the child withdraws from this purpose (see case example on "aurorism"; also Beier 1994, 2000).

Furthermore, there are differences in the ego-proximity of symptom formation: ego-syntone would be, for example, the imagined pregnancy and ego-dystone the unperceived pregnancy. In women, too, disturbance aspects of the personality would have to be considered, which affect the female identity (especially with regard to confidence in the **functional completeness as a woman** and her reproductive potency) as well as a disturbed assessment of self-experience (narcissistic aspect) and disturbances of dominance/submissiveness and thus of the relational capacities (Beier et al. 2021). An overview of the disorders of sexual reproduction can be found in Sect. 43.3.6.

44.2.4 Clinical Case Studies

44.2.4.1 "Unbearable Pressure"

A 58-year-old, wealthy restaurant owner presents to the university outpatient clinic with a 38-year-old wife (who runs a fashion boutique) because of existing (secondary, situational) disorders of sexual desire and erection (also secondary, situational) with a strong desire for children on her part. Anamnestically, the wife's abruptio at the age of 35 years was reported ("did not fit at that time").

They had undergone five unsuccessful IVF cycles and presented at the recommendation of the fertility center because both showed increasingly depressive symptoms. The spermiograms made it clear that his sperm quality had increasingly deteriorated.

He considered himself too old to have a baby of his own and was also skeptical about the option of adoption (of a child who would then be older). This had led to a persistent severe irritation on the relationship level, which was initially mitigated by sexual therapy intervention. An intimate rapprochement took place under the consensual agreement of not having sexual intercourse, which relieved him greatly, but she was only able to accept this for a limited period of time, although she also recognized and enjoyed the positive effects for the partnership. Thus, she pushed for continuation toward coital intimacy, which puts pressure on him and again brought up the sexual appetence issue with him describing the "pressure as unbearable." This was understandable in that the woman announced that she wanted to separate from her husband in order to find a partner who would be able to "father a child" for her, which highlighted the extent to which the reproduction dimension had overridden the dimension of attachment. (Note: This is often not true for the desire dimension. In many cases of male sexual dysfunction, women are more likely to maintain the relationship, often being more able to accept limiting the desire dimension.)

From an andrological point of view, the decreasing sperm quality in the course of reproductive efforts is a phenomenon observed again and again ("reproductive stress"). A simple possible solution with the aim of relieving the stress would be the temporary renunciation (e.g., 3–6 months) of targeted sexual intercourse at times of the ovulation optimum, undergoing medical examinations and methods of assisted reproduction. Such recommendations were easier to implement 20 years ago than today. Data from the German IVF Registry provide one reason for this. The age of couples seeking reproductive medical treatment has continued to increase in recent years. The resulting objective time pressure is also perceived as such by the couples and is not infrequently justified when the partner is older, so that the abovementioned recommendation cannot be implemented. In addition, there is time pressure due to regulations of the health insurance system; when the woman reaches the age of 40, statutory health insurance companies no longer cover the proportionate costs for methods of assisted reproduction, so that time delays are not only unacceptable for biological reasons when the partners are of a corresponding age.

In addition to a tendency toward decreasing sperm quality in the course of reproductive medical clarification, short-term, situation-related deteriorations in sperm quality also occur. Thus, it could be shown that essential variables of the spermiogram were worse and the anxiety of men was higher on the days when the ejaculate was collected for methods of assisted reproduction. If delivery was only for investigational purposes, sperm quality was better (Vellani et al. 2013).

However, the essential aspect in the casuistry described above is the **ambivalence of the desire for a child in both partners**, which is repeatedly reported by men in personal conversations during the andrological consultation. It often concerns couples with a considerable age difference. The older man understands his younger partner's wish to have children and does not want to deny her this wish out of affection for her and fear of losing his partner. Nevertheless, the men themselves do not really want children anymore, especially if there are already adult children from a previous relationship. This ambivalence regarding the desire for children is usually not addressed out of fear of escalation. The problem is exacerbated when andrological causes for the unfulfilled desire to have children are discovered in the man and he becomes even more involved in reproductive medical efforts. The burden of being the "cause" of the unfulfilled desire for a child seems all the more threatening to him because he now "has to" accept examinations and possibly interventions that he actually wanted to avoid.

44.2.4.2 "Pregnant Even as a Man"

In a 35 year-old "trans man" (condition after change of first name due to transsexual gender dysphoria clearly present here) with gynephilic sexual orientation (and erotic responsiveness to the adult female body scheme), the wish for having a child had arisen in the long-term partnership with a woman 2 years younger. This was to be done by the "cup method" (sperm donation by a work colleague, friend) with the partner, but failed.

The patient then decided to discontinue his testosterone treatment, which had been ongoing for 6 months, and by means of the "cup method" produced a pregnancy in himself, which led to the birth of a healthy son.

The family situation is characterized by a clear division of roles: "My wife is the mother, I am the father"). The course of pregnancy had been "hardly noticed"—"somehow disconnected." The same had applied to breastfeeding, which was carried out for half a year because it was "good for the child."

From the point of view of sexual medicine, it is particularly impressive that the **desire to have children is stronger than the sense of identity**, because very many trans men would categorically reject pregnancy in themselves and press for surgical removal of the uterus and ovaries ("having children is something for women").

44.2.4.3 "It's Now or Never".

A 37-year-old woman (IT specialist) presents to the university outpatient clinic because of a sexual aversion (secondary and situational) that has been going on for 3 years. She desperately wants a child of her own, has been doing cycle monitoring for 2 years, but cannot allow coital intercourse exactly during the fertile days. She does not understand this herself. Her husband (43 years old) was able to cope with it at the time and had been able to put up with the repeated rejections, since there was coital intimacy in between. He had not known that there was a connection with her cycle.

The couple had gone to a fertility center because of their unfulfilled desire to have children, where they were advised to undergo insemination treatment because time was pressing due to their age and it was "now or never" that they could achieve their goal to have children. This had put her under even more pressure and it had also annoyed her that she had been asked about the duration of her desire to have children, but that her sexuality had not been discussed, even though this was her problem.

During the sexual therapy sessions, it quickly became clear that the couple had hardly ever discussed their desire to have children. She did not want to put him under pressure "with it," because he already had two children of his own from his first marriage and it was therefore certainly not so important for him. She herself had ambivalent feelings about having children, because it did not really fit into her current life, but because of her age it had to be now. He, on the other hand, reported that he had not assumed that starting her own family was important to her, since to him her life seemed fulfilled professionally as well as in her relationship. However, he himself wanted another child with her without

question. The **lack of exchange about (re)starting a family** ultimately led to an estrangement that was quickly resolved in the sexual therapy sessions. The couple finally agreed to postpone starting a family together for another year and then, if necessary, to present at a fertility center for insemination treatment if sexual aversion should arise again until then. The couple quickly found the change from the reproduction dimension with omission of cycle monitoring and turning to the attachment dimension to be very relieving, even though they continued to be unable to allow episodic coital intimacy.

44.3 Expanded Concept of Sexuality in Fertility Treatment

The current state of **reproductive medical treatment options** (Sect. 44.1) makes it clear that in the course of therapy, **effects on the sexual and/or partnership experience of couples** are to be expected, if not unavoidable. In particular, treatment procedures of assisted reproduction with the various drug options for ovarian stimulation intentionally lead to a change in hormone metabolism (in the majority of cases through the use of antiestrogens and gonadotropins).

Antiestrogens used for hormonal stimulation can lead to temporary menopausal symptoms with hot flushes, loss of libido or vulvovaginal dryness; the same applies to the GnRH agonists and antagonists used for ovarian suppression. Progesterone, which is frequently used in the luteal phase, often leads to fatigue and depressive moods due to its central CNS effect, and to local irritation and dryness of the vagina and subsequent dyspareunia due to its mostly local vaginal application.

From the point of view of sexual medicine, this seems to be an ideal starting point to talk to the couple about their sexual/partnership (dis)satisfaction and to develop a biopsychosocial approach to integrated sexuality.

In accordance with the biopsychosocial model of sexuality in sexual medicine (Sect. 43.1), which combines its syndyastic, i.e., relationship-oriented (or social-communicative) dimension, its reproduction and desire dimensions, the relationship of these dimensions to each other is of initial interest.

This relational, precisely communicative dimension of sexuality persists throughout life.

It precedes and outlasts reproductive capacity, encompasses many gradations of body language expression, and is obligatory because of the "impossibility of not communicating" (Watzlawick et al. 1969). Rooted in it is the salutogenic meaning of gratifying relationships and their sexual embodiment in tenderness and coitus, but equally present is the pathogenic, suffering-creating, and destructive power of sexuality.

The reproduction dimension, in contrast, is present only during the period of reproductive capacity; for women between puberty and menopause, for men between puberty and the end of his sexual activity. It is limited not only in time but also in number, is subject to an "all-or-nothing" principle, and is also optional. Its meaning does not lie in the sensually experienced embodiment and thus consolidation of the relationship through sexual communication, but in the creative act of fathering and bringing one's own children into the world, which is generally taken for granted without reflection. Often, conscious reflection on the motivation of the desire for children begins only in the event of unwanted childlessness. "The desire for self-fulfillment, the need to give life and to recognize oneself and one's partner in the child, and thus to continue living on in some way" are, for example, motives that have been put forward by couples wishing to have children (Stauber 1989).

At this point at the latest, however, it is necessary to attach great importance to questions about the quality and stability of the relationship, about sexual satisfaction or dissatisfaction, also from the **point of view of the child's well-being.**

At the same time, these questions cover all those factors which can be expressed in the experience of sexuality within the partnership and which essentially determine the family atmosphere. This also includes the fundamental question already mentioned about the motives for wanting to have children. It is important to emphasize these "self-evident facts" because reproductive medicine is not directly confronted with the wishes or potential needs of the child to be conceived, but with the often imperative desire of the couple that it should help them (under all circumstances) to have a child.

Therefore, some self-evident principles should be recalled, which can become especially important in reproductive medicine (Loewit and Beier 2021):

- The child is the weakest part in the system, i.e., in weighing the interests of the adults against those of the potential child, it must not be forgotten that the child cannot defend itself, cannot help itself, also usually has no surrogate or representative, and is largely dependent in its further development on the given starting conditions.
- Children need parents and not "parents-to-be need a child." In this dichotomy, it depends on how mature and

realistic or how immature and illusionary the respective concrete wish for a child is and what goals it is actually supposed to serve. Has the couple found a common meaning of life which they are willing to share and which they want to pass on? And not the other way around, that the children are a **substitute for a lack of meaning in life**—"gap fillers," who are supposed to fill a maternal, paternal or partnership void.

- **Children need both parents** for a balanced development, especially for the acquisition of their gender identity and own partnership ability.
- Children have a **right to know their parents**; knowledge of their genetic ancestry must not be withheld from them.
- Partnership-related and narcissistic motives will always be at play more or less simultaneously in every person. Whether the measure of "normal" is exceeded depends on the magnitude of the respective shares, which, however, can be brought to light if this is provided for in an overall concept of fertility treatment.

44.4 Indication for Couple-Related Interventions

From the point of view of sexual medicine, it would be important above all to determine the **syndyastic level of functioning** of the couple, especially also with regard to the interaction of the three dimensions of sexuality (desire-reproduction-attachment) in the couple. This is to be seen independently of the sexual orientation of the individual partners and the forms of their cohabitation, and thus applies equally to same-sex couples or the most diverse conceivable couple constellations in connection with gender transpositions of one or both partners. Here, too, the decisive factor is always the question of the extent to which the child may have to assume functions which—for whatever reason—cannot be performed by the parents within their relationship (Sect. 44.3).

Similar to the treatment of preference disorders, (Beier 2010), the following five factors should be considered when planning treatment options (Sect. 43.5.3):

1. **Importance of the reproductive aspect** of sexuality for each partner. It makes a big difference whether the desire for children dominates the relationship experience or whether other meaningful aspects of the partnership become apparent, which could be taken on in a partner-related way.
2. **Additional occurrence of sexual dysfunctions**: Any sexual dysfunction may be caused by involuntary childlessness—or may have existed before. In both cases, this could be a symptom of stress within the relationship and a starting point for couple-related interventions, provided there is an interest in this and there are no other obstacles (see 3).
3. The **importance of the desire for a child** in inner experience: If the projected motherhood or fatherhood or the desired child has such a high inner value for psychological stability that without its realization life no longer seems to have any meaning, the limits of therapeutic interventions are described at the same time, especially if this significance exceeds that of the attachment to the real partner (see 5).
4. **Ability of self-restriction**: Not only the value of the desire for a child in self-experience (see above), but also—and by no means congruent with this—the ability to withdraw from oneself with regard to the relationship with the partner is to be regarded as a therapeutic limiting factor.
5. Existence of a **potential threat to the relationship** through the desire for a child: This can be recognized by the fact that the relationship would be terminated if the desire was not fulfilled.

Points 1–4 in particular make it clear why syndyastic focusing (Sect. 43.5.2) can be a useful therapeutic strategy in cases of involuntary childlessness—namely, if both partners are interested in an improvement of their sexual and partnership satisfaction and this can be combined with the necessary self-restriction within the relationship, which, however, is only the case if the desire for a child is not regarded as more important than the attachment itself in the inner experience of the person concerned. At the same time, this shows that exploration of the three dimensions of sexuality (desire, reproduction, attachment; Sect. 43.4.2) should be an indispensable element in diagnostics, because this makes it possible to assess the selection of therapeutic options and associated developmental opportunities.

The great **importance of psychological factors** was already evident in studies by Stauber (1989) in the fact that of the 30.9% of pregnancies achieved, only a good quarter occurred under therapeutic measures. The largest part (49.7%) developed outside of the medical treatment: during vacation, during breaks in treatment, after abandoning the desire for treatment or adoption of a child. Another 22.7% of gravidities occurred in close connection with diagnostic procedures (e.g., hormone determinations, but also laparoscopies).

In male partners, psychosocial stress has been shown to negatively affect semen quality in terms of sperm count, motility, and morphology alike. In an analysis of 69 recorded counseling sessions in cases of unfulfilled desire for a child, Kemeter and Fiegl (1999) found psychosocially stressful factors from the environment and the social life situation, the partnership, one's own biography and personality as well as

iatrogenic stress factors from the treatment as particular focal points of conversation in women and men. In particular, the treatment of psychological stress from the environment, the life situation, and the partnership (here, first and foremost, problems of disturbed sexuality as "efforts on behalf of fertility") led to acute relief for those affected. There can therefore be no doubt about the necessity of a biopsychosocial approach.

Key Points

- In cases of unfulfilled desire for a child, the task of biopsychosocial diagnosis and therapy is given, for which biomedical and psychosocial ways of thinking must be combined with a view to the couple.
- The various treatment procedures of assisted reproduction must also be considered with regard to their possible effects on the sexual and partnership experience of the couple.
- An expanded understanding of sexuality does not see it only as a means of reproduction, but also as a form of body language communication that expresses the quality of the relationship (fulfillment or frustration of basic needs).
- Reproduction can become the target site of conflict processing, in which inner-psychic coping strategies are mediated and primarily linked through the reproduction dimension of sexuality.
- Couple-related interventions in cases of unfulfilled desire for children focus on improving sexual and partnership relationship satisfaction and also address the question of the extent to which the child may have to assume functions that cannot be accomplished by the parents within their relationship.

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Part X

Male Contraception

Male Contribution to Contraception

45

Eberhard Nieschlag

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Abstract

One of the tasks of andrology is to provide methods for contraception on the male side. Such methods are necessary both to maintain a stable population in industrialized countries and to contain population growth in developing countries. In this chapter, the rationale for male contraception as well as social and demographic aspects will be discussed and the conventional methods of male contraception will be described: condoms, coitus interruptus, and periodic abstinence. Chapter "Approaches to Hormonal Male Contraception" is devoted to vasectomy and refertilization. To date, a reversible pharmacological method is not available. Research and development in this field will be described in Chaps. "Nonhormonal Approaches to Male Contraception" and "Legal Regulations in Andrology and Reproductive Medicine."

45.1 Introduction

Making contraceptive methods available to men is one of the tasks of andrology. Such methods are necessary both to maintain stable populations in industrial nations, as well as to diminish population growth in developing countries.

This chapter deals with the reasons for male contraception as well as social and demographic factors and the conventional methods for male contraception are described. A separate chapter is devoted to vasectomy and refertilization (Chap. 47), while research and development in the field of male contraception are dealt with in Chaps. 47 and 48.

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45.2 Requirements and Perspectives

45.2.1 Global Goal: "Sexual and Reproductive Health"

The WHO introduced the concept of "Sexual and Reproductive Health" as its stated goal: the right of all to lead a satisfactory sexual life to attain sexual fulfillment: Sexual and Reproductive Health requires that reproduction takes place under condition of "complete physical, mental, and social well-being" and signifies not only freedom from disease and from possible disturbances of reproductive functions. A child, once conceived, should have optimal conditions for undisturbed gestation and birth as well as for healthy childhood and development. Reproductive health implies the possibility of realizing the desire for offspring, as well as of controlling and planning fertility. **Contraceptive methods for women *and* men** are considered essential components of strategies which should lead to a high standard of reproductive health worldwide.

45.2.2 Contraception, Family Planning, and World Population

When advising on contraception and family planning as well as prescribing contraceptives, the physician must primarily concentrate on the desires and problems of the individual couple. Over and beyond this, he should be aware that contraception also has demographic implications and influences population dynamics. While the individual couple may have its own ideas about the number of children wanted, representative opinion surveys on the ideal size of the family show that these wishes have hardly changed in the course of the history of the German Federal Republic and have remained at about two children per family; however, the actual number of children per couple began to decline in the 60s with the introduction of oral contraceptives and other modern methods and for several years, the actual birth rate was even below the desired goal Only recently the desire for children matches the actual birth rate. This is not a uniquely German phenomenon but one that is ubiquitous. From the beginning of industrialization in the nineteenth century, this trend began in Germany and Europe even prior to modern contraception (Birg 2001).

The introduction of modern contraceptive methods led to a social revolution which has above all benefited women (Benagiano et al. 2007). Deliberate birth control, e.g., for economic reasons, was already practiced during earlier centuries (Bengtson and Dribe 2006). Today's contraceptive methods enable the couple to achieve their goals more precisely. Conversely, it is incorrect to assume that the mere availability of contraceptives leads to a reduction of population in lesser developed countries; the individual couple's motivation and desires remain the determining factors (Connelly 2008).

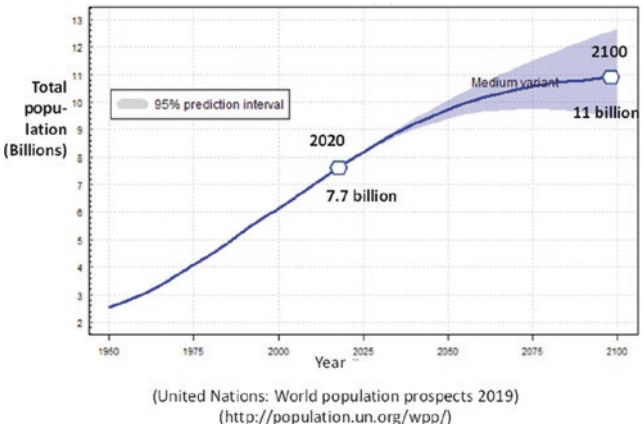


Fig. 45.1 Worldwide population growth from 1950 to 2100. Medium level projection by the United Nations World Population Prospects 2019

In 2020, the **world population** was **7.8 billion**, having quadrupled since 1950. Even conservative prognoses estimate that **11 billion** will populate the earth by 2100 (Fig. 45.1). Lesser developed countries, especially those in Sub-Saharan Africa and southern Asia bear the onus of this enormous population growth, while the populations of industrial nations are largely stable or even in decline (Fig. 45.2).

The population explosion creates hardly surmountable ecological and economic problems. India can be taken as an example, where one-sixth of the world's population occupy 2.5% of the earth's land surface, and where uncontrolled population growth wipes out the country's economic progress, which has in part been spectacular. Medical progress has decisively lowered mortality, particularly of children, so that average life expectancy worldwide is currently 70.4 years for men and 74.9 for women, albeit showing great geographic differences. In the EU, these figures are 76.3 for men and 83.9 for women. The highest life expectancies are found in Hongkong, Japan, and Switzerland, the lowest in Sub-Saharan Africa, where it lies in part below 40 years.

Above all, **life expectancy** has increased due to possibilities offered by effective medical intervention and programs for prevention. Ever more people reach the age for reproduction and thus contribute to greater population growth. In the meantime, the world is suffering from **climate change** largely of human origin, according to current knowledge. In discussions on greenhouse emissions, loss of resources and disappearing biodiversity on the one hand and the search for alternative energy sources on the other, it is often forgotten that **overpopulation** is the main problem in this scenario (Crist et al. 2017). For the more people must be nourished, use fossil energy and strive for higher standards of living, the

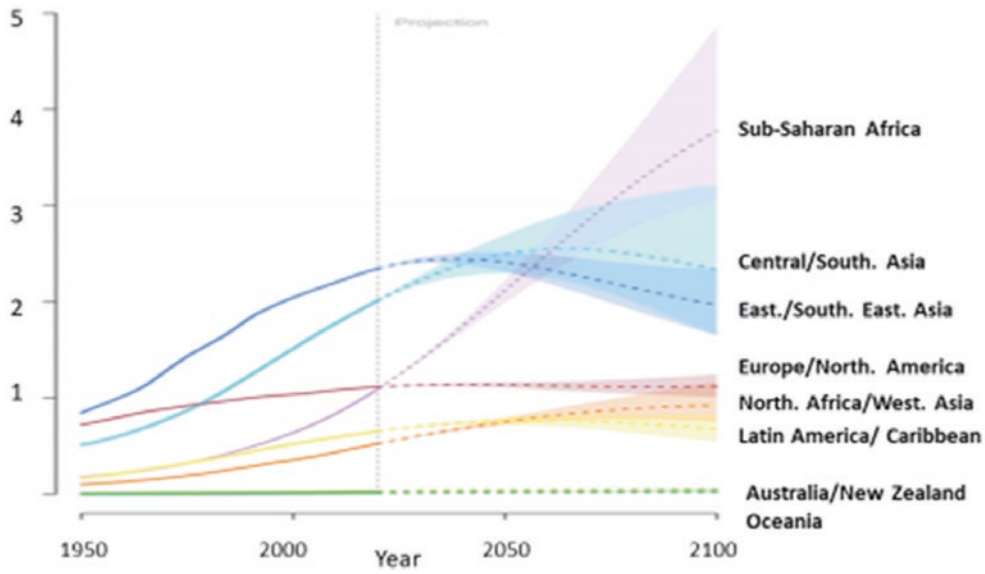


Fig. 45.2 Worldwide population growth 1950 to 2100. The projection shows that the largest growth will occur in Sub-Sahara Africa followed by Central-, South-, and East Asia while in Europe and North America growth will stagnate (United Nations World Population Prospects 2019)

more factors causing climate change are amplified. In these discussions, the enormous importance of contraception is largely neglected (Bongaarts and O'Neill 2018; Bongaarts and Sitruk-Ware 2019).

Although medicine offers possibilities for contraception, access to it is unevenly distributed around the globe. Thus, even today abortion, considered ethically and culturally unacceptable in many countries, is still used for "family planning." As a consequence, of the approximately 1,000,000 conceptions taking place daily, of which about 50% are unplanned and unwanted, approximately **150,000 are terminated daily worldwide by abortion.** Of these terminations, 45% are considered risky, of which 97% are performed in developing countries (Ganatra et al. 2017) and about 500 end lethally for the woman. Comparative investigations have shown unequivocally that the highest numbers of abortions are performed in those countries where effective contraceptive methods are not available. The low birth rates in western industrial nations and the generally stable demographic status are of decisive importance for prosperity and are largely due to contraceptive methods.

It is, however, of much concern that research in the field of contraception has greatly declined in recent years. Especially industrial nations have reduced their efforts. Leading pharmaceutical firms have drastically reduced studies on female methods and some have even terminated research programs dealing with male contraception (Strauss and Chaudhuri 2007; Nieschlag 2013). Publicly funded programs exist only in the USA at the NIH (on a modest level) and few philanthropic organizations such as the Population Council, (New York) have active research programs.

In view of the decline of funding for research on male contraception between 1996 and 2011, researchers met at annual "Summit Meetings on Male Contraception" to discuss results from studies. At the first Summit Meeting in Weimar, the "Weimar Manifesto on Male Contraception" was drafted addressing the public and the pharma industry (Nieschlag 2007, 2011). Later researchers gathered at the "International Consortium for Male Contraception" (ICMC) and issued two Paris Manifestos in 2016 and 2018 calling for the need for intensive efforts on behalf of new methods of male contraception. These appeals were directed to science, to the public and above all to the pharmaceutical industry (Serfaty et al. 2016, 2018; Wang et al. 2016). The latter failed to take up this demand as more lucrative fields were given preference.

45.2.3 Acceptability of Male Contraception

Recently public interest in male methods for contraception has notably grown. It is increasingly expected that men share with their partners not only the advantages but also the risks of family planning. As risks tend to increase with duration of use, sharing contraception between men and women would reduce dangers for each partner. Population conferences and women's world forums have explicitly called for new male contraceptive methods. Nonetheless no pharmacological method for male contraception exists to date.

Worldwide **28% of all couples practicing contraception rely on male methods,** albeit with varying preferences (withdrawal, condoms, vasectomy) (Ross and Hardee 2017). Overall, the proportion of men practicing contraception is increasing. However, the proportion of couples relying on male methods in Germany is only about 12.3%. There are great regional variations in vasectomy. Thus, the percentage of vasectomized men with female partners of reproductive

age in Germany is only 2.1% and just above the world average of 2.0%, while comparative figures from neighboring Switzerland are 2.9%, in the USA 4.3%, in Australia and New Zealand 7.7%, and are highest in UK with 10.4%. Similarly, the use of condoms for contraception varies from country to country with a worldwide average of 21% (Fig. 45.3).

It is to be expected that the percentage of men willing to practice contraception varies between cultures and with methods available. According to a survey in Hongkong and Shanghai 25 years ago, half the men interviewed were willing to take a daily contraceptive pill; in Edinburgh and Capetown two-thirds were willing to do so (Anderson and Baird 1997). After six decades of female oral contraception, the attitude of men toward **new** methods of male contraception has changed. Worldwide, and also in Germany, surveys showed men willing to use pharmacological contraceptive methods (Heinemann et al. 2005) (Fig. 45.4). Based on these results, it was estimated that alone in the nine countries surveyed, 44 million men would be prepared to use a pharmacological method and thus provide an incentive for the pharmacological industry (Dorman and Bishai 2012).

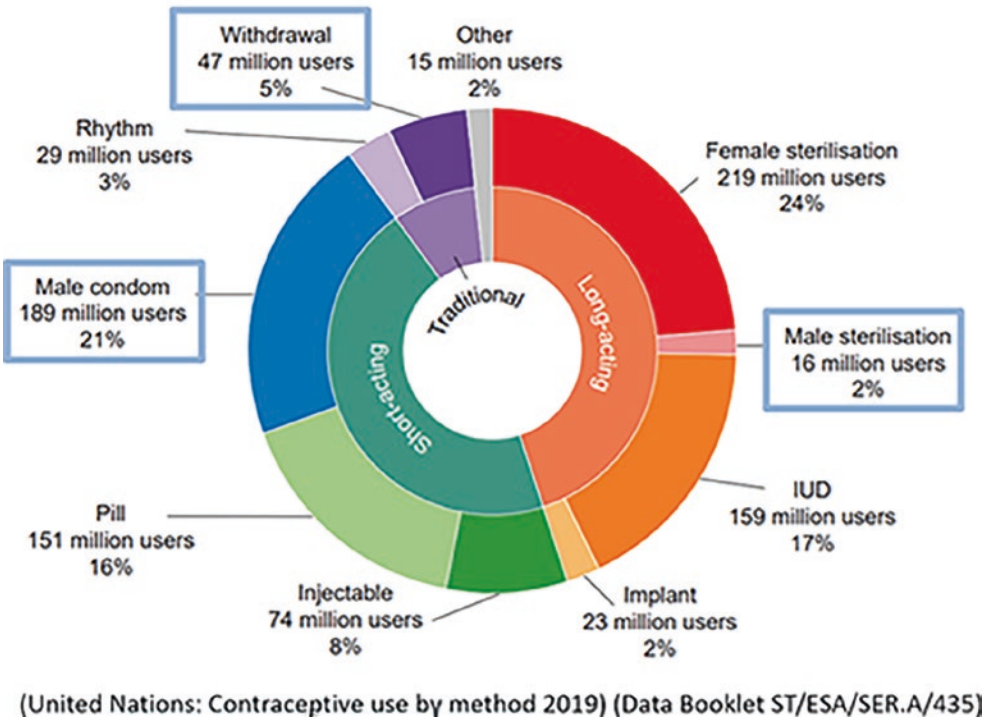


Fig. 45.3 Worldwide use of contraceptives methods by men and women in reproductive age 15–49 years (blue frames indicate male methods) (United Nations: Contraceptive use by method 2019)

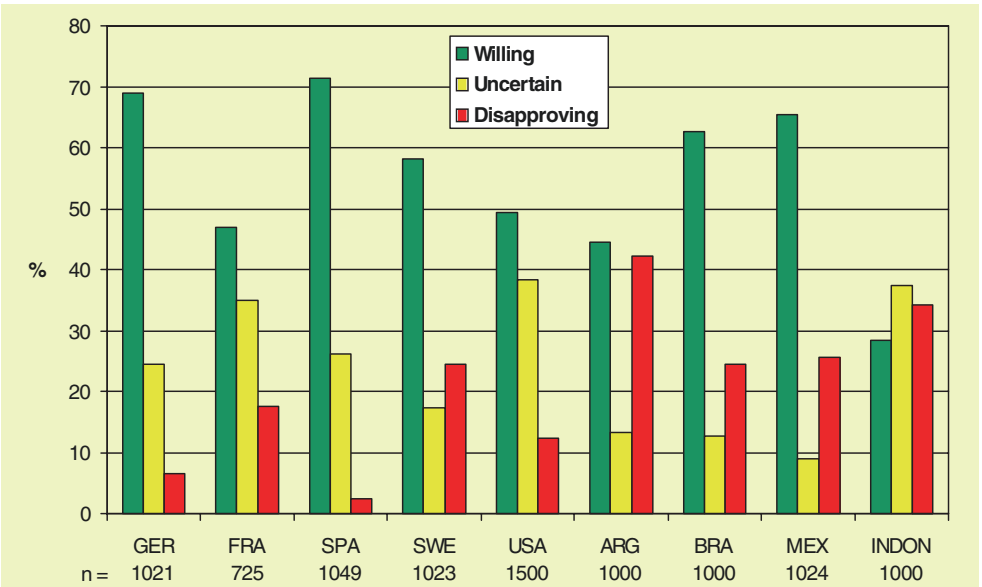


Fig. 45.4 Multinational male fertility control survey among 9342 men. *"If available would you be willing to use the new male fertility control method ?"* (Heinemann et al. 2005)

Prior to the "pill" which marked the advent of modern contraception and gave the woman responsibility for family planning, most available methods lay with the male. Condoms, coitus interruptus, and vasectomy are male methods demanding deliberate application by the male. During the past 60 years, "reproductive power" has largely devolved to women. Worldwide programs for family planning are largely directed toward women instead of including men (Hardee et al. 2017) which might help them to surrender prejudices and resistance to female contraception, especially in sub-Saharan Africa, i.e., in those countries with the greatest population growth (Kriel et al. 2019).

45.2.4 Possibilities

In general, men's willingness to contribute to contraception can be considered to be high. However, before men accept such a contraceptive method, certain prerequisites must be fulfilled. The method should:

1. Be as **effective** as comparable female methods
2. Be **acceptable** to both partners
3. Be **rapidly effective**
4. Be **free of side effects** and especially be without influence on masculinity, libido, and potency
5. Be **without influence** on progeny
6. Be **reversible** in regard to fertility
7. Be easily **available** and financially **affordable**

These criteria provide guidelines for evaluating existing methods and experimental approaches. Basically, these methods can be characterized as follows:

1. **Those preventing sperm transport into the female genital tract**
2. **Those suppressing spermatogenesis**
3. **Those preventing sperm maturation or function**

All existing methods belong to the first group, whereas current research concentrates on influencing germ cell development and maturation.

The possibilities of effective **and** reversible contraception are limited to female methods. **Periodic abstinence,** the **condom,** and **coitus interruptus**, as relatively unsafe methods, or **vasectomy**, as a method with limited reversibility, are **available to the male**. **Periodic abstinence** also requires strict cooperation by the male.

The distribution of male and female methods used worldwide and, for example in Germany, shows (Fig. 45.3) that basically only a narrow spectrum of methods is actively available. All pharmacological methods derive from **one** principle, namely **hormonal contraception**. The danger of overdependence on this method becomes apparent when one considers a possibly drastic reduction in its use. This should provide sufficient impulse to search for new methods. As no male pharmacological method for contraception yet exists, research in this field remains a major task (see Chaps. 48 and 49).

