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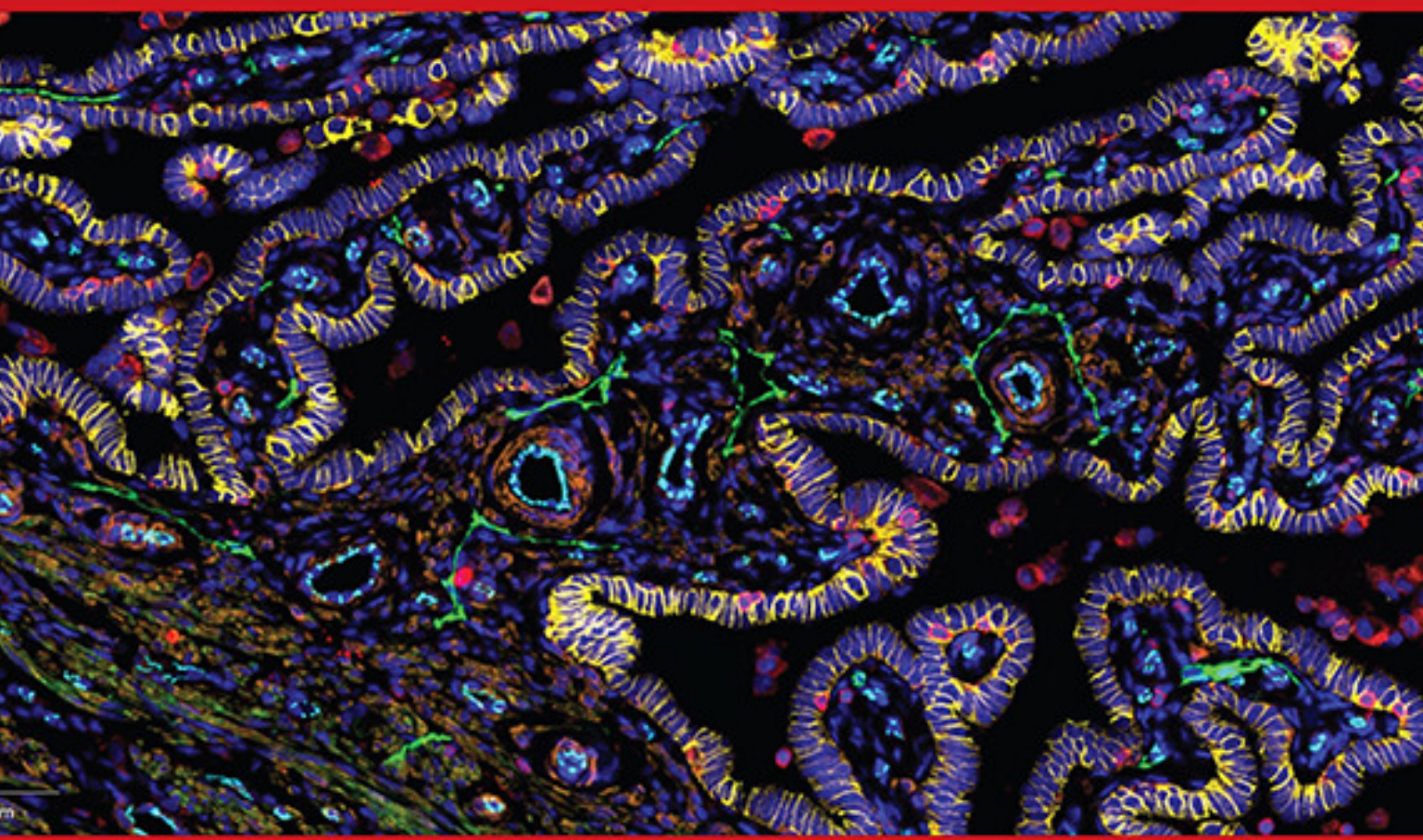

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AN OFFICIAL JOURNAL OF THE AMERICAN SOCIETY FOR REPRODUCTIVE MEDICINE

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ORIGINAL ARTICLES

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ASSISTED REPRODUCTION

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M. T. Lattin, A. S. Djandji, M. T. Kronfeld, T. Samsel, R. Ling, M. Ciskanik, S. Sadowy, E. J. Forman, and Z. Williams
New York, New York; Boston, Massachusetts; and Chicago, Illinois

The introduction of the assisted reproduction technology pipetting robot improves in vitro fertilization laboratory practices with increased precision and consistency in culture dish preparation.

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304 **World Endometriosis Research Foundation Endometriosis Phenome and Biobanking Harmonization Project: V. Physical examination standards in endometriosis research**

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Vancouver, British Columbia, and Montreal, Quebec, Canada; Ann Arbor and Grand Rapids, Michigan; Bethesda, Maryland; Palo Alto, California; Boston, Massachusetts; Oxford, United Kingdom; Aalborg, Denmark; Belgium; Clayton, Victoria, Australia; and Port of Spain, Trinidad and Tobago

An international collaboration of endometriosis research experts has developed standardized means of recording clinical physical examination findings in endometriosis and control patients to allow large-scale collaborative research.

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316 **Neighborhood deprivation in relation to ovarian reserve and outcomes of ovarian stimulation among oocyte donors**

T. Suresh, S. LaPointe, J. C. Lee, Z. P. Nagy, D. B. Shapiro, M. R. Kramer, H. S. Hipp, and A. J. Gaskins
Atlanta and Sandy Springs, Georgia

In our racially diverse cohort of vitrified oocyte donors, we observed no association between neighborhood deprivation and markers of ovarian reserve or outcomes of ovarian stimulation.

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326 **Effectiveness of preconception weight loss interventions on fertility in women: a systematic review and meta-analysis**

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Aurora, Colorado; and Kansas City, Kansas

Preconception weight loss interventions in women who are overweight or obese led to higher pregnancy rates compared with controls, but there was no impact on live birth or miscarriage rates.

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341 **Functional evidence for two distinct mechanisms of action of progesterone and selective progesterone receptor modulator on uterine leiomyomas**

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Bialystok, Olsztyn and Poznan, Poland; Bejing, People's Republic of China; London, United Kingdom; and Turku, Finland

This study showed the novel, distinct molecular mechanisms underlying the action of progesterone and the selective progesterone receptor modulator ulipristal acetate on uterine leiomyoma.

352 **Predicting risk of endometrial failure: a biomarker signature that identifies a novel disruption independent of endometrial timing in patients undergoing hormonal replacement cycles**

P. Diaz-Gimeno, P. Sebastian-Leon, K. Spath, D. Marti-Garcia, J. M. Sanchez-Reyes, M. d. C. Vidal, A. Devesa-Peiro, I. Sanchez-Ribas, A. Martinez-Martinez, N. Pellicer, D. Wells, and A. Pellicer
Valencia and Barcelona, Spain; Oxford, United Kingdom; and Roma, Italy

A biomarker signature for a novel endometrial disruption independent of endometrial luteal phase timing accurately identifies in vitro fertilization patients with poor endometrial prognosis and a >3-fold increased risk of endometrial failure.

REPRODUCTIVE SURGERY

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Cover image demonstrates the use of co-detection by indexing (CODEX) multiplexed immunofluorescence for visualizing various cell types in a cross section of a human Fallopian tube (DAPI - Bright blue, E-Cad: Yellow - Epithelial cells, Podoplanin: Green - Lymphatics and Fibroblasts, CD31: Cyan - Epithelial Cells and Endothelial Cells, CD45: Red - Immune cells, and SMA: Orange - Smooth Muscle Cells). Inset zooms in on the lumen, offering a detailed view of the cell types present in the intricate mucosal folds. Image courtesy of Kate O'Neill, M.D., Junhyong Kim, Ph.D., Addie, Alex, and Jean from the University of Pennsylvania as part of the NIH's Human BioMolecular Atlas Program (U54HD104392) and the Gift of Lift Donor Program (Philadelphia, PA). The investigators gratefully acknowledge the invaluable contributions of organ donors and their families, whose generosity make groundbreaking research and advancements in medical science possible.

Check for updates

A weighty issue

Eric A. Widra, M.D.

Shady Grove Fertility, Washington, D.C.

Interventions for management of obesity continue to expand. This Views and Reviews series discusses the impact of obesity on in vitro fertilization outcomes and practices and reviews emerging tools for weight management, specifically glucagon-like peptide-1 agents. (Fertil Steril® 2024;122:193. ©2024 by American Society for Reproductive Medicine.)
Key Words: Obesity, BMI limits, GLP-1

As the investigators of this issue's Views and Reviews eloquently point out, "obesity and infertility have been on a collision course for the past 40 years." Although we have been debating the granular—and indeed molecular—aspects of our field in these pages, there has been a revolution occurring in obesity medicine. Traditional medication regimens have been refined, expectations after bariatric surgery have become clearer, and newer, minimally invasive surgical approaches have evolved. Perhaps most importantly, the development of glucagon-like peptide agonists (glucagon-like peptide-1) has given new hope and opportunity to patients struggling with weight management.

Although the root cause of obesity may be straightforward, its physiological ramifications are widespread and complex. In the arena of reproductive health, obesity impacts assisted reproductive technology outcomes and has manifold effects throughout pregnancy as reviewed by the investigators in this issue. These concerns and others, including safety in the outpatient setting, have resulted in limited access to care in some settings. Given the extremely high and increasing prevalence of obesity in the United States, these limits impact a growing number of patients desiring care.

This Views and Reviews issue challenges us to reconsider our approaches to obesity. These challenges include a new look at body mass index limits as well as a review of the emerging role of glucagon-like peptide-1 medications in the care of the patient with infertility. This may be a revolution worth joining.

CRediT Authorship Contribution Statement

Eric A. Widra: Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Interests

E.A.W. has nothing to disclose.

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Check for updates

The impact of obesity on reproductive health and metabolism in reproductive-age females

Samantha B. Schon, M.D., M.T.R.,[a] Hannah E. Cabre, Ph.D., R.D.N.,[b] and Leanne M. Redman, Ph.D.[b]

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Obesity is a highly prevalent chronic disease that impacts >40% of reproductive-aged females. The pathophysiology of obesity is complex and can be understood simply as a chronic energy imbalance whereby caloric intake exceeds caloric expenditure with an energy surplus stored in adipose tissue. Obesity may be categorized into degrees of severity as well as different phenotypes on the basis of metabolic health and underlying pathophysiology. Obesity and excess adiposity have a significant impact on fertility and reproductive health, with direct effects on the hypothalamic-pituitary-ovarian axis, the ovary and oocyte, and the endometrium. There are significant adverse pregnancy outcomes related to obesity, and excess weight gain before, during, and after pregnancy that can alter the lifelong risk for metabolically unhealthy obesity. Given the high prevalence and pervasive impact of obesity on reproductive health, there is a need for better and individualized care for reproductive-aged females that considers obesity phenotype, underlying pathophysiology, and effective and sustainable interventions to treat obesity and manage weight gain before, during, and after pregnancy. (Fertil Steril® 2024;122:194–203. ©2024 by American Society for Reproductive Medicine.)

Key Words: Obesity, adipokines, infertility, reproductive health, fertility treatment

EPIDEMIOLOGY OF OBESITY

Excess body fat mass, or obesity, is the most prevalent noncommunicable disease worldwide. The World Health Organization defines obesity as abnormal or excessive fat accumulation that introduces undue risks to health (1). Body fat status is classified clinically using the body mass index (BMI)—a ratio of weight to height^2—a value of ≥ 30 kg/m^2 is used to diagnose obesity. Prevalence rates of obesity have nearly tripled globally since 1975 (1), which contributes to increasing morbidity and mortality for many noncommunicable diseases, including cardiovascular diseases, diabetes, and at least 13 types of cancer. The United States (US) has one of the highest obesity prevalence rates in the world, with the latest estimates suggesting that >42% of the adult population is affected (2). Furthermore, the occurrence of adults who are diagnosed with class II (35–39.9 kg/m^2) and class III (severe obesity; ≥ 40.0 kg/m^2) obesity has doubled in recent years, with class III obesity projected to increase at the highest rate in the coming decade (3).

The health of females throughout their reproductive years is critical for optimizing fertility, maternal and infant outcomes associated with pregnancy and childbirth (4), and lifelong health risks for women and children (5, 6). Indeed, all-cause mortality and cardiovascular events are increased in the offspring of mothers with obesity (7). In US women of reproductive age (18–40 years), obesity rates increased fourfold from 7.4% in 1976 to 27.5% in 2014 (8); to date, >40% of women 20–39 years are estimated to be living with obesity (2). Maternal prepregnancy obesity has been associated with an increased risk of obesity and overweight at early ages in offspring conceived with fertility treatments (9). Maternal BMI may also affect the risk of birth defects, such as spina bifida, and intellectual disabilities in offspring (9, 10). Because obesity-related adverse chronic health and behavioral risk factors during preconception can have detrimental effects on fertility, pregnancy, and maternal and offspring health outcomes (6), the prevention and management of obesity in females of reproductive age is critically important.

Obesity pathophysiology

There are 2 prevailing models of obesity pathogenesis: the energy balance model and the carbohydrate-insulin model. Although each agrees that there is a fundamental metabolic reprogramming of energy partitioning that favors excess energy deposition in fat, they differ in their explanation of pathways leading to chronic energy imbalance. The energy balance model suggests

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overeating coupled with physical inactivity and untoward metabolic adaptations to the obesogenic environment drive excess adiposity, although the carbohydrate-insulin model theorizes that increased adiposity drives overeating (11).

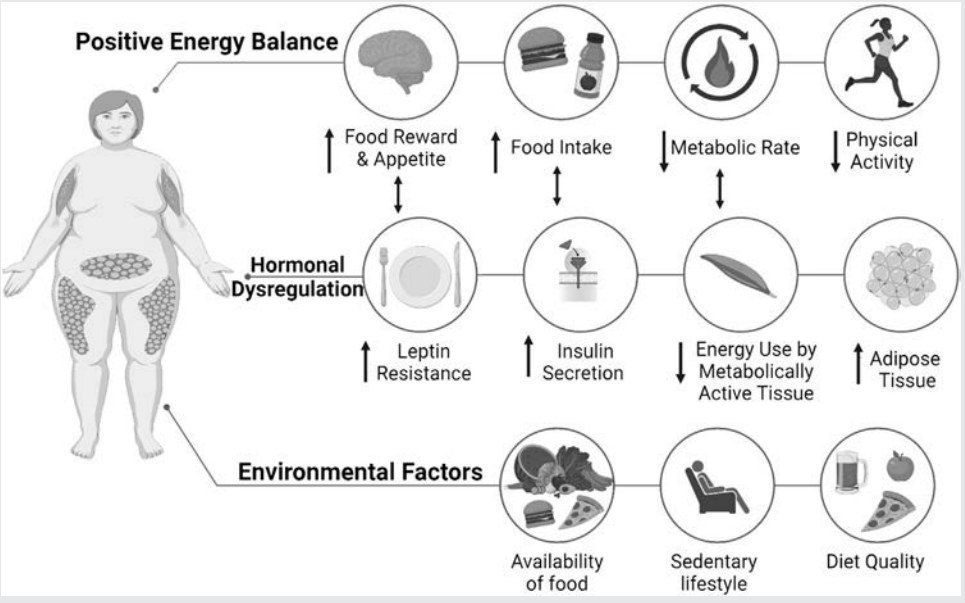
The energy balance model represents the most traditional and widely accepted concept for obesity, which states that obesity pathogenesis ensues from a chronic energy imbalance, whereby a chronic overconsumption of dietary energy that is not matched by basal metabolic rate and physical activity energy expenditure and excess energy is deposited in adipose tissue depots, leading to body mass expansion (Fig. 1) (12). In females of reproductive age, the relationship between energy balance and reproduction is tightly coupled. Reproductive processes are energetically expensive. Compared with males, females have a higher percent body fat mass and a greater tendency to store excess nutrients during surplus energy intake as a protective mechanism against undernutrition (13). Energy and nutritional status have direct effects on sex hormone production, ovulation, menstrual cycle regularity, fertility, conception, and early embryonic development (14).

The carbohydrate-insulin model of obesity, which is newly described, suggests that the chronic expansion of adipose tissue stores is driven by increased consumption of dietary carbohydrates because of their affinity for insulin secretion. Excess consumption of dietary carbohydrates and prolonged increases in circulating glucose stimulate a prolonged secretion of insulin, which directs dietary energy to fat storage in adipose tissue as opposed to oxidation by metabolically active tissues (11). In turn, cells are postulated to be "starved" of energy, driving an increase in dietary energy intake. In females of reproductive age, insulin is a key metabolic hormone with direct actions on the gonadotropin-releasing hormone neuronal network, which regulates reproduction (13, 15). Hence, variations in insulin secretion patterns influenced by diet composition and feeding frequency could subsequently impact energy partitioning to a greater extent than positive energy balance.

Increased intake of all 3 macronutrients (i.e., carbohydrates, fat, and protein) has been associated with insulin resistance, obesity, and metabolic diseases (16). A recent exploration regarding the composition of fat intake (e.g., low polyunsaturated fatty acids) has demonstrated that the excessive formation of free fatty acids and inappropriate lipid deposition in organs other than adipose tissue, also known as lipotoxicity, underlies the development of oxidative stress and mitochondrial dysfunction (17, 18). This most notably occurs through insulin resistance (18). Yet it is unclear whether increased consumption of one macronutrient is more detrimental than another regarding energy balance regulation.

Body weight is a central regulation. The brain relies on adipose- and gut-derived hormones, such as leptin, insulin, and ghrelin, to regulate appetite, satiety, energy expenditure, and hence energy stores (13, 19). It is also well established that these metabolic hormones are linked to fat stores, and they impact the control of body weight through long-term effects on energy balance (19). For example, the adipose tissue-derived hormone leptin regulates food intake and energy expenditure through hormonal signaling to the hypothalamus via a negative feedback loop to control adipose tissue mass (20). Individuals with obesity are often leptin-resistant, disrupting the normal central regulation of food

FIGURE 1



Overview of the pathophysiology of obesity.