Existing and experimental methods are described in the following section.

45.3 Existing Methods

45.3.1 Coitus Interruptus

The criteria given in Sect. 45.2.4 can be used to evaluate existing and new methods for male contraception. The method practiced longest, **coitus interruptus**, has the advantage of being completely free of side effects, requires nothing additional, and costs nothing. It does, however, require dexterity and self-discipline and reduces sexual pleasure. Moreover, **its failure rate is high**, as a pregnancy occurs in 19 out of 100 couples during the first year of practice (Trussell 1998). Other nonpenetrating sexual practices ("dry sex") probably have lower pregnancy rates, but no exact figures are available. Practice of coitus interruptus shows cultural and geographic variation, with high frequency supposedly in Rumania and Turkey. Of 1000 sexually active Turkish women interviewed, 43% admitted to using this method for contraception. Following counseling, 74% of these women switched to modern contraceptives (Demir et al. 2020).

45.3.2 Periodic Abstinence

Periodic abstinence, on which the various methods of **natural family planning (NFP)** are based, limits sexual activity to so-called "safe days" on which the likelihood of conception is low. Even if this method relies on observations based on the female cycle, it does require a male contribution as spontaneity and intercourse must be foregone for relatively long phases of the cycle. The methods' safety increases with the number of days of abstinence from intercourse. On average, a large number of failures are presumed in which 20 of 100 couples per year conceiving. Worldwide this method is used for contraception by 3.6% couples of reproductive age (Freundl et al. 2010).

If a portable monitoring device (Persona®) is used to indicate days on which abstinence is to be practiced by measuring LH and estrone-3-glucuronide in urine, the pregnancy rate drops to 12% (Bonnar et al. 1999). On average 13 days within a cycle must be considered "unsafe" or other contraceptive methods must be used during this time.

When evaluating coitus interruptus, periodic abstinence, and nonpenetrating sexual practices, it must be considered that despite their low effectiveness, in demographic terms these methods may contribute to population control, even if the risk of pregnancy remains high for the individual couple.

45.3.3 Condoms

The condom is the oldest contraceptive method available today. Early Egyptian drawings show men wearing condoms. In 1200 BC, fish bladders were used at the Minoan court on Cyprus to prevent disease and pregnancies. In 1564, the Italian anatomist Fallopio described linen bags saturated with medication to be used to avoid venereal disease. In seventeenth century, England condoms were first used for birth control. It is not certain whether the name derives from the English physician Condom, who was active at the court of Charles II from 1660 to 1685 and who recommended lamb intestines for contraception. The method was quickly exported to France and became widely used in Paris. Condoms were known there as "capote anglaise," while in England they were called "French letters." The latex-based condom was made possible by the American Charles Goodyear (1800–1860) after he invented the process of vulcanization. The first large-scale manufacturer was Julius Fromm, who produced 150,000 condoms daily in 1920 (Amy and Thiery 2015).

At present, condoms continue to be manufactured largely from latex, but polyurethane and polyisoprene condoms are also available. As these have a higher rupture rate and offer even less contraceptive protection than latex condoms, their use is limited to users allergic to latex. Recently spray-on condoms have been tested, they would solve the problem of size.

The manufacture of condoms is subject to standardized quality control and specifications of the WHO (2010). Since 1996, condoms sold in Europe must fulfill quality requirements as prescribed in the DIN EN 600, international EN ISO 4074 (since 2002). These norms prescribe a size of 17 or 16 cm length and a maximum diameter of 5.6 cm. In addition, the contraceptives must undergo serial tests for impermeability, inflatability, elasticity, and microbiological purity.

Even if manufacturing is subject to quality control, in a high percentage of cases, the condom may tear during intercourse. The older the condom, the higher the risk of damage. Apparently sexual practice also plays a role, as some couples always report a higher rate of tearing than others, as seen in trials with motivated and experienced couples who had no problems with tearing (Rosenberg and Waugh 1997).

Reports on contraceptive effectiveness of the condom show considerable variation. In general, it is assumed that 19 out of 100 couples will conceive during the first year of condom use (Trussell 1998). This is considerably better than the 85% of conceptions arising from unprotected intercourse, but ranges far behind the 3% probably achieved by female oral contraceptive methods. Contraceptive protection is lower for polyurethane condoms than for latex condoms. In addition, effectiveness drops if condoms are used for a longer time span. This depends not only on technical defects of the product but also on the fact that condoms must be used in direct connection with the sexual act and require constant motivation and attention. For this reason, many couples in stable relationships consider condoms only as a temporary contraceptive method.

Since the beginning of the AIDS epidemic and the call for "safe sex," the condom has greatly gained in popularity to avoid the risk of infection. Its effectiveness in avoiding HIV transmission is, however, markedly lower than in preventing pregnancies. This is not surprising in view of the technical failure rate and in view of the fact that the danger of infection exists not only at the time of ovulation but during every sexual act. Meta-analyses conclude that the failure rate in the prevention of HIV infections is about 31% (to 50%). Thus, the protective effect of the condom against this often lethal disease must be considered inadequate (Krishnaratne et al. 2016).

Key Points

- One of the tasks of andrology is to provide methods for contraception on the male side.
- Such methods are important both for maintaining a stable population in industrialized countries and for containing population growth in developing countries.
- There is a close connection between the ecological problems of climate change and overpopulation which must also be recognized by climate protectors in order to include contraception in their program.
- Worldwide, 28% of couples of reproductive age who use contraception already practice male methods (condoms, vasectomy, coitus interruptus, and periodic abstinence) and the willingness to use a reversible pharmacological method would be high if it were introduced to the market.

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Vasectomy

46

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Abstract

Vasectomy is a safe, standardized, and easy-to-perform method of male contraception. It bears only minimal risk of acute complications and no severe long-term complications. Moreover, vasectomy can be reversed through microsurgical refertilization procedures with a high rate of success and comparatively marginal expenses.

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46.1 History of Vasectomy

It is impossible to date the earliest vasectomy. At the end of the nineteenth century, such operations were not performed for sterilization, but for other medical indications. At the time of the earliest vasectomies, it was believed that severing the vas deferens would improve prostate disease, heal impotence, or extend life expectancy. Vasectomy was advocated as a **"fountain of youth"** (Isnardi 1896; Wolfers and Wolfers 1974).

Eugenic aspects also played an important role among the indications for sterilization, as did elements of **social control** as advocated by Ochsner (1899) and Sharp (1902). In the 1920s and 1930s, a series of nations passed laws justifying

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sterilization for eugenic reasons. Along with the other cruelties practiced by the Third Reich, compulsory sterilization for eugenic purposes resulted in the abrogation of the legal basis for eugenic sterilization in most states. Some states, however, maintained these laws and later some tried to apply them. Drake et al. (1999) describe the varied history of vasectomy and its changing indications over the course of time.

Vasectomy as a means of **contraception** became popular in the 1960s, first in the USA, and then in Europe and Third World nations. Countries with particularly rapid population growth such as India and Thailand (Nirapathpongporn et al. 1990) established vasectomy camps. In the USA, sterilization became the form of fertility control most frequently chosen by married couples over 30 years of age (Smith et al. 1985). According to a survey, in Germany a frequency of about 50,000 vasectomies per year can be extrapolated (Deindl 1990). In 2018, a representative questionnaire showed that 2% of the German male population utilizes vasectomy as a method of contraception (Bundeszentrale für gesundheitliche Aufklärung 2018).

46.2 Social and Demographic Relevance

Contraceptive vasectomy is not popular in all countries, and in some it plays no role at all. In India and China, and, to a lesser extent, in Korea, Sri Lanka, and Bangladesh, vasectomy is widely performed (Ross and Huber 1983). Generally, sterilization in the male always **competes directly with corresponding measures in the female**, i.e., tubal ligation, which ironically has become increasingly popular because of improved techniques, although vasectomy was always technically simple (Ross and Huber 1983). In any event, the introduction of **minimally invasive "no scalpel" techniques** serves to make vasectomy much more acceptable (Xiaozhang and Shungiang 1993; Reynolds 1994; Ozvaris et al. 1998). These are based on percutaneous electrocoagulation or chemical denaturing of the vas, or specialized puncture instruments, which are used to minimize surgical trauma.

Particular ethical considerations concerning male sterilization apply in developing countries (Rizvi et al. 1995) and explain the former preference for female over male sterilization (3:1) with the rationale that vasectomy is hardly reversible and thus unpopular. New and advanced technologies such as sperm cryopreservation are only available in wealthy countries.

67–88% of the American population use contraceptive methods for avoidance of pregnancy, as reported by the "Behavioral Risk Factor Surveillance System." According to this survey, women favor oral contraceptives, while men primarily rely on the surgical approach, i.e., vasectomy (Bensyl et al. 2002).

Another study found that about 25% of eligible couples in the USA rely on sterilization for birth control, with distinct differences between black and white populations: Among Caucasians, vasectomy and tubal ligation are performed about equally, while among Blacks tubal ligation is clearly preferred (Forste et al. 1995). In the USA, vasectomized men generally have above-average education, their family planning is complete, and they learned about vasectomy methods in newspapers or magazines (Kohli 1973). Thirty years later, no significant changes can be ascertained: vasectomized men in the USA are typically well-educated and married Caucasians with good medical insurance (Barone et al. 2004).

In England, only minimal differences between social classes were found with respect to the acceptance of vasectomy (Wright et al. 1977). The general acceptance of vasectomy as compared to tubal ligation has increased over the years in England (Rowlands and Hannaford 2003), as well as in Canada. The use of oral contraceptives and tubal ligation in the Canadian population is decreasing, while requests for vasectomies are steadily rising (Martin and Wu 2000). With advanced and ongoing aging of the population, vasectomy is no longer only an issue for young men. Older men also engage in sexual activity and favor vasectomy for contraception. In an Australian study of middle-aged males, 25.1% had been vasectomized, 5.6% of the vasectomized men did not have children, and 37% of men above 70 years of age were still sexually active (Holden et al. 2005).

It has been demonstrated that men who are prepared to undergo vasectomy usually live in **relationships with a strong emphasis on partnership**. Under certain conditions, positive and beneficial effects for a relationship can even be expected (Goebel et al. 1987). This influences patient selection made by counseling and operating physicians, resulting in the fact that in Germany predominantly men living in stable relationships are vasectomized. The number of vasectomized men varies in German-speaking countries: in Germany, 422 men per million inhabitants are vasectomized annually; in Austria, the number is only 81 (Engelmann et al. 1990).

46.3 Indications for Vasectomy

In contrast to formerly held but still widely accepted opinions, we believe that the indication for contraceptive vasectomy is easily established.

> Every man of legal age and able to give consent may decide in favor of vas ligation or occlusion for the purpose of sterilization.

No special medical or social indication is necessary, but many physicians performing vasectomy require certain preconditions, mostly to provide backup support for the physician. In such cases, for example, a certain number of children are required, a stable relationship with written consent given by the partner is desired; finally, the chances for refertilization are deliberately and falsely minimized. These measures represent a bias concerning the affected persons; only those highly determined to undergo vasectomy will be accepted. This procedure may be understandable, but it is not in the best interest of the responsible citizen and patient. He has the right to objective counseling about the chances and risks concerning the intervention he wishes. Well-meaning, but false-negative counseling of the patient must not deprive him of the possibility of deciding in favor of vasectomy. This does not limit the surgeon's right to decline to perform a vasectomy in individual cases.

46.4 Informed Consent

As in all other surgical procedures, the patient must be informed and his consent obtained at least 1 day prior to the operation itself. We observe **the procedures generally accepted for surgery** without any special modifications. Neither consent of the female partner nor even her existence is required. Information is provided about the procedure, which is almost always carried out on an outpatient basis. The **chances for subsequent refertilization** are explained according to statistics in the literature with patency rates of 70–90%. **Acute complications** consisting of bleeding and infections, usually negligible, rarely occur, but are mentioned. **Possible connections between vasectomy and other diseases**, e.g., arteriosclerosis or prostate cancer, discussed in the past and, again recently, are mentioned to the patient with reference to the relevant investigations. In cases of doubt, the physician's explanation and personal interpretation may be helpful. The need for **postoperative semen analysis** is stressed. As with most surgical procedures, written consent signed by the patient is necessary and should be documented.

Special considerations are involved when **vasectomies are performed on patients who are not able to consent to the procedure themselves**. Due to Germany's recent past, this is a particularly sensitive subject there. Various lobbies and the German Society of Medical Law (DGMR) have discussed this subject in publications, lectures, and workshops. In Germany, vasectomy policy for patients who are not able to consent to the procedure is regulated by law. Consent demands the authorization of a guardianship court. **Vasectomy of minors is forbidden** in Germany. Neither the underage patient nor his parents can consent to sterilization (Hiersche and Hiersche 1995).

46.5 Surgical Vasectomy Techniques

Vasectomy is a good example of how simple matters can be made complicated. What is to be achieved? The goal of the operation is to occlude the ducts reliably to prevent passage by spermatozoa, permanently or as long as the patient desires it. The following procedure has been established:

In the ambulatory patient, both spermatic cords are infiltrated with an anesthetic. Only in rare and exceptional cases is the intervention performed under general or regional anesthesia. The cords are digitally localized through the scrotal skin and, if necessary, local anesthesia is supplemented by deeper infiltration. After ascertaining the effectiveness of the anesthesia, the ejaculatory duct is clamped through the skin, using a small clamp, and the scrotal skin is incised over a distance of 0.5–1 cm. The vas is separated from the sheath and divided over two mosquito clamps. The vas is then dissected, and a piece of approximately 1 cm is excised and fixed in formalin. The vasal ends are ligated and electrocoagulated. The entrance wound is sutured with a single suture (Figs. 46.1 and 46.2). The identical procedure is performed on the contralateral side. The wounds are covered with two small dressings. After 1 h, the area is checked and the patient can be discharged to home care.

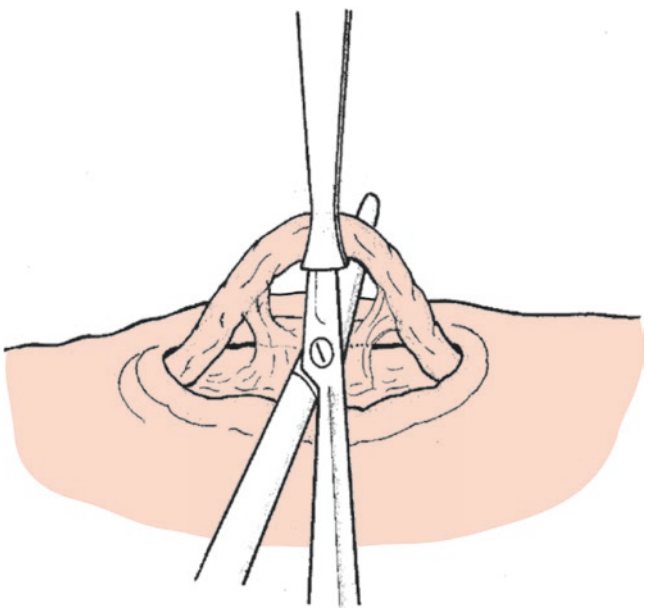


Fig. 46.1 Following a longitudinal incision of the scrotal skin along the vas deferens the vas is lifted by a small clamp

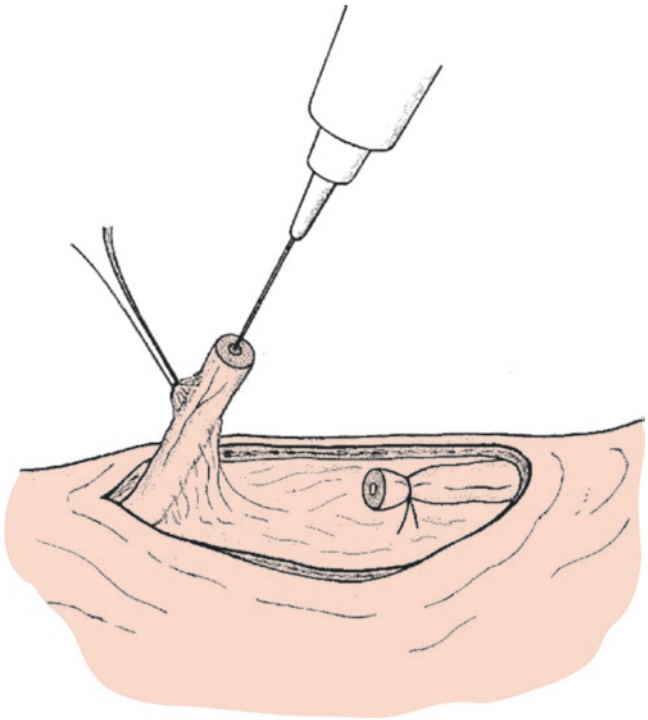


Fig. 46.2 Distal ligation of the vas deferens and cauterization of the proximal lumen

The following is important: The patient must be **well-informed** and not be afraid of the procedure. In dubious cases, **premedication** is highly effective. In sensitive patients, placement of a venous catheter is recommended. It should not be forgotten that even in such minor surgery emergency measures may become necessary. **Acute complications** are rare, but when they do occur, they are extremely irritating. Even minor bleeding can spread throughout the soft tissue, resulting in **scrotal hematoma** up to the size of a fist.

46.6 Technical Modifications

The technique described above, practiced and well-documented for over 30 years, represents a combination of several important features. The use of a special puncture device has obtained general acceptance as a "no scalpel" technique; the same is true for electrocoagulation of the cut edges of the vas deferens or the interposition of connective tissue (fascial interposition: FI). Labrecque et al. (2004) concluded in a systematic review that the "no scalpel" vasectomy is the safest surgical approach to the vas deferens and that a combination of FI and electrocoagulation yields the most effective occlusive results. Comparable data were reported by Cook et al. (2007a, 2007b) in two Cochrane Reviews: The "no scalpel" technique is associated with a lower rate of hematoma and infection and less postoperative pain compared to the conventional technique. Moreover, fascial interposition reduces the occurrence of vasectomy failures (Cook et al. 2014).

46.7 Effectiveness and Cost Efficiency

When correctly performed, vasectomy is among the **safest contraceptive methods**. With a pearl index of 0.1, vasectomy belongs to the safest contraceptive methods (Trussell et al. 2018). This makes vasectomy even more effective than tubal ligation (PI = 0.2–0.3). Failure is due to **recanalization** of the divided duct in up to 1.5% of cases (even possible in the form of late recanalization after two negative semen samples in 0.04–0.2% of patients), or to the extremely rare occurrence of a **double vas** or **incomplete division** of the duct. Failure rates increase with lack of experience, inferior operating techniques, and difficult anatomical circumstances. In Germany, as part of the health insurance modernization law, methods of sterilization such as vasectomy are not reimbursable. Therefore, the cost of such procedures must be borne by the patients themselves. When performed under local anesthesia, the costs usually amount to around 300–500€. (Deutscher Bundestag 2017).

Vasectomy is the most cost-efficient method of contraception (Nakhaee et al. 2002); thus, its implementation could achieve considerable cost savings in public health (Sonnenberg et al. 2004).

46.8 Complications

Acute postoperative complications must be distinguished from long-term complications. **Hematoma** of various degrees, **epididymitis,** or **wound infections** up to formation of an abscess occurs with a frequency of up to 1–6% (Alderman 1991; Schwingl and Guess 2000; Adams and Wald 2009). While this rate seems high, it should be remembered that vasectomy is a minor operation performed by many surgeons with only limited experience with this procedure. Its supposed harmlessness tempts some surgeons to perform it on an outpatient basis, quickly and inattentively. The price is necessarily an increased rate of acute complications. These lead to short-term **absence from work** of an average of 2.3 days (Randall and Marcuson 1985). **Serious complications virtually never occur**; deaths during or after vasectomy have not been reported, whereas in the USA 14 deaths/year are attributed to female sterilizations. Concerning **costs**, vasectomy is more eco-

nomical than tubal ligation, with the short-term costs of tubal ligation about 3–4 times higher (Smith et al. 1985; Hendrix et al. 1999).

Long-term surgical complications include **recanalization** on the one hand and the development of **sperm granuloma** on the other hand. Recanalization rates are reported to be about 0–3%. The actual rate depends on the surgical technique used. Resection of larger sections of the vas, burying the ends of the ducts in different planes of tissue, and the use of fibrin glue or fulguration of the lumen are all useful methods for minimizing the rate of recanalization. The use of minimally invasive techniques can, on the one hand, increase the chance of later refertilization, but, on the other hand, it can also greatly increase the chance of recanalization by up to 50% (Goldstein 1983). The occurrence of sperm granuloma is likewise correlated with surgical procedures used and varies considerably. The data in the literature vary between 3% and 75%. In terms of refertilization chances, a sperm granuloma can be evaluated positively as it reduces pressure in the epididymis and epididymal tubule and thus reduces the risk of a "blow-out." This is an intraoperative tubal rupture, requiring epididymovasostomy, which may be made impossible by the anatomical localization of the rupture.

It is important to explain **acute complications** to the patient and particularly to make him aware of the **possibility of failure**; he must be informed that, in individual cases, recanalization may occur even when postoperative semen analysis shows azoospermia on several occasions. If recanalization is to be completely excluded, the duct should be resected over several centimeters. Furthermore, **polyorchidism** and a **duplex vas** must be ruled out. For liability reasons, it is advisable to perform histological examinations of the resected parts of the duct to demonstrate the resection in complete tubal diameter.

46.9 Vasectomy and Long-Term Morbidity

Complaints of congestion, a feeling of obstruction or heaviness in the testis and epididymis, and **pain** of different degrees are occasionally described by the patient, but are usually temporary. Chronic epididymal pain is in all events rarely seen; its appearance is sparsely documented in the literature. **Sperm antibodies** in the serum can be found in up to 70% of those sterilized (Heidenreich et al. 1994; Adams and Wald 2009). Their presence is often associated with sperm granulomas. If refertilization is desired, we are not influenced by the presence of sperm antibodies nor by the titer, but perform refertilization in any case.

In the past, **various diseases have been causally attributed to vasectomy**. Based on monkey studies, in 1978 it was concluded that vasectomy could enhance arteriosclerosis. Several large-scale investigations involving over 10,000 men determined that vasectomized men bore no higher risk for **arteriosclerosis, diabetes mellitus, or immunological diseases** (Nieschlag 1987; Giovanucci et al. 1992). The risk of cardiovascular diseases after vasectomy is not increased in the long term, as Manson et al. reported in the "Physicians' Health Study" in 1999. A few years ago, a **possibly higher incidence of prostate carcinoma** in vasectomized males was suggested by retrospective investigations (Giovanucci et al. 1993). Further research on this topic has since followed. A large observational study by Byrne et al. found no correlation between vasectomy and prostate cancer (Byrne et al. 2017). Davenport et al. found a marginally higher rate of prostate cancer in vasectomized patients (hazard ratio 1.05; 95% CI, 1.01–1.11) (Davenport et al. 2019). However, a causal explanation could not be identified. An obvious explanation would be the closer urologic follow-up and treatment of vasectomy patients; hence, these patients have a higher rate of prostate cancer screening and PSA determination.

46.10 Psychosexual Effects

The history of vasectomy has already shown that psychosexual effects associated with vasectomy need not always be negative. On the contrary, the fact that the problem of contraception has been responsibly resolved usually has a positive effect on a relationship (Weidner and Weissbach 1992). The fear of unwanted pregnancy may disappear and sexual enjoyment may increase (Vaughn 1979; Miltsch and Senn 1999; Bertero et al. 2005). Conversely, those men who are pressured into being vasectomized (e.g., vasectomy camps!), whose religion discourages or even forbids such procedures, and whose partners are opposed to it or who are not sufficiently informed may experience vasectomy as associated with increased conflicts and complications. Thus, the initial situation of the person, the expectations he places in vasectomy, and how well informed he is are key factors for success. Correct information, pre-surgical counseling, and postoperative care and attention are important. These measures can prevent fear concerning a loss of male identity and masculinity.

> From the present state of knowledge, vasectomy does not promote a higher risk for the development of prostate carcinoma.

The long-term benefits and effectiveness of vasectomy continue to be discussed controversially (Jequier and Pryor

1998), even to the extent of spurious arguments that cryopreservation of sperm may replace refertilization or—even worse—replace information and counseling about vasectomy.

46.11 Refertilization

46.11.1 History of Refertilization Surgery

Isolated cases of vasovasostomy following accidental dissection of the vas deferens, mostly during hernia surgery, have been reported since the beginning of this century. After World War II, true refertilization surgery, namely vasovasostomy and epididymovasostomy, following deliberate ligation of the ducts became more frequent, and in 1948, O'Connor presented the first national survey on vasovasostomy. It is astonishing that at that early stage nationwide surveys were implemented to establish the indications, frequency, and success of refertilization surgery. In the USA, such surveys were repeated in 1973 (Derrick et al. 1973) and in 1979 (Wicklund and Alexander 1979).

In German-speaking countries, the following situation prevails (Engelmann et al. 1990): Along with the increase in vasectomies, there is a rising number of patients who wish to reverse the procedure. Of the many reasons, three concerns emerge. First, the higher divorce rate plays an essential role; the main reason for refertilization is **divorce followed by remarriage or a new partnership**. The second most frequent request comes from couples whose family planning had been completed by vasectomy, but who now have changed their minds or who have suffered the **unexpected death of one of their children**. The third reason is **improved economic standing** and the resulting possibility of being able to afford offspring.

Refertilization vasovasostomy has been performed for more than 40 years, but the technique has undergone major changes during that time. Initially, the macroscopic one-layer anastomosis technique, perhaps even with splinting of the small vas lumen, was applied. Increasing use of the operating microscope brought with it excellent postoperative results reported mainly by Silber (1977). This prompted the development of microscopic two-layer anastomosis without splints. Improved surgical results affected the mechanical patency of anastomosis, which must be considered the true control parameter of surgical quality. However, there is a discrepancy between surgical patency of the restored vas deferens and pregnancy rates, which are distinctly lower. Today, refertilization surgery is largely performed with optical devices, i.e., with loupe magnification or surgical microscopes. Patency rates of 90% are common and are achieved by many.

46.11.2 Current Demand and Frequency of Refertilization

The potential demand for refertilization surgery can be roughly extrapolated from the number of sterilization vasectomies performed, from the divorce rate and from other (secondary) parameters. It has been estimated to be 250,000–300,000 in the USA per year (Cos et al. 1983). In German-speaking countries, a survey taken in 1990 revealed that the vasectomy reversal rate was 3.5% and requests for reversal were twice as high (Engelmann et al. 1990). In Sweden, the refertilization rate was 0.5–5.4/100,000 residents (Ehn and Liljestrand 1997). In the USA, the refertilization rate has shown to be around 2% (Sharma et al. 2013).

46.11.3 Vasovasostomy

46.11.3.1 Indications, Counseling, Consent, and Costs

The typical patient desiring refertilization contacts his physician, usually his urologist, often the same one who performed the vasectomy. The indication is the patient's request, which in turn arises from the arguments described above. As for other surgery, refertilization requires truthful counseling. Invasiveness is comparable to that of vasectomy, with the exception that vasovasostomy requires more difficult surgical techniques. For this reason, general anesthesia is recommended and **hospitalization of 1–2 days** should be planned. As a general rule, we estimate **patency rates of 80%**; **pregnancy rates** are lower, at **60%**. If vasectomy was performed more **than 5 years prior**, we reduce these estimates by **a further 20%**. The surgeon's experience with this operation (both positive and negative) should be considered. The possibility of a "blow-out" in the epididymis or even in the rete testis should be mentioned. This would then necessitate **epididymovasostomy** or can make surgery impossible for technical reasons.

In most countries, including Germany, expenses for surgical refertilization are **not covered by insurers** as a general rule. In some special cases, such as the death of a child with concomitant parental distress, or persistent pain due to sperm granuloma, exemptions are granted. In these cases, cost transfer agreements should be arranged preoperatively. According to Heidenreich et al. (2000) and Schroeder-Printzen et al. (2003), the expenses per childbirth after vasovasostomy account for only a fifth of those after MEAS/TESE and ICSI.

46.11.3.2 Vasovasostomy Technique

The patient is placed in supine position; usually, a scrotal incision bilaterally or in the median of the raphe scroti is

chosen. Less frequently an inguinal or infrapubic approach is used. Usually, the surgeon operates while seated, avoiding interference with the operating table pillar; the operating microscope should be placed before the patient is positioned. Operating time of 2–3 h should be anticipated; for this reason, an indwelling catheter is sometimes inserted. Differing techniques have been described: initially macroscopic techniques with or without splinting of the lumen, followed by **microsurgical techniques** with loupe magnification or the operating microscope.

Macrosurgical techniques. Initially, surgical techniques without magnification and relatively coarse suturing material of 4-0 or 5-0 were used. Results were relatively poor because of complications such as sperm leakage and bad anatomical adaptation of the lumen. The advantages lay in simple and rapid performance of surgery and for these reasons some surgeons prefer macroscopic techniques even today. As late as 1999, Feber and Ruiz saw advantages in the shorter operating time, reduced costs, and the need for fewer surgical skills (despite a patency rate of 87% and pregnancy rate of 50%).

Silber (1977) made a great contribution when he described the anatomical peculiarities of the severed vas, with sizes differing between the dilated side proximal to the testis and the non-dilated side, and demonstrated that good operating results were best achieved with anatomically correct adaptation of the lumina.

Microsurgical techniques. In our view, using **solely macrosurgical techniques for refertilization is obsolete**. While they may be applied for vas anastomosis, although inferior in patency, patent epididymovasostomy is achieved purely by coincidence or not at all. Whether **loupes** with a two to eightfold magnification or an **operating microscope** with the advantage of variable magnification and increased field of vision is used depends on the personal preference of the surgeon, even though the best patency results are achieved with the operating microscope.

In the event of scrotal vasectomy, we dissect the ends of the vas deferens via a lateral scrotal incision. The scarred ends of the vas are excised. Patency of the proximal vas deferens is determined after insertion of a small flexible Teflon cannula by careful injection of saline solution. The fluid emerging from the distal vas end is assessed in the operating room under the operating microscope. If necessary, the epididymis is digitally massaged to obtain fluid. If no fluid is present, a "blow-out" must be assumed and the epididymis is examined. In such case, we perform epididymovasostomy. Typically, whitish or yellowish fluid emerges containing viable or dead sperm. In this event, surgery is continued as vasovasostomy.

The best anatomical adaptation of the lumen is achieved using a two-layer technique by which mucosal approximation is accomplished with six interrupted sutures using 9-0 or 10-0 suturing material. Special vasovasostomy needles that are doubly armed and whose degree of curvature is adapted to the size of the vas have proven useful. The vas ends can be aligned by using an approximator, facilitating placement of sutures. First, the inner mucosal layer is sutured and should ensure a leak-proof connection. Precise positioning of the sutures will ensure optimal patency rates; Goldstein et al. (1998) were able to achieve rates of 99.5% using their "microdot marking technique" and eight mucosa stitches. The mechanical strength of the anastomosis is achieved through the second muscular layer, also using 9-0 or 10-0 sutures, however, with a single-sided and spatulated cutting needle. The short scrotal wound is closed in two layers. A well-fitting dressing supporting the scrotum is preferred by most patients and is left in place for a few days (Figs. 46.3, 46.4, 46.5, 46.6, 46.7, 46.8, 46.9, and 46.10).