Schon. Obesity and reproductive health. Fertil Steril 2024.

intake and adipose tissue mass, particularly as the lower leptin levels decrease energy expenditure and inhibit appetite regulation (19, 21–23). Additionally, glucagon-like peptide-1 (GLP-1), a gut hormone vital to glucose homeostasis, acts through the GLP-1 receptor, which is expressed in the brain, gut, and pancreas (24, 25). Glucagon-like peptide-1 reduces blood glucose levels by stimulating insulin secretion, which is important in those who are insulin-resistant (26, 27). It also acts by inhibiting glucagon secretion, which reduces endogenous glucose production, subsequently reducing the drive for food intake and slowing gastric emptying. This interaction between the gut and brain is possibly mediated by neuropeptide Y, agouti-related peptide, and pro-opiomelanocortin neurons (24, 26). Obesity interferes with gut hormones (e.g., GLP-1) ability to secrete peptides (e.g., agouti-related peptide and peptide tyrosine), which are critical for the homeostatic control of body mass through regulating food intake (brain) and energy expenditure (metabolism) (24, 28).

Obesity phenotypes

With BMI arguably an imperfect measure of obesity (29), a wide heterogeneity of obesity-related comorbidities exists across individuals. Obesity that is present in conjunction with cardiometabolic complications (e.g., hypertension and hyperlipidemia) and metabolic disorders (e.g., prediabetes and diabetes) is linked with worsened disease progression and increased mortality (30, 31). When obesity presents in the absence of other comorbidities, it has been defined as metabolically healthy obesity. These individuals are likely in a transient state, with more chronic disease morbidities to develop eventually (32). Consequently, metabolically unhealthy obesity is defined as obesity plus risk factors for cardiometabolic disease (33). Although there is no clinical practice guideline for defining obesity phenotypes, the 4 diagnostic criteria for metabolic syndrome are most commonly applied, with the presence of ≥ 2 of the 4 diagnostic criteria (insulin resistance, impaired fasting glucose and/or tolerance, dyslipidemia, and hypertension) conferring a positive diagnosis of metabolically unhealthy obesity (33, 34). It is important to emphasize that although each mechanism of obesity development leads to chronic energy imbalance, none is the result of a person being lazy or lacking willpower. As discussed in detail below, evaluating when a patient of reproductive age has obesity plus other signs of cardiometabolic disease is important to understanding their reproductive abilities, future pregnancy complications, and long-term gynecological and general health (35, 36). Finally, evaluating obesity beyond weight can also reduce stigmatization often experienced by patients and allow for more holistic and personalized clinical management (37).

Beyond other existing comorbidities, obesity phenotypes have been also proposed for 1 of 4 different underlying pathophysiologies, and these can also be diagnosed clinically: hungry brain (defined by abnormal satiation), emotional hunger (defined by hedonic eating), hungry gut (defined by abnormal satiety), and slow burn (decreased metabolic rate) (38). To identify these pathophysiologies, clinical tests are utilized. Homeostatic eating behavior can be divided into 3 stages: hunger (desire to eat assessed using a visual analogue scale), satiation (calories consumed to reach fullness and terminate a meal assessed using an ad libitum buffet), and satiety (duration of fullness or return of hunger assessed using a visual analogue scale). Hedonic eating behavior can be assessed using the hospital anxiety and depression scale and a three-factor eating questionnaire, whereas energy expenditure can be evaluated using indirect calorimetry (38). In a 12-month pragmatic weight management trial with 450 adults, 32% of patients presented with a hungry brain, 32% with a hungry gut, 21% with emotional hunger, and 21% with slow burn (38). Understanding the underlying pathophysiology of each patient's obesity has allowed for a more personalized approach to care, particularly with medications. For example, individuals with a hungry brain phenotype may be more responsive to phentermine-topiramate, although those with a hungry gut may respond best to liraglutide, a GLP-1 agonist (38). Using these 4 clinical phenotypes in future research studies may help elucidate underlying mechanisms for successful interventions (38), thereby aiding in health promotion for at-risk populations such as reproductive-aged women.

OBESITY AND FERTILITY

Hypothalamic-pituitary-ovarian axis

The impact of obesity on reproduction is both complex and multifactorial. Clinically, females with obesity frequently experience irregular menses, with systematic observations of obesity-associated menstrual disturbances described for >70 years (39–44). The probability of menstrual irregularities corresponds with the degree of obesity, with an increasing risk for oligo- and amenorrhea associated with increasing BMI (41, 45). However, as discussed above, there are significant limitations to using BMI as the sole metric of pathologic adiposity. Studies have shown that outside of BMI, abdominal adiposity and subcutaneous abdominal adiposity are most significantly associated with anovulation (46, 47). Furthermore, the age of obesity onset also seems to impact the risk of anovulation. For example, overweight and obesity at age 7 and 23 years both independently increase the risk of menstrual dysfunction and subfertility at age 33 years (42).

Arguably the most well-established mechanism of obesity-induced menstrual dysfunction involves interruption of the hypothalamic-pituitary-ovarian (HPO) axis, resulting in anovulation. This is most evident in patients with polycystic ovary syndrome (PCOS), where excess adipose deposition, especially in central depots, results in hyperinsulinemia, increased androgen production, decreased sex-hormone binding globulin levels, and increased peripheral aromatization, all of which can have direct and/or indirect actions on the HPO axis (48, 49). A full review of PCOS and the associated metabolic derangements associated with obesity is outside of the scope of this review and has been reviewed recently elsewhere (50).

Among eumenorrheic females without PCOS, obesity often results in a relative state of hypogonadotropic

hypogonadism (43, 51, 52). Studies of eumenorrheic females with overweight and obesity compared with those with normal weight, report decreased serum follicular phase levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and inhibin B (51, 52). Recent work aiming to further understand the mechanisms of relative hypogonadism, assessed by simulating the pathologic milieu of obesity, found that hyperinsulinemia combined with hyperlipidemia acutely suppressed serum levels of both FSH and LH (53). Using a gonadotropin-releasing hormone agonist to suppress the HPO-axis followed by intravenous administration of FSH, a recent study found that there is likely an effect of obesity beyond acute pituitary suppression (54). Although equivalent serum levels of FSH and pharmacodynamics were achieved in women with and without obesity, women with obesity exhibited both decreased baseline levels of estradiol and inhibin B and a significantly attenuated response to FSH levels. This suggests that beyond the obesity-induced pituitary effects described above, there is likely a direct ovarian effect (54). Urinary levels of pregnanediol glucuronide are also reduced among women with obesity (52).

Ovaries

Obesity has a direct effect on the ovary and oocyte. Preclinical models of diet-induced obesity demonstrate primordial follicle depletion, follicle apoptosis, decreased mature follicle size and number, increased oocyte meiotic aneuploidy, and altered oocyte mitochondria (55–59). Although diet reversal improves the circulating metabolic milieu, meiotic and mitochondrial abnormalities persist, suggesting that obesity may induce an irreversible insult to the ovary (60).

Similarly, human studies demonstrate morphological oocyte abnormalities and decreased oocyte competence with obesity. Oocytes from females with obesity undergoing in vitro fertilization (IVF) treatment are often smaller compared with their normal weight counterparts, are less likely to complete development postfertilization, and in 1 study, were noted to have granular cytoplasm (61–63). In addition, obesity is associated with meiotic spindle abnormalities and fewer mature oocytes (64–66). Additionally, resulting blastocysts demonstrate altered metabolism, with reduced glucose consumption and increased levels of triglycerides (62). Moreover, the follicular fluid of females with obesity demonstrates significantly altered adipokine composition, inflammatory markers (e.g., C-reactive protein), and free fatty acids (67–70). Circulating inflammatory markers have been also shown to correlate with intrafollicular levels of free fatty acids and triglycerides (67).

The increased systemic leptin present with obesity is similarly evident in the follicular fluid of women with obesity, who have higher levels of intrafollicular leptin compared with women of normal weight (67, 71, 72). In vitro studies utilizing human granulosa cells obtained from women undergoing IVF treatment demonstrate that leptin can directly inhibit basal and FSH-stimulated estradiol and progesterone levels (73). Leptin has been also shown to directly suppress both antimüllerian hormone (AMH), a key hormone in developing fetal sex organs in vitro, and its receptor AMH receptor II's messenger ribonucleic acid levels in human granulosa cells (73).

There is some controversy regarding whether or not BMI is consistently associated with serum AMH levels, especially among women with regular menses (74). However, several recent large-scale studies demonstrated significantly lower AMH levels among women with and without PCOS (75–77). In the appraisal of body content study involving 1,706 eumenorrheic women without PCOS, obesity on the basis of either percent body fat or BMI was associated with lower serum AMH levels (76). Similarly, in a cohort of reproductive-aged African-American women ($n = 1, 654$), AMH concentrations were 23.7% lower in women with obesity compared with women with normal weight (75). In addition, this study suggested a possible cumulative effect of obesity: women with obesity present since age 18 years had AMH levels that were significantly lower than women with normal weight at age 18 years but who currently had obesity. Finally, a study using samples from the pregnancy in PCOS (PPCOS) II and ovarian aging studies (640 women with PCOS and 921 ovulatory control women) found that increasing BMI and waist circumference were associated with a reduction in AMH levels as well as AMH levels and antral follicle count (a marker of per-follicle AMH) (77).

Uterus

Obesity has a well-established association with endometrial cancer (78). Indeed, it not only increases the risk of endometrial hyperplasia and endometrial cancer but also increases the risk of mortality and recurrence posttreatment (79). Given the clear impact of hyperestrogenism on the endometrium, the impact of obesity on the endometrium because it relates to implantation, fertility, and infertility has come into view.

Clinically, models to assess the isolated impact of obesity on the endometrium use a donor oocyte model where embryos created from normal-weight oocyte donors are transferred into women with and without obesity. Although a meta-analysis including 4,758 women demonstrated no association between recipient obesity and the chance of pregnancy after IVF treatment (80), these results were recently contradicted by 2 large-scale studies that reported an independent endometrial effect associated with obesity (81, 82). Worthy of mention, a study using Society for Assisted Reproductive Technology data to assess live birth (LB) rates from frozen embryo cycles using preimplantation genetic testing for aneuploidy embryos and including 77,018 preimplantation genetic testing for aneuploidy cycles (6.266 of which were donor oocyte recipients) found a significant relationship between BMI and LB. This relationship was observed in both those using autologous and donor oocytes and was especially evident in patients with a BMI (≥ 40 kg/m^2) (82). Notably, the presented studies utilized BMI as the sole metric of obesity, which, as previously discussed, does not necessarily capture the complexity of obesity and may explain some of the contradictory findings above. Additionally, many practices have BMI restrictions, and thus higher levels of obesity may not be reflected (83).

Preclinical models of diet-induced obesity demonstrate an independent endometrial effect of obesity, with evidence of a decreased decidualization response to hormonal stimulation and decreased numbers of implantation sites (84). A high-fat diet has been shown to induce significant alterations to gene expression in mouse endometrial epithelia and stroma, with altered expression of genes related to innate immunity, leukocyte chemotaxis, and circadian rhythms (85). Studies of human endometrium report altered proteomic and transcriptomic profiles with obesity (86–88). It has been also suggested that obesity may displace the window of implantation, especially in the setting of stage II (BMI 35–39.9 kg/m^2) and III obesity (BMI $\geq$40 kg/m^2) (89). Finally, both leptin and adiponectin are expressed in the human endometrium, with altered protein and/or receptor expression found in women with recurrent implantation failure (90). In vitro studies using human endometrial cell lines suggest that leptin may also have a direct role in endometrial epithelial remodeling and endometrial receptivity during implantation (91, 92).

FERTILITY TREATMENTS

As reviewed above, obesity has a complicated and significant impact on all aspects of the HPO axis and also directly impacts the ovaries and uterus. Clinically, this culminates in longer-time-to pregnancy (even among eumenorrheic females) and increased risk for infertility (93–97).

Among females with anovulatory infertility, BMI was found to be an independent predictor of a decreased chance of LB with clomiphene citrate ovulation induction (98). In the PPCOS I study, participants with a BMI <30 kg/m^2 had significantly higher rates of LB compared with those with a BMI >30 kg/m^2 (99). Similarly, BMI remained a significant factor in rates of LB in PPCOS II (100).

Females with obesity require higher doses of gonadotropins, have lower estradiol levels, and are at increased risk for cycle cancellation (101–106). Despite similar percentages of euploid embryos (107, 108), most studies evaluating pregnancy and LB outcomes that include fresh embryo transfers (ETs) demonstrate an association with BMI. For example, using data from the Center for Disease Control and Prevention's National ART Surveillance System and 494,097 treatment cycles, females with obesity had a statistically decreased likelihood of both intrauterine pregnancy and LB (109). This same trend was recently confirmed in a non-US (Swedish) population using national data (110). Although it was suggested that the impact of obesity on LB may be less evident among women undergoing frozen ETs (111), 2 recent large-scale studies utilizing Society for Assisted Reproductive Technology data and only examining frozen ET cycles of euploid embryos did demonstrate an independent effect of BMI (82, 112) and women with a BMI >40 kg/m^2 had a 27% lower probability of LB compared with the normal weight reference group (82). It is extremely important to note, however, that although there is a consistent impact of obesity on IVF treatment outcomes, this effect is significantly modified by age (113, 114). Thus, although obesity may decrease the chance of LB, the powerful effect of age must always be considered.

OBSTETRICAL OUTCOMES

Miscarriage

A number of studies demonstrate an association between maternal obesity and an increased risk of miscarriage (115–120). This increased risk is apparent after pregnancies conceived spontaneously (115, 117) and those achieved after fertility treatments (116–120). It was also demonstrated that women with obesity have an increased frequency of euploid miscarriage compared with women with normal weight, suggesting nonchromosomal etiologies of pregnancy loss in this population (121, 122). Obesity is also an independent risk factor among populations of women with recurrent pregnancy loss and increases the risk of future pregnancy loss (122–124). As noted in the 2021 American Society of Reproductive Medicine committee opinion on obesity, the adjusted odds ratio for most studies describing these associations is modest and ranges between 1.2 and 1.9 (93). Thus, although a relationship between BMI and miscarriages is consistently demonstrated, there is a risk of confounding factors that are likely influenced by the heterogenous metabolic, inflammatory, and reproductive profiles associated with excess adiposity, as well as how obesity was defined in each study.

Adverse pregnancy outcomes

Females of reproductive age have an increased risk of longitudinal weight gain and consequent obesity (125). Longitudinal studies report that females gain, on average, up to 0.7 kg per year, with the greatest rates of weight gain occurring in females aged 18–50 years (125). Existing obesity-related cardiometabolic comorbidities before conception often digress into obesity-related adverse pregnancy outcomes, such as gestational diabetes and preeclampsia (5). In epidemiological studies of US national data, the occurrence of gestational diabetes increased exponentially from 3.6% in females with a normal weight BMI to 8.8%–13.9% in females with a BMI $\geq$30.0 kg/m^2 (126). Women with obesity who develop gestational diabetes are more likely to develop type 2 diabetes postpartum than those without gestational diabetes (127). Epidemiological data also demonstrate that hypertensive disorders affect approximately 6% of pregnancies in the US with a positive relationship with increasing BMI (5). Similarly, women who had hypertensive disorders related to obesity during pregnancy had a higher prevalence of chronic hypertension later in life compared with those who did not (49.6% vs. 21.5%, respectively) (128). Taken together, obesity-related adverse pregnancy outcomes can lead to a higher probability that women will develop metabolically unhealthy obesity postpartum, even if they were considered metabolically healthy before conception.

Weight gain in pregnancy compounds the effect of pregravid obesity on adverse pregnancy outcomes (5). Only approximately one-third of women gain the Institute of Medicine's recommended amount of weight during pregnancy,

with most women gaining weight outside the recommendations (21% too little; 48% too much) (129). Excess gestational weight gain in pregnant women with obesity, defined as >9 kg, may further exasperate adverse pregnancy outcomes (e.g., gestational diabetes mellitus, large for gestational age infants, nonelective cesarean delivery, and others.), leading to complications during gestation and delivery (5). The pervasiveness of obesity-related adverse pregnancy outcomes can affect subsequent pregnancies as well, indicating the need for effective weight management before conception, during gestation, and postpartum.

Implications of preconception weight loss

Numerous studies suggest that weight loss during prepregnancy and during gestation can significantly reduce the risk of obesity-related adverse pregnancy outcomes. Observational studies that followed 415,605 women for approximately 10.6 years (range, 2–22 years) demonstrated that weight loss with lifestyle interventions was associated with a reduced risk of hypertensive disorders during pregnancy and postpartum (130). Other studies observed a positive effect of weight loss (−4.54 kg) between pregnancies on the risk of gestational diabetes during the subsequent pregnancy, highlighting the benefits of weight loss preconception (30). Combining lifestyle interventions with other antiobesity treatments, such as bariatric surgery or weight loss medications, can improve health parameters before conception. Indeed, an evaluation of over 2.8 million women (of whom 8,364 have undergone surgery) found that bariatric surgery before conception was significantly associated with reductions in gestational diabetes, hypertensive disorders, and cesarean delivery during pregnancy (131). Bariatric surgery can also improve ovulation, reduce the risk of miscarriage, and improve perinatal outcomes (132). The major untoward effect of bariatric surgery appears to be the increased risk for small gestational-age infants because of weight loss throughout pregnancy, yet this risk can be minimized by waiting the recommended 12 months after bariatric surgery to attempt to conceive and by focusing on good nutrition intake and supplementation during pregnancy. It is important to note that although weight loss may improve obstetrical outcomes, randomized controlled trials do not demonstrate improvements in LB in the setting of preconception weight loss, especially among ovulatory individuals (discussed in additional detail in the accompanying review) (93, 133). However, treatments for obesity before conception in women of reproductive age can promote healthier pregnancies, reduce maternal and neonatal adverse outcomes, and have an intergenerational effect, potentially contributing to a lower incidence of childhood obesity (134).

RESEARCH GAPS AND CLINICAL NEEDS

Precision medicine

Despite the fact that over 40% of reproductive-aged women in the US are living with obesity, the current US obesity guidelines do not consider prepregnancy weight or the prevalence of obesity-related adverse pregnancy outcomes in the decision to escalate obesity therapies (2, 134). This lack of guidelines for women of reproductive age signifies a potentially missed opportunity to initiate meaningful weight-loss interventions preconception and postpartum to attenuate the effects of obesity on maternal health. Precision medicine is an emerging innovative approach to managing obesity that considers individual differences in genes (i.e., polygenic risk score and single nucleotide polymorphisms), environments, and lifestyles. Women of reproductive age represent a population that would greatly benefit from precision medicine, particularly in relation to nutrition and lifestyle (135). Identifying specific diet and physical activity components for preventing weight gain, excess adiposity, and obesity is important for creating a strong evidence base for tailoring intervention strategies aimed at improving health in reproductive-aged women with obesity. Studying the newly proposed pathologies of obesity (38) in a cohort of reproductive-aged females in conjunction with a neuroendocrine workup of their reproductive function would fill a major knowledge gap.