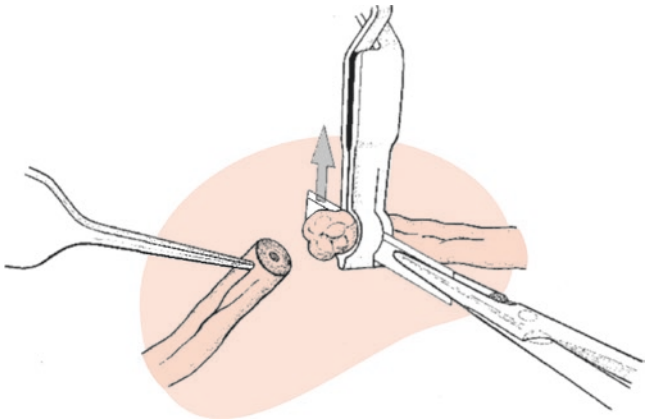


Fig. 46.3 Clean cuts of the two ends of the vas and careful bipolar hemostasis

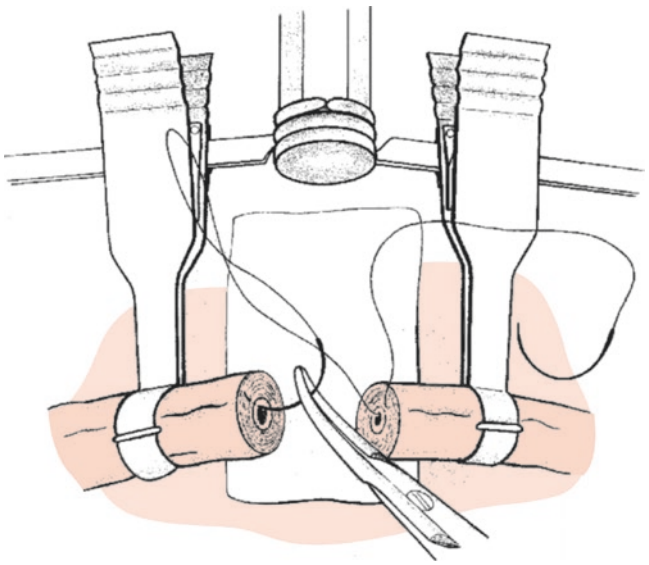


Fig. 46.4 Tension-free adaptation of the two ends of the vas by use of an approximator. Anastomosis of the mucosa is performed with nylon 10-0 double sutures

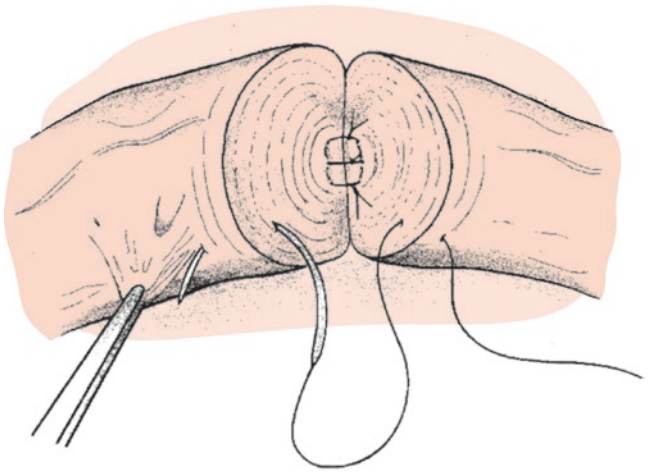


Fig. 46.5 Adaptation of the muscle layer of the vas by nylon 9-0 sutures

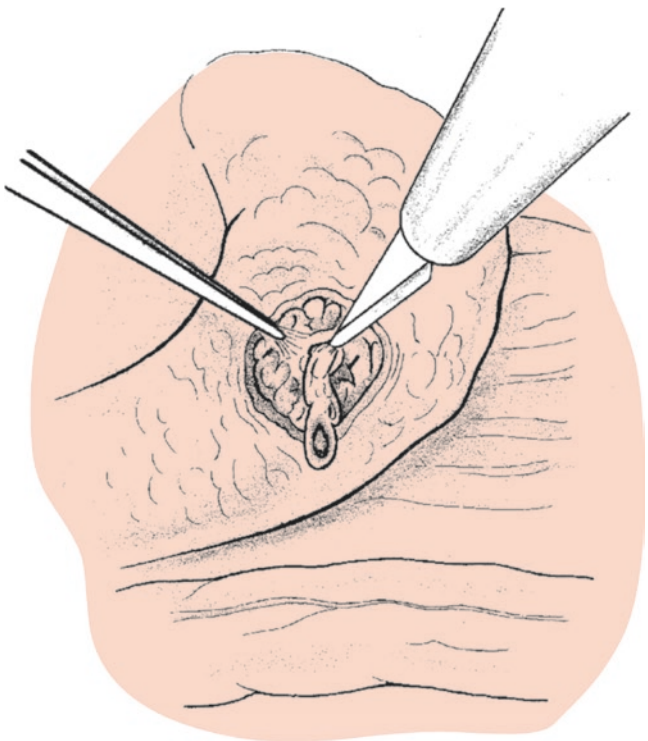


Fig. 46.6 A tubule is prepared and incised longitudinally

Robot-Assisted Technique. With the progression of operative procedures in the direction of robot-assisted operations, the vasovasostomy has also found its place in the array of robotic interventions. The postulated benefits include better overview, redundancy of tremor, and lower rates of fatigue of the surgeon. Marshall et al. described similar patency rates at 88% compared to traditional microscopic methods, however higher costs and longer operation times (Marshall et al. 2017; Gözen et al. 2020).

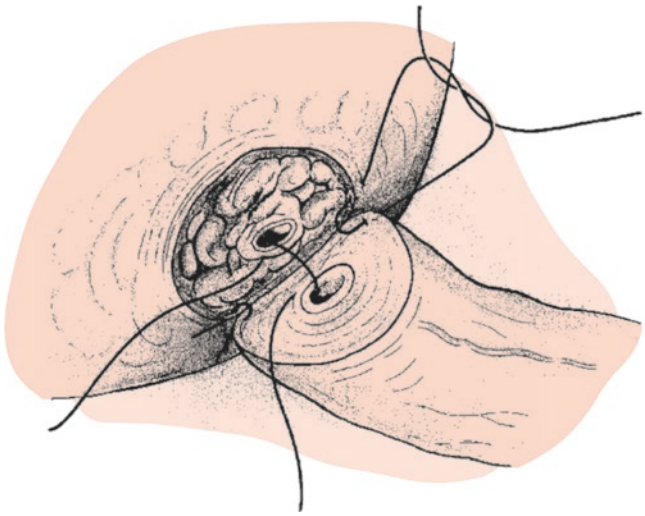


Fig. 46.7 Adaptation of the muscle layer of the vas and the tunica of the epididymis at the posterior circumference by nylon 9-0 single sutures

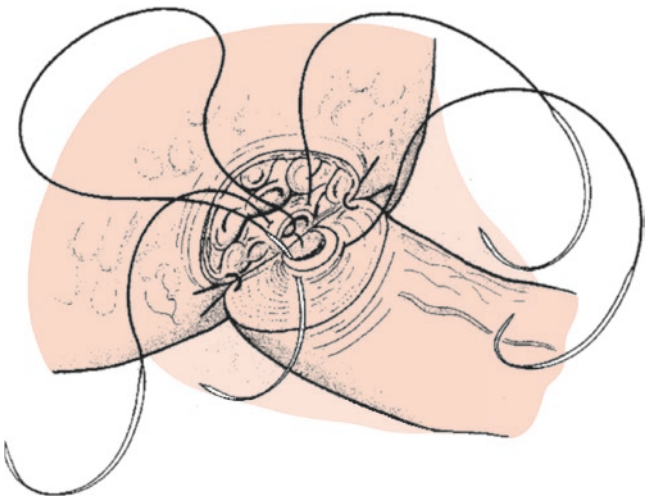


Fig. 46.8 Anastomosis between the tubule and the mucosa of the vas by nylon 10-0 doubly armed sutures

46.11.3.3 Results of Vasovasostomy

Results of vasovasostomy are quite variable, depending on whether one reads reports by individual authors or results from surveys. Similar variations are seen depending on whether patency rates are compared, describing surgical success, or pregnancy rates, which is the factor that most interests patients. Individual authors report patency rates of 100% and pregnancy rates slightly below. Nationwide surveys report average results more accurately: In 1948, O'Connor had a cumulative success rate of 38–40%; in 1973, Derrick calculated a patency rate of 38% and pregnancy rate of 11–26%. In 1990, Deindl found a cumulative patency rate of 73% in German-speaking Europe and a pregnancy rate of

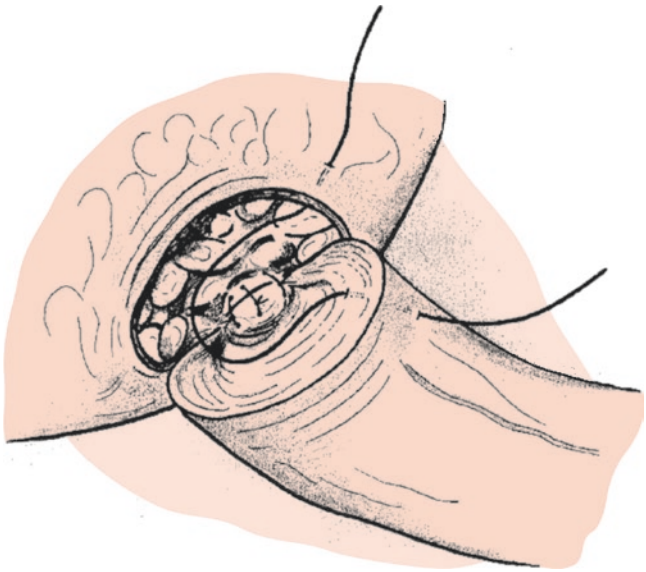


Fig. 46.9 Adaptation of the muscle layer of the vas and the tunica of the epididymis. Interrupted sutures at the anterior circumference with nylon 9-0 sutures

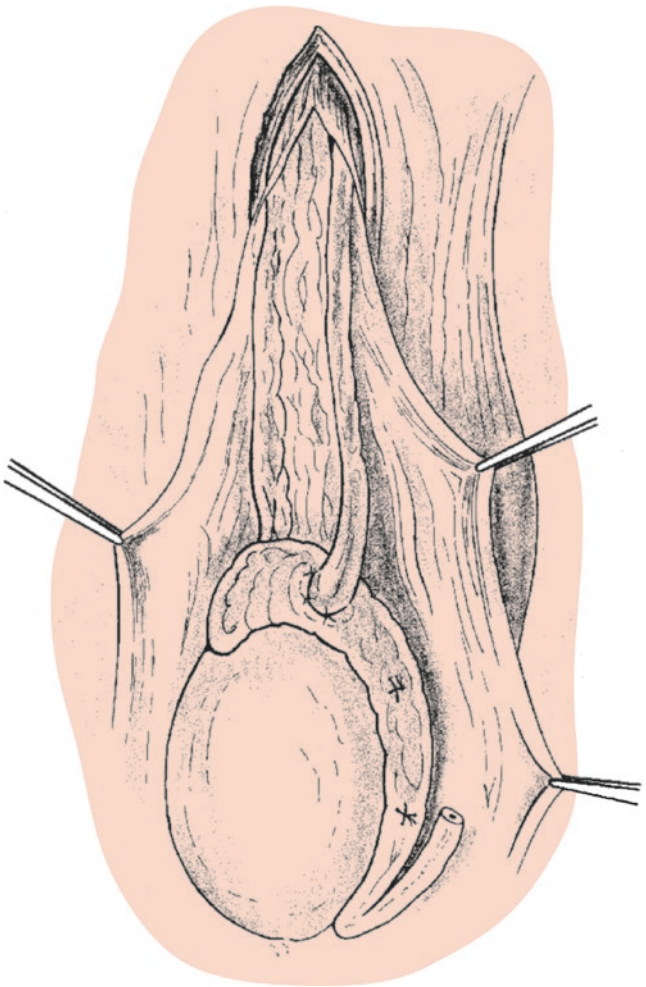


Fig. 46.10 Ligation of the epididymal tunic

47%. Centers with a high operating frequency, with skillful techniques and thin sutures, achieve better results. The time period between sterilization vasectomy and refertilization plays a role; even if these limits are arbitrary, the best results can be expected within the first 2 years, while 10 years after vasectomy the chances for refertilization are markedly reduced (Belker et al. 1991).

46.11.3.4 Complications Following Vasovasostomy

The rate of **acute complications** is comparable with that for vasectomy or scrotal exploration, but for different reasons. Specific **long-term complications** result from re-obstruction after initial patency. A 3% chance of such obstruction because of scar tissue formation must be expected. If patients remain azoospermic following refertilization, it is probably due to surgical failure or a more distal "blow-out" that had not been verified at the time of surgery. In these cases, re-operation is indicated. **Oligozoospermia** can be the result of partial obstruction—in such cases re-operation may be successful—or due to limited testicular production. If vasectomy dates back more than 5 years, we inform all patients about the option of concomitant **cryo-TESE.**

46.11.4 Epididymovasostomy

If the epididymal tubule is damaged by a "blow-out," epididymovasostomy must be carried out. For technical reasons, this procedure is only successful at the corpus or the cauda epididymidis. In the caput region, the diameter of the tubule is too small to accomplish fully patent anastomosis. **Epididymovasostomy** urgently requires microsurgical techniques, preferably using an operating microscope. We prefer to employ the end-to-side technique; others prefer side-to-side. After excising an oval window of the epididymal tunica, a single tubule containing sperm is chosen and is opened longitudinally. By placing four interrupted 11-0 sutures, anastomosis of the tubule with the mucosa of the vas deferens is completed. The anastomosis is then finished by means of further interrupted sutures between the epididymal tunica and the muscularis of the vas (Figs. 46.6, 46.7, 46.8, 46.9, and 46.10).

End-to-end anastomosis is more difficult. The patency rates of microsurgical tubular vasectomy are between 39 and 100%, including single-case reports. The average patency rate is 45% and pregnancy rates are 18% (Deindl 1990).

46.11.5 Future Developments in Surgical Refertilization

Refertilization surgery will become more common in future along with contraceptive vasectomy. The good results

achieved have reached a stable level and have not seen any further improvements during the past 10 years. Microsurgical training must continue to be emphasized for the surgeons involved.

The **intraoperative harvesting of sperm** which—cryopreserved—can be made available for later ICSI in case of a non-patent anastomosis (especially in the case of epididymovasostomy) should be discussed with patients even during the initial consultation (Djerassi and Leibo 1994). For those who wish to have children after vasectomy, surgical refertilization remains the method of choice from the standpoint of costs and chances for success.

We do not believe in abstaining from refertilization surgery and instead first harvesting testicular sperm by TESE or—even worse—epididymally for use with ICSI. This method can by no means measure up to "conventional" refertilization surgery—either in terms of pregnancies achieved, the cost and effort involved, or with regard to complication rates.

46.12 Future Development of Vasectomy

Both from a demographic and political point of view, as well as for the individual selecting a contraceptive method, vasectomy represents a highly safe option that can be performed rapidly at low cost, with few inconveniences for the patient. Without doubt, it is superior to the equivalent female operation. It is hoped that changes in the understanding of the male role and better knowledge will bring about wider acceptance of vasectomy, especially in view of the fact that it is no longer to be considered final as in a high percentage of patients refertilization can be achieved by vasectomy reversal.

Key Points

- Vasectomy.
- Sterilization.
- Contraception.
- Vasovasostomy.

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Approaches to Hormonal Male Contraception

47

Hermann M. Behre, Diana L. Blithe, and Eberhard Nieschlag

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Abstract

Currently, the most advanced approach to male contraception is a hormonal method. In principle, the suppression of spermatogenesis required for effective male contraception—while maintaining androgen-dependent functions—can be achieved with androgens alone, androgens in combination with GnRH antagonists, and androgens in combination with progestins. The closest to actual availability of such a method is the combined administration of testosterone and a progestin.

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47.1 Principle of Hormonal Male Contraception

Of the various experimental approaches and pharmacological trials aimed at male contraception, hormonal methods best meet the requirements mentioned in Chap. 46. **Hormonal male contraception** is based on the **feedback mechanism** that exists between the hypothalamus, pituitary gland, and testes. The goal is to suppress spermatogenesis and sperm concentrations in the ejaculate to azoospermia or at least sperm concentrations low enough (≤1 million sperm/mL ejaculate) to provide adequate contraceptive protection.

Spermatogenesis and secretion of testicular testosterone are so closely intertwined that all hormonal attempts to inhibit spermatogenesis without affecting testosterone production have failed to date (see Chap. 2 and Fig. 47.1). As studies in nonhuman primates have shown, inhibition of FSH alone, e.g., by antibodies, leads to a reduction in sperm concentration but not to azoospermia or complete infertility. Suppression of FSH and LH together, on the other hand, would result in azoospermia but at the same time induce the symptoms of hypogonadism or testosterone deficiency, affecting libido, erectile function, performance, male role behavior, and general metabolic processes such as erythropoiesis, protein, mineral, and bone balance. Therefore, suppression of the two gonadotropins LH and FSH must always be accompanied by exogenous **androgen substitution**.

The principle of hormonal male contraception is based on:

- Suppression of LH and FSH.
- Decrease in intratesticular testosterone and reversible stop of spermatogenesis.
- Substitution of peripheral testosterone to maintain androgenicity.

Initially, **testosterone** itself appears to be the ideal steroid to suppress gonadotropins and simultaneously maintain androgenicity. However, relevant studies have shown that the use of testosterone alone—unlike in Chinese populations studied (see below)—leads to azoospermia in only about 2/3 of Caucasian subjects, so that another gonadotropin-suppressing agent must be added to suppress spermatogenesis as completely as possible. GnRH analogues and a wide variety of steroid combinations and oral, transdermal, subcutaneous, and intramuscular forms of application have been tried, all with their specific advantages and disadvantages. This chapter provides a concise review of the various approaches to hormonal male contraception. A distinction must be made between efficacy and surrogate studies; in **surrogate studies**, ejaculate parameters—predominantly sperm concentrations—serve as endpoints, whereas in **efficacy studies**, actual contraceptive protection serves as the target parameter, making them very costly. Therefore, there are only seven completed efficacy studies (Table 47.1), but more than 60 surrogate studies.

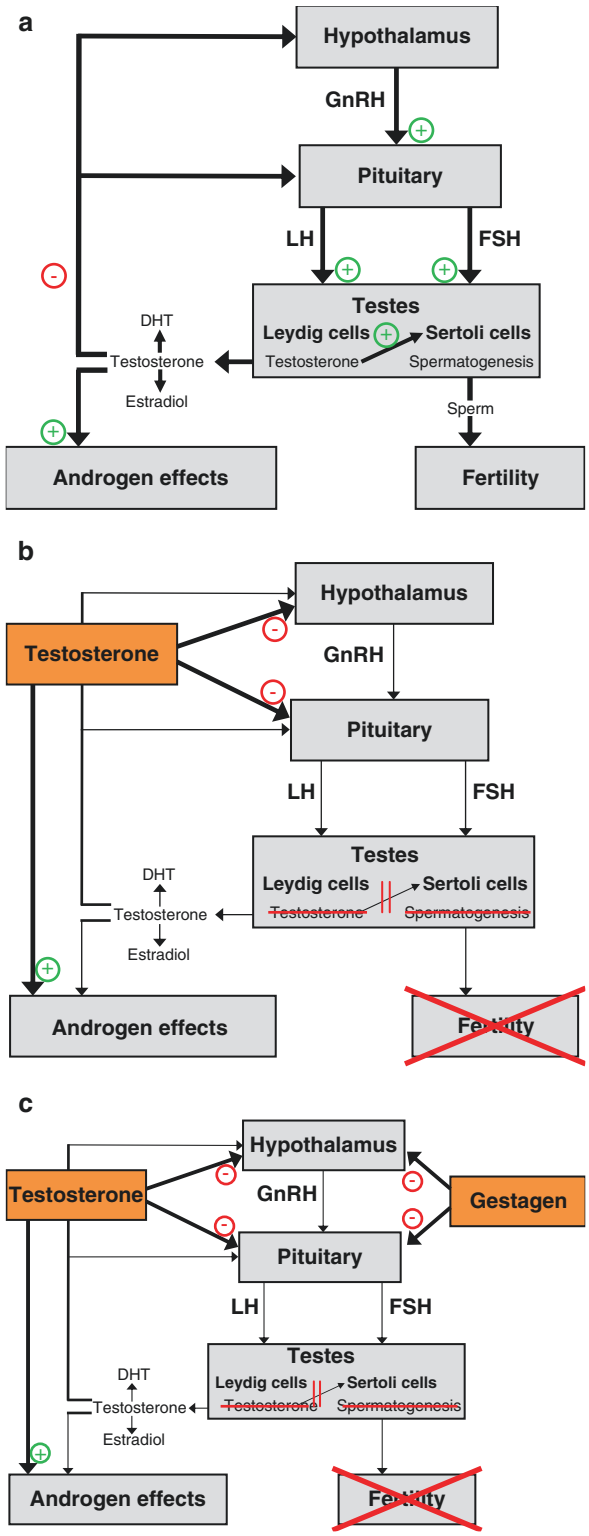


Fig. 47.1 (**a**) Schematic representation of the normal endocrine feedback mechanism of testicular function. (**b**) Principle of hormonal male contraception using testosterone. (**c**) Principle of hormonal male contraception when testosterone is used with a progestin

Table 47.1 Efficacy studies on hormonal male contraception

Sponsors/authors	Year of publication	Hormonal preparations	Couples in the efficacy phase
WHO	1990	TE	157
WHO	1996	TE	349
Turner et al.	2003	TI + DMPA	51
Gu et al.	2003	$TU_{(tso)}$	296
Gu et al.	2009	$TU_{(tso)}$	855
Soufir et al.	2011	T-gel + DMPA	25
CONRAD + WHO (Behre et al.)	2016	$TU_{(co)}$ + NETE	266

DMPA depot medroxyprogesterone acetate, *NETE* norethisterone enanthate, *TE* testosterone enanthate, *T-gel* testosterone gel, *TI* testosterone implant, $TU_{(tso)}$ testosterone undecanoate in tea seed oil, $TU_{(co)}$ testosterone undecanoate in castor oil

47.2 Androgens Alone

47.2.1 Testosterone Enanthate

Shortly after testosterone was synthesized and available for clinical use in the 1930s, its spermatogenesis-suppressing effect was already noted (Heckel 1939), but it was not until the 1970s that systematic research on this phenomenon for male contraception began. In these early studies, sperm concentration and total sperm count were used as surrogate parameters for efficacy, as has remained the case for most studies to date.

The first true **efficacy trial** of hormonal male contraception was based on testosterone and was conducted, sponsored by WHO, in 10 centers on four continents (WHO 1990) (Table 47.1). Healthy fertile men received weekly i.m. injections of testosterone enanthate. Within 6 months, 157 participants (70%) achieved azoospermia and then entered the efficacy phase, during which no other contraceptive was used by the couple concerned. Only one pregnancy was detected in this proof-of-concept study. Despite this apparently high efficacy, in principle this study cannot be used to determine the effectiveness of testosterone alone as a contraceptive agent because only men who responded with azoospermia were included in the efficacy phase of the study and the others were excluded.

To address the question of whether not only men with azoospermia but also those with marked oligozoospermia would be protected from conception, a **second efficacy study** was conducted with 357 couples worldwide (WHO 1996). In this large study, azoospermia again proved to be the most reliable prerequisite for contraceptive protection. When sperm concentrations did not fall below 3 million/mL of ejaculate, the resulting pregnancy rates were higher than 12% with condom use. However, when sperm concentrations fell below 3 million/mL ejaculate, which was the case in 97% of participants within 6 months of suppression phase, protection was not quite as high as with azoospermia but was significantly better than with condom use (Fig. 47.2).

Although these **WHO studies** represented a breakthrough in the development of hormonal male contraception and confirmed the principle of the mechanism of action, they did not provide a practical method. This is because a method involving weekly intramuscular injections may not be acceptable or suitable for widespread use. Moreover, several months and in some cases even a year of pretreatment were required before sperm production could be suppressed into the range consistent with contraceptive protection. For these reasons, current research is focused on the development of long-acting testosterone preparations and on methods to bring about suppression of sperm more rapidly.

47.2.2 Testosterone Buciclate

Because long-acting testosterone preparations appeared more promising in terms of practicality and acceptability, WHO and NIH (Bethesda, MD/USA) initiated a steroid synthesis program for the identification of such preparations (Waites 2003), which resulted in the long-acting testosterone ester **testosterone buciclate**. When tested in hypogonadal men, this ester had a half-life of 29.5 days, many times longer than the half-life of testosterone enanthate of 4.5 days (Behre et al. 1995, 2004). After a single injection of 1200 mg testosterone buciclate, suppression of spermatogenesis occurred similar to that seen after weekly injections of 200 mg testosterone enanthate, and azoospermia was achieved in 3/8 volunteer subjects (Behre et al. 1995). Despite the promising pharmacokinetic profile, no partner in the pharmaceutical industry could be found to further develop this compound.

47.2.3 Testosterone Undecanoate

Originally, **testosterone undecanoate** was tested in **capsules for oral administration** in Caucasian subjects after it became clinically available for the treatment of hypogonadism (Nieschlag et al. 1978). Participants received a daily dose of 6 × 40 mg distributed throughout the day for 12 weeks, but only one of seven men showed a reduction in sperm production consistent with contraceptive protection. This low efficiency is probably related to the short half-life of the orally administered testosterone undecanoate. Even when this testosterone preparation is administered 4 times a day, the resulting testosterone peaks in serum are insufficient to consistently suppress gonadotropins and thus achieve azoospermia.

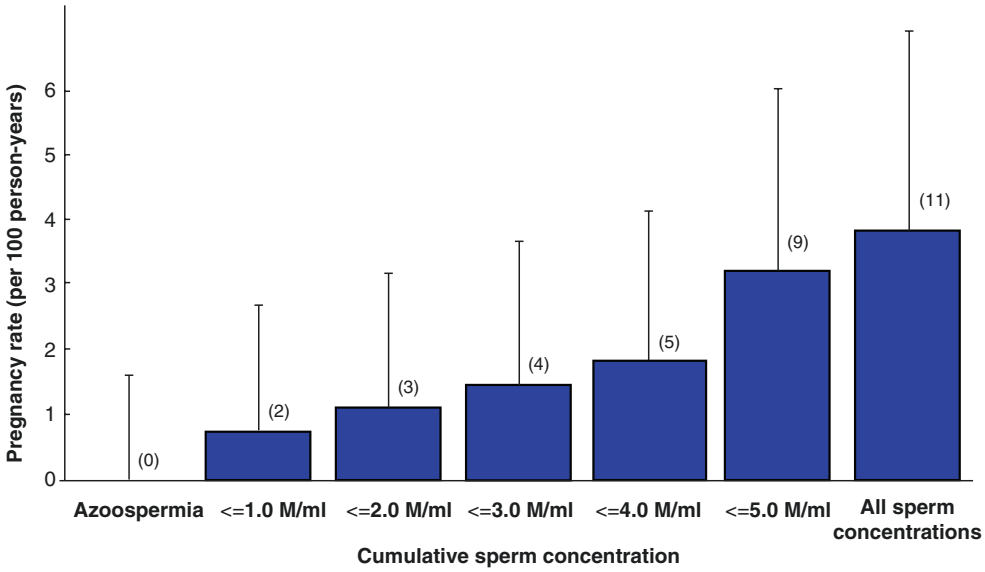


Fig. 47.2 Contraceptive efficacy of testosterone enanthate (250 mg weekly) in 346 volunteer trial participants: Pregnancy rates per 100 person-years in relation to sperm concentrations (WHO 1996)

After testosterone undecanoate was developed as an oral preparation in Europe (see Chap. 36), it was reformulated as an **intramuscular injection** in China by dissolving it in tea seed oil as a vehicle. Since the early 1990s, it has been used in China to treat hypogonadism and in male contraception studies. In Europe, the half-life of the Chinese preparation has been further extended by dissolving testosterone undecanoate in castor oil, and in this form, it is now available for clinical use in 750 or 1000 mg depot injections (see Chap. 36).

In clinical trials in China, all men receiving 1000 mg testosterone undecanoate i.m. every 4 weeks achieved azoospermia, and trial participants receiving 500 mg every 4 weeks showed 95% azoospermia or marked oligozoospermia after 4–5 months of suppression (Zhang et al. 1999). In a subsequent phase III study of 305 couples, the suppression phase was followed by an **efficacy phase** in which none of the men who showed azoospermia or marked oligozoospermia (<3 million/mL) induced a pregnancy (Gu et al. 2003). However, six participants showed sperm reappearance in the ejaculate during the efficiency phase, and one pregnancy was attributed to this **rebound phenomenon**. The few side effects observed were all typical of mildly elevated testosterone levels.

The largest **efficacy study** to date was also conducted in China, with monthly injections of 500 mg testosterone undecanoate. The efficacy study included 855 couples, during which only nine pregnancies occurred. This corresponds to a pregnancy rate of 1.1/100 for the 24-month efficacy phase (Gu et al. 2009). It should be noted that 43 couples were not enrolled in the efficacy phase due to inadequate suppression with sperm concentrations >1 million/mL. Anyhow, it can be concluded that testosterone undecanoate i.m. provides **better contraceptive protection than condoms in China**.

In an initial study with testosterone undecanoate in castor oil, 1000 mg was injected at 6-week intervals to 14 Caucasian subjects; 8/14 achieved azoospermia (Kamischke et al. 2000). Although this azoospermia rate is not different from that achievable with testosterone alone, the three times longer injection interval represents a significant advance. A subsequent study concluded that 8-week injection intervals of 1000 mg would be sufficient for contraceptive purposes (Qoubaitary et al. 2006).

Assuming that testosterone undecanoate injections of 1000 mg every 10–14 weeks are required for testosterone replacement in hypogonadal men, approximately one-third more testosterone is required for contraceptive purposes in normal men.

47.2.4 Testosterone Implants

Pellets consisting of pure testosterone are used in some countries to treat hypogonadism (see Chap. 36). In male contraception studies, the use of multiple subcutaneous implants, whose effects last for several months, was comparable to weekly injections of testosterone enanthate (McLachlan et al. 2000). The disadvantage of requiring a minor surgical procedure for implantation under the abdominal skin is compensated by the long efficacy and low price. However, spontaneous extrusion of the pellets and infection may occur.

47.2.5 19-Nortestosterone

In the search for a long-acting androgen preparation, **19-nortestosterone hexyloxyphenyl propionate** was tested. The spectrum of action of this androgen is similar to that of testosterone, and it has been used as an anabolic steroid in

the clinic since the 1960s. When 200 mg of the 19-nortestosterone ester was injected initially weekly and then every 3 weeks, the same azoospermia rate was achieved as with weekly testosterone enanthate injections. Accordingly, 19-nortestosterone ester is as effective as testosterone enanthate but can be applied at longer injection intervals (Knuth et al. 1985).

This synthetic androgen is as effective as testosterone in terms of suppression of spermatogenesis, and no noticeable side effects have been noted at the selected doses; however, long-term studies have not been conducted with this steroid to detect any adverse side effects with long-term use. Although the use of 19-nortestosterone is widespread among bodybuilders and high-performance athletes, the lack of reports of serious side effects, even with long-term use, cannot be evidence of potential harmlessness because systematic evaluations from this field have never been published.

47.2.6 7α-Methyl-19-Nortestosterone

The synthetic androgen **7α-methyl-19-nortestosterone (MENT)**, whose advanced development is supported by the Population Council of the United States for use as a male contraceptive, has a gonadotropin-suppressing effect approximately tenfold greater than testosterone. However, unlike testosterone, it is not able to undergo reduction in the 5α position, so its effects on the prostate may be less than those of testosterone. In an initial dose-finding study testing MENT in subcutaneous implants, it was found to be as effective as testosterone in terms of the azoospermia rate achieved (73% azoospermia in the four implant group at 6 months) (von Eckardstein et al. 2003). Clinical trials of **MENT implants** in combination with **etonogestrel implants** as an additionally administered progestin were disappointing in terms of suppression of spermatogenesis (Walton et al. 2007; Nieschlag et al. 2013). Whether reformulation of MENT implants can lead to better results in terms of highly effective suppression of spermatogenesis remains to be seen (Nieschlag et al. 2013).

47.2.7 Dimethandrolone Undecanoate and 11β-Methyl-19-Nortestosterone Dodecylcarbonate

The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) has advanced the development of new androgens that bind to both androgen and progesterone receptors and could potentially serve as **"single-agent" male contraceptives** (Long et al. 2019). Two drug candidates have been investigated in early-stage clinical trials: **dimethandrolone undecanoate (DMAU)** and **11β-methyl-19-nortestosterone dodecylcarbonate (11β-MNTDC)** (Attardi et al. 2006, 2011). The compounds are prodrugs with modifications that extend the half-life in blood to allow once-daily oral dosing. The prodrugs are hydrolyzed to release the active compounds DMA and 11β-MNT, respectively. Both compounds are derivatives of 19-nortestosterone.

Both compounds were tested in male volunteers, initially as a single dose and then as repeated oral dosing for 28 days. When administered orally or intramuscularly, DMAU is hydrolyzed to the active ingredient dimethandrolone (DMA). In phase I clinical trials conducted by NICHD's **Contraceptive Clinical Trials Network (CCTN),** oral DMAU (100, 200, or 400 mg) taken daily was well tolerated (Ayoub et al. 2017). Over a 28-day treatment period, serum gonadotropins were reduced below the detection limit in most subjects (Thirumalai et al. 2018). No serious adverse effects were observed. Most participants (80%) were satisfied with the method; 54% reported that they would use it as their primary contraceptive method if it were available (Nguyen et al. 2020). Similarly, results with a single oral administration of 11β-MNTDC (200, 400, and 800 mg) showed that the drug was well tolerated without serious adverse effects (Wu et al. 2019). In a 28-day study with daily oral administration of 11β-MNTDC (200 or 400 mg), gonadotropins were suppressed, also without serious adverse effects (Yuen et al. 2020).

These compounds are not aromatizable, which can lower serum **estradiol levels** when endogenous testosterone synthesis is inhibited. Potential long-term effects on bone health are not known, but in the 28-day study with oral DMAU, an increase in procollagen type 1 N-terminal propeptide (P1NP), a serum marker of bone formation, was observed (Thirumalai et al. 2021). Longer-term studies with these **progestogenic androgens** are needed to determine whether they are safe and can effectively suppress sperm production.

47.3 Androgens in Combination with GnRH Analogues

47.3.1 GnRH Agonists

The gonadotropin-suppressing effect of **GnRH agonists** is well known from clinical use in gynecology and prostate cancer therapy. An initial phase of gonadotropin stimulation lasting a few days is followed by downregulation of GnRH receptors, suppression of gonadotropins, and reduction in intratesticular testosterone and spermatogenesis. However, a number of studies on male contraception that attempted to apply this principle showed that the additional administration of GnRH agonists to testosterone administration, which is still required to maintain androgen-dependent functions, resulted in only weak suppression of

spermatogenesis, so that the agonists were found to be unsuitable for the purpose of male contraception (Behre et al. 1992; Nieschlag et al. 2004).

47.3.2 GnRH Antagonists

GnRH antagonists do not lead to the initial stimulation of gonadotropins because they do not act via downregulation but via blockade of the pituitary GnRH receptors, thus inducing an immediate drop in gonadotropins and correspondingly faster azoospermia. This was supported by several small clinical studies testing **GnRH antagonists** in combination with different testosterone preparations (summary in Nieschlag et al. 2004). Azoospermia was achieved in 47 of 55 volunteers participating in different clinical trials with different GnRH antagonists.

Despite these promising results, GnRH antagonists have not been further developed for male contraception because the need for daily to weekly parenteral injections and the currently **high cost** of available preparations stand in the way of widespread use. Also, approaches in which GnRH antagonists were used only initially until azoospermia was achieved and then continued with androgens alone (Swerdloff et al. 1998) have not been shown to be effective in further studies (Behre et al. 2001). Currently, **orally active GnRH antagonists** are being successfully tested in phase III trials for reproductive endocrinology indications (Schlaff et al. 2020; Shore et al. 2020; Al-Hendy et al. 2021), so the practicality of this effective approach to hormonal contraception may improve significantly. Thus, the development of an orally active GnRH antagonist in combination with an orally active androgen for male contraception serves as a strong potential candidate for a "**male pill.**"

47.4 Androgens Combined with Progestins

The gonadotropin-suppressing potential of **progestins** is well known from female contraception, where progestins are effectively combined with estrogens. Over the past 4 decades, numerous studies have been conducted on combinations of androgens (principally testosterone) with various progestins to identify a combination that would be suitable for male contraception. Approaches have compared four progestins [cyproterone acetate (CPA), levonorgestrel (LNG), norethisterone enanthate (NETE), and Nestorone®], of which CPA and LNG at the doses used in that study showed the strongest gonadotropin-suppressing effect (Zitzmann et al. 2017). Unfortunately, systematic investigation of the various progestins with regard to their contraceptive potential in men has not been intensified, while results from individual studies with different study designs are difficult to compare. Accordingly, a now outdated **Cochrane Review** analyzing 33 clinical trials also concluded that these studies included too few subjects to allow reliable conclusions on the differences between various hormone combinations (Grimes et al. 2012). Furthermore, not all studies followed the strict criteria for randomized controlled trials. However, it must be kept in mind that individual centers are often financially and logistically overburdened to include large numbers of volunteers in the studies and to comply with the criteria of the **regulatory authorities.**

47.4.1 Depot Medroxyprogesterone Acetate

From early studies in the 1970s conducted by WHO and the Population Council, **depot medroxyprogesterone acetate (DMPA**) emerged as a progestogen with great potential for male contraception (Barfield et al. 1979). The combination of DMPA with 19-nortestosterone injected at 3-week intervals was first tested in Caucasian men (Knuth et al. 1989), but then proved particularly successful in trials in Indonesia (WHO 1993).

One of the few **efficacy studies** that evaluated pregnancy rates rather than sperm as a surrogate parameter also used DMPA, but in combination with testosterone implants (Turner et al. 2003). In this Australian study, spermatogenesis was suppressed to azoospermia in 53/55 participants during the 1-year efficacy phase, and no pregnancy occurred. However, the discontinuation rate in this study was relatively high (49%) and it took several months for azoospermia to occur or to return to baseline levels after discontinuation.

To test whether at least one of the two steroids could be self-applied by the user, daily application of a transdermal testosterone gel with DMPA injections (300 mg/every 3 months) was tested (Page et al. 2006). The results were comparable to those of studies using DMPA with injectable testosterone.

47.4.2 Levonorgestrel

When testosterone enanthate was combined with oral LNG, enhancement of sperm suppression over testosterone enanthate given alone was noted [12/18 (67%) and 8/18 (33%), respectively, achieved azoospermia] (Bebb et al. 1996). However, the combination of daily oral LNG and intramuscular testosterone undecanoate given every 6 weeks compared to daily placebo and intramuscular testosterone undecanoate did not improve rates of sperm suppression in Caucasian men [8/14 (57%) and 7/14 (50%), respectively, achieved azoospermia] (Kamischke et al. 2000) but resulted in significantly improved efficacy in Chinese men [azoospermia achieved in 17/18 (94.4%)] (Wang et al. 2007).

When LNG implants were combined with testosterone implants in a comparative study, LNG was found to enhance the effect in Caucasian men (azoospermia increased from approximately 20% to 65% at 18 weeks). Chinese men respond well to testosterone implants alone but had greater suppression with the addition of LNG implants (azoospermia achieved in approximately 65% with testosterone alone at 18 weeks compared to approximately 78% with the addition of levonorgestrel) (Wang et al. 2005).

47.4.3 Norethisterone

The injectable sustained-release preparation, **norethisterone enanthate (NETE),** and the orally active **norethisterone acetate (NETA)** are hydrolyzed to release the active substance **norethisterone**. Norethisterone can be reduced to 5α-norethisterone and aromatized to ethinylestradiol. While norethisterone has androgenic activity (about 10% of testosterone), 5α-norethisterone simultaneously exhibits antiandrogenic properties. In a study in nonhuman primates (cynomolgus monkeys), NETE showed a protective effect on the prostate in that castrated animals treated with NETE and testosterone showed no prostate growth (Wistuba et al. 2012).

In a study in men, 13/14 males receiving 200 mg NETE in combination with 1000 mg testosterone undecanoate i.m. every 6 weeks achieved azoospermia (Kamischke et al. 2001). Further studies showed that injection intervals could be extended to 8 weeks (Meriggiola et al. 2005) and that testosterone undecanoate i.m. could be combined with oral NETA without loss of efficacy (Kamischke et al. 2002).

Based on these studies, **WHO** and the **CONRAD program** initiated a **prospective phase II efficacy trial** of injectable testosterone undecanoate and NETE, which enrolled 320 couples at 10 study sites worldwide (Behre et al. 2016). These healthy couples had no fertility limitation; men had a median age of 32 years, and female partners had a median age of 28 years.

Men received separate intramuscular injections of 200 mg **NETE** and injections of 1000 mg **testosterone undecanoate** every 8 weeks after enrollment in the treatment phase. In 95.9% of the study participants who completed the suppression phase, spermatogenesis was suppressed to a sperm concentration ≤ 1 million/mL ejaculate within 24 weeks (Fig. 47.3). During the subsequent **efficacy phase** of up to 56 weeks, during which the 266 included couples did not use other methods of contraception, four pregnancies occurred in the partners of the study participants, corresponding to a contraceptive **efficacy rate** of 1.57/100 (Kaplan–Meier method) (Fig. 47.3). At the end of the efficacy period, reversibility of suppression of spermatogenesis was 94.8% within 52 weeks (Fig. 47.3).

The most common **adverse events** during the study were acne, injection site pain, increased libido, and mood disturbances. Following the recommendation of an external safety review committee, recruitment of new study participants and further hormone injections were discontinued during the course of the study. From the perspective of many study physicians, this was unfortunate, and most couples in the study also indicated at the end of the efficacy phase that they would continue to use this contraceptive method if available (study participants 81.7%, partners 80.3%). At the time of writing this chapter, further study analyses are still being conducted with the aim of prospectively identifying those couples who are not expected to experience relevant side effects and who would then likely use this effective and acceptable male hormonal method.

47.4.4 Cyproterone Acetate

The oral **antiandrogen cyproterone acetate (CPA)** has potent progestogenic activity. In early studies in which CPA was combined with testosterone injections, there was a clear spermatogenesis-suppressing effect, but the antiandrogenic activity (manifested, e.g., by reduced hematocrit) was an undesirable side effect. However, when 1000 mg testosterone undecanoate was combined with 20 mg CPA p.o. daily every 6 weeks and only 2 mg CPA/day was administered in combination with testosterone undecanoate after an initial suppression phase, the spermatogenesis-suppressing effect was maintained [24/24 suppressed to <1 million/mL and 13/24 (54%) achieved azoospermia by week 12] without antiandrogenic side effects (Meriggiola et al. 2003).

47.4.5 Desogestrel and Etonogestrel

Desogestrel is an orally active progestin whose orally active metabolite **etonogestrel** is formed by conversion. Etonogestrel can also be applied directly as an implant. In combination with testosterone enanthate or testosterone implants, desogestrel showed good suppression of spermatogenesis (Wu et al. 1999; Kinniburgh et al. 2001).

Etonogestrel implants in combination with testosterone implants s.c. produced azoospermia in all participants ($n = 14$); however, it took up to 28 weeks to achieve this goal (Brady et al. 2004). In the first (and so far last) industry-sponsored **surrogate clinical trial,** Organon and Schering had chosen to combine **etonogestrel implants with testosterone undecanoate injections** at various doses (Mommers et al. 2008). This multicenter study enrolled 354 volunteers in six dosing groups, including a placebo group. Testosterone undecanoate at doses of 750–1000 mg was administered every 10–12 weeks with two different etonogestrel doses for

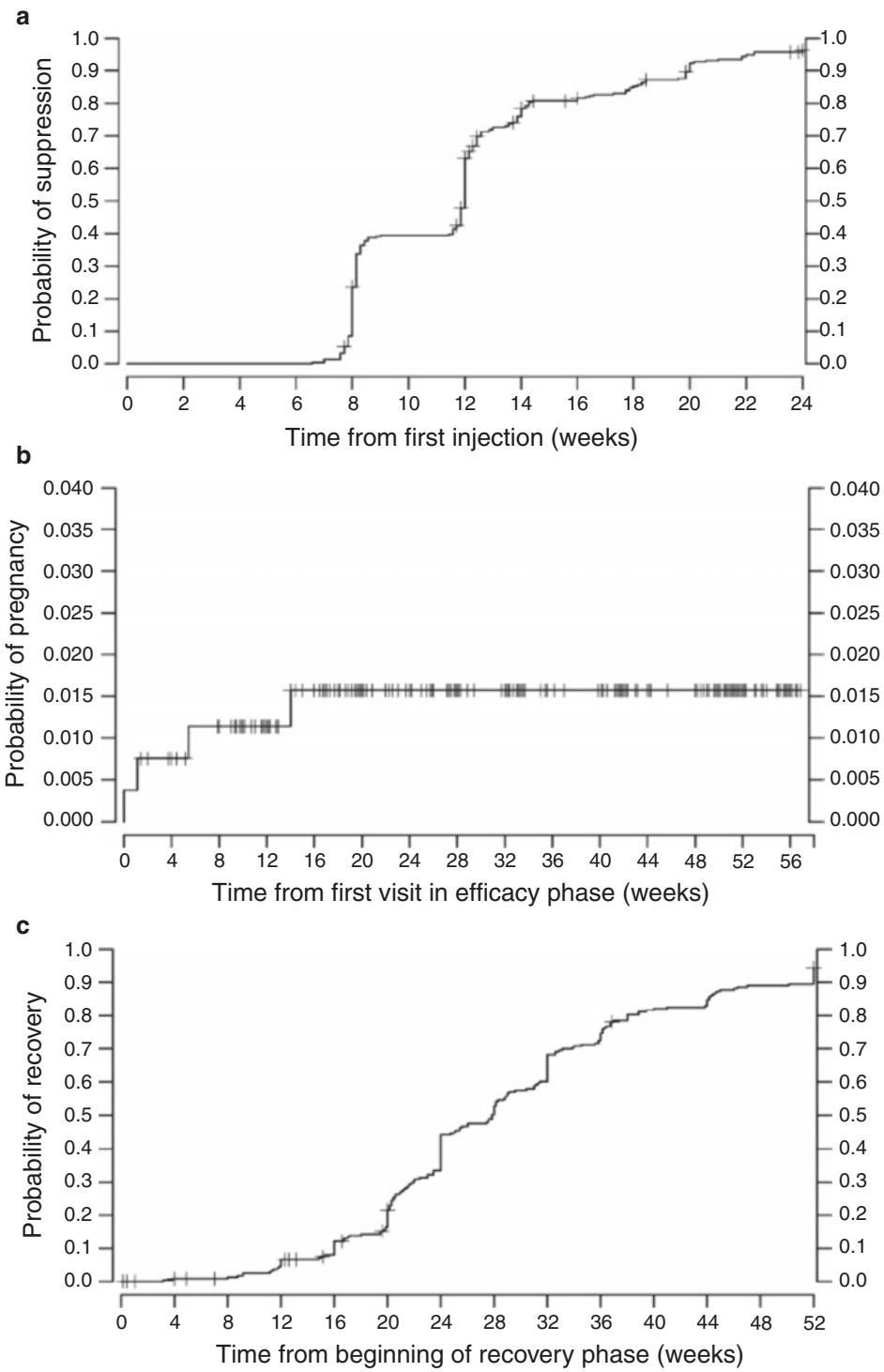


Fig. 47.3 **(a)** Cumulative suppression rate of spermatogenesis (Kaplan–Meier method) up to 24 weeks after the first injection (sperm concentration ≤ 1 million/mL, two consecutive times). **(b)** Cumulative pregnancy rate (Kaplan–Meier method). **(c)** Reversibility of suppression of spermatogenesis (Kaplan–Meier method) (Behre et al. 2016, modified authorized reprint)

42–44 weeks. Sperm concentrations were suppressed below 1 million/mL ejaculate in 90% of the trial participants (see Fig. 47.4). Although the combination of implants with injections does not seem particularly practical or attractive, the trial proved successful and could have provided the basis for a phase III trial. Unfortunately, both companies terminated their male contraception programs when they were acquired by other companies.

47.4.6 Nestorone®

The NICHD in collaboration with the Population Council has led the development of a new progestin/testosterone combination for hormonal contraception in recent years (Blithe 2016). The method currently furthest along in clinical development is a transdermal **gel formulation** containing both the progestin **Nestorone® (NES) and testosterone**. The

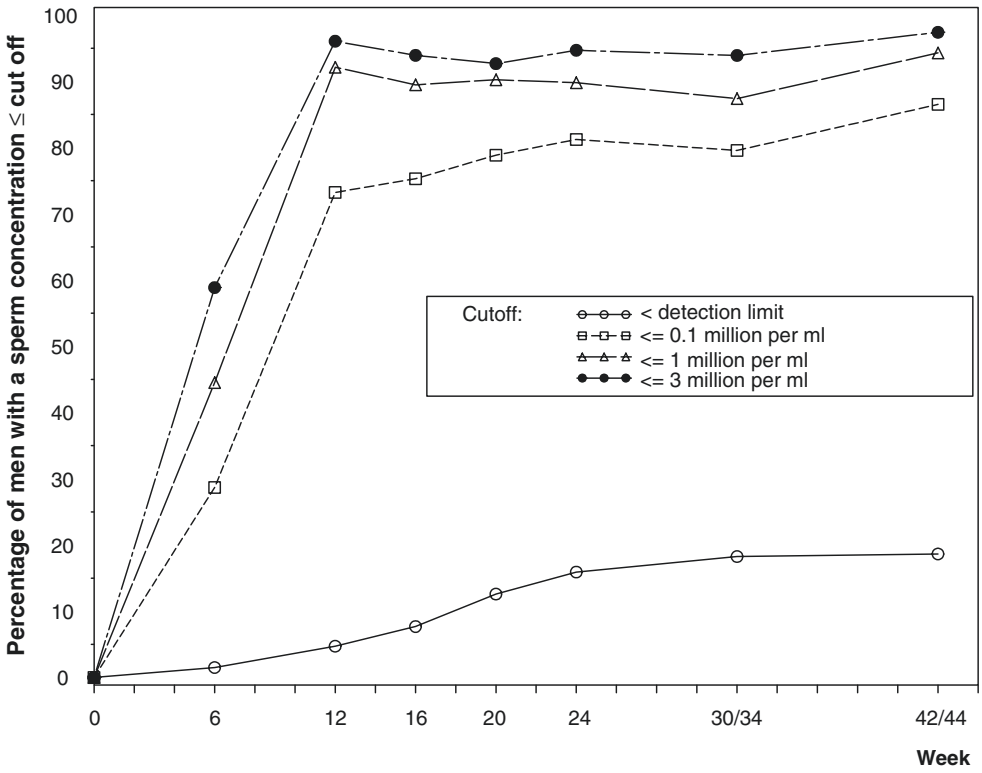


Fig. 47.4 Results of a double-blind, randomized, placebo-controlled multicenter trial of male contraception with a combination of etonogestrel implants and testosterone undecanoate injections. The percentages of study participants who achieved sperm concentrations below certain thresholds are shown (from Mommers et al. 2008, modified reprint with permission)

treatment requires daily application of the gel. An initial **proof-of-concept** study examined the use of separate formulations of NES gel (2, 4, 6, and 8 mg) and testosterone gel (100 mg). Subjects used the two gels for 3 weeks, with twice-weekly blood sampling to determine the effect on serum gonadotropins. A control group used a placebo gel with a testosterone gel instead of NES. A dose-dependent suppression of gonadotropins was observed over the 3-week period (Mahabadi et al. 2009). The treatment period was too short to detect any effects on sperm production. Based on the successful suppression of gonadotropins, a longer study was conducted with separate formulations of testosterone gel (100 mg) and NES gel (doses of 8 or 12 mg) or placebo gel to assess the suppression of spermatogenesis over 6 months of daily use. Azoospermia or severe oligozoospermia (<1 million/mL) was achieved in 89% of participants using testosterone gel and NES gel (8 or 12 mg), compared with 23% with testosterone gel and placebo gel (Ilani et al. 2012). The **gel combination** was well tolerated, and most men found it acceptable; however, some men found the use of 20 mL of gel per day impractical (Roth et al. 2014).

The two separate gels were subsequently reformulated into a **single gel preparation** with a higher concentration to reduce the volume of gel required for application. An initial study was conducted with the new formulation to confirm suppression of gonadotropins (Anawalt et al. 2019). In addition, a possible transfer of hormones to the partner was investigated, as well as the effects of showering after gel application (Yuen et al. 2019).

Based on the findings from these studies, a protocol was designed to evaluate combined NES/testosterone gel in couples who agree to use it as a contraceptive method after the male partner has achieved a sperm count <1 million/mL, the level that has been shown to provide contraceptive efficacy. The **efficacy study**, which is ongoing at the time of writing, is designed to evaluate the acceptability and efficacy of its use as a contraceptive method for up to 1 year. Thereafter, the man discontinues gel applications and sperm counts are monitored until two sperm counts in the normal range are observed. Study sites include seven locations in the USA, two in the UK, and one each in Sweden, Italy, Chile, and Kenya. The timeline for recruitment has been impacted by a global pandemic of COVID-19 requiring new enrollment to be halted temporarily due to institutional restrictions in the individual locations. Subjects enrolled prior to the pandemic were permitted to continue although clinics had very limited accessibility. Initial impressions from participants in the trial are that the new formulation (2.5 mL applied to each shoulder) is convenient and easy to use. The product effectively suppresses sperm production when used as directed. The time commitment to participate in this type of study is nearly 2 years. This is a demanding schedule in the best of times and periodic shutdowns due to the risk of viral infection have complicated the normal course of study conduct.

Once this phase IIb study is completed, if the results are promising, a **larger phase III efficacy study** will be required to collect sufficient data on safety and efficacy and ultimately to obtain **regulatory approval**. Since this would be a "**first-**

in-class" product, regulatory requirements have not yet been fully clarified. However, it is anticipated that such a product would need to have the same **user experience** required for approval of a new contraceptive method for women (20,000 cycles for safety and evidence of efficacy in 400 women using the product for 1 year).

47.5 Conclusion and Outlook

A variety of smaller and larger clinical trials testing testosterone alone or testosterone in combination with progestins or GnRH antagonists have been able to confirm the principle of hormonal male contraception. Beyond these multiple **proof-of-concept studies, seven efficacy studies** to date have shown pregnancy rates lower than those obtained with condom use (when used accurately) and equal to those obtained with oral contraceptives on the female side (WHO 1990, 1996; Gu et al. 2003, 2009; Turner et al. 2003; Behre et al. 2016). To date, however, no definitive hormone combination and dosage has been established, and the search for an **approvable hormone combination and delivery method** continues. This task falls into the domain of the pharmaceutical industry, which, however, has unfortunately left the field prematurely and has not yet resumed clinical research.

Under the impact of this vacuum, researchers active in the field initiated the **Summit Meetings of Male Contraception** in 1997 to provide a confidential forum for the exchange of ideas, study designs, and results (e.g., Nieschlag 2007). These have been continued by the **International Consortium for Male Contraception** (ICMC) since 2013 (Serfaty et al. 2016, 2018). Significant analyses of previously conducted studies emerged from these meetings. A multivariate analysis of the results of 1549 participants in 30 studies published between 1990 and 2005 demonstrated that **full reversibility of suppression of spermatogenesis** occurred in all studies conducted with a wide variety of hormones and combinations (Liu et al. 2006). The second analysis of the same studies also showed that the **rate at which suppression of spermatogenesis** is achieved can be accelerated by the addition of progestins to testosterone and that East Asian and younger men suppress more completely than Caucasian and older men (Liu et al. 2008).

Through these findings, Summit Meeting participants were encouraged to think of an effective and marketable product that could one day be presented to regulatory agencies. To open a dialog with regulatory authorities and prompt thinking about the criteria for potential approval, Summit Meeting participants drafted **recommendations for designing the appropriate studies** and **requirements for a male contraceptive** (Nieschlag 2007). The active participation of the pharmaceutical industry in the search for a male contraceptive in the meantime reinforces this approach (Mommers et al. 2008). This **first placebo-controlled study** also found that the **side effects** of a testosterone–progestin combination were quite tolerable.

Given these overall positive findings and the **good consumer acceptance** of a hormonal male contraceptive resulting from **opinion polls** or **study surveys** (Heinemann et al. 2005; Behre et al. 2016), the departure of industry from this area of research was a major surprise. The reasons for this withdrawal may be many, but financial considerations and patent protection issues for these public companies may have been paramount after all. Basically, the components of a hormonal male contraceptive are relatively inexpensive and could well compete with female methods; however, the approved preparations are currently sold at high prices for other indications. Under these circumstances, the only hope that remains is for governmental or philanthropic organizations to assume the final steps in the development of an actual available product to allow a **reasonable price** for the **final product**.

Key Points

- Currently, the most advanced approach to a male contraceptive is a hormonal method.
- In principle, the suppression of spermatogenesis required for effective contraception with a sperm concentration ≤ 1 million/mL ejaculate while maintaining androgen-dependent functions can be achieved with androgens alone, androgens in combination with GnRH antagonists, and androgens in combination with progestins.
- Androgens alone are effective only at a relatively high dosage.
- The combination of GnRH antagonists with testosterone at physiological doses appears promising.
- Clinical evaluation of contraceptive efficacy of a product containing a progestin and testosterone, delivered by a transdermal route, shows promise as an effective, reversible, user-controlled male contraceptive method.

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Non-Hormonal Approaches to Male Contraception

48

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Abstract

Technology for non-hormonal male contraception can range from application of biologics (large molecules) such as antibody vaccine and mRNA, vas deferens polymeric injection, or small molecules, including natural products as agonists or antagonists of reproductive tissue protein targets. The mechanism of action for these contraceptive agents is to affect sperm production, sperm function, or sperm transport. One prevailing rationale for pursuing non-hormonal contraceptive agents is the concept of little-to-no side effects. This remains to be seen as research and development into non-hormonal methods have proven difficult over the years and selectively or exclusively targeting reproductive tissue proteins as drug targets are in early stages.

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48.1 Introduction

The unintended pregnancy rate in the world is approximately 44% (Bearak et al. 2018). Although a variety of contraceptive methods is available to women, reversible contraceptive options for men are limited to condoms and withdrawal. With high discontinuation rates (~55%) for both methods, and typical failure rates of 13% and 20% for condoms and withdrawal, respectively, new methods of male contraception are needed to increase contraceptive options for men and

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women (Sundaram et al. 2017). Men express willingness to use a new method if available (Heinemann et al. 2005), and use of condoms, withdrawal, vasectomy, or shared participation in fertility awareness methods accounts for 25% of total contraceptive use worldwide (Bertrand et al. 2020).

48.2 Principles of Non-Hormonal Contraception

48.2.1 Systemic

The overall rationale for developing non-hormonal contraceptives is to avoid the hypothalamic–pituitary axis, potentially circumventing side effects associated with hormones. Non-hormonal male contraceptive methods can be broken down roughly into pharmaceutical intervention, physical intervention, or transport mechanism intervention. A pharmaceutical intervention in a hormonal pathway of male contraception is through feedback loop inhibition of the hypothalamic–pituitary axis, where the mechanism of action is to bring about suppression of spermatogenesis; thus, reducing or eliminating sperm production. Ideally, non-hormonal pharmaceutical intervention will bring about suppression of sperm production or sperm function while avoiding the hypothalamic–pituitary axis that might cause disruption of normal male hormone levels. Studies have shown that hormonal approaches take weeks or months for sperm numbers to be reduced sufficiently for contraceptive effect. As with the male hormonal contraceptive pathway (see Chap. 47), non-hormonal suppression of sperm production may be dependent on the life cycle of spermatogenesis (74 days for human sperm). Although sperm function is under similar cycle constraints, the contraceptive effect on sperm function could be faster, depending on the target. Mutations relating to sperm tail or flagella have shown infertility phenotypes in both animal and human sperm. In a similar manner, inhibition of sperm tail proteins that control sperm motility and/or hyperactivation can cause infertility phenotypes. Immunocontraceptives have been successfully used in animal models; however, translating this approach into humans has proven to be more challenging.

The development of non-hormonal small molecules derived from natural products and immuno-based contraceptives has had varying degrees of success in the clinic and in animal models. However, to date, systemic pharmaceutical approaches have not been successful in the clinic due to toxicity and/or efficacy. In this context, development of systemic reproductive tissue-specific targets is largely in the preclinical phase. Many proteins expressed in reproductive tissues affect either sperm production or sperm function. Targeting reproductive tissue-specific proteins may prove effective if high specificity is demonstrated to limit off-target effects. Studies exploiting iterative high-throughput screening (HTS), computational modeling, structural biology, and medicinal chemistry are being employed to move forward several potential non-hormonal male contraceptive agents.

For systemic pharmaceutical efforts that encompass sperm-specific antibodies, mRNA technologies, natural products, and reproductive tissue protein targets that cause sperm agglutination or loss of function, measurement of effectiveness may be difficult to observe or assess unless mating studies are conducted. Ultimately, regulatory approval of any method will require demonstration of safety and contraceptive effectiveness in large clinical trials conducted in couples.

48.2.2 Nonsystemic

In contrast to a systemic pharmaceutical approach, which could take weeks to months for contraceptive effect, differing application via local methods has shown varying degrees of success that could be more immediate. A physical stoppage of sperm transport through the vas deferens via surgical vasectomy (see Chap. 46) is a proven successful male contraceptive method, but a more permanent procedure. Another method involves polymer injected into the vas deferens to block seminal passage that is reversible. A more physically demanding and potentially damaging method is ultrasound or heat treatment of testes.

48.3 Sperm Production

48.3.1 Sperm-Specific Targets

48.3.1.1 Retinoic Acid Pathway

48.3.1.1.1 RARα

Deficiency of vitamin A (retinol) and all-trans-retinoic acid (tRA), and its physiologically active metabolite, first studied in male rats, have long been recognized for their role in human male sterility (Chung et al. 2016; Noman et al. 2020; and references cited therein). Retinoic acid (RA) is required for normal spermatogenesis. Studies have shown that retinoic acid receptor α (RARα) male knockout mice are infertile (Lufkin et al. 1993). The pathway of conversion of retinol to retinal and finally to retinoic acid (RA) provides prospects whereby inhibitors or antagonists can be implemented to stop RA synthesis and thereby stop spermatogenesis. In a series of studies with the pan-retinoic acid receptor antagonist, BMS-189453, reversible inhibition of spermatogenesis in a mouse model was demonstrated (Chung et al. 2016; Chung et al. 2011). Iterative screening, structure-based drug design (SBDD), and application of medicinal chemistry are

currently being employed to develop potent, specific antagonists to inhibit RARα activity in the RA synthetic pathway in order to inhibit sperm production (Noman et al. 2020).

48.3.1.1.2 BDAD and ALDH1A

The RA pathway involving suppression of spermatogenesis was demonstrated using a bisdichloroacetyldiamine analog (BDAD). In a clinical study of WIN18,446, a BDAD analog in the RA pathway, over 60 men were treated for 1 year (Heller et al. 1961). The drug was efficacious at inhibiting spermatogenesis and was well tolerated. However, the development of the drug was halted after the discovery that consumption of alcohol with the drug induced a severe disulfiram reaction. Disulfiram is a drug used in the treatment of chronic alcoholism by producing sensitivity to ethanol. Even a small amount of alcohol with the drug can produce varied reactions ranging from nausea, vomiting, flushing, throbbing headache, and respiratory difficulty, to more severe reactions that can cause heart attack, cardiovascular collapse, unconsciousness, and even death. The side effect caused by BDAD was due to off-target inhibition of a liver enzyme, aldehyde dehydrogenase-2 (ALDH2). In the liver, the enzyme ALDH2 detoxifies acetaldehyde during alcohol metabolism where ethanol is converted to acetaldehyde, which is then converted acetic acid. Inhibition of ALDH2 in alcohol metabolism by BDADs produces accumulation of toxic aldehyde. In humans, there are 19 aldehyde dehydrogenase (ALDH) isozymes that metabolize endogenous and exogenous aldehydes to their corresponding carboxylic acid derivatives (Vasiliou et al. 1999). Within this human ALDH family, an aldehyde dehydrogenase subfamily, ALDH1A, is involved in the synthesis of RA. A testis-specific member in this subfamily includes ALDH1A2, a primary RA-converting enzyme in spermatogenesis. Additionally, expression of ALDH1A2 is reduced significantly in the testicular tissue of some infertile men, inferring suitability for its development as a nonhormonal male contraceptive target (Amory et al. 2014). Research into discovery of covalent and non-covalent small-molecule inhibitors of ALDH1A2 is in progress. Through X-ray crystallography, binding, and enzymatic studies, mechanisms of action of human ALDH1A2 inhibition by WIN18,446 and reversible small-molecule inhibitors provide a framework for structure-based design of potent and selective inhibitors of ALDH1A2 (Chen et al. 2018).

48.3.1.2 Testis-Specific Bromodomain (BRDT)

A testis-specific bromodomain (BRDT) protein is a nonhormonal reproductive tissue-specific, chromatin-associated protein target. A subfamily of BET (bromodomain and extraterminal) proteins consists of four members: BRD2, BRD3, BRD4, and BRDT. BET proteins are comprised of two bromodomain modules. Testis-specific BRDT is critical for chromatin remodeling during spermatogenesis (Gaucher et al. 2012; Wisniewski and Georg 2020). It was demonstrated that male mice with homozygous BRDT null mutations are sterile (Shang et al. 2007). In an in vivo mouse study, JQ1, a small-molecule inhibitor of BRD proteins, was able to cross the blood–testis barrier and inhibit BRDT, causing complete, reversible contraceptive efficacy in male mice (Matzuk et al. 2012). Although JQ1 was effective for reversible contraception in the mouse model, it had off-target activity to other BRD proteins, thereby making JQ1 not suitable for further development as a non-hormonal male contraceptive. To alleviate this shortcoming, efforts are underway with high-throughput screening and discovery of different chemical scaffolds to develop specific inhibitors of BRDT. A study encompassing virtual screening, analytical testing, structure–activity relationship evaluation, and compound optimization through structure-based X-ray co-crystallography has resulted in the discovery of new chemical scaffolds with BRDT inhibitory activity (Ayoub et al. 2017). Although the critical role in spermatogenesis is mediated by the first bromodomain (BD1), the second bromodomain (BD2) module is a target for enhancing specificity. Library screening and follow-on medicinal chemistry optimization have produced potent BET inhibitor candidates with enhanced selectivity for BD2 (Law et al. 2018).

48.3.2 Small-Molecule Inhibitors

48.3.2.1 Inhibition of Sertoli–Germ Cell Adhesion (Adjudin, Gamendazole, and Indenopyridines)

Small-molecule drug candidates that target Sertoli–germ cell adhesion and cause release of immature spermatids from the seminiferous epithelium have been an active area in the discovery of non-hormonal male contraceptive agents. An indenopyridine derivative known as CDB-4022 inhibits mature sperm production in rat, feline, stallion, and nonhuman primate animal models. Full reversibility of sperm production, with no apparent side effects, was reported for this small molecule upon cessation of the drug treatment (Hild et al. 2007; Pozor et al. 2013; D'Francisco et al. 2019). Indazole carboxylic acid derivatives, gamendazole, H2 gamendazole, and adjudin, are additional contraceptive drug candidates targeting Sertoli cell–germ cell interaction. Oral doses of H2 gamendazole caused inhibition of fertility in rats (Tash et al. 2008). A problematic outcome of this contraceptive candidate is that, although the effect was reversible at low doses, at higher doses, the contraceptive effect was irreversible. The narrow therapeutic window for H2 gamendazole is a limitation to further progress with this candidate.