Tools to identify high-risk patients

Because obesity is considered a medical risk factor for a high-risk pregnancy, early prenatal care is recommended to evaluate the cardiometabolic profile and other possible risk factors often associated with obesity. Laboratory tests for assessment of nutritional status and metabolic profiles are recommended in addition to dietary assessments using a food frequency questionnaire (136). Personalized nutrition interventions focused on reduced caloric intake and addressing nutritional needs can aid in managing maternal weight preconception. Common tools utilized to evaluate high-risk women during pregnancy are blood and urine tests, ultrasonography, frequent blood pressure measurements, and regular follow-ups with medical staff. Additionally, the US Preventive Service Task Force issued a grade B recommendation for using behavioral interventions before conception and during pregnancy to manage obesity-related adverse pregnancy outcomes via gestational weight gain (137). Effective interventions for weight management focus on individual nutrition, physical activity, and/or lifestyle change delivered by clinicians, registered dietitians, qualified fitness specialists, physiotherapists, and health coaches across different settings (e.g., local community fitness centers) (137). However, in the absence of significant weight loss with the initiation of comprehensive lifestyle interventions, escalation of therapy should be considered.

Effective interventions and therapeutics for weight management before pregnancy

The use of obesity phenotypes may aid in addressing the limitations of BMI and the heterogeneity in obesity-related to varied weight loss in response to obesity interventions, such as diets, medications, devices, and surgery. First, evaluating when individuals have metabolically healthy or metabolically unhealthy obesity is a simple and available method that can guide practitioners in providing recommendations for personalized care (e.g., a low-fat diet for dyslipidemia).

Second, assessing obesity pathophysiology may further improve obesity care for females of reproductive age. Assessing homeostatic eating behavior (hunger, satiation, and satiety) can be completed through widely available questionnaires, although hedonic eating behavior and energy expenditure assessment require measures that may not be available clinically. Although differentiating between the phenotypes can provide a more in-depth assessment of potential mechanisms to guide individualized therapy for obesity (38), using accessible measures for homeostatic eating behavior can be a key tool clinicians can add to their obesity care. A multidisciplinary team, such as registered dietitians and behavioral specialists, may be an acceptable way to evaluate obesity in a more personalized manner. Understanding the key determinants of an individual's eating behavior and energy intake can be addressed with behavioral counseling in addition to therapeutic interventions such as weight loss medications and bariatric surgery. Before conception, antiobesity medications are approved for women of reproductive age. A prepregnancy weight loss goal of 5%–7% is a reasonable target and approximates the additional total body weight loss achieved by adjunct antiobesity medications (134). The use of medications may be an effective therapy to treat obesity that does not have as many long-term risk factors compared with bariatric surgery, but the use of medications should be paired with lifestyle interventions to prevent weight gain during pregnancy and postpartum. This is particularly important for females using medications before conception to ensure proper lifestyle skills are developed for managing weight during pregnancy and in the postpartum time period. Because obesity is multifactorial, practitioners should be aware that finding an effective solution will also be multidimensional and probably require lifelong approaches.

CONCLUSION

The health of nonpregnant women during their reproductive years is critical for optimizing fertility success and reducing maternal and infant mortality and pregnancy complications. Females of reproductive age with obesity may experience challenges to their reproductive health via direct effects on the HPO axis, the ovary and oocyte, and the endometrium. Thus, understanding the complex pathophysiology of obesity and utilizing phenotypes that go beyond the limitations of BMI is imperative for providing personalized clinical care for females of reproductive age.

CRediT Authorship Contribution Statement

Samantha B. Schon: Writing – review & editing, Writing – original draft, Conceptualization. **Hannah E. Cabre:** Writing – original draft. **Leanne M. Redman:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of Interests

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Addressing weight bias in reproductive medicine: a call to revisit body mass index restrictions for in vitro fertilization treatment

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The prevalence of obesity has doubled among reproductive-age adults in the US over the past 40 years and is projected to impact half of the population by 2030. Obesity is associated with a twofold to threefold increase in infertility, largely because of anovulation, and is associated with a lower rate of pregnancy with ovulation induction among anovulatory women. As a result of these trends and associations, in vitro fertilization (IVF) care will need to be adapted to provide safe, effective, and equitable access for patients with obesity. Research over the past 10 years has demonstrated safe sedation practices and effective procedure modifications for oocyte retrievals and embryo transfers in patients with obesity undergoing IVF treatment. We encourage IVF medical directors to revisit body mass index restrictions for IVF treatment in favor of individualized patient risk assessments to minimize weight bias and provide timely access to safe and effective IVF care for patients with obesity and infertility. (Fertil Steril® 2024;122:204–10. ©2024 by American Society for Reproductive Medicine.)

Key Words: Obesity, BMI restrictions, IVF, weight bias

Obesity and infertility have been on a collision course over the past 40 years. The prevalence of obesity, defined as a body mass index (BMI) >30 kg/m^2, has doubled from 20% in 1988 to 40% in 2020 among reproductive-age persons in the US (1, 2). Class III obesity, defined as a BMI >40 kg/m^2, has increased from <5% to 12%. Although the rate of obesity has increased in all races and ethnic groups, the prevalence is highest in non-Hispanic Black persons (56%) and Hispanic persons (44%). Obesity is associated with a two- to threefold increase in infertility, largely because of anovulation (3). In patients with polycystic ovary syndrome and infertility, obesity is independently associated with a lower rate of pregnancy (4). Subsequently, patients with obesity are more likely to require assisted reproductive technologies to conceive, yet access to in vitro fertilization (IVF) treatment is restricted in many IVF centers on the basis of BMI thresholds. A survey of IVF medical directors in 2016 found that 65% of IVF centers have policies on obesity, and 85% of centers with policies have a maximum BMI at which they will perform IVF procedures, ranging from 35 to 45 kg/m^2, with 1 center exceeding 50 kg/m^2 (5). The primary criteria for exclusion were anesthesia safety, followed by pregnancy rates, pregnancy complications, weight limits of equipment, and difficult visualization with ultrasound and speculum examinations. We review the evidence regarding anesthesia safety with oocyte retrieval in persons with and without obesity and examine how weight bias may contribute to upper BMI thresholds for IVF treatment. We encourage IVF medical directors to revisit upper BMI thresholds in favor of individualized risk assessment. We describe best practices to facilitate safe and inclusive IVF care for patients with larger bodies.

OOCYTE RETRIEVAL SAFETY

In the early 1980s, the first IVF live births for tubal factor infertility were achieved through laparoscopic oocyte retrievals performed under general anesthesia in hospital settings (6). The introduction of transvaginal ultrasound-guided oocyte retrieval in 1983 facilitated a minimally invasive approach that was quickly adopted (7). By the 1990s, most oocyte retrievals were performed with transvaginal ultrasound guidance in free-standing IVF centers (8). In a large US survey, 95% of oocyte retrievals were done

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under conscious sedation with meperidine and fentanyl used by department-trained staff or a combination of fentanyl with midazolam and/or propofol by anesthesiologists and nurse anesthetists (9). The remaining cases were done by general, regional, or local anesthesia, although deep sedation is a common modality used by anesthesiology personnel, and this was not separated out in the survey. Per the American Society of Anesthesiologists, patients under conscious sedation (also referred to as moderate sedation) are able to respond purposefully to verbal commands alone or accompanied by light stimulation (10). Spontaneous ventilation is adequate without interventions to maintain a patent airway. Deep sedation differs from moderate sedation in that "patients cannot be easily aroused but respond purposefully after repeated or painful stimulation. Patients may require assistance in maintaining a patent airway, and spontaneous ventilation may be inadequate (10)." Complications from retrievals and sedation were rare, and no life-threatening incidents were reported (9).

Overweight and obesity were first associated with poorer IVF treatment outcomes, such as higher cycle cancellation, and lower oocyte yield, in the early 2000s (11). The first report assessing the anesthesia experience of patients with obesity undergoing oocyte retrievals was based on data from a single hospital-based IVF center and was published in 2008 (12). Patients with overweight and obesity were more likely to have comorbid conditions, require alterations in anesthetic approach, and experience more intraoperative and postoperative events, including a greater incidence of desaturation and conversion to general anesthesia with general endotracheal intubation (GETA). Comorbid conditions included gastroesophageal reflux disease, depression and anxiety, hypothyroidism, diabetes, and hypertension, and at least 1 comorbid condition was present in 60% of patients with obesity. Spinal anesthesia and GETA were performed more often in patients with obesity than in patients without obesity (7% and 2%, respectively) and 3 out of 4 conversions from total intravenous (IV) anesthesia (TIVA) to GETA occurred in patients with a BMI >30 kg/m^2. Alterations in the oocyte retrieval approach were also described with more transabdominal oocyte retrievals performed in patients with obesity compared with patients without obesity (3% vs. 0.1%). No complications were reported. A large observational study from 2018 found that the complication rate from oocyte retrievals was 0.76% ,with hemoperitoneum, infection, and pelvic pain accounting for most complications (13). There were 14 anesthesia complications out of 23,827 retrievals (0.06%), and 10 resulted in hospital admission with resolution of the complications in all cases. The 14 anesthesia complications consisted of 12 cases of circulatory shock and nausea, 1 case of atrial fibrillation, and 1 case of cardiorespiratory insufficiency, agitation, and confusion. Of note, the mean BMI in the population was 22 kg/m^2, and a lower BMI was associated with a higher risk of complications. An observational study from 2019 compared the complication rate from oocyte retrievals in patients with a BMI ≥ 40 kg/m^2 (N $= 144$) to patients with a BMI <40 kg/m^2 (N $= 1,691$) in a hospital-based IVF center from 2012 to 2017 (14). Nearly 99% of patients received TIVA, and no patients with a BMI >40 kg/m^2 required conversion to GETA. Continuous positive airway pressure for oxygen desaturation was more common in patients with a BMI >40 kg/m^2 (17.6% vs. 2.7%), as was the need to place an oral or nasal airway (6.3% vs. 1%). Two patients with a BMI >50 kg/m^2 experienced desaturations that did not improve with a bag valve mask and required conversion to a laryngeal mask airway. All cases of spinal anesthesia or GETA were initiated before the procedure, and the rates were similar between patients with and without obesity. Oocyte retrievals were noted to be longer, and transabdominal retrieval was more common in patients with a BMI >40 kg/m^2 compared with patients without obesity (6.3% vs. 0). There was 1 complication that required hospital admission because of postoperative bleeding in a woman with a BMI <40 kg/m^2, and no complications that required hospital admission in patients with a BMI >40 kg/m^2. The investigators conclude that oocyte retrieval can be safely performed as an outpatient procedure in patients with class III obesity. Similarly, another report found no anesthesia or retrieval-related complications in patients with a BMI >40 kg/m^2 (N $= 121$) who received IV conscious sedation with midazolam and fentanyl under the direction of the physician performing the retrieval (15). All patients with obesity were screened for comorbidities before IVF procedure, and the procedures were performed in a hospital-based IVF center. Six sedation-related complications occurred in patients with a BMI <35 kg/m^2 and 10 procedure-related bleeding complications in patients with a BMI <40 kg/m^2. A higher rate of incomplete retrieval was observed in patients with a BMI >40 kg/m^2 compared with patients with a BMI <25 kg/m^2 (18.2% vs. 1.3%), and of the incomplete retrievals, 2 were unable to be initiated because of inaccessible ovaries in patients with BMIs of 40 and 42 kg/m^2. These findings are consistent with the literature regarding the safety of TIVA in patients with obesity undergoing other sedated procedures, such as first-trimester surgical abortions (16).

WEIGHT BIAS AND BMI RESTRICTIONS

Weight bias, or a negative weight-related attitude toward an individual with excess weight or obesity, contributes to weight discrimination or unequal treatment of people because of their weight (17). Explicit and implicit antifat bias is as common among physicians as among the general public (18). Less is known about weight bias in reproductive medicine, although a survey on BMI restrictions for fertility treatments administered by the reproductive endocrinology and infertility subspecialists (REIs) and maternal-fetal medicine subspecialists (MFMs) in 2019 provides insights (19). First, most REIs (97.2%) and MFMs (98.1%) who participated in the survey supported an upper BMI restriction for IVF treatment, but REIs were less likely to support an upper BMI restriction for ovulation induction than MFMs (64% vs. 87.6%, respectively). This suggests that REIs are more concerned about IVF procedure-related complications than pregnancy complications than MFMs. Further evidence of this is that REIs are less likely to have BMI restrictions for recipients of donor eggs than for autologous IVF treatment (5). Second, although lower BMI is a known risk factor for oocyte retrieval and pregnancy complications, REIs were more likely to

support upper BMI restrictions than lower BMI restrictions (72% vs. 56%), which is suggestive of an explicit antifat bias. Upholding upper BMI restrictions and ignoring lower BMI restrictions for IVF treatment despite the evidence that both extremes of weight are associated with risks has occurred in the United Kingdom as well (20). Third, REIs who disagree with upper BMI limits for IVF treatment were more likely to view these policies as stigmatizing and more likely to view BMI restrictions as a barrier to timely access to fertility treatment than REIs who agree with upper BMI restrictions. This suggests that REIs who support upper BMI limits may experience an implicit antifat bias because they are unwilling to reconcile that delaying IVF treatment to achieve weight loss may compromise outcomes in light of evidence that age is the best predictor of IVF treatment success. Finally, most respondents (99.3%) agree that REIs should recommend preconception weight loss to patients with obesity despite evidence that weight loss does not clearly improve live birth rates or good live birth rates, defined as a term delivery of a live-born infant with normal birth weight and no major congenital anomalies, in patients with obesity who undergo ovulation induction and IVF treatment (21). In summary, REIs appear less likely to provide evidence-based IVF care when caring for patients with obesity than patients who are underweight, possibly because of explicit and implicit antifat bias.

INDIVIDUALIZED RISK ASSESSMENT

Roughly half of IVF medical directors report that their affiliated anesthesia departments have a policy regarding BMI for elective procedures in an outpatient setting (5). Although most centers rely on BMI alone, some assess BMI with other criteria such as weight, neck, waist, and hip circumference, percent body fat, and comorbidities such as hypertension, diabetes, and obstructive sleep apnea. Some require anesthesia for preoperative assessments. Patients who are not approved for outpatient procedures are required to have their procedures performed in a surgery center or hospital setting. Oocyte retrievals may not be able to be performed in a surgical center or hospital setting given the time-sensitive nature of the procedure and the controlled environment needed to optimize IVF treatment outcomes. Additionally, few IVF centers are located in surgical centers or hospital settings, which limits the ability to refer to hospital-based IVF centers.

There is no standard policy regarding BMI restrictions for outpatient procedures in the US, so BMI upper limits vary widely from 35 to 50 kg/m^2 (22). The Accreditation Association for Ambulatory Health Care recommends that anesthesia providers should evaluate patients with obesity on an individual basis to determine whether they can safely undergo surgery in an outpatient setting (23). Intrinsic to the creation of patient selection is a thorough understanding of available resources to provide safe care for the patient (24). Individualized preoperative patient evaluations must ensure that the patient is matched to the resources that the site offers. However, the term "resources" is a broad one that deserves highlighting because it is the key driver of many, although not all, anesthesia patient selection decisions (25). Given that most oocyte retrievals are performed in free-standing IVF centers, careful cataloging of immediately available emergency supports is necessary, as is the possibility of leveraging resources such as adjacent health care clinics and emergency medical services. Free-standing IVF centers with a sole anesthesiologist or certified registered nurse anesthetist are at a disadvantage should there be an emergency, especially an airway emergency. Therefore, having staff trained in advanced life support is an absolute requirement. In addition, it is mandatory to have an emergency response plan in writing and easily accessible, should it be needed. Additional "resources" to consider include the equipment, layout, medications, and diagnostic testing available at an IVF center. This consideration encompasses everything from the procedural bed (i.e., weight limit, arm boards, safety straps), the procedural room layout (i.e., size, ability to maneuver, space for anesthesia equipment), available airway equipment (i.e., supraglottic devices, video laryngoscopy, fiberoptic scopes), available equipment to assist in IV placement, including alternatives for peripheral IV access (i.e., vein finder, ultrasound, central line kits), equipment to transfer the patient from one location to the next (i.e., transfer boards and mats, Hoyer lifts), and anesthesia equipment to manage the patient intraoperatively (i.e., anesthesia machine, IV infusion pumps, monitors, warmers, transducers, and others). Additionally, this consideration covers the equipment needed for postanesthesia care as well. Postanesthesia care unit (PACU) monitoring capability, formal electrocardiogram machines, and ventilatory support (i.e., positive airway pressure support, ventilators) are as important to consider as intraoperative equipment. The availability of point-of-care tests, such as hemoglobin, blood gas testing, basic electrolyte testing, and blood glucose testing equipment, is taken into consideration for patient selection criteria.

Despite these resource-based considerations, it is also important to weigh the pressures of patient selection criteria against the perceived negative effects on care, such as decreased access to care and delays in care. So, although it is critically important to have systems in place for the preanesthesia evaluation, it is equally important to have a consensus agreement from the anesthesiologists providing care in any location to guide the preanesthesia evaluation. It is equally critical to de-silo these decisions through multidisciplinary conversations with REIs and REI nursing leadership to discuss the rationale behind the patient selection criteria and brainstorm alternative resources to expand care in any given location. Developing patient selection criteria and individualized risk assessments, outside of BMI as a sole criterion, could minimize the impact of weight bias on access to IVF care. The goal of individualized risk assessments is to provide a consistent and predictable rationale for patient selection for safe anesthesia care for oocyte retrievals, with the aim of improving efficiency, reducing morbidity, and the rare event of a day of surgery cancellation.

BEST PRACTICES

Creating an inclusive IVF treatment environment for patients with larger bodies starts with respectful communication. Health care professionals can approach a discussion of weight

and infertility by asking permission (e.g., "May I discuss weight?") or by introducing the topic generally (e.g., "Patients with higher weight are more likely to experience infertility because they are less likely to ovulate"). Patients prefer that health care professionals use terms such as "weight" to discuss excess weight more than stigmatizing terms such as "morbidly obese," "fat," and "obese" (26). Patients report that they want their health care providers to avoid making generalizations about them and their lifestyles on the basis of their weight and to avoid associating all health conditions with weight by assessing and addressing comorbidities (27). It is important to use person-first language when referencing a patient's weight with other health care team members (e.g., "a patient with obesity" instead of "an obese patient"). Providing fertility care requires a full team, and thus, all faculty and staff should receive education regarding the prevalence of weight bias in health care and should be encouraged to use empathetic, unbiased language.