A challenge for any contraceptive agent to affect sperm production or function is the ability of a drug to cross the blood–testis–barrier. To overcome this challenge to reach the

Sertoli cell–germ cell junction, a strategy was developed to conjugate a recombinant FSH-binding fragment to the contraceptive candidate agent, adjudin (Mok et al. 2011). The strategy was successful in achieving increased specificity. However, achieving this increased specificity came at a cost of reduced oral bioavailability. From this series of studies of potential contraceptive agents targeting the testis Sertoli cell–germ cell junction, it is clear that safety, efficacy, and reversibility must be demonstrated in higher mammals prior to entry into a first-in-human clinical setting as a non-hormonal contraceptive agent.

48.4 Sperm Function

48.4.1 Sperm-Specific Targets

48.4.1.1 Ion Channels

Numerous ion channel proteins control sperm function in terms of motility, hyperactivation, chemotaxis, capacitation, and acrosome reaction among other activities. These ion channel proteins are expressed in the tail region of the sperm and in the head piece. Ions channels, including CatSper (cation channel of sperm), KSper (a potassium ion channel), sNHE (sperm Na+/H+-exchanger), and SLO3 (sperm-specific potassium channel), are expressed in sperm and have been shown to be required for male fertility (Lishko et al. 2012; Wang et al. 2007; Ren et al. 2001; Carlson et al. 2009; Chavez et al. 2014). Animal models incorporating mutations and deletions of these genes have shown male infertility without apparent systemic effects.

CatSper, a calcium ion channel, under normal function regulates sperm tail hyperactivation. Sperm enter the tubal isthmus through the utero-tubal junction and form a reservoir following intercourse (Suarez 2016). In this state, sperm can remain viable in the isthmus for several days. Physiologically, progesterone and other activators are secreted by the cumulus–oocyte complex after ovulation. When progesterone or prostaglandins bind and activate the α/β hydrolase domain-containing protein 2 (ABHD2), a cascade of events causes depletion of endocannabinoid 2-arachidonoylglycerol (2AG) from the spermatozoa plasma membrane. The process of removing 2AG by ABHD2 releases CatSper inhibition, resulting in calcium influx leading to sperm hyperactivation (Miller et al. 2016). Hyperactivated sperm detach from the sperm reservoir in the fallopian tube and swim toward the ampulla region to bind and penetrate the oocyte. An in vitro study with HC-056456, an inhibitor of CatSper, demonstrated that the drug prevented hyperactivation of sperm (Carlson et al. 2009). SLO3 (also known as KCNU1) is involved in calcium entry through CatSper. Genetic deletion of SLO3 in mice resulted in a male infertility phenotype (Chavez et al. 2014). A progestin steroidal analog, RU1968, causes dysfunction of CatSper's progesterone-mediated motility response in human sperm. The inhibitor is non-toxic to human sperm, and it also inhibits the potassium channel, hSLO3, with approximately 15-fold lower potency than CatSper (Rennhack et al. 2018). Since the inhibitor impacts progesterone function in the oviduct, it is unclear if this approach would be more appropriate for female use, either in a systemic pharmaceutical approach or in local application.

The mechanisms that regulate sperm function through inhibition of ion channels involve effects on motility, capacitation, and hyperactivation. It is not entirely clear how a systemic pharmaceutical approach to inhibit sperm ion channels would work as a non-hormonal male contraceptive, considering that these functional events occur post-ejaculation. Whether ejaculated sperm are functional or not functional remains an unknown in a clinical setting if the male uses inhibitors to systemically target these ion channels or other sperm-specific proteins that regulate sperm function in the female reproductive tract.

An enzyme that generates cAMP in sperm, ADCY10, was discovered in 1999 (Buck et al. 1999). Among the adenylyl cyclase family of proteins (ADCY1-10), ADCY10 is the only soluble adenylyl cyclase (sAC). A severe defect in sperm motility was observed for ADCY10 knockout mice with phenotypic male infertility (Esposito et al. 2004). Subsequent tissue enrichment profile showed that this protein is also expressed in non-reproductive somatic tissues (www.proteinatlas.org). In light of this analysis, off-target effects of inhibition of ADCY10 were a concern. However, whole-exome sequencing (WES) of two infertile men (from a consanguineous family) exhibiting severe asthenozoospermia (reduced progressive sperm motility) found that their phenotypic infertility was due to homozygous frameshift mutation in ADCY10. The deletion was upstream of the nucleotide-binding site, leading to sAC variants with impaired activity. The two men had complete loss of rapid progressive sperm motility and severe reduction in medium progressive sperm motility. Treatment of the sperm samples with cAMP analogs caused some improvement in both classes of progressive motility. These men lead normal lives except for infertility phenotype and the potential for developing calcium oxalate kidney stones. Other family members who were heterozygous with this variant had a history of calcium kidney stones (Akbari et al. 2019). The association of ADCY10 with dominant absorptive hypercalciuria has not diminished the enthusiasm for discovery and optimization effort for ADCY10 inhibitors as new non-hormonal contraceptive agents (Balbach et al. 2020).

Some of these targets have multi-modular functions. ADCY10 forms a complex with sperm Na+/H+ exchanger (sNHE), a transmembrane ion channel found on the principle piece of the flagellum; the complex is critical for sperm motility (Wang et al. 2007). Na+/H+ exchangers (NHE1-9)

are a family of transmembrane channels, with NHE1-5 residing in plasma membranes and NHE6-9 in organelles. Impairments to some of these transporters have been associated with disorders such as seizures, epilepsy, autism, diarrhea, or metabolic acidosis. The potential for off-target effects of inhibiting NHEs would be unwelcome; thus, any inhibitors developed for contraception must have very high sperm specificity. In a knockout model, *Nhe8*$^{-/-}$ male mice are infertile due to disruption in acrosome formation (Oberheide et al. 2017). Selective deletion of NHE8 in Sertoli cells did not impact fertility, whereas male mice with germ cell deletion of NHE8 were completely infertile. All homozygous female mice or heterozygous (+/−) males were fully fertile (Wang et al. 2003). Sperm NHE (sNHE) is mainly localized to the principal piece of the tail and is involved in regulation of sperm motility. A comparative study of patients with asthenozoospermia and normal control subjects showed that sNHE expression is decreased in sperm from asthenozoospermia patients compared with that from control subjects. (Zhang et al. 2017).

Na+/K+-ATPase (NKA) is a transmembrane ion transporter that maintains Na+ and K+ concentrations across the plasma membrane of cells. Four different isoforms of the NKA catalytic subunit are expressed in mammalian cells (NKAα1, NKAα2, NKAα3, and NKAα4). These ion channels are found in many somatic tissues, but NKAα4 is the isoform that is predominately expressed in the testis and is necessary for normal sperm function. NKAα4 is required for sperm motility and hyperactivation (McDermott et al. 2015; Syeda et al. 2020). NKAα4 knockout male mice are completely infertile (Jimenez et al. 2011). There are known cardenolide analog inhibitors of NKA that have been used clinically to treat congestive heart failure. Ouabain, in particular, a cardenolide analog, has higher affinity for the NKAα4 (ATP1A4) isoform than for other somatic forms (NKAα1, NKAα2, and NKAα3) in both mice and humans. Medicinal chemistry optimization using the ouabain scaffold as a starting point has yielded derivatives with specificity for the NKAα4 (Clausen et al. 2011; Konrad et al. 2011). Further, ouabain derivatives modified at the glycone (C3) and lactone (C17) domains show picomolar inhibition for the NKAα4 isoform with an excellent selectivity profile against NKAα1, NKAα2, and NKAα3. The in vitro and in vivo data demonstrate decreases in sperm motility for these new ouabain triazole analogs (Syeda et al. 2018).

48.4.1.2 Immobilization–Motility

A different class of a potentially druggable non-hormonal contraceptive targets is the sperm surface protein EPPIN (epididymal peptidase inhibitor). This protein is expressed only in male reproductive tissues: testis and epididymis (www.proteinatlas.org; www.citdbase.org). EPPIN is found on the sperm surface complexed with lactotransferrin and clusterin. Semenogelin binds to the EPPIN protein complex and inhibits sperm motility. After ejaculation, prostate-specific antigen hydrolyzes semenogelin and sperm attain progressive motility. A landmark study in male nonhuman primates (NHP) immunized with human EPPIN resulted in a high anti-EPPIN antibody titer leading to infertility (O'Rand et al. 2004). Another proof-of-concept NHP study showed that intravenous infusion of a small-molecule inhibitor of EPPIN, EP055, resulted in dramatic reduction in sperm motility to ~20% of pretreatment levels. EP055 is believed to affect sperm motility by causing rapid decrease in sperm calcium levels and internal pH (O'Rand et al. 2018).

A systemic immobilization of sperm transport via inhibition of serine proteases in ejaculated semen is another non-hormonal contraceptive discovery path. Disruption of sperm transport can cause reduction in fertility. In line with this strategy, pan-serine protease inhibitor, 4-(2-aminoethyl) benzenesulfonyl fluoride (AEBSF), inhibits semen liquefaction in vivo. This seemingly simple action drastically reduces the number of sperm in the oviduct, demonstrating inhibition of sperm transportation. In a systemic pharmaceutical application, female mice treated with AEBSF have lower fertilization rates and had significantly fewer pups per litter (Barton et al. 2020). Further investigation of the mechanism of semen liquefaction identified another potential non-hormonal contraceptive target, kallikrein-related peptidase 3 (KLK3) (Anamthathmakula and Winuthayanon 2020).

48.4.1.3 Sperm–Egg Interaction

In humans, there are 26 genes associated with members of a disintegrin and metalloproteinases (ADAMs) family of proteins. Several members of this protein family play an important role in late stage-sperm transport and interactions between sperm and egg zona pellucida. Several ADAMs are expressed exclusively or predominately in testis or epididymis and thus represent potential non-hormonal contraceptive targets (Cho 2012).

Species specificity is a critical feature of sperm–egg binding; however, within species, numerous alloantigenic proteins exist. Within a single species (swine), more than 20 potential unique alloantigenic sperm membrane proteins and five sperm raft proteins were identified. Among these, ADAM1, 2, and 3 were dominant sperm membrane alloantigens (Cormier et al. 2018). An ADAM-3 mouse knockout study revealed that sperm were not able to cross the uterotubal junction to enter the fallopian tube. In addition to loss of ability to migrate into the oviduct, impaired sperm–zona pellucida binding was observed (Yamaguchi et al. 2009). Mouse knockout data clearly demonstrate the validity of ADAM-3 as potential non-hormonal target for male contraception; however, it is unclear if human sperm have the same requirement for ADAM-3. Related ADAMs with thrombos-

pondin motifs (ADAMTS) are proposed to participate in sperm–egg adhesion (Russell et al. 2015).

The process of sperm–egg recognition and subsequent fusion of the two gametes during fertilization is the absolute requirement for sexual reproduction. Protein–protein recognition and interaction between egg and sperm are highly species-specific. A breakthrough in understanding of sperm–egg interactions at a molecular level occurred when the co-crystal complex of the sperm surface protein IZUMO1 and the egg protein JUNO (IZOMO1R) was solved (Aydin et al. 2016; Ohto et al. 2016). Knockdown of either protein produced infertile but otherwise healthy mice. The crystal structure and other studies reveal the importance of IZUMO1-JUNO interaction in sperm–oocyte membrane adhesion; however, underlying membrane fusion steps are more complex and require further consideration. Studies have shown that sperm proteins, SOF1, TMEM95 (Lamas-Toranzo et al. 2020), SPACA6 (Lorenzetti et al. 2014), and FIMP (Fujihara et al. 2020), are required for sperm–oocyte fusion in mice (Noda et al. 2020). Knockouts of these proteins produced mice that were completely infertile or severely subfertile.

Additional proteins continue to be identified as potential targets for male contraception. Cysteine-rich secretory proteins (CRISPS) are members of an abundant superfamily, widely expressed, including in the reproductive tract. CRISP2 is found in developing sperm head and tail and appears to be involved in acrosome reaction and normal flagellum waveform (Lim et al. 2019). ADAMS family proteins and the CAP (cysteine-rich secretory proteins, antigen 5, and pathogenesis-related 1 proteins) superfamily are rich targets for further study. Sperm complexes containing IZUMO and CAP members, such as GLIPR1L1, appear to play a role in optimal fertilization (Gaikwad et al. 2019). Further studies will continue to shed light on our understanding of sperm proteins and the complexity of sperm biology.

48.5 Sperm Production, Maturation, and/or Function

Testis-specific serine/threonine kinases (TSSK) are important for spermatogenesis and function. TSSKs belong to a six-member testis-specific serine/threonine kinase family in the human kinome: TSSK1, TSSK2, TSSK3, TSSK4, TSSK5 (pseudogene in humans and other primates), and TSSK6 (Salicioni et al. 2020). Male double TSSK1/TSSK2 knockout mice show phenotypic infertility (Xu et al. 2008; Shang et al. 2010). In addition, TSSK6 knockout mice resulted in a male infertility phenotype. This was due to severe impairment in motility and morphology of spermatozoa (Spiridonov et al. 2005). The ability to produce stable, enzymatically active recombinant human TSSK2 protein production represents a key achievement in progress toward targeting TSSKs in humans (Shetty et al. 2016). The potential importance of TSSK2 as a valid target for male contraception is observed for mutation screening in 494 men with azoospermia or severe oligozoospermia compared with 357 fertile controls. Analysis of the data indicates that a single-nucleotide polymorphism of the TSSK2 gene is associated with idiopathic male infertility (Zhang et al. 2010). In ensuing work, high-throughput screening of TSSK2 assays has produced potent inhibitors (<100 nanomolar) that show potential for targeting TSSKs with small-molecule inhibitors (Hawkinson et al. 2017; Salicioni et al. 2020).

The homeodomain-interacting protein kinase 4 (HIPK4) is a kinase that plays a role in later stages of sperm maturation. Validation as a male contraceptive target was established by HIPK4 knockout mice that exhibit a male infertility phenotype but are otherwise healthy. In the mouse model, HIPK4 is expressed predominantly in round and early elongating spermatids. HIPK4 mutation in sperm caused reduction in oocyte binding and incompetence for in vitro fertilization; however, intracytoplasmic sperm injection could produce viable offspring, indicating that sperm binding to the oocyte was the function disrupted by deletion of the kinase (Crapster et al. 2020).

48.6 Antibody, mRNA, and Immunocontraceptives

Immunocontraceptive approaches and the associated technology have been around for many years. In particular, application of monoclonal antibodies (mAb) against sperm antigen/protein has been a focus of research efforts for decades (Anderson et al. 1987; Norton et al. 2001). In a more recent study, a monoclonal antibody (mAb) technology platform directed against two sexually transmitted pathogens (HIV1 and HSV-2) has been developed as an antisperm mAb (Anderson et al. 2020). The antisperm mAb, H6-3C4, was originally isolated from the blood of a Japanese infertile woman. The antibody recognition site is a carbohydrate epitope on a glycosylphosphatidylinositol (GPI)-anchored glycoprotein, CD52g. This same epitope was found in abundance on the surface of human sperm (Isojima et al. 1987). It is reported that the investigators are seeking regulatory approval to conduct a phase I clinical trial with this contraceptive mAb (Anderson et al. 2020).

Synthetic mRNA technology has emerged as a novel approach to synthesize and deliver therapeutic antibodies. The application of this technology platform has been utilized to express PGT121, a well-established HIV-neutralizing antibody. Inoculation of the female sheep reproductive tract with synthetic mRNA for PGT121 resulted in high levels of antibody expression (Lindsay et al. 2020). This new paradigm of antibody production through mRNA technology is

another model for contraceptive development, potentially used by men or by women. In this scenario, mRNA for the sperm antigen is delivered to the female with the goal to produce antisperm antibodies in the reproductive tract. This technology is progressing rapidly as additional mRNA sequences encoding antisperm mAbs are being synthesized (Anderson et al. 2020). Recently, similar mRNA technology has been clinically validated and utilized to combat the COVID-19 pandemic, with synthesis and delivery of millions of doses of mRNA vaccines distributed worldwide.

48.7 Natural Products

Natural products encompassing herbal and traditional medicines have been around for centuries. Every culture, nation, and civilization have had, and continue to have, some form of natural product remedies for a variety of ailments. The application of natural products for dealing with these ailments ranges from minor headache to serious illnesses, such as hypertension, diabetes, heart disease, arthritis, blood disorders, and cancer. Natural products, and their derivatives, have also been used in contraception.

One notable natural product that was in the clinic as a non-hormonal male contraceptive is gossypol, the generic name given to polyphenolic aldehyde-containing compounds isolated from the cotton plant (Sato et al. 1985; Coutinho 2002). In the 1950s and 1960s, gossypol was implicated in causing infertility in rural areas of China when local residents began to press uncooked cottonseeds for cooking oil. Both women and men became infertile after consuming the raw untreated cottonseed oil. For women, fortunately, a gossypol-free diet resulted in eventual recovery of fertility. Sadly, some men did not recover from their infertility and impotency. The awareness that quantity and duration of cotton oil consumption influenced both the recovery rate and likelihood of permanent infertility led to the idea that gossypol could be used as a male contraceptive (Sato et al. 1985). In the 1970s and 1980s, large clinical studies of gossypol for male contraception were conducted in China. In total, more than 8000 subjects participated in these studies. Gossypol was highly efficacious; however, the narrow therapeutic window and frequent association with hypokalemia and irreversible sterility caused termination of further clinical development as a male non-hormonal contraceptive (Liu et al. 1987; Liu and Lyle 1987). In a recent animal study, ewes' consumption of a gossypol-rich diet during the critical period of fetal development and early neonatal life resulted in significant arrest of growth in offspring and reduced testicular weight in male offspring. In line with physical phenotype, physiologically, a significantly altered testis transcriptome and reduced testosterone levels were seen in male offspring. Many of these altered testis transcriptomes are implicated in sperm biology and testis development (Louvandini et al. 2020).

Extract from *Tripterygium wilfordii* Hook. f., commonly called Thunder God Vine, has been used in Chinese herbal medicine for many years. In particular, the refined extract was used to treat rheumatoid arthritis, chronic nephritis, chronic hepatitis, and various skin disorders for more than 50 years (Qian 1987; Zhen et al. 1995). Triptolide, a major component and the active ingredient of the extract, belongs to the class of chemicals called diterpene epoxides (Kupchan et al. 1972). By chemical nature, the epoxides are not stable. The discovery of contraceptive potential for triptolide was revealed when rheumatoid arthritis patients treated with the extract developed necrospermia or azoospermia (Yu 1983; Qian et al. 1986; Zhen et al. 1995). Studies in animal model showed extracts from *T. wilfordii* containing diterpene epoxides cause severe decrease in epididymal sperm count and motility in male rats (Qian et al. 1986; Matlin et al. 1993; Qian 1987; Zhen et al. 1995; Lue et al. 1998; Hikim et al. 2000; Huynh et al. 2000). In a similar manner to gossypol, prolonged exposure of *T. wilfordii* extract was associated with irreversible infertility in rats (Lue et al. 1998; Huynh et al. 2000). Additionally, triptolide's immunosuppressive properties could present a significant challenge from being developed as a systemic male non-hormonal contraceptive unless specificity for sperm effects could be developed (Ziaei and Halaby 2016).

An Indonesian traditional medicine from *Justicia gendarussa*, a commonly used plant in Jamu preparation, has been proposed as a male contraceptive in Papua. As with many other traditional medicines, this plant is used to treat a variety of ailments, including antibacterial, antifungal, anti-inflammatory, arthritis, and cancer, among other indications. Although, gendarussa is a commonly used term when referring to the extract of *J. gendarussa* for male contraception, gendarusin A and gendarusin B, flavonoid scaffold analogs, are thought to be active ingredients responsible for the contraceptive effect. It is proposed that the male contraceptive effect is mediated through a decrease in human sperm hyaluronidase activity (Widyowati and Agil 2018). A clinical trial was performed in Indonesia. Results have not been published, but some information was presented as an abstract and in media reports, claiming contraceptive efficacy from the extract of the *J. gendarussa* plant. The procedure involved ingestion of the extract by the male partner daily for at least 20 days before having intercourse during the female's ovulatory period. The authors claimed that the treatment was reversible within 30 days after the last treatment, with no serious adverse effects reported (Prajogo et al. 2014). Additional studies on its mechanism of action and evaluation of longer duration of use would be required prior to regulatory approval.

48.8 Sperm Transport–Physical Blockage

In the 1970s, India began development of a non-hormonal method to reversibly block the vas deferens. The procedure, called RISUG (reversible inhibition of sperm under guidance), encompasses injection of the solvent mixture of polymer styrene–maleic anhydride (SMA) and dimethyl sulfoxide (DMSO) into the vas deferens (Khilwani et al. 2020). The injected polymer is reported to form layers in the lumen and vas deferens as it precipitates, with positive charges and thus creating an acidic environment. Passage through this environment exposes sperm to ionic and pH stress that is thought to damage sperm, making them irreversibly ineffective. The procedure was used in rats, monkeys, and in first-in-human in 1989 in India. RISUG was evaluated in a phase III clinical trial in India with promising results (Sharma et al. 2019). However, further development was curtailed due to concerns regarding the studies' adherence to international standards. Reversibility of this technology is reported to be achieved by flushing the vas deferens with an injection of sodium bicarbonate–DMSO solution. The development of Vasalgel™, employing similar technology but using a mixture of SMA and styrene–maleic acid, showed rapid onset of azoospermia and durability that lasted 12 months in a rabbit model. Using this technology, contraceptive effectiveness was demonstrated in a NHP breeding study. The contraceptive effectiveness of male NHP lasted up to 2 years with a single injection of Vasalgel™ (Waller et al. 2016; Colagross-Schouten et al. 2017).

In the mid-1980s, percutaneous injection of polyurethane elastomer to vas lumen to form a plug in situ to achieve azoospermia was a clinical practice in China. The reported outcomes from these clinical studies suggest a safe and efficacious product (Zhao 1990; Zhen-Wen et al. 1992). Reversibility of this method through surgical removal of the plug from the vas deferens was demonstrated by return of sperm in the ejaculate. Return to fertility was further confirmed by reported successful birthing of offspring sired by the male partners (Sheng-Cai et al. 1992; Zhao 1990).

Similar to the other technologies, the development of a hydrogel, ADAM™, for injection to occlude the vas deferens to block the flow of sperm is in a preclinical stage. It is anticipated that this product will enter the clinic in 2021 as an investigational device.

48.9 Contraceptive Database

In 2018, a unique contraceptive–infertility target database–website was established and launched, encompassing a novel tool to identify male and female reproductive tissue-specific transcriptome and proteome targets. The database, Contraceptive Infertility Target Data Base (CITDBase: https://www.citdbase.org), is a downloadable curation of publicly available transcriptomic, proteomic, and immunohistochemistry data from human tissues mined from several databases. The degree of separation and distinction between reproductive and non-reproductive tissues in mining of these gene/protein targets of various databases was achieved through application of filters. These filters were designed in a stepwise fashion for catchment and delineation of listing of transcriptomic and proteomic reproductive tissue targets. This website provides a user-friendly interface and allows investigators to mine transcriptomic and proteomic resources to identify high-quality contraceptive/infertility targets. Additionally, numerous links provide direct and easy access to various databases enhancing information and data output.

48.10 Conclusion

Systemic non-hormonal natural products have been evaluated in humans. Thus far, no systemic non-hormonal reproductive tissue-specific contraceptive chemical entity has cleared the full preclinical assessments that stringent regulatory agencies require in order to approve a first-in-human clinical trial. However, the field of reproductive biology is bursting with exciting discovery and development research on mechanisms, identifying and validating targets and designing putative agents to regulate them. Many systemic non-hormonal male contraceptives are in early stages of development. In addition to protein targets and small-molecule inhibitors described above, active research is ongoing for novel non-hormonal contraceptive target discovery, validation, compound library screening, and chemistry. Numerous laboratories are engaged in discovery and optimization of small-molecule inhibitors. It is hoped that some of these systemic non-hormonal contraceptive agents would be successful in preclinical development and advance into clinical evaluation. Successful launch of these products will depend on further successful clinical studies that demonstrate safety, specificity, and effectiveness.

Hormonal male contraceptive agents have progressed to later-stage clinical trials and a combination of marketed drugs has been used off-label to demonstrate proof-of-concept male contraceptive efficacy. Many researchers conjecture that non-hormonal methods might have fewer side effects or a faster pathway to regulatory approval. A note of caution is needed in discussing development of a novel systemic non-hormonal male contraceptive method. These agents will be used by healthy individuals for long duration, so demonstration of safety is critical, especially since the user is not subjected to increased risk due to pregnancy. Current regulatory guidelines require large clinical trials to establish safety and effectiveness for new female methods. It is without a doubt that standards will be no less stringent for novel male methods. Additionally, unless there is investment

from pharma, it will be challenging for male contraceptive product to reach approval.

For male-centric contraception, condoms, withdrawal, and vasectomy are the only existing methods. To date, there are no systemic or nonsystemic male contraceptive agents approved for use. Introduction of an effective reversible male contraceptive method will address a major unmet need and has potential to significantly impact and reduce unplanned pregnancy rates. The socioeconomic impact of introducing an effective male contraceptive method could be invaluable. The concept of male contraceptive products other than condoms would afford choices and would provide an opportunity for men to better engage and manage in reproductive decision-making.

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Part XI

Law and Ethics in Reproductive Medicine

Legal Regulations in Andrology and Reproductive Medicine

49

Mark Makowsky and Jochen Taupitz

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Abstract

The chapter on "Legal regulations" presents the basic legal framework of medical practice before dealing with specific aspects. The general prerequisites of lawful medical treatment and medical liability are discussed first. Concerning prevention of pregnancy, the legal prerequisites of sterilization and castration are specifically discussed. Sections on the induction of pregnancies through techniques of assisted reproduction deal with their legal basis, the law of parenthood, and the assumption of costs by statutory and private health insurance. This is followed by explanations on preimplantation diagnostics, which has been governed by the Embryo Protection Act since 2011. An overview of the legal basis of research with embryos and embryonic stem cells follows a discourse on cryopreservation.

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49.1 General Part

49.1.1 Regulations on Medical Law

There is no special "physicians' law" that coherently regulates all legal issues connected with this subject area. In principle, the actions of physicians are to be measured against the law that applies to everyone. Fundamental value decisions are derived first of all from the **Basic Law** or Constitution **(GG)**. In particular, the patient's right to self-determination (Art. 2 (1) GG in conjunction with Art. 1 (1) GG) and to life and physical integrity (Art. 2 (2) GG) is anchored in constitutional law. These essential rights fundamentally forbid, for example, medical treatment against the patient's will. The physician can invoke freedom of profes-

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sion (Art. 12 GG) against state intervention. Most of the regulations governing medical law are found in the **German Civil Code (BGB)** and the **Criminal Code (StGB)**. Of particular importance from the BGB are the provisions on the treatment contract (§§ 630a et seqq. BGB) and the elements of tort (§§ 823 et seqq. BGB), which were included in the Patients' Rights Act (2013). These provisions regulate, for example, the obligation to pay damages in the event of treatment errors. In the Criminal Code, the offenses of a violation of private secrets (§ 203 StGB), bodily harm (§§ 223 et seqq. StGB), and negligent homicide (§ 222 StGB) are particularly relevant to medical practice.

In addition, there are some **special laws** for particular areas of regulation, e.g., the Embryo Protection Act (ESchG), the Transplantation Act (TPG), the Transfusion Act (TFG), the Act on Voluntary Castration and Other Methods of Treatment (KastrG), and the Medicines Act (AMG). For **professional law**, the most important sources of law are the Federal Medical Order (Bundesärzteordnung, BÄO), the chamber and health care profession laws of the federal states, and the statutes issued by the federal states' medical associations. The professional regulations of the chambers specify in more detail the duties to be observed by the physician in the exercise of his profession. A violation may result in disciplinary sanctions.

49.1.2 Prerequisites for Medical Treatment

Even if a medical treatment is carried out professionally and successfully, according to the established case law of the civil and criminal courts, the offense of **"bodily harm"** (§ 823 BGB, §§ 223 et seqq. StGB) is fulfilled (BGHZ 29, 46). This repeatedly irritates medical practitioners. In the legal literature, too, there have always been voices opposing the classification of a lege artis healing intervention as "bodily harm." However, the view of jurisprudence is supported by the fact that the patient must be able to decide in a self-determined manner about interventions by third parties in his or her legal interest. The physician must respect the patient's autonomous decision against (even necessary) treatment. Incidentally, the classification as "bodily harm" in the sense of the law does not imply either a moral or a legal unworthiness of the doctor's actions. Whether the "bodily harm" is lawful or unlawful must rather be examined in a second, independent step.

The medical measure is **unlawful** if it violates specific prohibitions (e.g., ESchG) or good morals in general (e.g., a cosmetic operation with disproportionately high risk). Apart from that, a medical measure is lawful if it is **medically indicated**, the sufficiently informed patient has **effectively consented** (informed consent), and the treatment is **carried out professionally** (lege artis).

Before carrying out a medical measure, the doctor must obtain the **patient's consent** (§ 630d BGB) and **inform the patient** of all relevant circumstances (§ 630e BGB). The capacity to consent is not bound to rigid age limits. A person is capable of giving consent if he or she has the natural capacity for insight and judgment and can comprehend the nature, significance, scope, and risks of the medical measure and to direct his or her will accordingly. In principle, adults are capable of giving consent. However, in individual cases, they may lack the capacity to consent due to temporary (e.g., due to drugs) or permanent disorders (e.g., due to mental illness). Conversely, **minors** can also effectively consent if they are capable of fully comprehending the significance and scope of the intervention and its consequences according to their mental and moral maturity (e.g., as a rule from the age of 16 in the case of minor interventions; see also OLG Hamm, NJW 2020, 1373 on the consent of a 16-year-old to an abortion). In the case of more severe interventions, however, the courts additionally require the consent of the parents as holders of parental custody (so-called co-consensus, see, for example, OLG Frankfurt a.M., MedR 2020, 282 on consent to circumcision). The physician is therefore well-advised to always obtain the **consent of the legal guardians** in cases of doubt. If the patient cannot consent, the consent of a person authorized to do so must be obtained. In the case of minors, this is usually both parents (§ 1629 (1) BGB); in the case of adults who are incapable of giving consent, it is the responsible guardian (§§ 1896 et seqq. BGB), whereby in serious cases the guardian must also obtain the approval of the guardianship court (§ 1904 et seq. BGB: life-threatening intervention, sterilization). The parents of a male child who is not yet capable of giving consent may also consent to a medically unnecessary circumcision conducted lege artis (§ 1631d (1) BGB). However, neither the child itself nor its parents can effectively consent to the sterilization of a minor (§ 1631c BGB). A guardian can only consent to the sterilization of a patient incapable of consent under strict conditions and with the approval of the guardianship court (§ 1905 BGB).

In principle, the following applies: The more severe the intervention, the higher the requirements for the capacity to consent.

Effective consent requires that the patient or the person authorized to give consent has been properly **informed** beforehand (§ 630d (2) BGB). In particular, the physician must inform the patient about the type, scope, implementation, expected consequences, and risks of the measure as well as its necessity, urgency, suitability, and prospects of success regarding the diagnosis or therapy (cf. § 630e (1) BGB). In the case of several medically equally indicated and

customary methods that may lead to substantially different burdens, risks, or chances of cure, the **alternatives** must also be pointed out. In detail, the scope and intensity of the information provided by the physician to elicit informed consent depend on the circumstances of the individual case. The physician is subject to particularly comprehensive duties of disclosure if he departs from the corridor of medical standards (so-called novel methods, cf. BGH, NJW 2020, 1358). The information must be provided **orally** by the physician himself or by a person who has the necessary training to perform the procedure; written information sheets may be referred to as a supplement. The information must be provided in a comprehensible and timely manner. In addition to informing the persons entitled to consent (parents, guardians, etc.), the essential circumstances of the intervention must also be explained to the patient who is incapable of giving consent, taking into account his or her stage of development, his or her ability to understand, and his or her well-being (cf. § 630e (5) BGB).

The physician's duty to provide **economic information** must be distinguished from the so-called self-determination **information**. To protect the patient from financial surprises and to make the patient aware of the economic consequences of his or her decision, the physician must inform the patient in advance about the expected costs of treatment if it can be assumed that they will not be fully covered by health insurance (§ 630c (3) BGB; recently BGH, NJW 2020, 1211). Information on self-determination is also to be distinguished from **"safeguarding information"** about all circumstances essential for the treatment to ensure that the patient's behavior is appropriate for the treatment (§ 630c (2) BGB).

If consent cannot be obtained for a medical **(emergency) measure** that cannot be postponed, e.g., due to the patient's unconsciousness or the inaccessibility of persons authorized to give consent, the measure may be carried out without consent if it corresponds to the presumed will of the patient (§ 630d (1) 4 BGB). Informing the patient in advance is also not required in this case (§ 630e (3) BGB). If possible, the physician must ascertain the patient's presumed will by questioning relatives.

Effective consent requires timely, comprehensible, and verbal informing the patient about the nature, significance, and scope of the intervention. If consent cannot be obtained, the principles of presumed consent apply.

49.1.3 Prerequisites for Medical Research

The admissibility requirements for measures of medical research are only expressly regulated by law for certain special **fields**, in particular by §§ 40 et seqq. AMG, §§ 19 et seqq. Medical Products Act (MPG) and §§ 31 et seqq. Radiation Protection Act (StrlSchG). For research projects interfering with the psychological or physical integrity of a human being or using bodily materials or data that can be traced to a specific human being, the professional regulations contain a supplementary standard based on **§ 15 Model Professional Regulation Code of the German Federal Medical Association (MBOÄ)**. According to this, participating physicians must ensure that, prior to the start of the research project, advice is given by the competent ethics committee on questions of professional ethics and professional conduct. This obligation to obtain advice under professional law does not apply if a vote of an ethics committee in accordance with the AMG or MPG is available. In the case of research on human beings, there is also an obligation under professional medical law to observe the ethical principles laid down in the World Medical Association's **Declaration of Helsinki** as amended by the 64th General Assembly in Fortaleza in 2013. Furthermore, publications of research results must disclose any business relationships between the physician and the sponsor and its interests.

The special laws on clinical trials(e.g., §§ 40 et seq. AMG—note, however, Regulation (EU) No. 536/2014 on clinical trials on medicinal products for human use, which will soon enter into force) distinguish between purely scientific (nontherapeutic) research on healthy subjects and therapeutic research on diseased subjects. The legislative evaluations underlying the regulations can also be used to assess other, not specifically regulated research projects on humans.

A **purely scientific trial** does not provide any (health) benefits to the healthy subject, but is solely aimed at gaining new knowledge for medical science. It is therefore permissible only under strict conditions according to § 40 AMG (Deutsch and Spickhoff 2014, marginal no. 1703 et seqq.). In particular, the risks and disadvantages—in relation to the benefits for the subject and the probable significance of the medicinal product for medical science—must be medically justifiable. Scientific trials on subjects in institutional care are generally inadmissible. Scientific trials on minors are only permitted under very limited conditions (§ 40 (1) 1 no. 4, (4) AMG). The subject must have consented in writing or electronically after being fully informed about the nature, significance, risks, and implications of the clinical trial (in the case of minor, the legal representatives and, if applicable, the minor himself/herself in accordance with his/her capacity to consent). Consent can be freely revoked at any time. Further requirements include management of the clinical trial by a physician with at least 2 years of experience, a previous pharmacological/toxicological trial, a scientific trial plan, the conclusion of a no-fault trial subject insurance policy, the consenting assessment of the competent ethics committee, and approval by the competent higher federal authority.

A **therapeutic trial**, on the other hand, is carried out on subjects with a relevant disease and, in addition to gaining new knowledge for medical science, also aims to benefit the subjects (saving their lives, restoring their health, and relieving their suffering). For such clinical trials, § 41 AMG contains a special provision that supplements and modifies the general requirements of § 40 AMG for the protection of trial participants (Deutsch and Spickhoff 2014, marginal no. 1720 et seqq.). According to this, the clinical trial may only be conducted if the use of medicinal product to be tested is medically indicated to cure or alleviate the subject's disease or if it provides a direct benefit to the group of patients suffering from the same disease. Further restrictions apply in this respect to minors and adults who are incapable of giving consent.

In clinical trials, the requirements of the Ordinance of the Application of Good Clinical Practice in the Conduct of Clinical Trials of Medicinal Products for Human Use (GCPV) must also be observed.

49.1.4 Medical Liability and Insurance

If the doctor culpably carries out a medical measure without provision of sufficient information and thus without effective consent (**error in informing**) or in violation of the physician's duty of care (**medical malpractice**) and the patient, therefore, suffers injury to body or health as a result, this triggers **contractual liability** according to § 280 (1) BGB as well as **tort liability** according to § 823 BGB for all material damage and additionally for compensation for pain and suffering according to § 253 (2) BGB. While the tort claim for damages is directed against the treating medical professional personally, the contractual liability affects only the patient's **contractual partner**, i.e., depending on the case, the owner of the individual practice, the treating physician in the case of a group practice, the owner of a joint praxis (e.g., GbR, GmbH), or the clinic. According to § 278 BGB, the contracting party is also liable for the misconduct of all support staff involved in the treatment (e.g., medical assistants, nursing staff, etc.). The treatment contract also gives rise to certain secondary obligations on the part of the physician. In addition to the aforementioned duties to provide informed consent (§ 630 BGB), there is, for example, the duty to **document** treatment by keeping a patient file and to record all measures and results essential for current and future treatment (§ 630f (1), (2) BGB). The patient has the right to inspect the patient file without delay (§ 630g BGB). The physician has a **duty of confidentiality**, the culpable breach of which may also result in sanctions, including criminal sanctions (cf. § 203 StGB).

In the event of an action for damages, each party must prove the circumstances favorable to him or her. Accordingly, the patient has the burden of proof for all the facts on which the claim is based (malpractice, physical injury or damage to health, and causation). The physician must prove that he obtained the patient's consent and properly informed him beforehand, i.e., that the "personal injury" was justified. However, due to the typical lack of evidence in medical malpractice suits, the courts have helped patients early on by **facilitating evidence.** This case law has now been codified for contractual liability in § 630h BGB. Thus, a fault on the part of the treating party is presumed if the patient is injured by a general treatment risk that is fully controllable by the treating party (e.g., functionality of medical technical devices, compliance with hygiene standards). Furthermore, there is a presumption that measures which are not documented in the patient's file, but which are required to be recorded, were not taken. If the patient proves a **gross treatment error,** i.e., a significant breach of medical standards, there is also a presumption that this error caused the injury to life, body, or health.

The doctor's risk of being exposed to claims for damages that threaten his or her existence due to a culpable error can be secured by taking out **professional liability insurance**. This also guarantees the injured patient a solvent debtor. In Germany, physicians in all federal states are obligated to take out liability insurance according to the state law or statute law. In the context of drug research on humans, the mandatory and strict liability insurance for volunteers under the AMG takes precedence over malpractice insurance.

In the context of medical treatment, the treating physician may be exposed to contractual and tortious claims for damages. Within the framework of contractual liability, the physician is also liable for any faults of support staff. The physician is obligated to take out malpractice insurance.

49.2 Special Legal Aspects

49.2.1 Preventing Pregnancies

49.2.1.1 Legal Framework

There are various methods of **contraception = pregnancy prevention,** e.g., sterilization or vasectomy, the use of intrauterine devices, the use of ovulation inhibitors, and soon perhaps the "male pill," although the development of such a contraceptive continues to be difficult. In principle, medical assistance with such forms of contraception is legally permissible if the general prerequisites of medical practice (Sect. 49.1.2) are fulfilled, i.e., the treatment is carried out in accordance with the standards and with the patient's informed

consent. However, castration and sterilization require further discussion.

49.2.1.2 Castration

Castration is a treatment by which the gonads of a person are deliberately removed or rendered permanently dysfunctional. Such a serious intervention, which, because of its permanence, is more drastic than sterilization, may not be carried out for the sole purpose of pregnancy prevention or for sociocultural reasons, even with the consent of the patient. Rather, castration is only permitted for purely medical reasons or—in accordance with the KastrG—to curb an abnormal sex drive. Castration of women or men for **purely medical reasons**, e.g., for the treatment of carcinoma, is not subject to special regulation by the KastrG and therefore is governed by general principles (Sect. 49.1.2; Laufs et al. 2019, § 128, marginal no. 3).

Voluntary castration of males against the effects of an abnormal sex drive, however, is governed by the **KastrG**. The law also applies to other medical treatments of men or women that are directed against the effects of an abnormal sex drive and could result in a permanent dysfunction of the gonads (e.g., medical treatments with estrogens or antiandrogens). According to § 2 KastrG, castration is only permitted under two alternative conditions: either the treatment must be necessary in order to prevent, cure, or alleviate serious illnesses, mental disorders, or suffering in the person concerned that are related to their abnormal sex drive (**medical indication**), or it must be indicated in order to counter the risk of criminal offenses (**criminological indication**). In addition, the person concerned must have reached the age of 25 and have given his or her effective informed consent (cf. § 3 KastrG). No disproportionate physical or psychological disadvantages may be expected from the castration. Furthermore, the treatment must be carried out by a physician according to the findings of medical science. Finally, an expert body must confirm that the prerequisites for castration and the consent of the person concerned have been met and that a medical member of the expert body has examined the person concerned and informed the person concerned, his guardian, or his caretaker. If the person concerned is **not capable of giving effective consent,** but can at least understand the immediate consequences of castration, his or her **consent** is sufficient after appropriate provision of information if the informed guardian also consents with the approval of the guardianship court. If, however, the person concerned does not even understand the immediate consequences of castration, it may only be carried out, even with the consent of the guardian, if there is a medical indication due to a life-threatening illness of the person concerned (in this respect without age limit). In this event, too, the consent of the guardian requires the **approval of the guardianship court,** which must hear the person concerned personally. Forced castrations are not compatible with the values of the Basic Law (**GG**), in particular **human dignity** (Art. 1 (1) GG) and the right to physical integrity (Art. 2 (2) GG) and are punishable as grievous bodily harm (§§ 223, 226 (2) StGB).

49.2.1.3 Sterilization

Sterilization is a procedure by which the vas deferens in men (vasectomy) or the fallopian tubes in women (tubal ligation) are interrupted or rendered nonfunctional to eliminate the ability to conceive. In contrast to castration, however, libido and the ability to have sexual intercourse are retained. There is no special law as there is for castration. Nor does the MBOÄ contain any statement on this subject.

As for any other surgical intervention, the individuals concerned must be informed about the nature of the intervention, its consequences, possible side effects and complications, and must have given their free consent. The general rules (Sect. 49.1.2) apply with the following provisions: forced sterilization is always inadmissible (and punishable) for the same reasons as forced castration. **Voluntary sterilization** is in any case permissible and not punishable if there is a medical, genetic, criminological, or social indication for it (cf. Laufs et al. 2019, § 127, marginal no. 4 et seqq., 24 et seqq.). According to case law of the Federal Court of Justice (BGHZ 67, 48; BGHZ 124, 128), sterilization **with the consent** of the person concerned is in principle lawful even without a special indication. Special circumstances are required for the sterilization to appear immoral and therefore unlawful (Deutsch and Spickhoff 2014, marginal no. 1052). With respect to the irreversible loss of reproductive capacity, **intensive efforts to obtain consent** and an **examination of the capacity to consent** is always necessary, taking into account the age, lifestyle, and mental constitution of the patient. The consent of the spouse is not legally required (BGHZ 67, 48).

Statutory health insurance only covers the costs of sterilization according to § 24b SGB V if it has become necessary due to illness.

The **sterilization of a minor** is prohibited without exception (even with the parents' consent) according to **§ 1631c BGB**. Persons who are under care but are still capable of giving effective consent themselves can only consent to their sterilization themselves. Consent cannot be substituted by the guardian. For persons who are permanently incapable of giving consent, i.e., who cannot comprehend the nature, significance, and scope of the medical measure, a guardian must be appointed to decide on consent to sterilization. The guardian can then apply to the guardianship court for approval of the sterilization under the strict conditions of § **1905 (1) nos. 1–5 BGB**, which must be met cumulatively. Sterilization may only be carried out two weeks after the court's approval has become effective. Accordingly, the court must issue a certificate to the guardian showing when the authorization

became effective. The treatment method that allows refertilization is always to be preferred.

49.2.1.4 Medical Liability

The physician is liable according to the general principles (Sect. 49.1.4) for culpable malpractice and errors in eliciting informed consent that result in an **unwanted pregnancy** that thwarts economic family planning (Müller 2003, p. 697; Ziegler 2021, p. 34). According to established case law, in such cases, the parents are entitled to a contractual claim for damages due to the **expenses for child support** (pecuniary loss). This also applies if the treatment contract was only concluded with one parent. This is because the spouse or nonmarital partner is included in the contractual scope of protection if the purpose of the contract is to avoid economic burdens caused by a child. The physician may be liable if **sterilization fails** due to an error (BGHZ 76, 249; BGHZ 76, 259), if the **necessity to conduct a semen analysis** when performing a vasectomy is not sufficiently explained (BGH, NJW 1995, 2407; cf. also OLG Hamm, VersR 2002, 1562; OLG Cologne, MedR 2015, 522), if the treatment with **contraceptives is carried out incorrectly** or if incorrect advice is given on this (BGH, NJW 1998, 155; BGH, NJW 2007, 989), or if an error occurs during **genetic counseling** (BGHZ 124, 128). If such a culpable medical malpractice or error in eliciting consent leads to the birth of an unwanted child, the physician must, according to established case law of the civil courts, compensate the child support as damage. Admittedly, the second Senate of the Federal Constitutional Court (BVerfGE 88, 203; BVerfGE 96, 409)—on the legal reform of abortion—had generally taken the view that the legal qualification of the existence of a child as a source of damage was out of the question on constitutional grounds (Art. 1 (1) GG) and that it was therefore out of the question to assess child support as damage. However, the Federal Court of Justice (BGHZ 124, 128; BGH, NJW 1995, 2407; BGH, NJW 1997, 1638) did not follow these—generally held—arguments. It was not the existence of the child, but only the parents' economic burden of child support that constituted the damage. The physician's obligation to compensate was limited to a purely pecuniary significance, without the child being thereby tainted or its right to life being called into question. The affirmation of such an obligation to compensate did not constitute a violation of the Constitution. The first Senate of the BVerfG (BVerfGE 96, 375) has endorsed this view. Accordingly, today the physician must expect liability for damages in such cases (Quaas et al. 2018, § 68 marginal no. 168). The obligation to pay compensation is limited to the amount necessary for the child's subsistence according to average requirements (BGHZ 76, 259). The cash maintenance plus an appropriate compensation for care is to be compensated (BGHZ 76, 259). In addition, the burden of an unwanted pregnancy constitutes **bodily harm to the mother** within the meaning of § 823 (1) BGB, which leads to a claim for compensation for pain and suffering (§ 253 (2) BGB) to compensate for the discomfort associated with the pregnancy and childbirth (BGH, NJW 1995, 2407).

Unsuccessful sterilization can lead to the physician's liability for damages in the amount of the parents' maintenance burden. At the same time, claims for compensation arise from the associated bodily harm to the woman.

49.2.2 Inducing Pregnancy (Assisted Reproduction)

49.2.2.1 Introduction: Diversity of Applicable Legal Norms

The aim of reproductive medicine is to treat unfulfilled desire to have children. Today, a wide range of treatment options is available to compensate for absent or limited biological functions. There is no comprehensive legal regulation of all legal issues arising in this context in Germany. Major areas are still governed by the Embryo Protection Act (ESchG), which is more than 30 years old and came into force in 1990. The law focuses on preventing abuses in the field of reproductive medicine and human genetics, and, in particular, on preventing the instrumentalization of embryos. According to the explanatory memorandum, the law is limited to countering possible abuses of new reproductive techniques and to providing for prohibitions under criminal law only where they appear indispensable for the protection of particularly high-ranking legal interests. With its more than 28 criminal offenses, the EschG contains a wealth of detailed regulations.

Insofar as assisted reproduction measures are not prohibited by the ESchG, a large number of scattered regulations are applicable: the Transplantation Act (TPG) is primarily relevant for the donation and removal as well as the transfer and processing of human germ cells (egg and sperm cells), including the preparation of these measures, since human germ cells qualify as tissue as defined in § 1a no. 4 TPG (BT-Drs. 16/3146, p. 23). Further regulations can be found in the TPG Tissue Ordinance (TPG-GewV), in the German Medicines Act (AMG, there in particular §§ 1–4, 4a, 20b-d, 21a, 63, 63a, 63i, 64, 67, 72b, and 142) and in the German Drug and Active Substance Manufacturing Ordinance (AMWHV, there in particular §§ 1, 2, 5a and, if applicable 5b). Also relevant are the Sperm Donor Register Act (SaRegG), the Gene Diagnostics Act (GenDG), the provisions of §§ 630a et seqq. of the German Civil Code (BGB) on the treatment contract (Sect. 49.1.2), and the professional regulations of the states' medical associations.

In addition, the German Medical Association was authorized pursuant to § 16b (1) TPG, in agreement with the Paul Ehrlich Institute as the competent higher federal authority, to establish the generally accepted state of knowledge of medical science regarding the removal of tissues and their transfer in guidelines supplementary to the provisions of a legal ordinance pursuant to § 16a TPG (= TPG-GewV). This includes, in particular, the requirements for the medical assessment of medical suitability as a sperm donor, the examination of sperm donors, and the collection, transfer, and use of human germ cells. Pursuant to § 16b (2) TPG, compliance with the state of the art of medical science (as required, inter alia, by § 8c (1) no. 2 TPG) is presumed if the guidelines of the German Medical Association have been observed. For the field of assisted reproduction, the 2018 guideline on the collection and transfer of human germ cells in the context of assisted reproduction is relevant (https://www.bundesaerztekammer.de/fileadmin/user_upload/downloads/pdf-Ordner/RL/Ass-Reproduktion_Richtlinie.pdf. Accessed 12/30/2020).

When performing assisted reproduction, a plethora of different and scattered regulations must be observed.

49.2.2.2 Sperm Donation in the Homologous and Heterologous System

In contrast to egg donation (Sect. 49.2.5), sperm donation is not prohibited by the Embryo Protection Act. Some special legal regulations exist on the performance of sperm donation and on the consequences under the law of parentage, in addition to the regulations mentioned in the introduction, (only) for medically assisted heterologous insemination, since the law regulating the right to know the parentage in the case of heterologous use of semen came into force on July 1, 2018. This article law, which does not apply to cases in which a woman performs the insemination herself (so-called private system or cup donation), contains the Semen Donor Registry Act (SaRegG) in Article 1, and amends the parentage law of the German Civil Code (BGB) in Article 2 to the effect that the sperm donor cannot be established as the father of the child in the case of a medically assisted heterologous insemination (performed after July 1, 2018) (§ 1600d (4) BGB). The SaRegG contains a wealth of obligations affecting the sperm donor and the intended mother, as well as obligations regarding data collection, data storage, and data forwarding to the German Institute for Medical Documentation and Information (**Deutsches Institut für Medizinische Dokumentation und Information—DIMDI**).

Case law has ruled (1989) that anonymous sperm donation is inadmissible on account of the **right of the child to know its own parentage**, which is derived from Article 2 (1) in conjunction with Article 1 (1) of the Basic Law (BVerfGE 79, 256 [268 et seq.]. In the case of fertilization carried out with donated sperm, it obliged the treating physician carrying out the treatment to provide the subsequent child with information about the identity of the sperm donor on request. Accordingly, the physician or the sperm bank not only had to establish this identity, but also had to ensure that the relevant information was carefully stored for at least 30 years since a 2007 amendment to TPG (previously 10 years according to medical professional law), so that it would actually be available in the event of a possible assertion of the right to information (Taupitz and Theodoridis 2018, 457 et seq.).

Since the SaRegG came into force, this **documentation period** is 110 years (§ 8). In addition, in the case of medically assisted sperm donation, the data must be forwarded to the German Institute for Medical Documentation and Information (DIMDI) and is managed centrally there. This is because a person who suspects that he or she has been conceived through heterologous use of sperm in medically assisted artificial insemination has a claim against DIMDI for **information** from the sperm donor register in accordance with § 10 (1) SaRegG. According to § 10 (2) SaRegG, the claim is aimed at obtaining knowledge of the stored personal data of the sperm donor; however, the person entitled to information does not obtain knowledge of any half-siblings (critically Taupitz and Theodoridis 2018, p. 462). After reaching the age of 16, a person can personally demand information; before that, parents as legal representatives can assert the claim on behalf of their child.

Requirements for the partnership status of the intended parents. In the past, a strict distinction was made between **homologous insemination**, i.e., the use of the sperm of the woman's own spouse/partner on the one hand, and **heterologous insemination**, i.e., the use of foreign sperm (in the case of non-united or same-sex or single persons) on the other, because only artificial insemination in the homologous system was considered permissible. This differentiation depending on the status of the intended parents is now obsolete as far as the basic permissibility of the measure is concerned (but see above the comments on the law regulating the right to know the parentage in the case of heterologous use of semen and Sect. 49.2.2.5 on reimbursement of costs). This is because in Germany there are no longer any legal regulations as to which group of people (single, married, living in a partnership, heterosexual, **homosexual**) for whom medically assisted fertilization may be performed (Taupitz 2021). Corresponding regulations are found neither in the Embryo Protection Act nor in the Sperm Donor Registry Act, where one would most likely assume such a regulation (Taupitz and Theodoridis 2018, p. 466). However, surrogate motherhood is prohibited under § 1 (1) 7 of the ESchG (Sect. 49.2.5). This means that homosexual couples in Germany are prevented from founding a family with a

child who is genetically linked to one of the partners, provided that the woman carrying the child is willing to permanently leave her child to a third party after birth.

There was indeed the (model) guideline of the German Medical Association on the performance of assisted reproduction (Bundesärztekammer 2006). According to point 3.1.1, "methods of assisted reproduction … should in principle only be used in married couples, taking into account the best interests of the child." It went on to say that "[they] may also be used in the case of a non-married woman, …. if the attending physician has come to the conclusion that the woman is living with a non-married man in a committed partnership and that this man will acknowledge paternity of the child thus conceived." In a commentary also included in the (model) guideline, which, however, was "intended to be merely an aid to interpretation of the above (model) guideline without sharing in its binding character", it was stated that "in the case of couples who are not married to each other, […] heterologous insemination should be approached with particular caution; it is explained by the aim of ensuring a stable relationship with both parents for the child thus conceived. For this reason, heterologous insemination is currently excluded in women who are not in a partnership or in a same-sex partnership." The consequence of these requirements was that sperm donation should not be used in female couples and should not be used in single women. The previous version of 1998 had declared medically assisted fertilization "not permissible" even more sharply in the case of single women and in same-sex relationships (3.2.3).

However, a guideline issued by the German Medical Association—contrary to the view expressed in the cited commentary on the (model) guideline—is not binding on physicians unless there is a corresponding statutory order. Only the law of the respective state's medical association is binding. In addition, the Board of the German Medical Association decided in 2018 that the (model) guideline was "irrelevant" (Bundesärztekammer 2008). The guideline on the collection and transfer of human germ cells in the context of assisted reproduction (Sect. 49.2.2.1), which was issued instead in accordance with § 16b TPG, does not comment on the group of persons for whom reproductive medical measures may be performed.

Artificial insemination is permitted regardless of whether the person concerned is living in a same-sex or opposite-sex marriage or partnership, or alone.

However, it should be expressly pointed out that medically assisted fertilization is always a decision of conscience on the part of the physician: According to § 10 ESchG, no one is obliged to carry out artificial insemination or to participate in it. The couple therefore has no corresponding claim against the physician.

Prerequisites for artificial insemination. Initially, the general rules on the admissibility of medical measures apply to the performance of artificial insemination (performance of a standard measure lege artis following sufficiently prior informed consent (see Sect. 49.1.2). § 4 (1) no. 1 ESchG also expressly requires the consent of both the woman and the man whose gametes are being used. At an early stage, the physician should work to ensure that the couple receives competent counseling about the possible psychological burden of the treatment and the conditions that are significant for the welfare of the child (Leopoldina 2019, p. 92 et seqq.).

§ 9 ESchG stipulates that only a **physician** may perform artificial insemination. However, this does not preclude the **delegation** of certain activities and thus a **division of labor**, e.g., with a reproductive biologist (Taupitz in: Günther et al. 2014, C. II. § 9, marginal no. 7). However, if a woman performs artificial insemination on herself contrary to the doctor's reservation, neither she nor the man whose sperm is used for artificial insemination will be punished under § 11 (2) ESchG (so-called personal ground for exclusion from punishment).

Despite the man's consent, no egg cell may be artificially inseminated with his semen after his death (§ 4 (1) no. 3 ESchG); the widow may also not demand the semen from the cryobank in order to use it to carry out a fertilization that is permissible abroad after the man's death (OLG München, NZFam 2017, 957 with comment by Biermann). The **prohibition of post-mortem fertilization** is based on the fear that the idea of being descended from someone who has already died at the time of creation could have an incriminating effect on the child's identity. However, it is disputed when artificial insemination after the death of the man exists within the meaning of the provision: In the opinion of the OLG Rostock, the point in time at which the sperm is introduced into the egg (impregnation of the egg) is decisive (OLG Rostock, MedR 2010, 874), because at this point in time the sperm is used, while the literature predominantly focuses on whether the man at the time of completion of fertilization ("nuclear fusion" in the diction of § 8 (1) ESchG) has died (because then the egg cell is "fertilized" after his death according to the wording of § 4 (1) no. 3 ESchG, see Taupitz in: Günther et al. 2014, C. II. § 4 marginal no. 35). The different opinions are particularly relevant if the egg cell was impregnated with the man's sperm during his lifetime, then stored cryopreserved as a 2-PN cell (PN = pronucleus) in the pronuclear stage, and only thawed after his death and further cultivated until the embryo is created. This is permissible in the opinion of the Rostock Higher Regional Court.

§ 3 S. 1 ESchG prohibits the selection of sperm cells intended for fertilization according to sex, which is intended

to prevent any entry into the manipulation of the genetic makeup of children. An exception applies only if the selection serves to protect the child from certain defined serious sex-linked diseases (§ 3 S. 2 ESchG).

Rule of Three versus German Middle Way. According to § 1 (1) no. 3 ESchG, anyone who "undertakes to transfer more than three embryos to a woman within one cycle" is liable to prosecution. This provision limits the maximum number of embryos that can be transferred to a patient per cycle, which have previously been created by IVF, to three. In addition, according to § 1 (1) no. 5 ESchG, whoever "undertakes to fertilize more oocytes of a woman than are to be transferred to her within one cycle" is punished. According to some opinions, the provision of § 1 (1) no. 5 ESchG merges with that of § 1 (1) no. 3 ESchG to form the so-called rule of three. Against the background that only three embryos may be transferred, it is concluded that the same numerical restriction also applies to egg fertilization. This can lead to the fact that in unfavorable cases, no successful fertilization takes place and thus no embryo can be transferred in the respective cycle. If necessary, the woman must be stimulated in several cycles to ultimately help her become pregnant.

In the opinion of the representatives of the **German Middle Way** (Taupitz and Hermes 2015), on the other hand, the reproductive physician may estimate, on the basis of a prognosis, with how many oocytes he must attempt fertilization depending on the individual characteristics of the couple, in order to obtain as many viable embryos as are to be transferred. If the prognosis is correct, he also has the intention from the beginning to avoid a (forbidden) "surplus" of viable embryos. However, since the prognosis of how many fertilized oocytes will not develop does not always prove to be accurate, more fertilized oocytes may be capable of development in individual cases, resulting in so-called surplus embryos. The German Middle Way has become widely accepted in reproductive medicine practice in Germany. In the meantime, several courts and public prosecutors have also come to the conclusion that a strict "rule of three" cannot be inferred from the Embryo Protection Act (BFH, judgment of May 17, 2017, VI R 34/15; OLG Hamburg, judgment of September 14, 2017, 9 U 71/15; Taupitz and Hermes 2015, p. 269 et seqq.).

Elective Single Embryo Transfer (eSET). Experience abroad shows that adequate pregnancy rates can be achieved with the transfer of only one embryo if the oocytes are observed for a few days after fertilization before transfer to the woman is carried out. The ESchG does not state that all eggs fertilized in one cycle must also be transferred and that selection is prohibited. Rather, it depends on the woman's will which and how many embryos are transferred to her—only the maximum number of three is specified. The reproductive physician is also obliged, among other things on the basis of the treatment contract, to inform the woman before the transfer about the degree of developmental capability of the respective embryos recognized by him (for example, on the basis of morphological observation of the embryos, e.g., using modern microscopic laboratory techniques such as "timelapse" microscopy); the same applies to the risks of multiple pregnancy associated with a transfer of several embryos. It is then her decision (cf. § 4 ESchG) how many of a maximum of three embryos and which embryos she wishes to have transferred. In doing so, the woman can certainly make a choice with regard to the developmental capacity; she can even freely refuse the transfer (Taupitz in: Günther et al. 2014, C. II. § 1 (1) no. 3 marginal no. 7). It is also undisputed that all stages may be examined that are not yet embryos within the meaning of § 8 ESchG; this applies, in particular, to the pronuclear stage. Furthermore, it is undisputed that, on the basis of morphological considerations, entities that do not develop ("arrested" embryos) may also be removed from culture. This does not only follow from the fact that the ESchG only protects living embryos. Rather, and in particular, it is not reasonable for the woman to have an "embryo" transferred that cannot lead to implantation.

However, insofar as it is planned from the outset, in the context of Eset, to fertilize more oocytes than to transfer embryos later, this is not permissible due to the associated "stockpiling" in accordance with § 1 (1) no. 5 ESchG.

49.2.2.3 Law of Parenthood

In legal terms, the child created by artificial insemination is completely equal to a child conceived by natural means. Even in the case of sperm donation, the family law relationships between the parties are governed by the provisions of the Civil Code on parentage (§§ 1591–1600d of the Civil Code). The mother is the woman who gives birth to the child (§ 1591 BGB); it is not possible to challenge maternity. If the mother of the child produced by sperm donation is married, her husband becomes the legal father of the child (§ 1592 no. 1 BGB). If she is not married, her partner becomes the legal father if he acknowledges paternity with the mother's consent before or after the birth (§§ 1592 no. 2, 1594, 1595 BGB). In both cases, the parents themselves can no longer challenge paternity if procreation by sperm donation took place with their consent (§ 1600 (4) BGB). In this case, only the child can challenge paternity.

If there is no legal paternity of another man (any more), the donor can become the child's legal father either by formally acknowledging paternity with the mother's consent or by being judicially established as the father (§ 1600d BGB). However, judicial determination of paternity is not possible if the child was conceived after July 1, 2008 by medically assisted artificial insemination in a health care facility using heterologous semen provided by the donor to a collection facility according to the Semen Donor Registry Act (§ 1600d (4) BGB).

If the sperm donor has been established as the father of the child or has effectively acknowledged paternity, the child is entitled to **claims for maintenance and inheritance** against the donor. The donor cannot counteract these claims in his external relationship with the child, but only through appropriate release agreements with the intended parents, the natural mother, and the physician, i.e., within the internal relationship (Taupitz and Schlüter 2005).

In order to be able to assert its rights, the child must know who its genetic father is. Since the SaRegG came into force, the child born after a medically assisted heterologous insemination (performed after July 1, 2018) has a **claim to information** against **DIMDI**. Other children may have a **claim** against the mother for **naming the natural father** (BVerfG, NJW 1997, 1769). In addition, they have a claim against the physician who performed the artificial insemination for information about the identity of the sperm donor.

If a sperm donation is used in the case of a **female couple** or a **single woman**, the legal mother is, as always, the woman who gave birth to the child (§ 1591 BGB). In any case, the father can only be a man. Also, legal co-motherhood, e.g., by acknowledgement, is not possible in Germany so far. This means that if the woman is not married to a man and no man acknowledges paternity and the paternity of the sperm donor is not established by a court, the child has only one parent and only one family line. At best, the child can obtain a second parent after birth through the complex procedure of (stepchild) adoption (also on the part of the mother's partner). These family law consequences do not argue against the admissibility of assisted fertilization in the case of an expected **solo motherhood** (Taupitz 2021). However, it should then be foreseeable that disadvantages associated with solo motherhood can be compensated as far as possible by the mother's own arrangements or by third parties (friends, parents of the mother) who share responsibility in caring for the child. This would then also have to be the subject of psychosocial counseling (Leopoldina 2019, p. 62).

49.2.2.4 Physician Liability

According to general principles, when performing artificial insemination, the physician is liable for any culpable treatment error and any culpably omitted or insufficient information, insofar as this results in damage to the wife, husband, or sperm donor. **Culpable errors** in treatment may include, for example, damage to sperm or ova or fertilization not performed lege artis or implantation of a recognizably damaged embryo without appropriate informed consent. Liability may also be considered if the physician performs artificial insemination without the necessary consent of all parties involved. In the case of heterologous insemination, the physician may also be liable if he violates the child's **right to information on his parentage**, e.g., by destroying treatment records or failing to fulfill his obligations to forward the data to DIMDI (see Sect. 49.2.2.2). Liability toward the sperm donor may be considered if anonymity is assured and the physician is subsequently forced to disclose the name of the donor to the child.