Additionally, IVF treatment staff can thoughtfully provide size-inclusive care in properly equipped centers. Waiting room chairs in larger sizes should be available. Medical assistants can obtain weight measurements when medically necessary and in a private setting with scales that measure higher body weights. Blood pressure (BP) cuffs and gowns should be available in an array of sizes to obtain accurate BP readings and to avoid leaving patients feeling exposed. Examination and procedure rooms should be equipped with tables that securely accommodate heavier weights. Ultrasonographers can optimize the resolution and quality of transvaginal images by altering the frequency setting and can identify sonographic windows by respectfully asking to adjust a pannus or asking a patient to assist with repositioning during a transabdominal scan. Phlebotomists can attempt to palpate a vein when not visible or apply a warm compress to aid with vein identification for a blood draw. An array of speculums with varying widths and lengths should be readily available with proper lighting when performing a pelvic examination. Providers should start with a standard speculum and make adjustments only when needed because not all patients with obesity will require a longer or wider speculum, and patient comfort should be prioritized.

ANESTHESIA MODIFICATIONS

There are many anesthesia options for patients presenting for oocyte retrieval that have all proven successful, including regional techniques and all levels of sedation up through general anesthesia (28). The goal is to choose the safest anesthesia plan for a patient given the patient's current medical condition and wishes; limitations because of facility resources may also play a role. All anesthesia plans should include monitoring of oxygen saturation, electrocardiogram, exhaled carbon dioxide, BP, and heart rate, and the immediate availability of emergency medications (including reversal agents) and equipment.

Patients with obesity presenting for surgery have increased risks from anesthesia, including aspiration, oxygen desaturation, and increased need and difficulty of airway assistance, such as positive pressure mask ventilation and intubation (29–31). This may be magnified in oocyte retrieval because the patient is in the lithotomy position and the procedure times are prolonged in patients with obesity (14). The preoperative interview and examination should identify risk factors in obese patients for perioperative respiratory compromise, such as known or suspected obstructive sleep apnea (OSA) (e.g., loud snoring, daytime tiredness, observed apnea, large neck), uncontrolled gastroesophageal reflux disease, central distribution of adiposity, and a Mallampati score >2.

Patients with class III obesity or significant OSA do well with moderate sedation for oocyte retrieval. Rapid-onset medications such as midazolam and fentanyl can be carefully titrated while keeping the patient responsive and at minimal risk of airway compromise from obstruction or aspiration. A paracervical block is not sufficient by itself but may be a helpful addition to this technique. Nonopioid sedation with ketamine or dexmedetomidine has been also used for moderate sedation, although dexmedetomidine's relatively slow onset makes it difficult to titrate and may prolong recovery discharge times (32, 33). Dexmedetomidine, however, has resulted in improved quality of recovery scores when replacing fentanyl in a fentanyl and propofol anesthetic (34).

Propofol infusions with fentanyl often cross the line into general anesthesia because the dose necessary to keep the patient immobile for oocyte retrieval prevents the patient from purposefully responding to stimulation (35). This technique works well in patients without obesity and OSA because they are kept spontaneously breathing without an artificial airway and wake up rapidly. There are also reports of successful use of fentanyl plus propofol in patients with obesity and class III obesity (12, 14). However, there are concerns that patients with class III obesity and/or OSA are more likely to need increased oxygen delivery (nonrebreather) and airway maneuvers such as jaw thrust or an oral airway; a laryngeal mask airway or endotracheal tube could on occasion be needed, so propofol and fentanyl should be used cautiously at the discretion of the anesthesia team.

Spinal anesthesia has been used successfully in patients for oocyte retrieval and reduces the likelihood of airway manipulation, but this technique is more challenging in patients with obesity and would be dependent on the skill and comfort of the anesthesia team and the ability to recover a patient with regional anesthesia in the PACU (12).

Safe recovery and expedient discharge of patients with obesity require an understanding that they are more susceptible to post-procedure pain, oxygen desaturation, and the need for supplemental oxygen. The anesthesia plan should include non-sedating adjuncts to control pain, such as 1,000 mg oral acetaminophen preoperatively (although 1 randomized controlled trial of IV acetaminophen failed to show a benefit), 30 mg IV ketorolac at the conclusion of the procedure, and lower abdominal heating pads in the PACU (36–38).

RETRIEVAL MODIFICATIONS

Inaccessible ovaries are the most common challenge to oocyte retrieval in patients with obesity. Thoughtful preparation before the start of stimulation is the most critical component

TABLE 1

Procedural techniques to improve success in difficult oocyte retrievals.

Anesthesia	Adequate anesthesia is essential and mechanisms to optimize for patients with obesity are described in the text.
Ultrasound	Ensuring high-quality ultrasound technology can be especially important during difficult procedures. Being able to adjust frequency and depth, and having high penetration presets can enhance visualization.
Bladder	Fully emptying the bladder can improve visualization and ovary positioning. If preoperative voiding is not sufficient, a sterile catheter can be used.
Pressure	Transvaginal probe manipulation with simultaneous abdominal pressure. This technique often requires assistance from a circulating nurse or medical assistant, ideally one who has some experience assisting in similar cases.
Positioning	Patients should be at the edge of the bed to allow a full range of motion for the transvaginal wand. Additionally, anti-Trendelenburg position can help guide ovaries back into the pelvis when abdominal pressure is insufficient.
Access	A transvaginal transmyometrial approach may be needed and helpful when the above techniques have not yielded success. Transabdominal approach: the transvaginal transducer with the needle guide can be applied abdominally with high confidence and success with this technique. Alternatively, a transabdominal transducer could be used.

Boots. Individualized risk assessment for IVF. Fertil Steril 2024.

of this. Often, patients with a higher BMI have ovaries that are easily visualized and accessible. In fact, 1 center described no increase in the number of difficult retrievals requiring alternative maneuvers (39). However, ovarian access should be assessed as part of the initial evaluation that determines the treatment plan and whether IVF is a reasonable treatment option. In the event that ovaries are difficult to visualize and access, these patients need additional counseling about the potential for less accurate monitoring during the stimulation and for an incomplete retrieval. This is the same counseling we would provide to patients with endometriosis, large and multiple fibroids, or ovaries that are adhered high or out of the pelvis.

Similar to the approach in patients with endometriosis or fibroids, several techniques can be employed to improve oocyte yield and decrease complications (Table 1). Thoughtful preparation should also include scheduling considerations, such as scheduling challenging cases as the last retrieval of the day in the event that additional time is needed to employ techniques to improve oocyte yield.

EMBRYO TRANSFER MODIFICATIONS

Optimizing embryo transfer (ET) in patients with obesity relies on advanced preparation as well. A trial ET with a full bladder and ultrasound guidance should be utilized to ensure that the ET is as efficient and least traumatic as possible. This opportunity allows for optimal speculum selection and cervical visualization. It confirms abdominal ultrasound visualization is possible and can guide optimal ultrasound settings (i.e., gain, depth, and penetration). Occasionally, people with obesity have longer vaginal canals, and thus, an array of ET catheters with varying lengths can facilitate the ease of ET. When even the longest transfer catheter still does not reach the ideal location within the cavity, a ring forcep can be used to extend manual reach. Trial ET can also identify patients who might benefit from a cervical stitch being placed in advance of an ET to facilitate visualization of the cervix and navigation of the cervical canal in cases where transabdominal visualization may be limited by habitus. When an abdominal ultrasound approach is simply not adequate, an ET can be performed with a transvaginal transducer. A large open-sided Graves or similar speculum should be placed, and the ET catheter should be inserted into the cervix. The transvaginal transducer can then be placed through the speculum, and the catheter is then advanced into the endometrial cavity. Alternatively, transrectal ultrasound could be considered to optimize visualization. Although not traditionally used, both transvaginal and transrectal ultrasounds have been successful with more difficult procedures. Lastly, when patients poorly tolerate the discomfort of a difficult trial of ET, we can also ensure that partner support is present and/or consider the use of anxiolytics.

CONCLUSION

Unless the trends observed over the last 40 years are halted or reversed, nearly half of the population will have obesity, and nearly 1 in 4 will have class III obesity by 2030, with higher rates observed in non-Hispanic Black and Hispanic populations (40). Although glucagon-like peptide 1 agonists and similar antiobesity medications provide promise for safe and effective weight loss over a short timeframe, the cost of glucagon-like peptide 1 agonist treatment for 1 year exceeds the cost of IVF treatment in many regions of the country (41). Limited access to cost-prohibitive antiobesity medications before IVF treatment will further exacerbate inequitable access to infertility care, particularly among non-Hispanic Black patients and Hispanic patients. To provide safe and equitable access to IVF treatment for a growing population of patients with infertility, we need to adopt individualized risk assessments, recognize our explicit and implicit antifat biases, and adapt our approach and equipment to support people with larger bodies. Larger data sets are needed to truly assess the risks of rare sedation and procedure-related complications so that individualized risk assessments are evidence-based and stratified by comorbidities, not BMI. We need to move beyond BMI, an imperfect measure of health, to meet the family-building needs of our population in the decades to come.

CRediT Authorship Contribution Statement

Christina E. Boots: Conceptualization, Writing – original draft, Writing- review & editing. **Marjorie Gloff:** Conceptualization, Writing – original draft, Writing- review & editing. **Stewart J. Lustik:** Writing – review & editing, Writing –

original draft. **Wendy Vitek:** Conceptualization, Writing – original draft, Writing- review & editing.

Declaration of Interests

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Treating obesity and fertility in the era of glucagon-like peptide 1 receptor agonists

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The objective of this narrative review is to advocate for improved comprehensive care of patients with obesity and infertility. Persons with an increased body mass index have less successful reproductive outcomes, and recently, new medications to treat neuroendocrine hormone imbalances are producing meaningful weight loss akin to surgical interventions. For the first time, obesity is publicly being recognized as a disease. These medications contain the newest generation of glucagon-like peptide 1 receptor agonists and deserve our attention for several reasons: regardless of body mass index, many patients will be using them; it is necessary to understand the mode of action, side effects, and implications for anesthetic procedures and pregnancy; and it is important to evaluate when they could be used to improve health outcomes and/or access to fertility care. (Fertil Steril® 2024;122:211–8. ©2024 by American Society for Reproductive Medicine.)

Key Words: Fertility, glucagon-like peptide 1 agonist, obesity, weight loss

The objective of this narrative review is to advocate for improved comprehensive care of patients with obesity and infertility. Persons with an increased body mass index (BMI) have less successful reproductive outcomes. This patient population presenting for infertility treatment is unique; they are motivated, compliant, educated, and eager to learn more. However, simply telling them to lose weight by "eating less and exercising more" is insufficient in addressing the obesity epidemic. No woman who has reached reproductive maturity and has not, at some point, attempted to modify her weight, which is why the new category of antiobesity medications has created a firestorm in society. For the first time, obesity is publicly being recognized as a disease because medications to treat neuroendocrine hormone imbalances are producing meaningful weight loss akin to surgical interventions. These medications contain the newest generation of glucagon-like peptide 1 (GLP-1) receptor agonists (RAs) and deserve our attention for several reasons: regardless of BMI, many patients will be using them; it is necessary to understand the mode of action, side effects, and implications for anesthetic procedures and pregnancy; and it is important to evaluate when they could be used to improve health outcomes and/or access to fertility care.

PRECONCEPTION CARE

Weight loss may be one mechanism to improve reproductive outcomes; however, it is not the only tool in elevating the care provided to patients with obesity. Before initiating fertility care to any patient, enhanced preconception counseling should be conducted. This is perhaps even more important for patients with a higher BMI. They should be screened for comorbidities (i.e., diabetes, hypertension, and thyroid dysfunction), which extends beyond screening for overt disease to also the precursors of these diseases. For example, many of our young patients have early insulin resistance (diagnosed by a hemoglobin A1c [HbA1c] level of 5.7%–6.4% or abnormal fasting glucose or response to oral glucose load) but are not yet in the diabetic range (HbA1c level of >6.5%). Counseling patients on the spectrum of diseases and improving their trajectory are a unique and meaningful opportunity for preconception providers. Regardless of BMI, counseling on nutrition and physical activity should be performed. Although BMI can be predictive of poor lifestyle choices, normal and high BMIs can also be realized with poor and healthy habits, respectively. We should not assume that big equals bad or that lean equals good, or we will miss the opportunity to affect change.

In addition to optimizing physical health, screening mental health and well-being should also be a focus of the preconception conversation. Patients with obesity are at higher risk of depression, anxiety, and disordered eating, and these are only exacerbated

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by adding the diagnosis of infertility. Screening for these mental health disorders and offering reliable referrals to mental health resources, including psychiatrists, psychologists, and peer groups specialize in perinatal mental health, are critical to providing excellent care (1).

FERTILITY CARE

The relative risks of poorer reproductive outcomes are higher in patients with an increased BMI, whereas the absolute risks are generally low. To date, there are no high-quality prospective studies showing that acute weight loss improves fertility treatment outcomes. For these 2 reasons, BMI alone should not be used to restrict access to fertility care (2), and instead, our field should be working to improve the care for patients in higher BMI ranges. Although BMI alone should not restrict access to fertility care, it should be considered when an individualized care plan is created. This care plan should consider how BMI may be associated with difficult visualization of the ovaries and/or increased complexity of procedures such as saline ultrasound examinations, intrauterine inseminations, and embryo transfers. There may also be a role for higher medication dosing during ovarian stimulation and/or altered administration techniques, such as intramuscular vs. subcutaneous trigger medications (3, 4). Anesthesia colleagues have set strict BMI limits in an effort to predict airway complications during monitored anesthesia care, and often, this is the BMI that ultimately limits access to in vitro fertilization (IVF) (5, 6). It is possible that airway prediction may be improved with additional variables (i.e., neck circumference or previous anesthesia events) beyond BMI alone. Further discussion about expanding IVF access to a higher BMI is in the third article of this Views and Reviews. When restrictions are limited beyond our specialty, our focus should be on the following: referrals to IVF centers with advanced anesthesia options; improving other non-IVF aspects of fertility care; and, for some patients, helping them achieve a BMI that both improves health and increases their access.

WEIGHT LOSS

Weight loss may be the "right" answer for some patients as part of their infertility treatment plan. The treatment plan should consider the patients' age, ideal family size, other aspects of their health, and their desire for and commitment to weight loss. Maternal age and its influence on oocyte quality remain the overarching predictor of fertility success, and thus, pausing fertility treatments to focus on weight loss and improved health should balance the risks of reproductive aging and consider the ideal number of children (7). The mechanisms of weight gain and obesity are vast and complex, as well as the weight loss strategies. However, these strategies are routed in 5 tenets: nutrition; physical activity; behavioral therapy; medications; and bariatric surgery. When weight loss is recommended, education, resources, and ongoing support should be offered to both increase effectiveness and ensure that the weight loss is achieved safely.

To properly counsel patient about weight loss, it is important to have a solid and up-to-date understanding of the evidence surrounding weight loss and fertility (8). Recent large prospective studies have mostly not demonstrated an increased live birth rate (Table 1) (9–13). Five randomized controlled trials, each with sample sizes of >100 women, have explored whether acute weight loss before fertility treatment improves success (9–13). In these studies, the weight loss interventions were successful in achieving the goal of approximately 7% loss (i.e., 9–20 lb on average) using caloric restriction, sometimes with increased physical activity, and/or antiobesity medications. The intervention groups had higher rates of spontaneous conception and improved ovulation in the setting of ovulation induction. However, in the general infertility and unexplained infertility population, weight loss did not improve the live birth rates. In 3 of the studies, the weight loss intervention was associated with a nonsignificant increase in the miscarriage rates. Although the lack of statistical significance should be noted, the trend suggests caution and a potential increased risk of miscarriage for some patients.

There are no prospective studies on bariatric surgery and subsequent fertility outcomes; however, in the setting of anovulation, resumption of menses has been documented after the weight loss associated with bariatric surgery (14, 15). A recent large retrospective study compared IVF outcomes in patients who had previously undergone bariatric surgery with those in age- and BMI-matched controls (16). There was no difference in the cumulative live birth rates; however, those who underwent bariatric surgery retrieved fewer oocytes and had fewer frozen embryos and lower birth weights. Additional evidence showing worse obstetric outcomes has cautioned that conception should not happen within at least 1 year of surgery (14, 15).

Finally, there is a new class of antiobesity medications on the market that is demonstrating effective and meaningful weight loss—GLP-1 RAs, such as semaglutide (17) and tirzepatide (18). A landscape of medical interventions to aid in weight loss before pregnancy have been recently reviewed (19, 20). The approval of GLP-1 RA agents to treat obesity irrespective of diabetes has led to an increase in uptake of these medications and raises concerns about heightened prenatal exposure (21). Although there are no studies correlating weight loss achieved with these medications with improved fertility outcomes, we anticipate seeing data in the near future. Several trials are registered; however, data have not yet been published. The remainder of this article focuses on these new medications that reproductive endocrinologists will be seeing more of and, we would argue, should be using as the most effective, least invasive means of weight loss.

GLP-1 AND GASTRIC INHIBITORY POLYPEPTIDE RAS IN FERTILITY CARE

Implementing obesity management into a reproductive endocrinology practice is realistic in both academic settings and private practice. Most reproductive endocrinologists have experience treating polycystic ovary syndrome and, thus, have managed similar medications with similar side effects, such as metformin. Additionally, as infertility specialists, we are familiar with prescribing medications that require specialty pharmacies and insurance prior authorizations.

TABLE 1

Randomized controlled trials investigating weight loss and fertility treatment outcomes.