What constitutes the damage to be compensated in each case depends on the specific individual case. In the case of culpably caused **failure of artificial insemination**, the costs of the medical treatment and the loss of earnings incurred in vain are to be considered first. The physician may be subject to an obligation to pay or compensate for child support in the case of fertilization measures performed without sufficient consent, as well as in the case of a violation of the child's right to information regarding its biological father by preventing the establishment of paternity. If a child's **disability** is due to a culpable medical error during artificial insemination, the physician is liable for the additional needs caused by the disability (BGHZ 86, 240; "wrongful birth", see Bach 2019). In cases of culpably failed treatment, claims for compensation for pain and suffering may arise in addition to claims for damages. According to case law of the Federal Court of Justice, these also come into consideration in the case of damage to or destruction of body components that are removed from the body but are later to be returned to it, such as in the removal of oocytes for extracorporeal fertilization, but also in the case of destruction of sperm that was cryopreserved to maintain the ability to reproduce (BGH, NJW 1994, 127; critical of the dogmatic derivation Taupitz 1995).

Medically assisted fertilization in a **female couple** or in a **single woman** does not in itself give rise to a claim for damages against the reproductive physician unless, according to general principles, there has been an error in informed consent or treatment. Assisting in the procreation of children of single women or female couples is not an act that gives rise to an obligation to pay damages (Taupitz 2021).

49.2.2.5 Cost Coverage by Private and Statutory Health Insurance

In recent years, the highest courts have repeatedly had to deal with the question of whether and to what extent the costs of reproductive medical measures are reimbursable under **private health insurance (PKV)** and **statutory health insurance (GKV)** (see Becker and Kingreen 2020, commentary on § 27a SGB V; Knispel, in: BeckOK 2020, commentary on § 27a SGB V). Reimbursement of treatment costs for assisted reproduction measures continues to be shaped by different insurance principles. In statutory health insurance, the principle of body-relatedness applies, i.e., statutory health insurance generally pays for treatment(s) of the body of the insured person as well as extracorporeal treatment measures (BSG, NJW 2005, 2476), regardless of who or what is the "causer" and regardless of the presence of an illness. In private health insurance, on the other hand, the

causation principle applies. Here, an illness present in the insured person (inability to procreate or conceive) justifies entitlement to benefits. However, the entirety of medical measures, including co-treatment of the spouse, then counts as medical treatment (BGH, NJW 2004, 1658). The prerequisite for cost coverage is in any case that the measure is permissible under German law, even if it is performed abroad (BGH, BeckRS 2017, 114753; BSG, NJW 2002, 1517). In particular, the ESchG must be observed.

According to **§ 27a of the German Social Code (SGB) V**, the statutory health insurance funds cover the costs of medical measures to induce pregnancy under certain conditions. The conditions for benefits for measures to induce pregnancy are conclusively regulated in § 27a SGB V. The provision was amended by the Health Care Modernization Act effective from January 1, 2004. The obligation of the GKV to provide benefits was significantly restricted at the expense of the insured, in that only three instead of four attempts are now paid for and only 50% of the costs of the measure approved with the treatment plan are covered instead of 100%. Furthermore, **age limits** were introduced, according to which the couple must be at least 25, the woman no older than 40 and the man no older than 50. § 27a SGB V is concretized by the **Guidelines of the Federal Joint Committee (Gemeinsamer Bundesausschuss—G-BA)** on artificial insemination from 1990, the last amendment of which became effective in 2017 (BAnz AT 01.06.2017 B4). The guidelines specify further requirements for cost coverage. The G-BA may also define indications for individual measures, although it is not required to define the limits in such a way that a method is available in every individual case (BSG, BeckRS 2011, 76087).

In order to alleviate **funding hardships**, the Federal Ministry for Family Affairs, Senior Citizens, Women, and Youth has launched a federal initiative "Help and Support for Involuntary Childlessness," which grants a federal subsidy to the states within the framework of the "Guideline on the Granting of Allowances for the Promotion of Assisted Reproduction Measures," if the states also financially support infertility treatment. This is now the case in a number of states. In addition, according to the GKV-Versorgungsstrukturgesetz (SHI Care Structure Act) of December 22, 2011, the health insurance funds may finance more than three attempts on their own and/or to reduce the couples' own financial contribution.

In addition to **insemination** (insemination in the woman's body in a spontaneous cycle or after hormonal stimulation), **in vitro fertilization** with embryo transfer and **intratubal gamete transfer**, the benefits catalog of the guidelines now also includes **intracytoplasmic sperm injection (ICSI)** after the BSG held the exclusion previously included to be invalid (BSGE 88, 62). § 27a (4) SGB V additionally creates an entitlement to (full funding of) cryopreservation if there is a risk of infertility due to disease-related treatment by means of a germ cell-damaging therapy and **cryopreservation** of egg or sperm cells or germ cell tissue is necessary to enable future artificial insemination using the cryopreserved egg or sperm cell or germ cell tissue (in more detail Knispel, in: BeckOK 2020, § 27a (4) SGB V § 27a marginal no. 32b et seqq.). The Federal Joint Committee shall specify the details in guidelines (§ 27a (5) SGB V).

Preimplantation diagnostics and **polar body diagnostics** to exclude oocytes affected by a genetic defect from embryo transfer are not included in the services covered by § 27a SGB V, since only medical measures to bring about a pregnancy are financed (BSG, BeckRS 2015, 66531; BSG, BeckRS 2015, 72580).

§ 27a SGB V requires that the measure must be necessary as determined by a physician, i.e., there must be a **medical indication** for the measure. Priority is given to measures of medical treatment to overcome infertility. Only if such measures do not offer a (sufficient) prospect of success or are impossible or unreasonable (and, moreover, the desire for a child "unintentionally" cannot be realized naturally), measures according to § 27a SGV come into consideration (BSG, NJW 2005, 2476). In addition, there must be sufficient prospect that a pregnancy can be brought about.

Cost coverage can only be considered for **married couples** and only if **their egg and sperm cells** are used exclusively. Before the procedure is carried out, the spouses must seek advice on the treatment from a physician who is not carrying out the treatment himself, taking into account the couple's medical and psychosocial aspects. The physician will then refer the spouses to a physician or facility that has been granted authorization to perform artificial insemination in accordance with § 121a SGB V. Furthermore, the insured persons must be within the age limits specified in § 27a (3) SGB V (already shown above). Since involuntary childlessness is decisive for the insured event according to § 27a SGB V, a claim for benefits also exists if neither of the spouses is demonstrably ill and the couple's infertility cannot be medically explained (cf. Knispel, in: BeckOK 2020, SGB V § 27a marginal no. 2).

According to the BVerfG ruling of February 28, 2007 (FamRZ 2007, 529 et seqq.), it is compatible with the Basic Law for the legislature to limit the SHI obligation to provide benefits to persons who are **married to each other** in § 27a (1) no. 3 SGB V (criticized by Taupitz 2021, applicable also to same-sex couples and single women). An extension of benefits to unmarried couples is also not possible through a regulation in the statutes according to § 11 (6) SGB V (BSG, NJW 2015, 1903). The exclusion of heterologous in vitro fertilization does not constitute a violation of either constitutional or European Community law (BSG, NJW 2002, 1517). The age limits of 40 years for women and 50 years for men introduced in 2004 in § 27a (3) SBG V are also considered

constitutional by case law (most recently BSG, NZS 2008, 256; BSG, BeckRS 2009, 61854).

If all the requirements of § 27a SGB V are cumulatively met, the health insurance funds must bear 50% of the costs for artificial insemination within the body of their insured member as well as for extracorporeal treatment measures. According to case law, **the fifty percent rule** is constitutional (BSG, BeckRS 2007, 48148; a constitutional complaint was not accepted by the BVerfG: BVerfG, BeckRS 2009, 32364). There is no obligation to pay benefits for measures performed directly and exclusively on the body of the spouse who is not insured with the health insurance fund (BSG, NJW 2005, 2476). Insofar as both spouses are legally insured, each has a claim against his or her statutory health insurance for payment of the costs of the nonpersonal measures. Since, of course, the benefits are to be provided only once, as a result the costs must be shared. In the absence of a legal solution, the practice follows a recommendation of the leading associations that the woman's insurance company bears all costs of extracorporeal measures (Knispel, in: BeckOK 2020, SGB V § 27a marginal no. 30). The number of pregnancies induced by medical measures of § 27a SGB V is not explicitly limited. Since § 27a (1) no. 2 SGB V only limits the number of unsuccessful therapy attempts, there is a renewed entitlement to benefits under § 27a SGB V after a successfully induced pregnancy (Knispel, in: BeckOK 2020, SGB V § 27a marginal no. 3a).

Reimbursement of costs in **private health insurance** is not based on a statutory claim, as is the case with statutory health insurance, but on a **contractual claim** against the private health insurer. According to § 1 (2) of the Model Conditions for Health Insurance (currently in the 2009 version; MB/KK) following § 192 (1) of the German Insurance Contract Act (VVG), the insured event is the "medically necessary treatment of an insured person due to illness or the consequences of an accident." After the Federal Court of Justice (BGH) first decided in its ruling of December 17, 1986 (BGH, NJW 1987, 703) that organically induced sterility is an **illness according to § 1 MB/KK** and that homologous in-vitro-fertilization is a **medically necessary treatment**, the costs for corresponding measures are covered by private health insurance if all prerequisites are met. The "**causation principle**" applies to privately insured persons. According to this, an illness within the meaning of the conditions only exists in the case of the spouse who is incapable of reproduction (BGH, VersR 1998, 87). A prerequisite for reimbursement by private health insurance is also that artificial insemination is medically necessary. Medical necessity is assessed by taking into account the prospects of success of artificial insemination and is affirmed if there is a **probability of success** of 15% (BGH, VersR 2005, 1673; in more detail Waldkirch 2020, 325). The number of attempts is not limited per se in the area of private health insurance, but numerous unsuccessful earlier treatment attempts reduce the individual prospect of success (BGH, VersR 2005, 1673). The BGH has also clarified that even after the birth of a child due to artificial insemination, the illness continues to exist within the meaning of the insurance conditions (BGH, VersR 2005, 1673). As far as the medical prospect of success of an IVF/ICSI treatment is given, in contrast to the GKV, in the PKV there is, in principle, no rigid border concerning age of the future parents (BGH, VersR 2005, 1673). However, it is still disputed whether the claim against the private health insurer necessarily requires that the partners are **married** (cf. on the state of opinion Waldkirch 2020; Taupitz 2021); in view of the increasing social acceptance also of nonmarital cohabitation, a refusal of cost coverage is no longer up-to-date (OLG Karlsruhe, BeckRS 2017, 127991; OLG Hamm, NJW-RR 2017, 284 (286); LG Berlin, r + s 2004, 203; LG Dortmund, VersR 2008, 1484; Taupitz 2021). If the prerequisites are met, the private health insurance must cover the entire costs of artificial insemination. This includes both the costs for treatment of its policyholder and the costs of the policyholder's partner (Waldkirch 2020, p. 324). It does not matter whether there are also reasons for the childlessness of the policyholder's partner if the artificial insemination is medically necessary solely due to the undisputed sterility of the policyholder (BGH, VersR 2010, 1485; BGH, VersR 2006, 1351). **Polar body diagnostics** and **preimplantation diagnostics**, which serve to avoid the transmission of a genetic mutation to the embryo, are not considered medical treatment of the insured person, unless they are used to identify genetic defects that may trigger or increase a fertility disorder of the insured person and thus increase the probability of a successful pregnancy (Waldkirch 2020, p. 324).

For cost coverage under **civil service law aid** on the basis of the corresponding aid regulations of the states or the federal government, see the information sheet of the Federal Office of Administration [as of September 29, 2020], available at https://www.bva.bund.de/SharedDocs/Downloads/DE/Bundesbedienstete/Gesundheit-Vorsorge/Beihilfe/Merkblaetter/kuenstliche_befruchtung.pdf?__blob=publicationFile&v=5. Accessed on December 30, 2020) s. BVerwG, NVwZ-RR 2014, 240; BVerwG, NVwZ-RR 2011, 193; VGH Kassel, Judgment of September 24, 2019, 1 A 731/17; OVG Münster, BeckRS 2012, 48697; OVG Münster, NVwZ-RR 2013, 193; OVG Koblenz, NJOZ 2009, 4228; VG Regensburg, BeckRS 2013, 58180; Beckhove 2008, p. 72 et seqq.

Reimbursement of treatment costs for reproductive medical procedures in Germany is still characterized by inequalities between statutory and private health insurance as well as by gaps in financing.

49.2.3 Preimplantation Genetic Diagnosis

Preimplantation genetic diagnosis (PGD) is the targeted genetic testing of embryonic cells after IVF prior to embryo transfer. It is used to clarify the suspicion of a serious genetic disease or chromosomal disorder. A positive test result should give the couple the opportunity to withdraw their consent to embryo transfer. Without the woman's consent, the physician may not transfer the remnant embryo.

After the Federal Court of Justice ruled in a judgment of July 6, 2010 that PGD was not prohibited by the Embryo Protection Act in force at the time, the legislature inserted a regulation of PGD into the ESchG effective from December 8, 2011 (§ 3a). § 3a (1) ESchG contains a fundamental **prohibition of PGD** (for exceptions see below) and at the same time a definition of PGD: According to this, PGD is the genetic examination of embryonic cells in vitro before the intrauterine transfer of the embryo. The cells of an embryo also include the cells of the trophectoderm, from which the placenta is later formed (after nidation) (BVerwG, judgment of December 2, 2020, 3 C 6.19; Taupitz in: Günther et al. 2014, C. II. § 3a marginal no. 17).

§ 3a (2) ESchG provides for two **exceptions** to the general ban on PGD; if these conditions are met, PGD is not unlawful and therefore not punishable. Paragraph 2, sentence 1 regulates the case where, due to the genetic disposition of the woman from whom the egg cell originates or the man from whom the sperm cell originates, or both, there is a **high risk of a serious hereditary disease** for their offspring. With regard to the "serious hereditary disease," the legislator has deliberately refrained from a catalog of specific diseases, apparently in order to avoid discrimination and stigmatization of persons living with certain clinical diseases and because the weighting of a disease or disability, as well as the assessment of the ability to face a life with it, cannot be made in an objectively universal way. According to the explanatory memorandum to the law, a hereditary disease is "serious" "in particular" if it "differs significantly from other hereditary diseases due to a low life expectancy or severity of the clinical picture and poor treatability" (BT-Drucks. 17/5451, p. 8). In contrast to § 15 (2) Genetic Diagnostics Act (GenDG), according to which a prenatal genetic examination in the course of a pregnancy may not be directed at a **late manifesting disease**, § 3a ESchG does not prohibit PGD with regard to such a disease. With regard to the disease in question, there must be a "high genetic risk" in the couple to be treated. According to the explanatory memorandum, the risk must be related, on the one hand, to the usual risk of the population living in Germany. "On the other hand, the probability of occurrence is to be assessed genetically according to the laws of transmission and combination of hereditary predispositions. A probability of 25–50% is estimated as a high risk" (BT-Drucks. 17/5451, p. 8). The admissibility of PGD must be decided in each individual case. If it is questionable whether the hereditary disease is already sufficiently serious because of the genetic disposition of one of the parents, further aspects connected with this disposition must also be taken into account, such as the fact that the parents already have a child with the serious hereditary disease or that the woman has had an abortion after prenatal diagnostics and medical consultation in accordance with § 218a (2) StGB, or that the parent with the genetic disposition has the disease himself (BVerwG, judgment of November 5, 2020, 3 C 12.19).

§ 3a (2) 2 ESchG contains the second exception to the general prohibition of PGD. According to this, PGD is not unlawful if it is carried out to determine serious damage to the embryo that is highly likely to lead to a **stillbirth or miscarriage**.

A further prerequisite for both exceptions is the written **consent** of the woman from whom the egg cell originates. It is also necessary for the requirements of § 3a (2) ESchG for the case to be examined and approved by an ethics committee.

The jurisdiction of the ethics committee depends on which center is designated to perform PGD.

The performance of PGD has been permitted in Germany since 2011 under very narrow conditions.

49.2.4 Cryopreservation

§ 9 ESchG places the cryopreservation of embryos and pronuclei (= oocytes after penetration of the sperm cell, but before nuclear fusion) pursuant to a **physician's reservations** because of the associated dangers for the embryo, but does not otherwise regulate the conditions of cryopreservation as such. However, it follows from § 1 ESchG that the further cultivation of such a pronucleus may only take place for the purpose of transfer to the woman from whom the egg originates and only with the consent of both parents (BayObLG, judgment of November 4, 2020, 206 St RR 1459–1461/19; Taupitz 2019). For legal protection, an agreement should be made with the parents that pronuclei may neither be cryopreserved nor further cultured after cryopreservation if only one parent requests this or if one parent is deceased. Cryopreservation of sperm and testicular tissue is permitted (with appropriate consent) without other restrictions. Cryopreservation is performed by specialized companies or institutions on the basis of a custody contract concluded with the patient. This contract must regulate the duration of preservation and the question of what is to be done with the material after the end of the preservation period. Neither is regulated by law; both storage for an unlimited period and active dis-

carding are permissible (on leaving embryos to die or actively discarding them, cf. BGH, NJW 2010, 2672 [2676 marginal no. 38]). In the event of culpable destruction or damage to the preserved material, claims for compensation for pain and suffering may arise anyway if the preservation was intended to compensate for lost reproductive capacity (BGH, NJW 1994, 127; Taupitz, NJW 1995, 746).

According to case law of the BSG, cryopreservation of eggs and sperm preserved for the possible repetition of an attempt at artificial insemination is not covered by statutory health insurance funds, so that the costs must be borne by the patient (BSG, BeckRS 2000, 41117). The situation is different according to § 27a (4) SGB V if there is a risk of infertility due to a disease-related treatment by means of germ cell-damaging therapy and cryopreservation of egg or sperm cells or germ cell tissue is necessary to enable future artificial insemination with the help of the cryopreserved egg or sperm cell or germ cell tissue (on the reimbursability under civil service law aid, see BVerwG, BeckRS 2007, 20035).

49.2.5 Egg Donation, Embryo Donation, and Surrogate Motherhood

Egg donation. Egg donation (more precisely: the involvement of a reproductive physician in egg donation; neither the donating woman nor the recipient is punished: § 1 (3) ESchG) is prohibited under the ESchG. This is to prevent so-called split motherhood, where the genetic mother and the gestating mother are not identical. The reason given was primarily the welfare of the child. There were fears of psychological conflicts or impairments as well as problems in determining the child's identity (BT-Drs. 11/5460, p. 7 et seq.). However, so far empirical research has been unable to prove any substantial difference in the evaluation of so-called split paternity and maternity by children conceived in this way (Leopoldina 2019, p. 66), so that the ban on egg cell donation can hardly be justified (also from a constitutional point of view). Specifically, the ESchG prohibits transferring to a woman a foreign **unfertilized** egg (§ 1 (1) no. 1 ESchG), artificially **fertilizing** an egg for a purpose other than to induce a pregnancy in the woman from whom the egg originates (§ 1 (1) no. 2 ESchG), or **removing** an embryo from a woman before completion of its implantation in the uterus in order to transfer it to another woman (§ 1 (1) no. 6 ESchG). The prohibition also includes the thawing and further cultivation of an impregnated oocyte if at this point in time of thawing and further cultivation there is the intention to transfer the subsequently developing embryo to another woman (BayObLG, judgment of November 4, 2020, 206 St RR 1459–1461/19; Taupitz 2019, p. 339).

In contrast, there is **no** ban on transfer to another woman in the event that extracorporeal fertilization occurs but the (already existing) embryo can no longer be transferred to her, e.g., because of the death of the woman from whom the egg originated or because of her change of mind. In such a case, it is permissible to transfer the embryo to another woman in order to save it, if she wants to carry the child to term as her own (Taupitz and Hermes 2015, p. 1802 et seq.). However, the procedure of this so-called **embryo donation** (from the perspective of the donating parents) or **embryo adoption** (from the perspective of the receiving parents) is completely unregulated (proposals on this at the German Ethics Council [Deutscher Ethikrat 2016]).

Surrogate motherhood. According to § 13a of the Adoption Placement Act (AdVermiG) in the version applicable from January 1, 2002, a surrogate mother is a woman who, on the basis of an agreement, is willing to undergo artificial or natural insemination or to have an embryo not originating from her transferred or otherwise to carry it to term and to leave the child to third parties after birth. The law prohibits, on penalty, surrogate mother placement and punishes public advertising of it (e.g., through newspaper advertisements or newspaper reports) as well as the search as a misdemeanor. § 1 (1) no. 7 ESchG prohibits artificial insemination or the transfer of a human embryo to a woman who is willing to leave her child permanently to a third party after birth.

Both egg donation and surrogate motherhood are prohibited. Embryo donation is permitted only if a circumstance occurs after the embryo has been created that prevents the embryo from being transferred to the woman from whom the egg was derived.

49.2.6 Research with Embryos and Embryonic Stem Cells

The **ESchG** and the **Stem Cell Act** (StZG), which came into force in 2002, aim to protect the right to life and the human dignity of embryos both preventively and repressively by establishing prohibitions with restrictive exceptions and related criminal provisions. However, they differ, among other things, in their **scope of application**: while the ESchG, according to the prevailing opinion (OLG München, NZFam 2017, 957; Frister 2016), also penalizes acts of aiding and abetting as well as complicity, provided that the corresponding act (e.g., removal of the oocyte with the knowledge that it will later be donated) is committed in Germany and the main act (transfer to another woman) violates the ESchG, the StZG (since amendment of the law in 2008) only covers the **import** of embryonic stem cells to Germany and the use of embryonic stem cells that are **located in Germany**.

The provisions of the ESchG are characterized by the view that human life begins with the fusion of egg and sperm, that from this point on it already enjoys the protection of the Basic Law and that its human dignity is to be respected (Art. 1 and 2 GG). However, the Basic Law does not contain any explicit statements on the constitutional protection of human embryos. To date, the Federal Constitutional Court has also only dealt with the constitutionally mandated protection in connection with abortion issues, i.e., in relation to embryos and fetuses in utero. In doing so, the court has expressly left open the question of whether "human life already comes into being with the fusion of egg and sperm cell" (BVerGE 88, 202 et seq., marginal no. 151). Above all, the court very clearly emphasized that "the legislature is not in principle obliged to take the same measures of a penal nature to protect unborn life as it considers appropriate and necessary to safeguard born life" (BVerfGE 39, 1 et seqq. marginal no. 45). Thus, in questions of embryo protection in vitro, the legislature **could** certainly provide for **different provisions** than those contained in the ESchG of 1990.

Irrespective of the question, which is still disputed in the constitutional literature, as to what value is attached to human dignity (Art. 1 (1) GG) and the right to life (Art. 2 (2) GG) of the embryo in vitro (for the now boundless literature, see Taupitz, in: Günther et al. 2014, § 8 marginal no. 4), the ESchG prohibits both the production of embryos for **research purposes** and any use of permissibly produced embryos for the purposes of **consumptive research**. Only **curative experiments** with the aim of preserving the life and health of the specific embryo are possible. However, the law cannot be interpreted as prohibiting "**allowing to die"** or **active discarding** of an embryo (BGH, NJW 2010, 2672 [2676 marginal no. 38]). The law also contains no obligation to preserve the embryo outside the womb or by transferring it to a woman (Taupitz, in: Günther et al. 2014, B. III. marginal no. 20). Therefore, the question is justifiably raised whether it would not make more sense to use surplus embryos for high-level research instead of "just" discarding them or cryopreserving them for "eternity." Since PGD has been permitted in Germany, there are even numerous surplus embryos that are not transferred to the intended woman precisely because of their disposition to a serious hereditary disease or because of serious damage most likely leading to a stillbirth or miscarriage. Especially, these supernumerary embryos would be particularly suitable to contribute to research of the corresponding genetic predisposition inherent in them. But even they, which would not be transferred to a woman anyway, are covered by the ban on research with embryos. All **totipotent cells** removed from the embryo are equal to the embryo (§ 8 (1) ESchG). The use of sperm and oocytes for research purposes is not covered by this prohibition, nor are impregnated oocytes in the pronuclear stage, even if their production for this purpose is prohibited under § 1 (2) ESchG. The rights to the components separated from the body belong to the person from whom they originate, so that use for research purposes is in principle only permissible with this person's consent, and in the case of impregnated oocytes, with the consent of both gamete donors.

§ 5 ESchG contains further regulations, which prohibit the artificial modification of the genetic information of human germline cells as well as the use of human germ cells with artificially modified genetic information for fertilization (§ 5 (1) and (2) ESchG). Excluded from this prohibition of **germline intervention** are manipulations if it is excluded that the cells will be used for fertilization or transfer to an embryo, fetus, or human being, as well as alterations that are not intended and are based on measures such as vaccinations, radiation, or chemotherapeutic treatments (§ 5 (4) ESchG). Finally, the Embryo Protection Act contains explicit prohibitions on cloning (§ 6 ESchG) and on **chimera and hybrid formation** (§ 7 ESchG).

To supplement the ESchG, the "Act to Ensure Embryo Protection in Connection with the Import and Use of Human Embryonic Stem Cells" (Stem Cell Act/**Stammzellgesetz**—StZG) was enacted in 2002 (for the development see Gerke and Taupitz 2018). Basically, this law prohibits the **import and use of pluripotent cells** from previous embryos. In addition, the ESchG makes it a criminal offense to place orders for the production of embryonic stem cells abroad. However, the StZG contains exceptions in which the import and use of stem cells in Germany are possible if certain conditions are met. Initially, researchers in Germany were only allowed to use stem cells obtained abroad before the cutoff date of **January 1, 2002**, according to § 4 (2) no. 1a StZG. The Act Amending the Stem Cell Act abolished the former cutoff date and replaced it with **May 1, 2007**. Thus, the import and use of stem cell lines produced before May 1, 2007, can be approved for research objectives that cannot be achieved in conjunction with adult or animal cells, provided that the production or use does not conflict with the ESchG. Before the Robert Koch Institute, the responsible body, can grant approval, the Central Ethics Committee for Stem Cell Research must have issued an opinion (§ 6 StZG). If embryonic stem cells are introduced or used without the required approval, this constitutes a criminal offense under § 13 (1) no. 1 StZG.

Researchers in Germany are allowed to use embryonic stem cells obtained abroad before the cutoff date of May 1, 2007 for high-priority research objectives according to § 4 (2) no. 1a StZG. Research with embryos including the derivation of embryonic stem cells is prohibited to them.

Key Points

- The chapter on "Legal regulations" presents the basic legal framework of medical practice before dealing with specific aspects.
- The general prerequisites of lawful medical treatment and medical liability are discussed first.
- Concerning prevention of pregnancy, the legal prerequisites of sterilization and castration are specifically discussed.
- Sections on the induction of pregnancies through techniques of assisted reproduction deal with their legal basis, the law of parenthood, and the assumption of costs by statutory and private health insurance.
- This is followed by explanations on preimplantation diagnostics, which has been governed by the Embryo Protection Act since 2011.
- An overview of the legal basis of research with embryos and embryonic stem cells follows a discourse on cryopreservation.

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Ethical Criteria of Reproductive Medicine

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Hartmut Kreß

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Abstract

Reproductive medicine is a dynamic branch of today's high-performance medicine. It shares an interdisciplinary link with andrology. With regard to the treatment options of reproductive medicine, three of the relevant ethical criteria overlap with basic human rights, i.e., the right to individual self-determination including the right to reproduce, the right to health protection, and the welfare of the child. The health and welfare of children who are born with the help of reproductive medicine have to be given consideration before such reproduction takes place. In view of these three ethical criteria, controversial and hypothetical options also have to be considered, such as surrogacy or the theoretically imaginable usage of stem cells for reproductive purposes. On a research level, it is imperative that more information be gathered concerning dangers for human reproduction, e.g., from chemicals. Also, in light of ethical responsibility toward future generations, research should be conducted as to the impact of global warming on reproductive health.

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50.1 Questions Under Consideration

Within the space of a few decades, reproductive medicine has become an expanding branch of today's high-performance medicine. It enables the fusion of sperm and ovular cells in case of infertility but also for other medical, biographical, and social reasons. The first time a child was born following such fertilization outside the body (in vitro fertilization) was in England in 1978. By 2016, 8.3 million children had been born worldwide based on IVF treatment (Diedrich et al. 2020). One of the pioneers of 1978, Robert Edwards, won the Nobel Prize in Medicine in 2010.

However, there is a dispute about reproductive medicine. After Edwards was awarded the Nobel Prize, the Vatican reiterated its opposition to IVF. As early as 1987, the Roman Catholic magisterium prohibited men or women from making use of IVF (Congregation for the Doctrine of the Faith 1987). Subsequently, this prohibition was renewed, inter alia in the CDF's 2008 instruction "Dignitas personae." The prohibition extends to Catholic medical professionals and institutions. The Vatican went as far as calling for "passive resistance" against states and laws allowing assisted reproduction.

Beyond the faith-based community, other groups such as feminists have voiced their opposition against modern reproductive medicine, which, according to them, subjects the female body to "bio-power." Feminist criticism is also directed at the use of IVF in cases where it compensates for male infertility/subfertility. Insofar as "one 'alleviates' the infertility of a man through interventions on a healthy woman", women become "shadow service-providers of the reproductive industry" and are thereby degraded to being "bio-reactors" (Gehring 2005).

These references amplify the great extent to which reproductive medicine has caused controversies. Notwithstanding, internationally reproductive medicine has become firmly rooted in both medical and societal everyday life. However, on a national level, legal regulations vary significantly, i.e., from a very liberal approach (UK, Israel) to strong limitations and prohibitions (Italy, Germany). The law that presently (2021) governs reproductive medicine in Germany, i.e., the embryo protection law (Embryonenschutzgesetz), dates from 1990. Its effect was intended to be extremely restrictive. As a consequence, elective single-embryo transfers or oocyte donations are prohibited. However, this restrictive prohibition has by no means led to the population making less use of reproductive medicine, on the contrary. If required, patients go abroad in order to undergo procedures (e.g., obtaining an embryo through oocyte donation), which are not permitted within the country. In the Federal Republic of Germany itself, no less than 408,000 children were born with the help of IVF between 1982 and 2016. Thus, 3% of children in Germany are born each year following IVF treatment.

Andrology focuses particularly on the reproductive capacity of men. This fact explains its interdisciplinary connection to reproductive medicine. The following reflections focus repeatedly on procedures of reproductive medicine that relate to andrology or are of particular interest regarding male reproductive health and the realization of the desire of men to have children.

By way of introduction, a cursory mention should be made of the cultural–historical background to the topic.

50.2 Aspects of Medical and Cultural History

Andrology, like reproductive medicine, has only developed as a discipline in its own right since the second half of the twentieth century. However, it has much older roots. In 1677, the Dutch scholar Antoni van Leeuwenhoek (1632–1723)—a pioneer in the use of the microscope—observed spermatozoa in seminal fluid. At the time, this resulted in the realization that the absence of sperm in the seminal fluid meant an inability to conceive. The anatomist Regnier de Graaf (1641–1673), who also lived in Delft, had dissected ovaries shortly before. The observations of the two scholars also influenced fundamental discussions about the origin of the human being that were being conducted at the time. From a philosophical standpoint, it was thought that they confirmed the doctrine of preformation, according to which the embryo, the new living being, already pre-existed as a finished form.

Thus, following the findings of A. van Leeuwenhoek and R. de Graaf, two different preformist theories were formulated. On the one hand, the view was put forward that the new life was already present in the egg and was activated by the seminal fluid. In addition to this theory of the ovists, which was based on R. de Graaf, the school of the animalculists developed. It was based on the research of A. van Leeuwenhoek and claimed that the embryo was pre-formed in the sperm (Bernard 2014, pp. 27 ff., 35 ff).

In addition, a doctrine that can be traced back to Aristotle had become effective in the history of Western thought. Christianity adopted and modified it. It claimed that male sperm contained the soul of the human being. During procreation, the semen transmitted the soul to the offspring (theory of traducianism or generatianism). In the Middle Ages, a different view of the origin of the soul finally prevailed in Christianity. According to this view, it was God himself who gave a human being its soul (theory of creatianism). The Church Father Augustine (354–430) was still undecided. He was inclined toward creatianism, but also expressed his approval of traducianism. Traducianism, with its idea that the soul is transmitted through male semen during the act of procreation, was attractive to Christian theology because it could explain the presence of original sin in every human

being. For Christianity, sexuality and sin were intimately connected.

However, the Christian Church was uncertain about the exact time when the divine ensoulment actually took place. There was talk of either the 40th, 80th, or 90th day after conception. Eventually, the view prevailed that the divine bestowal of the human soul took place on the 90th day. This definition is still used today in secular law. In the Federal Republic of Germany, the provisions of section 218 of the Criminal Code, which declare abortion acceptable up to the end of the first 3 months of pregnancy under certain conditions ("time-phase solution"), are a reminder of the 90-day rule.

This means that in intellectual history, the human or male sperm had been the subject of religious and moral speculation for centuries. The preformist idea of an embryo pre-formed in the semen was finally abandoned in the nineteenth century. Since then, medical science has no longer been based on religious, theological, or natural philosophical speculations, but rather on empirical natural sciences and experiments.

Consequently, the pathologist Rudolf Virchow (1821–1902) no longer considered the soul but the cell to be the origin of life. Nowadays, in order to justify modern science-based options of reproductive medicine discussions they must be informed in an ethically appropriate way by normative criteria, which are based on post-religious thinking. They must take into account ideological pluralism, meet the requirements of a secular discourse and be rationally plausible. The philosopher Paul Ricoeur has appropriately emphasized that ethics today must be understood as "argumentation ethics" (Ricoeur 1996). Moreover, it is important that ethics take into account the special features distinguishing andrology and reproductive medicine from other branches of medicine.

50.3 Particular Aspects of Reproductive Treatment Options

Medical diagnosis and therapy usually emerge from discussions between a doctor and a patient. For a standard case of medical and clinical practice, the guiding principle of "patient-centered medicine" has become established in medical ethics. The doctor has to respect the will of the patient and must orientate himself toward his well-being. Andrology and reproductive medicine, however, differ from the standard situation of the duality of doctor and patient. The diagnoses and therapies they carry out transcend the counterpart of a physician with only a single patient. This is because, as a rule, it is ultimately about two persons who want to have a child together. In addition, other persons may come into play, e.g., if a sperm donor or an egg donor is willing to aid a couple with infertility problems by providing them with their gametes.

In yet another way, the andrological or reproductive medical treatment constellation points beyond the "normal" doctor–patient relationship. Their examination procedures and therapy options by no means only affect the directly treated patients themselves, but at the same time another person, namely the hoped-for child that is yet unable to express itself.

Because the birth of a child is the desired goal, its interests must be taken into account from the outset in reproductive medical treatments.

In relation to these multi-personalities of the treatment constellation, three ethical criteria are of primary importance for andrology and reproductive medicine: the personal rights and the right of self-determination to which every human being is entitled, the protection of health, and the best interests of the child. These ethical criteria are also legally relevant. They overlap with individual fundamental and human rights.

50.4 Normative Criteria

50.4.1 Self-Determination

The desire to reproduce is one of the essential characteristics of human existence. In the philosophy of the Middle Ages, this fact was expressed in the formula that procreation was an inclinatio naturalis, i.e., a "natural aspiration" or a "natural inclination" that humans share with nonhuman life. Part of being human is that the individual deals with such natural inclinations in the sense of his human moral reason.

Following the philosophy of the Enlightenment (e.g., Immanuel Kant) and today's constitutional law, every individual has the right to self-determination and the free development of their personality because of the dimension of reason and the dignity of being human. Connected with the right to self-determination are their freedom of conscience and ideology, but also their personal right to found a family or to protect their private life. Nowadays, it is generally recognized in both the ethical and the legal spheres that the right to self-determination and the right of personality also entail the right to reproductive freedom or, in other words, reproductive autonomy. This includes that every individual has the right to

make use of medical assistance in procreation, including the offers of technically advanced reproductive medicine (Gassner et al. 2013, p. 31 ff.; Kreß 2020, p. 660 ff.).

50.4.2 Health Protection

A second criterion that is central to reproductive medicine is the protection of human health. In terms of intellectual history, it can be traced back to antiquity. According to the Hippocratic Oath, physicians were obliged to strive for the patient's well-being (salus aegroti) and to do him no harm (nil nocere). In 1948, the Declaration of Geneva revisited the idea. The revised version, adopted by the World Medical Association in 2017, comprehensively mentions "health and well-being" of patients as the standard of medical ethics. Along the same lines, in 1966 the United Nations recognized "the right of everyone to the enjoyment of the highest attainable standard of physical and mental health" in Article 12 of its International Covenant on Economic, Social and Cultural Rights. Fifty years later, this human rights convention was officially adopted and declared binding by 160 states.

Andrology and reproductive medicine focus on stabilizing the reproductive health of their patients or—as far as possible—on restoring it. It forms a sub-element of the right to protection of health and to health care, which every individual can claim for himself.

50.4.3 Child Welfare

As a third consideration, it should be emphasized: Doctors working in the context of reproductive medicine have to bear in mind the best interests of the child. They contribute to the existence of children who would probably not have been born without the medical measures they perform. Therefore, they are responsible for the health and well-being of these children.

To be more precise: Physicians practicing reproductive medicine have to consider the two criteria mentioned above, the right of every human being to self-determination and to the protection of their health, not only in view of the patients they encounter concretely—the woman, the man with the desire to have children—but also in an abstract and anticipatory way. The two criteria already apply pre-conceptually and prenatally. Insofar as children are produced with medical assistance, their potential rights to self-determination and their personal rights must be respected even before they are produced (Kreß 2018, p. 278 ff.; National Academy of Sciences Leopoldina 2019, p. 57). For example, they must not be denied the opportunity to obtain knowledge of their genetic origin as adolescents. For this reason, anonymous sperm or egg donations in which the name of the donor is not known cannot be ethically justified. Likewise, reproductive medical measures must not cause damage or health burdens to the child after its birth, which could have been avoided or which are disproportionate. If it becomes apparent that a reproductive medical measure will harm a child to an alarming degree, it must be refrained from.

These three criteria of ethics—the individual right to self-determination, the protection of health, and the best interests of the child—form the normative basis when some options for action in reproductive medicine are examined. Prior to this, embryo protection should be mentioned, which has often been the dominant topic in the ethical, legal, and political debates on reproductive medicine in recent decades.

50.4.4 A Further Aspect: The Status of Embryos Prior to Nidation

Reproductive physicians fertilize oocytes with sperm outside the woman's body. They then transfer the fertilized ovum or early embryo into a woman's uterus so that a pregnancy takes place and a child is born. For technical reasons, this results in embryos that remain "surplus" and are not implanted in the woman. They are left to die. Alternatively, they can be cryopreserved or, depending on the national legal situation, donated to other people who wish to have a child or are used for research purposes. These current possibilities of reproductive medicine to create and access embryos have given rise to the ethical and legal question of whether embryos are already "human beings," "persons," or "individuals" in the narrower sense and what claim to protection should be attributed to them.

In today's ideological pluralism, the question is answered in different ways. The Roman Catholic Church has categorically rejected the creation of embryos outside the womb. To this end, it even revised its own teachings in 1987. For many centuries, it had consistently held that prenatal life was animated by God on the 90th day. Instead, in 1987, it officially stated that ensoulment takes place on day 1 at the union of sperm and egg. Because of its animation by God and the dignity conferred by God, the fertilized egg cell is absolutely inviolable. Therefore, the Catholic Church maintains that research on embryos or the creation of supernumerary embryos is reprehensible under all circumstances (Congregation for the Doctrine of the Faith 1987, Introduction 4, 5).

In states such as Poland, Italy, or even the Federal Republic of Germany, the Catholic point of view has strongly influenced state legislation. However, it is a matter of religious speculation that cannot claim general validity. Nor is the Catholic doctrine shared by other religions. Voices from post-religious humanism or philosophers hold quite different views at any rate. Ethically and jurisprudentially, the idea has largely prevailed that prenatal life is to be protected all the

more strongly the further it has developed. Therefore, embryos or fetuses have a greater right to protection after implantation in the uterus has taken place around day 7, or when organs or neuronal structures begin to form from the second month of pregnancy, or when pain sensation can gradually be assumed from around the 20th week (Herring 2016, p. 338; National Academy of Sciences Leopoldina 2019, p. 27). The early embryos with which reproductive medicine is concerned constitute "human life," but not yet a "human being", i.e., the embryos are not yet human individuals in the narrower sense. This is supported by the fact that prior to nidation neither the final genetic identity is determined nor whether it will result in the birth of one or several individual humans (twins). It follows from this that the creation of supernumerary embryos or, for example, the removal of embryonic stem cells from them is ethically and legally acceptable.

In recent decades, the moral and legal status of fertilized eggs or early embryos has often been at the forefront of debates on reproductive medicine. However, this topic does not concern a whole range of reproductive medical treatment options at all. For example, it is irrelevant for mitochondrial replacement therapies because the mitochondrial exchange is performed before the fertilization process is completed (Human Fertilisation and Embryology Authority 2016, p. 10). The same applies to andrology because it does not deal with embryos but with male germ cells.

In the following, a selection of possibilities for action that today's reproductive medicine opens up is addressed. Treatment options are also mentioned that arise specifically in the context of andrology or that are relevant for male reproductive perspectives. First, reproductive medicine therapies are discussed that can be realized in the homologous system, i.e., in the context of a couple's relationship with each other. Subsequently, treatments in the heterologous system will be discussed. In these cases, third persons are needed in addition to the couple wishing to have children, such as a sperm donor, an ovum donor, and a surrogate mother.

50.5 Treatment Options of Reproductive Medicine from an Ethical Standpoint

50.5.1 Extracorporeal Creation of Embryos in a Homologous Setting

50.5.1.1 Intracytoplasmic Sperm Injection

In its "classical" form, first successfully practiced in 1978, sperm and ovum are brought together in such a way that sperm can penetrate the ovum in a culture dish. Such in vitro fertilization (IVF) serves to compensate for female infertility. Later, male infertility could also be treated based on this approach. In intracytoplasmic sperm injection (ICSI), on which publications have been available since 1992 (Bernard 2014, p. 410), the physician injects selected sperm into oocytes, as these are not capable of doing so on their own due to poor quality and motility.

If the ICSI procedure is used because of male subfertility, the woman's rights of self-determination and health protection must be fully respected. This applies particularly since the woman concerned must accept the accompanying circumstances of out-of-body fertilization, namely hormonal stimulation and the puncture of the oocytes, so that a sufficient number of oocytes capable of fertilization become available.

In medical terms, it has been objected that the ICSI procedure was rushed into becoming clinical routine in the 1990s and 2000s after only inadequate preliminary testing. The objection also served as a warning against rashly declaring other innovations in reproductive medicine, such as uterus transplantation, to be the norm (Olausson et al. 2014, p. 41). As far as ICSI is concerned, the objection could be refuted. As things stand today, the procedural safety and success of IVF and ICSI are comparable. If a child is conceived extracorporeally, there is a 1.3-fold or 30% higher risk of malformation with both methods compared to natural conception (Ludwig and Ludwig 2020, pp. 559, 563).

In general, therefore, the "all-clear" could be given regarding the procedural safety of ICSI. Nevertheless, from an ethical point of view it must be emphasized that, depending on the andrological findings, in each individual case it must be examined whether using ICSI is justifiable from the standpoint of the child's welfare, namely the child's health. If fertility deficits of the man are the cause, there is also a risk of sperm and fertility problems for his male offspring, which may be produced with the help of ICSI. One individual problem may be mentioned as an example: If microdeletions of the Y chromosome are present in the man wishing children, potential sons will inherit the structural aberration and be infertile themselves (Schuppe et al. 2020, p. 448). Apart from this, increased chromosomal abnormalities have been observed in ICSI fetuses due to the subfertility of their genetic producers (Ludwig and Ludwig 2020, p. 554).

Andrologists and reproductive physicians must inform, educate, and advise men and their partners about such risk factors. In addition, it should be ensured that patients can receive psychosocial counseling independently of the medical practitioner. In such psychosocial counseling sessions, the danger of health risks for the child will also have to be addressed. The potential parents should reach a decision that they can justify to the child—if a birth should occur after ICSI.

50.5.1.2 Medical Freezing

While ICSI is frequently used in quantitative terms, a different type of reproductive medicine, i.e., **medical freezing**, affects only a small group. It is aimed at younger patients who are undergoing oncological treatment. Recently, the life and future prospects of patients who have overcome cancer as adolescents or younger adults are quite favorable. They may later want to enter into a partnership and realize a wish to have children. Oncological therapy, namely chemotherapy, which is necessary to combat the disease and restore their health, threatens to cause germ damage. Nevertheless, the opportunity is now opening up for those affected to produce their own children. Reproductive medicine offers them the possibility of cryopreserving their germ cells before they start chemotherapy, so that they can then be stored and thawed out for fertilization if necessary.

For women, cryopreservation of germ cells has only been a viable option since the second decade of the twenty-first century. Before that, it failed technically because of the very poor survival rates of eggs after thawing. Cryopreservation of germ cells has been practiced for a long time in male patients suffering from tumors in their younger years—such as testicular tumors, Hodgkin's disease, non-Hodgkin's lymphoma, or leukemia. Their sperm can be thawed and used to fulfill a wish for a child, provided a partner is then available. In addition to the cryopreservation of sperm, testicular biopsies can also be considered (Schuppe et al. 2020, p. 479).

In individual cases, medical freezing involves difficulties that must be addressed in each case. For example, germ cells from tumor patients may already be damaged before chemotherapy due to the disease itself.

> From an ethical point of view, however, it should be emphasized that medical freezing serves to protect the health of younger patients and to provide them with the best possible health care. Provided that their tumor disease is successfully treated, they retain reproductive autonomy for their life planning, i.e., the possibility of deciding to have their own child.

In this respect, the offer of reproductive medicine serves their existential well-being ("salus aegroti") in a comprehensive, holistic sense.

50.5.1.3 Other Quasi-Homologous Case Constellations

50.5.1.3.1 Social Freezing

IVF, ICSI, and medical freezing are applications of reproductive medicine in the homologous system. IVF and ICSI are usually requested by two people who wish to have a child together out of an existing partnership. The situation is different with medical freezing: Sperm or eggs of younger patients, who often do not yet have a life partner, are frozen due to a disease. The cryopreservation of their germ cells is done preventively in order to keep a partnership—as a homologous system—with children open for their biographical future.

Now, in addition to the case constellation of a homologous system, which could possibly exist in the future, other reproductive medical interventions are conceivable. One of these is social freezing. Women have their eggs removed because they hope for a partnership and a pregnancy later in life. The topic has attracted a great deal of public attention. It is not to be discussed in detail here, also because this article concentrates on male reproductive perspectives. On a fundamental level, it should be emphasized that the three ethical criteria mentioned—right to self-determination, health protection, and best interests of the child—are equally relevant to social freezing. On the basis of these criteria, critical questions arise, above all concerning the best interests of the child. If a woman who is over 35 or even over 40 has her eggs removed and the pregnancy is then to take place later, there are health risks for the child. In addition, the male partner whith whose sperm the thawed eggs are later to be fertilized may be older. Advanced age may have a negative effect on the quality of the sperm. In addition, the idea of having a child grow up with unusually old parents is questionable from a psychosocial and developmental psychological point of view.

Even in Israel—a state with particularly liberal legislation on reproductive medicine—a maximum age of 55 years to thaw and fertilize eggs has been set for women (Shkedi-Rafid and Hashiloni-Dolev 2011, p. 292).

50.5.1.3.2 Postmortem Insemination

The situation is different if a woman wishes to be inseminated with sperm that originates from her deceased partner's cryopreserved sperm. In such cases, it must be ascertained whether the deceased had consented to this, because his individual right to self-determination and personal rights continue to apply postmortem.

Subsequently, the welfare and interests of the child that the woman wants to bring into the world with the help of the deceased's sperm must be considered. The child must not become a mere means to an end to compensate for the death of the deceased and to let him "live on." Such projections and pre-expectations threaten to violate the intrinsic value of the child and its subject status in a problematic case.

In terms of intellectual history, since the Enlightenment, and especially since the late twentieth century, the insight has prevailed that a child is to be regarded from the outset and from the beginning as an independent individual with its own subjective rights. From antiquity to modern times, the

opinion had prevailed that children were only a subordinate part of the parental or paternal "household" status; they were the "property" of their parents. This viewpoint was finally overcome in 1989 by the United Nations Convention on the Rights of the Child.

It would be an ethical step backward if certain recent offers of reproductive medicine, of all things, had the effect of alienating children by making them the object or projection screen for the wishes of their producers—be it a woman or a man.

The topic of postmortem insemination exemplifies that this problem, the danger of overalienation of children through the prior expectations of their producers, needs to be discussed and critically addressed with regard to today's reproductive medicine in general (Kreß 2018, pp. 266 ff., 280 ff.).

50.5.1.4 Preimplantation Diagnostics

In the case of medical or social freezing, reproductive physicians act so that in future years of life patients can enter into a partnership and produce a child together with a partner (procreative care). In contrast, postmortem fertilization mentioned last refers to a homologous system that had existed in the past. As a rule, however, reproductive physicians treat patients who are currently in a partnership (homologous system). Since the 1990s, it has been possible to perform extracorporeal fertilization in the homologous system not only to treat infertility problems of the woman or the man, but also for genetic reasons. If a couple—either the woman or the man or both—are known to have family-related genetic disorders, preimplantation genetic diagnostics (PGD) can be considered. After extracorporeal creation, embryos are tested for the hereditary disease in question, e.g., cystic fibrosis or severe muscle diseases, so that the woman is then implanted with an embryo that is unaffected by the genetic defect.

The approval of PGD has been met with considerable resistance in some countries, especially in the Federal Republic of Germany. The main objection has been that PGD violates the protection of life of early embryos. In contrast, the three criteria highlighted above—the right of self-determination and reproductive freedom of people who wish to have children, their right to protection of their health and health care, and the best interests of the child—lead to the position that PGD merits ethical support.

This results in particular from the point of view of the best interests of the child. The procedure has the good sense to spare a child serious disease from which it would probably have had to suffer after birth. Moreover, PGD helps to avoid abortions, which can be carried out later in the pregnancy after a prenatal diagnosis has been carried out by means of amniocentesis or chorionic villus sampling.

On the basis of their personal rights and their freedom to reproduce, a couple has the right to decide for themselves whether they use PGD if there is a risk of serious chromosomal or genetic diseases affecting the child they hope to have. Both the state and society must respect such personal decisions, which are made in favor of a child and its health.

It is therefore ethically extremely problematic that in the Federal Republic of Germany each individual application of PGD must be approved in advance by a state-appointed ethics committee. The woman must submit an application and bear the costs for the commission together with her partner. According to state regulations, the ethics committee has to decide on the circumstances of the individual case and on its ethical justifiability. This German regulation is an expression of authoritarian state paternalism that overrides the individual citizen's right to self-determination and reproductive autonomy. It is not to be found in any other state. The regulations that apply in Great Britain are preferable. Clinics are licensed to perform PGD by a state-authorized institution, the Human Fertilisation and Embryology Authority (HFEA). The moral responsibility and the individual decision remain with the couple wishing to have children (Dücker 2019; Kreß 2021).

50.5.2 Extracorporeal Creation of Embryos in a Heterologous Context

Various offers of reproductive medicine are such that, in addition to the couple wishing to have a child, other persons are involved. Ethically, the normative objectives, i.e., self-determination—health protection—child welfare, are also relevant for these cases.

50.5.2.1 Sperm Donation

If a couple's lack of male fertility cannot be remedied by andrological or reproductive medical support, if the use of ICSI does not promise success, or if there is a risk that a male offspring in particular will be born with health problems after a pregnancy facilitated by ICSI, sperm donation can be considered. Furthermore, sperm donation is now of interest to lesbian women and couples.

The creation of children with another person's sperm (heterologous sperm donation) has been discussed even before andrology or reproductive medicine became established as medical disciplines. In the past, rejection was often

expressed. In Germany in the 1950s, sperm donation was considered a violation of the husband's human dignity even if it was consensual. In 1962, draft penal legislation of the West German government raised the objection of adultery against sperm donation. It was to be feared that "marriage in its significance as the morally predetermined germ cell of the people and of humanity … would be undermined and degraded" (quoted from Erbel 1971, p. 316).

In recent decades, ethical standards have changed completely for good reasons. At that time, argumentation was still based on Catholic natural law. Thus, they were often based on the idea that individuals and society were bound to timeless, unchangeable laws, to which the institution of a patriarchally construed marriage belonged. Nowadays, however, the argument is quite different. Ethical criteria such as the right to self-determination, the protection of health, and the welfare of the child are of central importance. These criteria are based on human rights and the personal interests of the individual. Hence, sperm donations to which those involved and affected have consented are legitimate in principle. For reasons of health protection, infections through donor sperm must be medically excluded.

Anonymous sperm donations, however, are problematic. This is because they cannot be reconciled with the personal rights of the child, namely with its right to know its genetic origin. Information about the genetic father can also become relevant for health issues of the child.

Ethically and psychologically, it is important that the parents with whom the child grows up after sperm donation—i.e., the "social parents"—practice frankness and transparency and talk to the children in good time and in a manner appropriate to their age about how they were conceived.

50.5.2.2 Oocyte Donation

Oocyte donation is to be discussed analogously. In this case, it is a matter of female reproductive autonomy and reproductive health. It is considered in cases where a woman is unable to produce her own eggs. It must be taken into account that egg retrieval is an invasive procedure that carries a certain risk for the donor. She must have been informed, educated, and counseled about the medical circumstances and psychosocial aspects in order to be able to consent to the voluntary donation of her eggs. The prohibition of egg donation under criminal law, which is currently in force in the Federal Republic of Germany (as of January 2021), is untenable from an ethical point of view. State legislators disregard women's fundamental rights to self-determination when they prohibit them from removing oocytes and voluntarily donating them to other women.

Some states, such as Spain, stipulate that oocyte donations must be anonymous. Such a requirement is also ethically unacceptable. This is because anonymous oocyte donation, like anonymous sperm donation, conflicts with the best interests of the child. In principle, however, the fact that a child owes its existence to an oocyte donor, i.e., a third person outside the homologous system, is not detrimental to its personal development and identity. This view was frequently used as an argument against egg donation in the past. Today, it can no longer be upheld on the basis of empirical studies.

50.5.2.3 Surrogacy

In addition to oocyte donation, mitochondrial donation, uterine donation, or surrogacy are now practiced. In these cases, too, in addition to the couple wishing to have a child other individuals are involved. Surrogacy will only be mentioned here, especially because it is relevant for male reproductive autonomy, specifically for the realization of reproductive desires of homosexual men.

In Israel, surrogacy was already legally permitted in 1996. Since then, women there have been allowed to carry a child for heterosexual couples. On February 27, 2020, Israel's highest court ruled that the state must amend the law to allow homosexual couples and single men to use surrogacy in the future. Previously, they had had to choose the alternative of having a child they had produced carried by a surrogate mother abroad. With its decision, the Israeli Supreme Court affirmed that in same-sex couples men may no longer be discriminated with regard to their reproductive freedom.

When reflecting on the issue of surrogacy, it is important to distinguish between different forms of abuse. Abuse occurs when women who are in economic need are exploited as surrogate mothers. In this case, women are threatened with objectification, in extreme cases with bioslavery, which can be compared to the reprehensible trade in organs. India is one of the Third World countries in which women have been deliberately instrumentalized by a medical surrogacy industry. Incidentally, in India, women from socially disadvantaged sections of the population are also put under highly reprehensible pressure to give up their oocytes in order to sell them to prospective parents who "order" such oocytes (Bärnreuther 2020). The Indian state intervened against commercialized surrogacy in 2016 and legally prohibited it.

A distinction must be made between commercial surrogacy and the kind of surrogacy where a woman, out of her own free decision and for altruistic motives, agrees to carry a child to birth for another woman or for a heterosexual couple or homosexual persons. Conceptually, this form of surrogacy can be characterized as pregnancy donation. In substance, it can be compared to the voluntary living donation of organs. A woman agrees to make her uterus temporarily available to

persons who, for medical or biological reasons, are unable to bear a child they hope to have themselves. Some states—apart from Israel, e.g., Great Britain or the Netherlands—have addressed the issue and created legal frameworks for this (Ditzen and Weller 2018).

From an ethical point of view, it is essential that the personal and health interests of the women who make themselves available for such a pregnancy donation are safeguarded. In addition, comprehensive psychosocial counseling and support must be guaranteed over a long period of time for all those involved—for the contracting prospective parents, for the gestating woman, the "surrogate mother", and especially for the growing child.

50.6 Hypothetical Treatment Options

Approaches to reproductive medicine that cannot be realized at present, but may be ready for discussion in the future, require their own ethical reflection. As a precautionary measure, the need for clarification must be emphasized, especially with regard to procedural safety, health protection, and the welfare of the child.

50.6.1 Germline Therapy

Thanks to the genome editing method developed in 2012 (CRISPR-Cas; "gene scissors"), the genetic information of individual genes can be exchanged in a targeted manner. Theoretically, the procedure can be used to help men or women with a monogenetic hereditary disease to have a genetically own child. Genetic intervention could be carried out in sperm or oocytes or in an early embryo in order to exclude the respective disease for the human being produced and, moreover, for his or her potential offspring.

The method of genome editing is currently still prone to errors due to off-target and on-target effects, among other things. Thus, there is a risk of damage to the child due to the procedure, including the danger of tumor formation. It is completely open whether it will be possible in the future to ensure that no unintended consequential damage occurs in children born after germline interventions. In addition, PGD is available as an alternative to germline therapies for almost all conceivable indications, through which the hereditary diseases in question can also be prevented (Wolf et al. 2019, p. 896). PGD has the great advantage that there are no procedural or iatrogenic impairments to fear for the child after birth.

50.6.2 The Use of Stem Cells

Then, in the distant future, it could become conceivable to produce oocytes from induced pluripotent stem cells. The cells to be reprogrammed and triggered for reproductive purposes would then be taken from adults who wish to have children. This would allow individuals, such as women, who lack a functional uterus (Robertson 2016, p. 84) or are at an older age, or male same-sex individuals and couples to create and give birth to a genetically own child. Homosexual men would require a surrogate mother.

Technologically, the generating oocytes from stem cells is a highly complicated proposition. If it were to become feasible, it would require reflection not only on medical ethics but also on social ethics. Potentially, it would not only shape the personal life planning and design of individuals but would have the general social effect of further decoupling sexuality and procreation. The structures of family and intergenerational cohabitation would be significantly changed (Carbone 2016).

Now, in the twentieth and twenty-first centuries, the understanding of sexuality, marriage, life forms, and family has already changed substantially in Western societies. In the meantime, same-sex life forms and patchwork or rainbow families have become legally accepted. Recently, acceptance for co-parenting, i.e., for raising children together without sexual contact between the partners, has begun to emerge. Modern reproductive medicine has not triggered these processes of socio-familial change in recent decades, but it has strengthened them. It would be a naturalistic or culturally determined fallacy to reject additional shifts in the approach to sexuality and partnership per se.

Regarding the use of stem cells for reproductive purposes, the decisive problem is rather that it is forbidden to carry out incalculable human experiments. The anticipatory rights of children must be taken seriously; they must not be physically harmed. In terms of medical technology, it is uncertain whether, in the future, the creation of children from pluripotent stem cells will ever be feasible without risk.

50.7 Research on Reproductive Health: Ethically Required

At this stage, germline therapies or the generation of oocytes from stem cells are "science fiction". However, it is quite obvious to use stem cells, namely human embryonic stem cells, in other respects for andrological and reproductive medical research. These can be used as biological material to gain new insights into the genesis of male germ cells and the causes of male infertility (Report 2017, p. 7). Such basic research projects could even be initiated in the Federal Republic of Germany, although in principle the German

Stem Cell Act has made research on human embryonic stem cells on the domestic territory a punishable offence. In addition to basic research, the great importance of applied research, which is committed to preventive reproductive health, must be emphasized in ethical and health policy terms. Specifically, how damage to male fertility by environmental chemicals or pharmaceuticals can be minimized needs to be clarified (Schuppe et al. 2020, p. 454).

Another research goal has received too little attention so far. The effects of climate change on male reproductive health are largely unknown. It is uncertain to what extent the warming of the earth's atmosphere will affect sperm quality and male fertility. The German Society of Urology pointed out in 2020 that there is currently no systematic research on the consequences of global warming for the kidneys, urinary tract, and male reproductive organs (Wahlers 2020). The development of appropriate research is ethically required because those existing today are responsible for the living conditions that future generations will have to cope with. Since the environmental crisis of the 1970s, philosophical ethics has drawn attention to the responsibility for future generations and the need to come to terms with the threats to human existence posed by a highly technical civilization. The philosopher Hans Jonas updated lines of thought developed in antiquity by Aristotle and in the Enlightenment by Immanuel Kant and developed the thought that those alive today bear a "duty towards later generations" (Jonas 1979, p. 85). It is a matter of guaranteeing conditions for subsequent generations to exist on a basis that at least does not represent a substantial deterioration compared to present-day conditions.

> The "duty towards later generations" necessarily includes taking precautions for their reproductive health. Therefore, medical research on the horizon of andrology and reproductive medicine has to analyze the effects of global warming on male reproductive health.

In conclusion, it is necessary to return to the current approach to issues of andrology and reproductive medicine.

50.8 The Role of Religions

Religious beliefs continue to play a role in people's personal convictions. Sexuality and reproduction are topics in which religions have been particularly interested for thousands of years. Even today, patients, doctors, and politicians are still guided by statements made by churches or religions on the subject of reproduction. This happens despite the fact that churches and religions have largely lost their former dominance and formative power in Western secularized societies.

50.8.1 Opposing Views

In terms of their content, the statements of individual religions on modern biomedicine are disparate. As far as reproductive medicine is concerned in particular, the Roman Catholic Church on the one hand and Judaism on the other hand are to be mentioned as opposite poles.

The Roman Catholic Church rejects any form of extracorporeal fertilization because, according to it, a child within marriage must have been produced "naturally" in an act performed in the flesh. To reject embryo research and IVF, the Church also stated in 1987 that God endows embryos with a soul as soon as they are procreated, so that they are inviolable (Congregation for the Doctrine of the Faith 1987). Sometimes the Catholic position has even been radicalized. It has been argued that even germ cells must be accorded a special status of protection (see Hilgendorf 1999, p. 155; Bobsien 2016, p. 228).

Judaism represents a completely different point of view. In Jewish religious history, life, health, and reproduction were always considered particularly important goods. Therefore, it was considered a religious duty to produce offspring in marriage, which was a sign of God's blessing. In the Talmud, it was said, "He who has no children is like a dead man" (quoted from Jütte 2016, p. 216). Due to a pronatalist intellectual and religious history, the use of reproductive medicine including preimplantation diagnostics or research on human embryonic stem cells are consistently affirmed in contemporary Judaism. Today, support for reproductive technology is found in both religious and secularized Judaism.

However, the traditional views of the Jewish religion also result in lines of thought that are inconclusive. With reference to the Jewish Halakhic tradition, oocyte donations are considered permissible in today's Judaism, whereas sperm donations are questionable because it is difficult to distinguish them from adultery (Weisz 2011, p. 39). Incidentally, a similar view is found in Shiite Islam (Ilkilic 2011). It was groundbreaking that Israel permitted surrogacy in 1996 (Birenbaum-Carmeli 2016)—but with disconcerting individual provisions. Against the background of Jewish maternal law, the surrogate mother is required to belong to the same religion as the couple for whom she carries the child. Exceptions must be approved by a commission. The surrogate mother should be unmarried.

In addition, the state of Israel allows sex selection, which is strictly forbidden in many countries. With the help of PGD, a couple is allowed to select embryos of a particular sex they desire, provided they already have four or more chil-

dren of the opposite sex growing up. This shows the paradox that a religious affirmation of "progressive" medical technology can be motivated by anti-modernism. In Israel, of all places, backward-looking, orthodox religious groups make extensive use of medical progress. There, especially religiously conservative Jewish and Muslim parents use sex-selecting to produce male offspring after several girls have already been born (Lavi 2010, pp. 89, 94). Reproductive technology is used to preserve a traditionalist understanding of gender.

50.8.2 The Difficulties of Religious Viewpoints

A fundamental problem of religious viewpoints is based on the logic of their argumentation. As a rule, they invoke the authoritative validity of ancient sacred writings or the authority of the church or religious tradition. This is very often done without taking a critical historical look at the old texts or traditions, and without relativizing them in terms of intellectual history or critique of ideology. This problem is evident across the various Christian churches and other faiths. Therefore, a two-sided conclusion must be drawn.

In today's religiously and ideologically pluralistic societies, religiously conditioned ideas still have an effect. If people feel bound by them, this must be respected—both by doctors practicing reproductive medicine and by the state legal system. People of faith can legitimately invoke their personal right to self-determination and their positive freedom of religion.

On the other hand, boundaries must be drawn. This applies in particular where religiously based views violate ethical insights that are generally recognized today for good reasons. These include the personal rights and reproductive autonomy of women and the non-discrimination of the female sex.

Key Points

- It has been demonstrated that treatments offered by reproductive medicine can be particularly well evaluated on the basis of three ethical criteria, which are to be understood in their correlation to individual fundamental rights: the personal right to self-determination and the reproductive freedom of each individual person, the protection of human health, and the welfare of the child.
- It is not just the everyday practice of andrology and reproductive medicine that is to be measured against these three criteria. Rather, conceivable future treatment options, including the hypothetical use of stem cells for reproductive purposes, must also be considered in their light.
- Furthermore, they are relevant for the definition of research goals. The problem of what impact climate change or global warming will have on the reproductive health of future generations deserves separate attention.
- In order to support people in making responsible use of their right to self-determination and reproductive freedom, the offers of psychosocial counseling should be structurally expanded. If people are considering conceiving a child with medical assistance, they should be able to seek psychosocial counseling in addition to communication with the consultant physician.
- Competent psychosocial counseling is particularly necessary when other people, such as a sperm donor, an oocyte donor, a uterus donor, or a surrogate mother, are involved in the treatment process.
- The health and well-being of children who are to be born with the help of reproductive medical assistance must already be taken into account preconceptually. The welfare of the child should also play a particularly important role in psychosocial counseling and support.

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