Trial name	Lead investigator	Publication year	Study site	Study population	Study duration	Intervention 1	Intervention 2	Control	Fertility treatment	Weight loss	Live birth rate	Miscarriage rate
OWL-PCOS	Legro et al. (9)	2015	United States, multicenter	PCOSAge, 18–40 y; BMI, 27–42 kg/m^2	16 wk	Caloric restriction with meal replacements, medication (sibutramine or orlistat), and increased physical activity	Both intervention 1 and OCP	OCP alone	Ovulation induction with timed intercourse for 4 cycles	−6.2% and −6.4% −1.0% $P < .001$	Intervention: 25.0% (12/48) and 25.5% (12/47) Control: 8.5% (4/47)	20% (3/15) and 14.3% (2/14) 42.9% (3/7)
	Mutsaerts et al. (10)	2016	The Netherlands	InfertilityAge, 18–38 y; BMI, >29 kg/m^2	6 mo	Caloric restriction, increased physical activity, and coaching	None	Direct to fertility treatment	Standard of care Dependent on indication	−4.4 kg −1.1 kg $P < .001$	Intervention: 43.9% (123/280) Control: 53.9% (153/284) RR, 0.82; 95% CI, 0.69–0.97	23.4% (41/175) 14.5% (27/186) RR, 1.54; 95% CI, 0.98–2.43
	Einarsson et al. (11)	2017	Sweden, Denmark, and Iceland	InfertilityAge, 18–38 y; BMI, 30–35 kg/m^2	12 wk of weight loss2–5 wk of weight stabilization	Strict low-calorie liquid formula diet	None	Direct to fertility treatment	1 cycle of IVF	−9.44 ± 6.57 kg +1.19 ± 1.95 kg $P < .00001$	Intervention: 29.6% (45/152) Control: 27.5% (42/153) 95% CI, 12.9 to −8.6; $P = .77$	30.3 (20/66) 23.2% (13/56) Not significant
	Wang et al. (12)	2021	China	InfertilityAge, 20–40 y; BMI, >25 kg/m^2	4–12 wk	Orlistat (weight loss medication) plus recommendations for low-fat diet and high-quality physical activity	None	Placebo medication plus recommendations for low-fat diet and high-quality physical activity	1 cycle of IVF	−2.49 kg −1.22 kg P .005	Intervention: 25.5% (112/439) Control: 25.6% (92/438) RR, 1.0; 95% CI, 0.8 −1.25; $P = .98$	19.4% (27/139) 15% (20/133) RR, 1.29; 95% CI, 0.76–2.19
FIT-PLESE	Legro et al. (13)	2022	United States, multicenter	Unexplained infertilityAge, 18–40 y; BMI, >30 kg/m^2	16 wk	Caloric restriction with meal replacements, orlistat, and increased physical activity	None	Increased physical activity without weight loss goals	Ovarian stimulation with intrauterine insemination for 3 cycles	−6.6 ± 5.4% −0.3 ± 3.2% $P < .001$	Intervention: 12.2 % (23/188) Control: 15.2% (29/191) RR, 0.81; 95% CI, 0.48–1.34; $P = .40$	33.3% 14/59)23.7% (21/63) RR, 1.40; 95% CI, 0.79 −2.50

Note: BMI = body mass index; CI = confidence interval; OCP = oral contraceptive pill; PCOS = polycystic ovary syndrome; RR = relative risk.

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TABLE 2

Summary of the 2 newest medications containing glucagon-like peptide 1 receptor agonist.

Name	Mechanism of action	Weight loss efficacy after 1 y (placebo-subtracted)	Dose titration	Safety concerns/ contraindications	Duration to stop before anesthesia	Duration to stop before pregnancy
Semaglutide	GLP-1 receptor agonist that suppresses appetite and delays gastric emptying	−14.9% (−12.5%)	0.25 mg weekly × 4 wk and then 0.5 mg; can increase to 1, 1.7, or 2.4 mg as needed	Family or personal history of medullary thyroid cancer or MEN2 syndrome and pregnancy	At least 1 wk per anesthesia guidelines	At least 2 mo before conception per manufacturer
Tirzepatide	GIP/GLP-1 dual agonist that suppresses appetite and delays gastric emptying	−15% (−11.9%) at 5 mg −19.5% (−16.4%) at 10 mg−20.9% (−17.8%) at 15 mg	2.5 mg × 4 wk and then increase to 5 mg; can increase by 2.5 mg every 4 wk to a maximum dose of 15 mg as needed	Family or personal history of medullary thyroid cancer or MEN2 syndrome and pregnancy	At least 1 wk per anesthesia guidelines	At least 1 mo before conception per manufacturer

GLP-1 = glucagon-like peptide 1; MEN2 = multiple endocrine neoplasia type 2.

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Referrals to clinics that specialize in weight loss management can be a great resource to our patients. However, several patients will not have efficient access to this resource because of geographic, financial, or wait time constraints. Thus, once a patient with obesity and infertility presents to a fertility office, we either would ideally have expedient referrals to multidisciplinary practices or should provide this care as part of our comprehensive reproductive treatment plan. Let us walk through a patient scenario.

A 32-year-old woman presents with 1 year of primary infertility. Her cycles are every 32–38 days, and the BMI and antimüllerian and HbA1c levels were 41 kg/m^2, 5 ng/mL, and 5.9%, respectively. After thorough preconception testing and a fertility evaluation determined no clear etiology for her fertility, her reproductive endocrinology and infertility specialist offers comprehensive treatment options. First, we assess the patient's potential and willingness to improve lifestyle and mental health status. We discuss the basic concepts and importance of nutrition and mental health. Online resources and a referral to a dietician and psychologist are provided. Second, we discuss the role BMI may play on infertility and briefly review the data on weight loss, ovulatory function, and the fertility treatment, such as IVF. After further discussion, the patient describes several years of weight loss attempts with various calorie-restrictive diets and the inability to sustain meaningful weight loss. Given her good ovarian reserve and relatively young reproductive age, she is motivated to pursue weight loss again before fertility treatment and is particularly excited to consider one of the new antiobesity medications that she has heard so much about.

Medication choice

To select the right medication, we need to better understand GLP-1 and gastric inhibitory polypeptide (GIP) (Table 2). Glucagon-like peptide 1 and GIP are 2 incretin hormones that are secreted by L-cells in the gut after food intake (22). They bind to their respective receptors to stimulate insulin secretion in a glucose-dependent manner. Glucagon-like peptide 1 and GIP RAs mimic the effects of incretin by binding the receptor of GLP-1 or GIP, leading to prolonged physiologic actions. Both have central effects to reduce appetite and decrease gastric emptying to increase satiety, which can lead to reduced food intake and lowering of body weight (23). In addition to influencing multiple organs such as the brain, pancreas, skeletal muscle, and kidneys, GLP-1 RA can increase glucose uptake and lipolysis in white adipose tissue and thermogenesis in brown adipose tissue (22).

Short-acting (daily administration) GLP-1 RAs, such as exenatide (Byetta), liraglutide (Victoza), and dulaglutide (Trulicity), are approved for use in diabetes. After approval for glucose control, liraglutide was approved and commercialized for weight loss (marketed as *Saxenda*). The newest generation of these medications is longer acting (weekly administration). Semaglutide is a GLP-1 RA and is marketed as *Ozempic* for diabetes use and approved at higher doses, under the name Wegovy, for chronic weight management. The enhanced efficacy of the weekly administration of GLP-1 RA (17) compared

with its predecessors has caught media attention and catapulted its popularity. Most recently, a medication that combines the dual stimulation of both GLP-1 and GIP receptors received Food and Drug Administration approval. Tirzepatide leads to further improvement in both glucose reduction and weight loss in patients with and without type 2 diabetes compared with semaglutide (18). Tirzepatide for the treatment of diabetes is marketed as Mounjaro and received Food and Drug Administration approval for weight loss in patients without diabetes in late 2023 under the name Zepbound. Given that semaglutide and tirzepatide are the most effective and most common at the time of this publication, our recommendation to our hypothetical patient is to start with tirzepatide.

Prescribing

Semaglutide and tirzepatide are subcutaneous injections that are administered weekly by the patient at home. The medication is started at a low dose and then titrated at the manufacturer's recommended schedule. The starting dose of semaglutide is 0.25 mg for 4 weeks and then increased to 0.5 mg. Depending on clinical response and side effects, the dose can be gradually increased to 1, 1.7, or 2.4 mg and then maintained.

Tirzepatide is also a subcutaneous weekly injection. Dosing principles start at 2.5 mg, increasing to 5 mg weekly after 4 weeks. Further titration by 2.5 mg every 4 weeks depends on the response as well as the ability to tolerate the adverse gastrointestinal symptoms. If losing weight, then the dose does not need to be increased. There is no specific biochemical monitoring recommended for either medication. When initiating tirzepatide, patients should be counseled regarding the potential reduction in efficacy/absorption of oral contraceptives (24). Thus, women are advised to use nonoral contraception for 4 weeks after each dose escalation. This has not been noted by manufacturers when prescribing semaglutide (25).

Finally, there are 2 other considerations when prescribing these medications: cost and medication shortages. Several insurance companies require prior authorization for these medications. A significant administrative burden is placed on the clinical office to submit this and often resubmit with alternative brands if the first request is denied. However, fertility offices have some familiarity with this process given the need for authorization for several fertility medications and procedures. Because of their popularity and manufacturer delays, several patients have difficulty accessing these medications, especially at certain doses. Delay of doses often causes delay of results, and these medications may need to be retitrated. Additionally, there are no clear guidelines about switching between these medications and whether starting at the lowest dose and titrating from the beginning are necessary. The lack of generic formulations keeps the cost of these medications high, and without insurance coverage or special coupons, monthly costs can be thousands of dollars in the United States. Given the limited data on safety and efficacy, the use of compounded agents is not recommended.

Efficacy and expectations

The efficacy of semaglutide has been studied in the Semaglutide Treatment Effect in People with Obesity (STEP) trials. The last study, STEP 8, described a mean weight loss of 15.8% in 338 subjects after 68 weeks. Most participants were women (78.4%) (26). Tirzepatide use in individuals without diabetes but with a BMI of >30 kg/m^2 was evaluated in the SURMOUNT-1 trial (18). The mean changes in weight after 72 weeks with 5-, 10-, and 15-mg doses were 15%, 19.5%, and 20.9% loses, respectively (vs. placebo, −3.1%). Extension trials from STEP 1 and SURMOUNT documented utility in the longer use of GLP-1 and GIP RAs. The STEP 4 extension trial illustrated that weight loss plateaued around week 60 of semaglutide use with a total expected weight loss of 17.4% (26). When tirzepatide use was extended (SURMOUNT-4), the weight loss continued until week 76 and averaged 25.8% until the trial was stopped at week 88 (27). *Excitingly, more than 30% of patients lost >25% of their body weight, which is approaching the estimated rate of approximately 30% after bariatric surgery* (28). There are no current guidelines on the standard interval and frequency of monitoring visits. General recommendations would be to offer check-ins every few months with increasing intervals over time. These check-ins should be to assess the pace of weight loss, offer support in cases of side effects, encourage healthy nutrition and continued exercise, and monitor mental health. These visits could be in person or virtual and could be provided by the physician or other clinical providers, such as nurses, pharmacists, and/or nurse practitioners.

Side effects and contraindications

Semaglutide and tirzepatide medications have similar adverse reaction profiles predominantly manifesting as gastrointestinal symptoms such as nausea, vomiting, and constipation, which can be severe at times (17). As noted in the phase 3 trials, these symptoms were daily for some people. Over-the-counter medications, such as antinausea or antacid medications, can be used to manage these side effects. Reports of gallstones have been documented (25), and rare, serious instances of gastroparesis have been postulated to be associated with semaglutide (29). Long-term implications associated with weight loss, including reductions in lean muscle mass (17) and/or the development of osteoporosis, are areas of concern (30).

The only clear contraindications to using these medications are pregnancy, lactation, and individuals with a known genetic predisposition to medullary thyroid cancers or associated syndromes (e.g., multiple endocrine neoplasia type 1). Caution is also advised against the use of these medications if there is a personal history of pancreatitis.

Glucagon-like peptide 1 RAs are considered class C for use in pregnancy and are not recommended. Manufacturers of semaglutide recommend abstaining from the medication for 8 weeks before pregnancy because of observed teratogenicity in animals treated with semaglutide. Manufacturers of tirzepatide recommend abstaining for 4 weeks before pregnancy in the Canadian product guidelines; however, the US product guidelines do not provide a recommended amount of time between discontinuation and conception (24). Preclinical animal studies suggest a higher risk of early pregnancy loss, reduced fetal weight, and increased skeletal and visceral malformations. However, the challenge lies in differentiating whether observed fetal outcomes result from an energy deficit or specific drug effects, given that pregnancy should not be a hypocaloric state.

Human gestational exposure to long-acting GLP-1 RA is limited in the published literature. Muller et al. (31) summarized 26 records of exposure of short-acting GLP-1 RA (liraglutide or exenatide) during pregnancy or lactation, and Minis el al. (32) reported 2 cases of exposure to liraglutide up to 13 weeks' gestation and dulaglutide up to 15 weeks. After cessation of the medication and initiation of insulin, no fetal malformations, comorbidities, or maternal complications were reported, and neonatal weight was appropriate for gestational age. In the original drug application trials for semaglutide (SUSTAIN 1–6 and STEP 1–5 and 8), 46 pregnancies were exposed to the drug for a short duration until pregnancy was confirmed. There was a single report of a congenital anomaly of the external ear. All semaglutide-treated women had healthy children with no pregnancy losses or confirmed teratogenicity (25). In 2023, Skov et al. (33) reported a case where semaglutide was discontinued at 3 weeks and 4 days of gestation. The pregnancy resulted in an infant with macrosomia, and the investigators attributed the infant size to the "rebound weight gain" because the patient did not have gestational diabetes. Finally, a 2023 review of a noninsulin antidiabetes medication in the *Journal of the American Medical Association* found that 461 individuals filled GLP-1 RA prescriptions in the first trimester. There was no statistically significantly increased risk of malformations (21).

There are no clinical trials or case reports regarding tirzepatide use in pregnant patients. Studies in animals (i.e., rats and rabbits) have shown harm to fetal development when there was maternal weight loss (24).

In a patient with infertility, a crucial consideration would be the use of these medications before procedures requiring anesthesia, such as an oocyte retrieval or hysteroscopy. The American Society of Anesthesiologists Consensus currently recommends discontinuing the long-acting GLP-1 and/or GIP RA 7 days before the procedure, regardless of the indication of the medication, procedure being performed, or type of anesthesia being planned (intravenous sedation and general anesthesia both require 1-week abstinence) (34). This recommendation is due to the known delay in gastric emptying and, thus, increased risk of aspiration.

Medication discontinuation

Weight regain is commonly observed after a reduction in BMI whether achieved through medical or lifestyle modifications. Weight regain after discontinuation of GLP -RA is anticipated because of the resurgence of physiologic response to weight loss and adaptations in gut hormones aimed at maintaining weight homeostasis. This observation has been documented in the extension of the STEP 1 trial (35). One year after stopping semaglutide 2.4 mg, participants had regained two thirds of their weight loss. Furthermore, the STEP 4 study compared those who stopped semaglutide 2.4 mg after 20 weeks with those continuing for an additional 48 weeks. Those who continued the medication had sustained weight loss, whereas those who switched to placebo experienced weight gain to less than their baseline weight. However, the weight regain had not yet plateaued at week 68 when the study was concluded (36). Similar observations were described in the SURMOUNT-4 study, where withdrawal of tirzepatide led to regain of lost weight and continued treatment maintained or progressed weight loss (27).

When GLP-1 RAs are discontinued before conception, it is imperative to assess possible ramifications of acute and possibly rapid weight regain before and in early gestation, as well as the resulting untoward influence on mental health. As observed in 1 case report (33), weight regain can manifest in an overshoot effect leading to potentially higher than expected weight gain in pregnancy. The profound return of hunger cues has been reported and will require psychological management. Potential consequences of excessive preconception weight gain or "weight cycling" are excess gestational weight gain and associated negative outcomes including early pregnancy loss, gestational diabetes, preeclampsia, and nonelective cesarean delivery (37). It is crucial, but challenging, to mitigate the risk of weight cycling through strategies including counseling on behavioral changes and nutritional support and physical activity. The adoptions of multidisciplinary and multifaceted approaches, when available, are highly recommended for the best chance of long-term weight management (38).

The optimal recommendations on how to stop semaglutide or tirzepatide are not known. Weaning the dose of the medication may have less obvious appetite stimulation than an abrupt cessation. However, this has not been studied. A preliminary unpublished study (39) suggested that bridging with metformin offered some improvement in weight regain, although improved cardiometabolic parameters did revert toward baseline once semaglutide was switched back to metformin or no medication.

Specific considerations of GLP-1 RA in fertility treatment

There are nuances to using this medication around the time of fertility treatment. Notably, the recommendations currently encourage discontinuation before conception; however, the preconception use of this medication may have important benefits and possible repercussions. The reproductive effects of GLP-1 RA are not well understood. Although it has been suggested that GLP-1 receptors exist in the reproductive system (40), to date, there is no human clinical data describing fertility impacts. The benefits of using these medications preconception include improved insulin sensitivity and other

metabolic parameters associated with higher BMIs, such as blood pressure and inflammation. In a single prospective study of 27 participants, an improved embryo implantation rate after pretreatment with a short-acting GLP-1 RA (liraglutide) before IVF was reported. The investigators hypothesized that the anti-inflammatory properties of this medication may positively affect the endometrium and enhance receptivity and implantation (41).

However, detrimental effects could be postulated as well. Nutritional deficits and the altered metabolism noted during acute weight loss may affect pregnancy and neonatal outcomes. Perhaps, similar to the randomized controlled trials using lifestyle and other weight loss medications, acute weight loss may be associated with improved ovulation rates and spontaneous conceptions but not with assisted reproductive technology outcomes. We need to carefully monitor the rates of miscarriage, and similar to the results noted after bariatric surgery, we need to carefully monitor birth weights and the metabolic health of children. The rate of preconception weight loss should be monitored, and thoughtful nutritional counseling to ensure nutritional adequacy should be provided to persons taking this medication preconception. Although not yet identified, there may benefits to assessing nutritional status before conception by taking a dietary history, ensuring prenatal vitamin adherence, and considering screening the serum levels for common vitamin deficiencies (iron, vitamin D, and vitamin B12) and anemia.

Before initiating these medications, mental health support should be provided, and screening for disordered eating behaviors should be performed. Weight loss, especially required weight loss, can trigger new events for several persons who have struggled with body image, weight, and food. Ensuring that they have specialized support during this time is important to providing comprehensive and compassionate care to our patients.

One of the most compelling uses for these medications is for those undergoing ovarian stimulation for planned oocyte or embryo cryopreservation. There is currently a lack of data regarding the use of GLP-1 RA medication during oocyte stimulation. Data collection is ongoing. One of the primary advantages of continuing this medication around the time of ovarian stimulation in the setting of required weight loss is to access this treatment. As shown in the data earlier, weight regain is common after discontinuation of the medication, and for persons just under the BMI cutoffs, prolonged time without the medications incurs the risk of not meeting these restrictions before their first cycle or in the event of needing a second or third cycle. Finally, counseling regarding the anesthesia recommendation to discontinue long-acting GLP-1 RA is critical to ensuring that this process is completed in a timely and safe manner.

CONCLUSION

Reproductive endocrinology and infertility specialists should consider integrating obesity management into the care of our patients with appropriate collaboration and resources.

As compassionate and comprehensive caregivers, it is our responsibility to ensure that patients with high BMIs are receiving unbiased, evidenced-based treatment. This is most likely to occur using a multidisciplinary approach. For any patients considering or already taking antiobesity medications, we should be offering nutritional counseling and mental health support and ensuring that they discontinue appropriately before conception and/or procedures requiring anesthesia.

Weight loss before pregnancy in some persons living with obesity may restore ovulation and improve maternal and fetal outcomes. However, long-term data are not yet available, and thus, careful attention to ongoing, upcoming research is needed.

CRediT Authorship Contribution Statement

Alyse S. Goldberg: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Christina E. Boots:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing.

Declaration of Interests

A.S.G. reports honoraria from Biosyent. C.E.B. has nothing to disclose.

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The assets and risks of expanded carrier screening in gamete donation

Carrier screening in reproductive medicine is aimed at detecting deoxyribonucleic acid variants that are linked to a recessive genetic disease. Done before or during pregnancy, couples and individuals can be identified who are at risk of passing on genetic conditions to their offspring. When both partners carry the same autosomal recessive disease, there is a 1 in 4 chance in each pregnancy of having an affected child. For X-linked disorders, half of the male children of a carrier mother will be typically affected. This knowledge can guide reproductive decisions for those at high risk.

Carrier screening programs have existed since the 1970s and were initially focused on ethnic groups with high rates of specific conditions. However, genetic conditions do not occur solely in specific ethnic groups, and our increasingly multiethnic society makes it ineffective to focus on populations on the basis of ancestry. In fact, most affected children are born into couples with no known family history. Carriers are typically healthy and lack a family history of genetic conditions; they are usually unaware of their reproductive risk until their child is diagnosed with a genetic disorder (1). It has been estimated that 2 in 100 couples are at risk of having a child with a recessive genetic condition. Consequently, in the past decade, carrier screening has become more common for the general preconception and prenatal populations.

EXPANDED CARRIER SCREENING

With advancements in genotyping and sequencing technologies, expanded carrier screening has emerged. In expanded carrier screening, many disorders are screened using a single sample. Companies that offer expanded carrier screening create their own lists of disorders that they test for. This list is called a screening panel and enables the simultaneous assessment of 100 different conditions (2). Particularly in gamete donation, expanded carrier screening is adopted widely because donors' genetic backgrounds are often unknown, self-reported ethnicity can be inaccurate, and donations frequently occur across borders.

Expanded carrier screening is relatively easy but comes with costs and may pose a significant challenge in gamete donation. How are donor privacy and rights protected? What about genetic counseling when the screening panels include variants of unclear significance? The question is whether these and other potential disadvantages provide sufficient reason not to do these tests standardly. The goal of this "fertile battle" is to have our combatants help us understand the pros and cons of expanded carrier screening in gamete donation, and I feel the investigators did a wonderful job (3).

The proponents, Drs. Capalbo, Pla, and Janssens state that expanded carrier screening should be offered as part of reproductive medicine, including gamete donation cycles. The investigators argue that expanded carrier screening ensures the health of future offspring and provides psychological reassurance to recipients but acknowledge there are some barriers and make some suggestions to overcome these barriers. They suggest concerns about donor privacy and rights to be mitigated through opt-out options for receiving results, and to use genetic matching protocols to prevent donors from being rejected solely on the basis of carrier status. Comprehensive screening could be used to support dynamic donor-recipient matching, this could maximize the use of available gametes.

The opponents, Drs. Accoe, Pennings, and Mertes warn us about the risks of expanded carrier screening. The investigators do endorse carrier screening but explain that expanded carrier screening can cause psychological distress, misunderstandings about health, and increased financial burdens, leading to reduced access and fewer donors. It is argued that the benefits of expanded carrier screening are limited because a significant reduction in risk can be achieved with a smaller panel of screenings. Expanded carrier screening is also criticized for potentially reinforcing negative attitudes toward disabilities, promoting discriminatory societal norms, and not respecting donor autonomy. The investigators suggest that to enhance autonomy, recipients should be informed about the genetic risks without automatically disqualifying donors on the basis of expanded carrier screening results.

Our battling teams have both done a superb job presenting a succinct and persuasive argument. Both teams come up with suggestions on how to deal with barriers and risks. This discussion will hopefully prove useful in providing a richer understanding of the risks and benefits of expanded carrier screening and how we can move forward while best serving the needs of both donors and recipients.

CRediT Authorship Contribution Statement

Madelon van Wely: Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Interests

M.v.W. has nothing to disclose.

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Should we use expanded carrier screening in gamete donation?

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Disclaimer: Authors for "fertile battles" are chosen to represent the full breadth of opinions. Individual authors, even within one side of the debate, do not necessarily agree with all viewpoints expressed.

PRO – Expanded Carrier Screening Should Be Used In Gamete Donation

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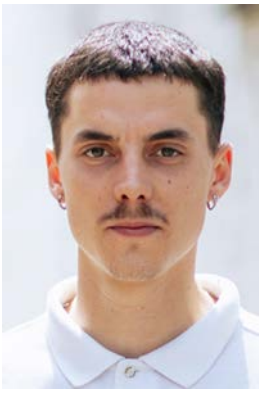

CON – Expanded Carrier Screening Should Not Be Used In Gamete Donation

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PRO – Expanded Carrier Screening Should Be Used In Gamete Donation *(continued)*

Sandra Janssens, M.D., Ph.D.

Recent advances in sequencing technologies and enhanced understanding of clinical genetics have spurred the development of universal carrier screening (CS) programs, i.e., screening for multiple severe recessive disorders simultaneously regardless of family history or ancestry (1–3). This shift has revolutionized the field of reproductive medicine, empowering individuals and couples to make more informed decisions about family planning and providing an equitable screening opportunity for all.

In this light, the American College of Medical Genetics recently highlighted the need for genetic screening paradigms to center on ethnic- and population-neutral standards in the interest of equity and inclusion, recommending that the phrase "expanded carrier screening" (ECS) be replaced simply by "CS" (4). As such, the term "equitable CS" has been suggested as the most appropriate nomenclature (5) by an evidence-based practice guideline from the National Society of Genetic Counselors. They also suggest that CS should be offered to all individuals who are currently pregnant, considering pregnancy, or might otherwise biologically contribute to pregnancy.

In terms of reproductive risks, the burden of severe recessive disorders is known to be substantial, with a prevalence of approximately 1 of 300 pregnancies and accounting for approximately 20% of infant mortality (1, 6). If the main genetic reproductive risks are plotted against female age, particularly at younger ages, the risk of having an infant affected by one of the many severe early-onset recessive disorders can outweigh the risk of having offspring affected by viable aneuploidies (7–9). In many countries, the National Health System offers all pregnant women a variety of options for prenatal screening and testing for trisomy 13, 18, and 21 (10). Therefore, CS, which can also be performed at the preconception stage, potentially allowing for even higher reproductive autonomy, should be made equally available to individuals seeking to have a family.

However, despite its general acceptance and proven benefit, widespread adoption of CS remains limited because of the practical, educational, and regulatory barriers that exist

CON – Expanded Carrier Screening Should Not Be Used In Gamete Donation *(continued)*

Heidi Mertes, Ph.D.

In their practice guideline on gamete donation, the American Society for Reproductive Medicine recommended CS for a limited number of common autosomal recessive conditions for all nonidentified (anonymous) candidate donors (35). Candidate donors who test positive or refuse screening are usually considered ineligible (36). The purpose of this recommended screening program is to prevent harm by identifying and reducing the risk of transmitting genetic conditions to donor-conceived offspring. In an effort to further reduce the risk for autosomal recessive and X-linked conditions, ECS in candidate donors is increasingly advocated for. Although the goal of preventing some severe genetic conditions is laudable, it does not logically follow that the current limited screening should be expanded to include hundreds of conditions. It is important to recognize that the complete elimination of risk is impossible (37), and that screening also generates harm. As Gray et al. (38) famously phrased it: "All screening programmes do harm; some do good as well, and, of these, some do more good than harm at reasonable cost." This raises the question of where the optimal balance between the benefits and drawbacks of donor screening is to be found.

Expanded carrier screening involves targeted screening for a large number of genes (1). Different screening panels exist and can be implemented in various ways (26). As nicely illustrated in a study by Silver et al. (36), different donors will be either approved or rejected depending on which screening panel is adopted. In their cohort of 27 donor applicants (who all tested negative for cystic fibrosis), the different carrier panels excluded either 1, 4, or 6 applicants, but "no single donor was uniformly identified as carrier positive by all three panels," and next-generation sequencing analysis revealed that all donors were carriers of 1–8mutations associated with severe monogenic pediatric diseases (36). Expanded carrier screening is then an elaborate way of confirming what is already known: the existence of a genetic risk. Thus, rejecting all donors who happen to test positive on one particular panel is either a kind of false reassurance or results in an empty donor bank.

PRO – Expanded Carrier Screening Should Be Used In Gamete Donation *(continued)*

worldwide. In many settings, the lack of preconception consultations, insufficient resources for genetic counseling, and a general lack of knowledge and awareness about reproductive genetics and screening options, both among the general population and healthcare providers, have limited the access to CS. Consequently, in recent years, CS has been used primarily in individuals and couples undergoing in vitro fertilization (IVF), where all these capabilities are more commonly available and where reproduction is already a medicalized process.

In gamete donation, CS has seen almost global adoption for several specific reasons. Unlike in natural conception, gamete recipients usually do not know their donor's genetic background. Considering that ethnic background, in our multiethnic society, is often inaccurately self-reported (11) and the growing trends of cross-border gamete donation, CS provides donor selection policies that can be normalized with respect to the reproductive risk of recessive disorders (12). Therefore, CS has become a valuable tool for identifying potential risks of transmitting genetic disorders that might not otherwise be predicted and provides reassurance to prospective recipients. Concomitantly, clinics and gamete banks hold a heightened ethical, and often legal, responsibility to ensure that the gametes they provide are free from medical risks. This duty of care extends to both the recipients and the future children conceived through these means, aiming to safeguard their health and well-being. Knowing that CS has been conducted can provide psychological reassurance to prospective parents and reduce the overall anxiety associated with the risk of genetic disorders and the uncertainty surrounding the health of the future child (13, 14). Additionally, unlike couples who conceive naturally, gamete donors can be selected without major implications by genetic matching procedures to identify a lower transmission risk of a genetic disease. Finally, a further unique element of donation cycles is that a single donor can generate several children with different recipients. For all these reasons, it has been considered particularly relevant and appropriate to provide more stringent screening policies in gamete donation cycles, including CS.

Occasionally, concerns about the asymmetry between CS application to gamete donation cycles and natural conception have been raised as an argument against its use (15). However, couples undergoing IVF and even individuals within the general population who are seeking CS are increasingly driven by a desire for informed reproductive choices, making this argument no longer valid in today's reproductive medicine (16–19). Where governmental policies have been put in place to deliver CS to the public, for instance, in Australia with the Mackenzie mission, or when insurance companies have developed policies to cover CS before IVF cycles predominantly in the United States, CS has become a well-received reality by all stakeholders. This trend is further supported by the global expansion of preimplantation genetic testing capabilities,

CON – Expanded Carrier Screening Should Not Be Used In Gamete Donation *(continued)*

An alternative approach to reduce genetic risk is to also screen recipients and avoid pairing gametes of carriers for the same autosomal recessive condition. Recipient-donor matching can be achieved by screening recipients with the same panel as donors or by targeting only the condition carried by the donor. Whether this matching—made possible by ECS—is morally desirable depends on various factors. Our argumentation addresses considerations related to harm, attitudes toward disabilities, and respect for autonomy. We argue that ECS panels do not outweigh the drawbacks and that screening programs should remain proportional.

Drawbacks of genetic risk reduction

First, ECS can cause psychological distress when a finding reveals previously unknown health information about the individual undergoing the test. This is especially true if the test result lacks actionable implications beyond providing information about the risk to future offspring. Although for preconception screening of couples, anxiety can be reduced by only reporting couple results (1), this is less appropriate in a donor conception setting because the donor is much more likely to reproduce with other people in the future and as an explanation may need to be given when a donor is excluded (39). Even when there is a possibility to withhold information from donors—either because of clinic policy or because donors exercise their "right not to know"—it remains burdening for medical staff to judge whether disclosure or concealment of significant findings results in the least harm (40).

Aside from psychological distress, misunderstandings regarding the scope of the screening and the implications of the results can also lead to tangible harms, either by overestimating ("I've been genetically screened and cleared from genetic conditions so I cannot have a hereditary form of breast cancer") or underestimating ("I'm a carrier of a cardiac disorder, so I need regular heart monitoring and better not play sports") one's own health status. As a general rule, counseling of donors about the scope and impact of screening becomes more complicated as more conditions are being screened for, and misunderstandings become more prevalent.

Furthermore, increased screening also increases the financial burdens for recipients. These costs include not only those for screening itself but also for counseling and follow-up appointments. This raises concerns about justice and reduced access. Likewise, imposing additional requirements on donors could decrease the supply of donor gametes. Potential donors may find the added requirements overwhelming or undesirable, leading them to opt out of the donation process. Limited access to regulated gamete donation may lead potential recipients to seek riskier alternatives, such as finding donors online, which compromises overall safety (41).

A crucial step in judging whether these disadvantages of ECS are acceptable is weighing them against the benefits of

PRO – Expanded Carrier Screening Should Be Used In Gamete Donation *(continued)*

which complement CS by enabling embryo selection when a genetic risk is identified.

There have been arguments from certain quarters regarding donors' right not to be tested when CS becomes mandatory in a clinical setting (20). In this scenario, donors who decline testing would not be eligible to proceed with the donation (21). The inclusion of CS can therefore be perceived as a violation of the donor's privacy, right to donate, and right not to know. However, those violations can first be justified by the health benefits for the future offspring, and second, they can be overcome (for donors who are not willing to receive their CS results) by simply opting out option for receiving results, preserving the donors' autonomy, and making this argument weak at best. When opting out is practically impossible (like for X-linked conditions), the health of future children and the health of donors should be prioritized. Similar considerations apply to the potential for incidental findings in CS, which are a relatively common occurrence with today's genomic analysis. Similar to other medical evaluations, such as imaging, physical examinations, and laboratory tests, CS can reveal unanticipated information that, although not directly related to the aim of the test, could be relevant to an individual's healthcare (22). In line with the recent American College of Medical Genetics considerations (23), which state that staff possess ''a fiduciary duty to prevent harm by warning patients and their families about certain incidental findings and that this principle supersedes concerns about autonomy'' including the ''right not to know,'' within CS programs, donors should be made aware of this possibility through pretest genetic counseling and informed consent. It has been reported that most donors (24) typically wish to be informed about genetic screening outcomes (25), and often view CS as a valuable tool for their own future family planning.

Another argument against CS in gamete donation that no longer holds is the ethical conflict of discontinuing or discarding donors if they are identified as carriers for a given autosomal recessive disorder. This is a valid concern if unadjusted clinical workflows or regulatory policies are followed. For instance, in a recent report from a Danish donor bank (26), 17.6% of donors were rejected based on their carrier status because, in Denmark, it is the interpretation of the law that donors cannot be accepted if they are carriers of a recessive disorder (27, 28). This could evidently lead to a substantial shortage of gametes, undermining principles of justice by limiting the availability of gametes to a smaller number of recipients. However, in modern reproductive genetics, there is not a single scientific justification why a donor should be rejected based on their individual carrier status for an autosomal recessive disorder. Numerous studies reveal that almost all healthy individuals are carriers of autosomal recessive pathogenic gene variants (29, 30). This barrier can easily be avoided using genetic matching protocols. This involves the assignment of a gamete donor that is not a carrier for recessive mutations in the same gene as the recipient, thus

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ECS and comparing this balance to alternative approaches. After all, the degree of risk reduction should be proportional to the associated disadvantages, including psychological distress, misunderstandings, and reduced access (20). The overall absolute reduction in risk is limited for ECS. For example, Abulí et al. (42) demonstrated that the use of ECS reduces the risk of a child being born with a genetic condition by 0.75%. More importantly, most of this risk reduction can be achieved by implementing a limited panel. As illustrated by Ben-Shachar et al. (43), although 2%–3% of couples are at risk for a severe autosomal recessive or X-linked condition, 84% of at-risk couples can be identified by screening only for the 18 conditions with a prevalence above 1/100. Thus, although substantial relative risk reductions can be achieved with selective screening, the incremental gain of moving from a limited panel to an expanded panel is small, whereas the potential harms keep increasing. Therefore, the added risk reduction from ECS is too small to justify its drawbacks.

Addressing disability critiques

The question of what (not) to include in a screening panel to reduce genetic risk is further challenged by what is often referred to as the "disability critique" (44). It emphasizes that screening programs require some notion of which future people are more or less desirable and reflect value judgments based on discriminatory attitudes toward individuals living with genetic conditions, illnesses, or disabilities (45). In addition, these programs may reinforce harmful societal norms and biases against these individuals, promoting the belief that they have lesser value or no place in society (44, 45).

The goal of preventing the birth of people with genetic conditions is considered particularly controversial when it comes to mutations that are not associated with severe and life-limiting conditions. An example is nonsyndromic hearing loss (46). Although many ECS panels include genes associated with nonsyndromic hearing loss, some argue that it is misguided to equate this condition with suffering or with a diminished level of human flourishing. From this perspective, it does not constitute a limiting or unfortunate condition nor a pathology or defect in need of correction or avoidance. Instead, they posit that it is part of a different, cultural community and that this community would be better served with more inclusive measures than with exclusionary screening programs (46).

To avoid a discriminatory message, the goal of preconception genetic screening has shifted away from "prevention" to the so-called "autonomy paradigm" (15, 37). Rather than aiming to prevent the birth of people with genetic conditions, screening is performed to support the reproductive decision-making of recipients. The limited presence of the autonomy paradigm in current donor programs is probably because it conflicts with practices that automatically reject candidate donors with positive screening results. Respect for autonomy cannot be invoked if recipients are not given the opportunity

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reducing the reproductive risk without rejecting donors. Remarkably, variants' pathogenicity and classification can also be updated with evolving knowledge, allowing dynamic and timely matching at the donation timepoint. A recent case report of a carrier donor of a potentially lethal disease, adrenoleukodystrophy (31), is a prime example of the benefit of performing CS in donation cycles with dynamic variant reclassification. In this instance, dynamic reclassification of a variant initially deemed a Variant of Uncertain Significance to pathogenic allowed two families, who had conceived using gametes from the same donor, to be notified in time to start life-saving treatment for their children.

Therefore, contrary to the belief that more extensive CS could lead to discriminatory practices, comprehensive screening exemplifies how thorough analysis can support dynamic donor-recipient matching without excluding donors based on genetic findings. Expanding comprehensive screening to recipients can also increase the use and availability of gametes in countries where domestic demand is not always met, and gametes are imported from abroad. Therefore, wider adoption of proportional CS policies is expected to lead to more equitable and efficient global gamete donation policies and maximize donor gamete utilization (32).

Certain sources have voiced concerns over the possible adverse effects that the dissemination of genetic information might entail in CS (15). Their caution is seemingly based on the concept of genetic exceptionalism - the idea that genetic information is intrinsically unique and warrants special handling compared with other types of health data. This perspective is however becoming increasingly outdated, with genetic information now regarded as just another category of health data, without being inherently more intrusive. In practice, US gamete banks, adhering to the American Society for Reproductive Medicine guidelines, collect comprehensive psychiatric profiles and lifestyle details from donors. This includes intimate aspects such as sexual behavior, and in some cases, evaluations of potential donors' intelligence are conducted. Moreover, because of Food and Drug Administration regulations, there are restrictions against sperm donations from men who have same-sex sexual encounters, including advisories to screen for physical indicators of such activities. Therefore, the screening processes in place today are profoundly personal and invasive by nature, making genetic testing for CS a relatively passive addition within this framework. Furthermore, genetic laboratories are required to protect all data in accordance with national or supranational regulations. As a result, the risk of compromising donor anonymity and the confidentiality of genetic data is significantly minimized. These stringent data protection measures ensure that the personal and genetic information of both donors and recipients remains secure. Additionally, research involving genomic data is always subject to evaluation by Ethics Committees, where rigorous requirements are enforced to ensure the anonymization of data and informed consent for data utilization by any subject. These protocols are designed to protect individuals.

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to choose. However, as previously stated, for ECS to be efficient and feasible, a known carrier status alone should not be used to disqualify a donor. Retaining donors and providing recipients with information about genetic risks (i.e., the mutation found in the donor) can enhance their autonomy.

Concerns related to the autonomy paradigm

Before addressing further arguments against the idea that increased screening improves autonomy, it is important to recognize that the autonomy paradigm does not render disability critiques moot. Although respecting autonomy is important, societal norms that shape decisions regarding screening programs and the collective impact of these decisions should not be neglected (45). Merely offering screening for certain mutations conveys the message that there are valid reasons for avoiding having a child with such conditions, potentially nudging recipients into making "preventative" reproductive choices. Moreover, it is remarkable how sudden respect for reproductive choices diminishes when a choice is made for rather than against a genetic mutation, as exemplified in the case of the deaf lesbian couple looking for a sperm donor with a family history of deafness (47).

The idea that more screening increases autonomy presents additional challenges. The inclusion of more genes in a screening panel leads to information overload and makes it increasingly difficult to obtain valid informed consent (48). Recipients may also mistakenly believe that ECS guarantees healthy children (41). Yet, for reasons mentioned above, many genes (for autosomal recessive and dominant conditions), as well as genetic markers for multifactorial conditions are not included. Moreover, cases caused by de novo mutations or gonadal mosaicism can still occur (49).

Carrier screening is only useful if both parties are screened. Indeed, for donor screening to be relevant, recipients need to be screened for at least the mutation(s) found in the donor. However, if the goal of supporting the reproductive decision-making of recipients is pursued, screening cannot be mandatory. As argued by de Wert et al., (37) imposing ECS as a precondition for access to donor treatment "would be disproportionate, as in absolute numbers, the risk of being a carrier couple of not yet identified recessive disorders would still be very low." Also, such a requirement would be inconsistent with the low uptake of preconception CS in the general population, especially considering that the a priori risk is already lower in donor conception (given the standard screening criteria that are already in place) (15). Thus, routine ECS of donors is overreaching. In addition, it is unclear why the donors' autonomy should not be respected when they decline ECS, either due to the drawbacks discussed above or due to health privacy concerns if they are later identified (20). We hold that neither the autonomy paradigm nor the prevention paradigm justifies an unrestricted expansion of genetic testing.

PRO – Expanded Carrier Screening Should Be Used In Gamete Donation *(continued)*

vidual privacy and uphold ethical standards in the handling of sensitive genetic information.

From a broader perspective, reducing the incidence of genetic disorders through CS in gamete donation cycles can have significant social and economic benefits. It can lead to a reduction in healthcare costs associated with managing genetic disorders and improve the quality of life for individuals and families. The costs associated with caring for children with inherited conditions have increased significantly because of new pharmacologic products, approaches to prophylactic and targeted treatments and advancements in healthcare that lead to longer lifespans. Meanwhile, genetic testing costs have decreased. All cost-effectiveness analyses performed so far in different economic settings including the perspective of the public payer have highlighted the significant benefit of screening over disease management and treatment. Carrier screening in gamete donors is particularly more cost-effective because gamete donors have on average more offspring and donor screening is done only once (33).

Carrier screening represents a transformative advancement in reproductive medicine, including gamete donation cycles, by enhancing donors' genetic screening, expanding reproductive options, preventing inherited disorders, providing psychological relief, and upholding ethical principles. It is envisioned that preconception CS will further evolve in the coming years, boosting its clinical utility as new genes and pathogenic genomic variations are being identified as causes of early or postimplantation embryonic lethality (34). It is foreseeable that in the near future, prospective parents as well as gamete donors and recipients could be provided with an additional level of clinically useful information from preconception CS to prevent negative pregnancy outcomes. If the field of CS does not evolve to overcome current barriers that limit prospective parents' equitable access to education and screening options, the medical disparity will only widen. Hence, the current debate should not center on whether CS is appropriate in gamete donation cycles, but rather on how it should be optimally implemented and how to enhance the existing capabilities of genetic screening. This includes patient education, the selection of conditions screened, coverage and technological performance, interpretation of variants, consent procedures, costs, and matching criteria. Efforts should now focus on providing fair access and equity in up-to-date screening across diverse sociopolitical and economic contexts, rather than questioning the benefits of CS itself. Furthermore, the attitudes of donors toward CS should be and can effectively be respected by offering them the option to opt in to receive genetic results, and dynamic matching protocols must be employed to ensure that a donor's willingness to donate is not compromised by their carrier status.

CON – Expanded Carrier Screening Should Not Be Used In Gamete Donation *(continued)*

Conclusion

When ECS is used, the added reduction in genetic risk is small. At the same time, there are several drawbacks, including those related to psychological and financial burdens, reduced access, discriminatory attitudes toward people living with genetic conditions, and autonomy. To address these concerns, the genes included in the panel must be limited by their prevalence and severity. In this light, the notion of "expanded" screening is questioned, because it may not go beyond existing recommended practices.

CRediT Authorship Contribution Statement

Dorian Accoe: Conceptualization, Writing – Original Draft. Guido Pennings: Conceptualization, Writing – Reviewing and Editing. Heidi Mertes: Conceptualization, Writing – Reviewing and Editing. Antonio Capalbo, Josep Pla, Sandra Janssens: Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Interests

A.C. is employee of Juno Genetics. J.P. is employee of IVIRMA clinics and Coordinator of the Special Interest Group in Reproductive Genetics of the Spanish Fertility Society. S.J. has nothing to disclose. D.A. has nothing to disclose. G.P. has nothing to disclose. H.M. has nothing to disclose.

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Reproductive endocrinology and infertility in the year 2035

We often hear about the shortage of subspecialists in reproductive endocrinology and infertility (REI). Solutions include shortening the fellowship period to 2 years and "upskilling" specialist gynecologists and advanced practice providers. In fact, it has been argued that there is no value to the 3-year fellowship and we should shorten the training period to allow fellows to learn in vitro fertilization (IVF) and enter practice more quickly because this is what most are doing currently (1). Let us follow this through to its natural conclusion. Will this lead to achieving the stated goals and what will the field look like in 10 years?

I have no doubt that with proper training, a gynecologist who can do a vaginal hysterectomy can perform an egg retrieval, or an advanced practice provider proficient in intrauterine insemination can do an embryo transfer. As a corollary, certified nurse midwives have increased access and delivery for many patients with obstetrics. With certified nurse midwives and specialist obstetricians doing most deliveries, the maternal-fetal medicine (MFM) subspecialist can focus on more complex cases. Perhaps, the parallel in REI will increase access and allow specialization in the field of REI. However, MFM fellowships are not shortening, and it could be argued that more active research is now coming from MFM and presented at national meetings than novel research in the field of REI. Will current and future REI training prepare us for a role as the "expert"? Is the answer a decrease in training, limited to IVF, and a reduction in endocrine expertise and research acumen?

Alongside the shifts in workforce demands, there is a growing emphasis on artificial intelligence (AI) and its anticipated impact on our field. This includes the ability to recognize patterns and establish correlations with clinical outcomes. Algorithms are being refined to provide guidance on various aspects of controlled ovarian stimulation such as the type of stimulation, dosing, and when to trigger. In the embryology laboratory, AI assistance for fertilization and embryo selection is already available and completely automated IVF is not far away. This perhaps begs the question, how much training really is critical to function as an REI specialist – maybe less?

At first glance, a shift toward shortening the fellowship training period and/or using generalists, with the incorporation of AI, holds the promise of increasing capacity, standardizing treatments and lowering the costs. However, these benefits will only truly serve the society if these low-cost centers pass lower costs to patients and move into underserved areas (something that has yet to happen). In addition, this shift may lead to a *decrease* in the demand for fellowship-trained REI specialists. So, we could go quickly from a purported shortage of REI specialists to a glut of the very professional numbers we now are trying to increase. What will the job search in 10 years from now look like for REI graduates? Likely gone will be commitments in early fellowship and signing bonuses with promises of high salaries and partnership. The large corporations and private equity, who increasingly own these facilities, prioritize maximizing profits over maintaining allegiance to their staff (and the REI specialists *will* be the "staff").

Let us contemplate the implications of ongoing workforce changes within the context of increasing the integration of AI into our field. When data are inputted into the black box of an algorithm, with outputs that lack explanation, how do we challenge what will become "dogma"? Who will explain the pathophysiology – the why and how of the conclusions? Moreover, although a strength of AI is the identification of *new* patterns with possible clinical implications, how will we understand, explain, and use these newly identified patterns? This concern has surfaced recently in the field of specialty pathology where the interpretation of histology – perhaps in new and novel ways – risks leaving the field without the experts to understand the implications or the underlying pathophysiology (2). Without foundational understanding, the ability to use these new data will not be available to move knowledge forward and develop new treatment strategies.

This is not to say there is no value to AI. Artificial intelligence is already having a huge impact on precision medicine and if used properly to "augment" human decisions, rather than replace them, the potential for a positive impact on human health is enormous. The synergy of "artificial intelligence" and "human intelligence" will be critical to moving health care forward (3). We need to remember, as in all research interpretations: "garbage in, garbage out". Algorithms rely on retrospective data to identify patterns and develop their output. Therefore, the source of these data, and its diversity, will be important. In addition, we are still in a time of limited clinical external validation (using a new data set different from that which trained the algorithm). Clinical usage will require transparency and published clinical trials. Are we actually improving the clinical outcomes? And, how do we measure this? The "foundation model" for AI – with the integration of image interpretation *and* medical history and available literature and self-supervised learning – will take us certainly beyond simple pattern recognition if done correctly (4). This may supply "answers," but who supplies the "questions" that allow the field to move forward?

So, what should REI training look like in this rapidly developing environment? Does a shortening of the fellowship period, decreasing focus on core principles, and more focus on IVF serve our field? With the above changes, our field will be confronted with an increase in information – not a decrease. Although there has appropriately been a push to increase training in genetics and embryology, we should introduce also training in the interpretation and use of AI. In addition, we should train fellows to be partners in the AI revolution. Just as our understanding of physiology and disease pathophysiology allows us to ask important questions and be better partners in translational research (a requirement beyond simply collecting specimens for a basic scientist), we will need to be more than the source of the "input" for AI. We will be called on to combine "human intelligence" with AI to optimize the interpretation and usage if we are to improve the care we give (3). This will require us to know *more* about endocrinology, pathophysiology, and basic principles of care – not less. Will we be ready and able to meet this challenge?

I have spent a large portion of my career with a heavy focus on IVF. Therefore, I understand its profound impact on our field and how important it is for clinical care. Importantly, I fault our government for restricting access to research dollars in this area (made only worse in our current climate – think Alabama), often separating clinical care from basic and translation research compromising novel innovation in the field. Remember the collaboration between a scientist and a clinician – and the years of basic research that lead to the birth of Louise Brown. I am concerned an increased focus on the *clinical practice* of IVF and a devaluing of time spent learning endocrinology and research principles will not provide the knowledge to be active participants in the future of our field and will lessen the potential for innovation.

For example, the classic references for REI fellows were developed originally to include articles that formed foundational knowledge for our field. One such article, the studies of Knobil, led to our understanding of how the pulsatility of gonadotropin releasing hormone (GnRH) is critical to normal function. This knowledge allowed for the development of pulsatile GnRH treatment for patients with hypogonadotropic hypogonadism and the introduction of the GnRH analogues used daily in gynecological and REI practice today. Without understanding the underlying nature of GnRH, we would not have these clinical tools. The current reference list is dominated by IVF and clinical practice articles. Although these are important for evidence-based practice, they do not form foundational knowledge and will not help move the field forward. They give the best available data today, but these may not be the best available data tomorrow and do not help us question "how" or "why." They do not challenge our way of thinking or approaching problems. They homogenize us. This may be good for clinical care and for managing large practices. But, to move our field forward, I would argue we need trainees who feel free to question our current practices, to understand how changing the input for AI may change the output, and how the identification of "patterns" might lead us to the development of new theories of pathophysiology and new drug development and/or treatment paradigms.

It has been argued that those interested in these more in-depth questions and studies could do longer, more specialized fellowships (5). Moreover, we should save resources and increase access by training a cadre of excellent "competent" clinicians to meet the demand and to reflect most jobs after fellowship. I would caution those who argue this – but particularly, I would caution the younger generation. The skills you think you need now can be replaced easily. Where will the jobs be in the future? Who will be the teachers of the future? Who will move our field forward? What type of research will we be trained and capable of performing? To save REI, we need to learn more – not less. We need to challenge more and question more. This cannot be done without foundational knowledge.

CRediT Authorship Contribution Statement

Marcelle I. Cedars: Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Interests

M.I.C. has nothing to disclose.

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Ovarian hyperstimulation syndrome should be dead, we are just waiting on the obituary

HISTORICAL PERSPECTIVE

In a not-so-distant past, ovarian hyperstimulation syndrome (OHSS) was the most dreadful and frequent complication of assisted reproductive technology (ART). Ovarian hyperstimulation syndrome consisted of a massive modification of vascular permeability resulting in extravasation of fluid in the so-called third space with the following two primary consequences: accumulation of fluid in the pelvic cavity and ascites formation, and hemoconcentration and reduction of the vascular volume leading to possible anuria as a result of prerenal failure and increased pressure in renal veins due ascites tension.

The proper treatment of severe forms of OHSS consisted of providing rehydration for fluid expansion together with compensation for losses (albumin and salt). If diuresis fell below 30 mL/h and/or patient discomfort was excessive, hospitalization was required for intravenous hydration and paracentesis for ascites drainage. The latter required compensation for albumin losses. If ascites recurred and diuresis again fell below critical levels, paracentesis had to be repeated, which was often needed for two to four times until the phenomenon resolved. Prophylaxis against venous thromboembolism (VTE) was also provided with low molecular weight heparin at the first signs of OHSS, even if hospitalization was not necessary.

Catastrophic and sometimes fatal consequences of OHSS have been reported. These almost always resulted from improper management particularly, when patients were treated in peripheral facilities not familiar with OHSS. One inappropriate OHSS treatment too often seen was the prescription of diuretics, such as furosemide (Lasix), and fluid restriction, which could seriously aggravate the clinical situation.

MILD STIMULATIONS: A WRONG-GOOD IDEA

Originally, the cause of OHSS was believed to result from an excessive number of follicles and E2 levels per se. One measure logically taken was to propose reducing the number of stimulated follicles by using so-called "mild ovarian stimulation" protocols. This approach was also rooted in the erroneous belief that the magnitude of ovarian response to ovarian stimulation (OS) not only increased OHSS risk but also affected oocyte quality (and ultimately, ART outcome). Today, we know that these justifications for favoring mild stimulations in ART were actually erroneous for the below two reasons.

First, we realized over a decade ago that OHSS is not linked to the actual number of follicles alone, but rather to the co-occurrence of administering a high dose of human chorionic gonadotropin (hCG). Indeed, in the early days of OS—dating back from pre-ART times—hCG was used to trigger ovulation in replacement for luteinizing hormone (LH) surge. The early developers of OS had indeed recognized that there was no spontaneous functional LH surge in OS induced with gonadotropins. However, substituting hCG for LH surge in OS was seen as of menial importance for decades. Indeed, hCG was believed to be equivalent to LH surge and thus, little attention was paid to how hCG effects differed from those of LH.

Second, we now know that the magnitude of the ovarian response to OS—number of oocytes retrieved—does not alter oocyte quality (1). Certainly, strong responses to OS do alter endometrial receptivity—hence, ART outcome after fresh transfers—but not oocyte quality. This has now been amply verified after the advent of embryo vitrification by analyzing the outcome of frozen embryo transfers in relation to the number of oocytes collected (1).

THE LONG-IGNORED CULPRIT: hCG

We have long known that OHSS symptoms follow a single- or double-"hump" course. The first hump of symptoms is related to exogenous hCG used for triggering ovulation. It generally peaks 7 days after triggering ovulation. The possible second hump in OHSS symptoms is linked to endogenous hCG produced in case of pregnancy. The onset of the second hump of OHSS symptoms was classically detected 1–2 days before the scheduled pregnancy test in fresh embryo transfers. Altering the timing of embryo transfers only became a practical option after mastering embryo vitrification.

We now know that the core pathophysiological process of OHSS—the extravasation of plasma fluid in the third sector—is indeed mediated by an effect of hCG and E2, on ovarian follicles. The difference in half-lives between hCG and LH is the likely cause of the different consequences of triggering ovulation with hCG or gonadotropin-releasing hormone agonist (GnRH-a; inducing an LH surge). Whether the follicle-stimulating hormone (FSH) elevation, which also occurs after a bolus of GnRH-a plays a role in the differences observed in vascular endothelial growth factor (VEGF) release after GnRH-a or hCG has not been explored.

THE NO-hCG, NO OHSS ART OPTION

The solution for avoiding OHSS is therefore to opt for a no-hCG OS protocol. This option became possible by using a bolus of GnRH-a instead of hCG when follicles reach maturity in an antagonist OS protocol. This option described more than three decades ago (2) had been ignored when GnRH-a was nearly routinely used in OS, either in down-regulated or flare-style stimulations. However, with the advent of GnRH antagonist protocols, the use of hCG as a trigger can be replaced by a bolus of GnRH-a. Endometrial alterations encountered after triggering ovulation with GnRH-a mandate, however, to adopt a segmented ART strategy, with freezing of all embryos and deferred embryo transfer.

As outlined in an article by Miller et al. (3) vasoactive substances such as notably, VEGF are markedly lower after triggering of ovulation with GnRH-a compared with hCG. This effect of hCG on VEGF production by ovarian follicles and high E2 levels are seen as the primary mechanisms causing OHSS.

Unfortunately, there are patients who will not, or may not properly, respond to GnRH-a trigger. This includes women who suffer, or have suffered in the past, from hypothalamic amenorrhea. Improper response to GnRH trigger has also been reported in women with low body mass index (<19) or who have received GnRH-a treatment in the recent past (months). The latter is most likely encountered in women who suffer from endometriosis. To detect an improper response to GnRH-a triggering, it is recommended to measure serum LH levels on the day after triggering. Ideally, LH should be ≥ 15 mIU/mL. If LH levels are lower, it is recommended to retrigger with a low dose of hCG, which therefore may cause OHSS, or abandon the cycle. In people at risk of OHSS who are bound to not respond to GnRH-a trigger, a smaller amount of hCG can be added together with GnRH-a, but this is not without risk of OHSS.

RISK OF VTE IN NO-hCG ART?

Classically, the risk of VTE identified in OHSS was attributed to the high levels of serum E2 encountered in excessive OS responses. Indeed, several coagulation factors synthetized in the liver are activated by E2, with the risk that overexposing the liver to E2 might stimulate coagulation.

Our current understanding of the pathophysiology of OHSS has changed our views, however. Today we believe that the hemoconcentration induced by VEGF, rather than E2 levels, is the primary cause of increased VTE risk in OHSS. Hence, the proper way to address the OHSS-linked VTE risk is to opt for no-hCG ART cycles, in which VEGF-related hemoconcentration is markedly reduced. The no-hCG ART option is therefore also indicated in women at an increased risk of VTE because of hemostasis disorders.

The formal proof that hemoconcentration is the sole factor increasing VTE risk in women who have strong responses to OS has not been formally established, however. Therefore in the absence of such proof, it is reasonable to follow a conservative management. Hence, it is still recommended to use low molecular weight heparin for VTE protection when E2 levels are exceedingly high, even in no-hCG ART cycles and therefore, in the absence of clinical OHSS.

CARING FOR RARE CASES OF OHSS

The available option of triggering ovulation with GnRH-a rather than hCG and easy access to embryo vitrification have nearly totally eradicated severe cases of OHSS. Today, the use of yesteryear's agonist protocols—preventing the use of GnRH-a trigger—should be proscribed as first-line therapy in any new ART patient. Long agonist protocols could only be considered in documented poor responders, but their superiority in these cases has not been documented.

One needs to acknowledge that mild cases of OHSS can occur in no-hCG protocols. In addition, very rare cases of severe OHSS have been reported in no-hCG ART cycles (4). These occurrences, which are fortunately very rare, have not been fully evaluated for possible FSH receptor mutations or other forms of alteration. Indeed, OHSS can also occur in case of rare FSH receptor mutations, even in natural cycle conception (5).

Finally, one should also be aware that management errors may happen and therefore, OHSS may still be encountered. The incidence of OHHS has however markedly decreased, declining from 0.4% in 2002 to 0.1 % in 2016 (https://www.focusonreproduction.eu/article/News-in-Reproduction-OHSS). Society for Assisted Reproductive Technologies data are even more eloquent, reporting an even sharper, >10-fold decrease in severe OHSS from 0.28% in 2007 to 0.02% in 2021 (https://www.sart.org/globalassets/_sart/infographics/ivf-risks.png). Hence, young reproductive doctors, who are rarely exposed to OHSS, need to know the fundamental steps of its management. Simulation models can be used for training for OHSS management given the rarity of these cases, emphasizing the importance of applying proper clinical measures if this occurs.

CONCLUSION

The long-ignored role of hCG in the genesis of OHSS has now been recognized. Antagonist—or Progestin-Primed Ovarian Stimulation—protocols allow the sole use of GnRH-a for triggering ovulation in lieu of hCG. This, however, practically implies opting for freeze-all and deferred embryo transfer. Today, the no-hCG ART option is clearly preferred in all patients at risk of OHSS because of their OS response and/or number of follicles or antimüllerian hormone levels and in women with VTE risk. However, rare cases of severe OHSS still occur, mainly because of treatment errors.

CRediT Authorship Contribution Statement

Dominique de Ziegler: Conceptualization, Writing – original draft, Writing – review & editing. **Sean Sokteang:** Writing – original draft, Writing – review & editing. **Paul Pirtea:** Writing – original draft, Writing – review & editing. **James P. Toner:** Writing – original draft, Writing – review & editing.

Declaration of Interests

S.S. has nothing to disclose. P.P. has nothing to disclose. J.P.T. has nothing to disclose. D.D.Z. has nothing to disclose.

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Assessment of obstetric characteristics and outcomes associated with pregnancy with Turner syndrome

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Objective: To assess national-level trends, characteristics, and outcomes of pregnancies with Turner syndrome in the United States.
Design: Cross-sectional study.
Setting: The Healthcare Cost and Utilization Project's National Inpatient Sample.
Subjects: A total of 17,865,495 hospital deliveries from 2016–2020.
Exposure: A diagnosis of Turner syndrome, identified according to the World Health Organization's International Classification of Disease 10th revision code of Q96.
Main Outcome Measures: Obstetrics outcomes related to Turner syndrome, assessed with inverse probability of treatment weighting cohort and multivariable binary logistic regression modeling.
Results: The prevalence of pregnant patients with Turner syndrome was 7.0 per 100,000 deliveries (one in 14,235). The number of hospital deliveries with patients who have a diagnosis of Turner syndrome increased from 5.0 to 11.7 per 100,000 deliveries during the study period (adjusted-odds ratio [aOR] for 2020 vs. 2016; 2.18, 95% confidence interval [CI] 1.83–2.60). Pregnant patients with Turner syndrome were more likely to have a diagnosis of pregestational hypertension (4.8% vs. 2.8%; aOR 1.65; 95% CI 1.26–2.15), uterine anomaly (1.6% vs. 0.4%; aOR, 3.01; 95% CI 1.93–4.69), and prior pregnancy losses (1.6% vs. 0.3%; aOR 4.70; 95% CI 3.01–7.32) compared with those without Turner syndrome. For the index obstetric characteristics, Turner syndrome was associated with an increased risk of intrauterine fetal demise (10.9% vs. 0.7%; aOR 8.40; 95% CI 5.30–13.30), intrauterine growth restriction (8.5% vs. 3.5%; aOR 2.11; 95% CI 1.48–2.99), and placenta accreta spectrum (aOR 3.63; 95% CI 1.20–10.97). For delivery outcome, pregnant patients with Turner syndrome were more likely to undergo cesarean delivery (41.6% vs. 32.3%; aOR 1.53; 95% CI 1.26–1.87). Moreover, the odds of periviable delivery (22–25 weeks: 6.1% vs. 0.4%; aOR 5.88; 95% CI 3.47–9.98) and previable delivery (<22 weeks: 3.3% vs. 0.3%; aOR 2.87; 95% CI 1.45–5.69) were increased compared with those without Turner syndrome.
Conclusions: The results of contemporaneous, nationwide assessment in the United States suggest that although pregnancy with Turner syndrome is uncommon this may represent a high-risk group, particularly for intrauterine fetal demise and periviable delivery. Establishing a society-based approach for preconception counseling and antenatal follow-up would be clinically compelling. (Fertil Steril® 2024;122:233–42. ©2024 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Pregnancy, Turner syndrome, trend, characteristics, outcomes

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Turner syndrome is a genetic disorder characterized by the partial or complete loss of one X chromosome and it impacts one in 2,500 female live births (1, 2). Most cases involve the karyotype "45,X" or complete loss of one sex chromosome, but mosaic karyotypes with a percentage of normal cell lines also occur. Physical features of Turner syndrome include short stature, a webbed neck, and a broad chest with widely spaced nipples. There is also a high rate of premature ovarian failure, congenital heart defects, brain, renal, liver, and skeletal abnormalities (3).

Premature ovarian failure impacts as many as 95% of individuals with Turner syndrome. This is a result of follicular atresia occurring often before birth or early in life which results in high rates of infertility. Among mosaic patients, the follicular atresia may not be as pronounced, and spontaneous pregnancies can occur (4). Congenital heart defects are present in 23%–50% of patients with the most frequent defects including bicuspid aortic valve, aortic arch abnormalities, and coronary artery anomalies. Cardiovascular complications are the leading cause of death in individuals with Turner syndrome (5, 6). Equally important, complications of cardiovascular disease continue to be the leading cause of maternal mortality in the United States.

Historically, pregnancy has been discouraged in individuals with Turner syndrome because of the high rate of cardiac malformations and the concern for aortic dissection. Updated guidelines from the American Heart Association suggest that the cardiovascular morbidity among pregnant patients with Turner syndrome may be less severe in appropriately screened patients (6). Other adverse obstetrical complications such as an increased miscarriage, cesarean section, and intrauterine fetal growth restriction have also been associated with maternal Turner syndrome (4).

Newer, less restrictive recommendations along with increased access to assisted reproductive technology will likely result in an increasing number of patients pursuing pregnancy. An updated analysis evaluating the safety of pregnancy in this high-risk group is therefore warranted. The objective of the current study was to assess national-level trends, characteristics, and outcomes of pregnancies with Turner syndrome in the United States.

MATERIALS AND METHODS

Data

This cross-sectional study queried the Healthcare Cost and Utilization Project's National Inpatient Sample (7). The Healthcare Cost and Utilization Project is the United States health service data platform that is supported by the Agency for Healthcare Research and Quality, one of the 12 federal agencies within the United States Department of Health and Human Services. The National Inpatient Sample is publicly available and deidentified; hence, the University of Southern California Institutional Review Board deemed the current study exempt (HS-16-00481).

The National Inpatient Sample approximates a stratified sample of 20% of discharges in each center from all the participating hospitals across 48 States and the District of Columbia. Every year, the data set captures >7 million inpatient admissions. In 2020, a total of 4,580 hospitals participated in the program. In each encounter, the program captures a maximum of 40 diagnoses and 25 procedures during the index hospitalization. When weighted for national survey estimates, it covers >97% of the US population.

Study eligibility

The study population was patients who had a hospital delivery from 2016 to 2020. Identification of hospital delivery was based on the World Health Organization's International Classification of Disease, 10th revision (ICD-10) Clinical Modification and Procedural Coding Schema codes and Disease-Related Group codes that followed prior investigations (Supplemental Table 1, available online) (8, 9). Patient age was restricted to 15–54 years as following prior studies (8, 10).

Exposure

The exposure was a diagnosis of Turner syndrome in this study. The ICD-10 code of Q96 was used to identify pregnant patients with Turner syndrome. This coding schema followed prior investigation (Supplemental Table 1) (11). The code was consistent without change or obsoletion during the study period of 2016–2020.

Exclusion criteria included fetal abnormality including fetal chromosomal abnormality (Supplemental Table 1). Although the program captures maternal information for the index hospitalization for delivery but not neonatal information, this exclusion was to ensure that the diagnosis of Turner syndrome represents a maternal chromosomal condition.

Main outcome measures

The obstetric characteristics and outcomes related to Turner syndrome were the primary interest of analysis in this study. The following 5 areas of obstetric events were assessed: maternal conditions during pregnancy; fetal characteristics; placental factors; uterine and membranous factors; and delivery outcomes.

Maternal conditions during pregnancy included gestational hypertension, pre-eclampsia, gestational diabetes mellitus, and excess weight gain during pregnancy. Fetal characteristics included intrauterine growth restriction, intrauterine fetal demise including stillbirth, large for gestational age, multifetal gestation, and fetal breech presentation.

Placental factors included placenta previa, low-lying placenta, placenta abruption, placenta accreta spectrum, placenta malformation, and vasa previa. Uterine and membranous factors included uterine rupture, cervical insufficiency, premature rupture of membrane, and chorioamnionitis. Delivery outcomes included gestational age at delivery and cesarean delivery. Gestational age at delivery was grouped into the following: >40, 39–40, 37–38, 34–36, 26–33, 22–25, and <22 weeks (12, 13).

Identification of these obstetric parameters was based on the ICD-10 Clinical Modification and Procedural Classification Schema that followed prior studies (Supplemental Table 1) (8, 9, 14). These codes were consistent without change or obsoletion during the study period.

As the secondary outcome measure, diagnoses of aortic dissection were evaluated (Supplemental Table 1) (14, 15). This vascular complication was assessed because of the relevance of Turner syndrome and pregnancy.

Study covariates

Baseline nonobstetric characteristics assessed in this study were the following domains: patient demographics; medical conditions; substance use factors; mental health conditions; past pregnancy data; gynecological factors; and hospital parameters.

Patient demographics included patient age (<25, 25–29, 30–34, 35–39, and ≥40 years), year (2016, 2017, 2018, 2019, and 2020), race and ethnicity (Asian, Black, Hispanic, Native American, Other, and White) determined by the program, primary payor (Medicaid, private insurance including health maintenance organization, self-pay, and other), and census-level median household income (every quarter). Race and ethnicity were included as these factors are associated with pregnancy characteristics and outcomes.

Medical conditions included pregestational hypertension, pregestational diabetes, obesity, and asthma. Substance use factors included cigarette use, illicit drug use, and alcohol use. Mental health conditions included anxiety and depressive disorders. Past pregnancy data included prior cesarean delivery, pregnancy losses, and grand multiparity. Gynecological factors included uterine anomaly, uterine myoma, and polycystic ovary syndrome.

Hospital parameters included regions of the United States (Northeast, Midwest, South, and West), facility relative bed capacity (small, mid, and large), and facility location and teaching status (rural, urban nonteaching, and urban teaching). These hospital parameters were determined by the programs.

The program's default data and the ICD-10 Clinical Modification codes were used to identify these study covariates that were aggregated as similar to prior studies (Supplemental Table 1) (8, 9, 14). Missing values in each study covariate were grouped for analysis.

Analysis plan

The first step of the analysis was to assess the prevalence of pregnancy with Turner syndrome. The data were aggregated and summarized per 100,000 hospital deliveries. This was performed per the whole cohort level and the study covariates.

The second step of the analysis was to examine the temporal trends of pregnancy with Turner syndrome. A multinominal regression model was used to assess the temporal trend by comparing it to the year 2016.

The third step of the analysis was to evaluate the baseline nonobstetric characteristics associated with Turner syndrome. A multivariable binary logistic regression model with a conditional backward selection method was fitted for the analysis. This approach was preselected to avoid overfitting in the model because of the assumption that pregnancy with Turner syndrome is rare. All study covariates with a P value of $<.05$ level in univariable analysis were chosen for the initial covariate selection, and the least significant variable was removed from the model sequentially with the stopping rule of P value of $<.05$ in the final model. The magnitude for statistical association was expressed with adjusted-odds ratio (aOR) and a corresponding 95% confidence interval (CI).

The last step of the analysis was to assess obstetrics characteristics related to Turner syndrome. In this step analysis, an inverse probability of treatment weighting cohort was created to mitigate the baseline demographic differences between the 2 exposure groups (16). This approach was chosen to avoid overfitting and to consider the chronology of nonobstetric and obstetric events. A propensity score was generated by a binary logistic regression model entering the independent confounders between the exposure groups identified in the prior step analysis (area-under-curve 0.683; 95% CI 0.650–0.717).

Weights were assigned as 1 / (propensity score) for the patients with Turner syndrome and 1 / (1 − propensity score) for the patients without Turner syndrome. Stabilized weights and threshold technique at 10 were employed to avoid extreme weighting. Balance statistics between the 2 exposure groups in the weighted cohort were assessed by standardized difference and the value of >0.20 was considered clinically imbalanced modeled covariates between the 2 exposure groups.

Independent obstetric factors related to Turner syndrome were assessed with a multivariable binary logistic regression model with a conditional backward selection method as described above in the inverse probability of treatment weighting cohort. The effect size for Turner syndrome was expressed with aOR and 95% CI.

All statistical analyses were based on two-tailed hypotheses and a P value of $<.05$ was considered statistically significant. The national estimates were based on the weighted values provided by the programs. Statistical Package for Social Sciences (IBM SPSS, version 28.0, Armonk, NY) and R statistics (version 3.5.3, R foundation for Statistical Computing, Vienna, Austria) were used for the analysis. The STROBE guidelines were consulted for the performance of this study (17).

RESULTS

Study cohort

A total of 17,865,495 hospital deliveries for national estimates met the inclusion criteria (Supplemental Fig. 1, available online). The average age at delivery was 29.0 years. Cohort-level characteristics are shown in Table 1. Nearly half of the study population were White individuals (50.4%), followed by Hispanic (20.0%), Black (14.4%), and Asian (6.0%). Patients were most frequently privately insured (51.5%) followed by Medicaid (42.3%). Most deliveries were at an urban teaching hospital (70.5%). Over the 5-year study period, the annual number of deliveries decreased by 9.1% from 3.72 million in 2016 to 3.38 million in 2020 (Table 1).

Prevalence of pregnancy with Turner syndrome

During the study period, a total of 1,255 pregnant patients had a diagnosis of Turner syndrome and the remaining 17,864,240 pregnant patients did not have the diagnosis. This corresponds to the prevalence of pregnant patients

TABLE 1

Baseline demographics.

Characteristic	N (%)	Prevalence[a]	*P* value
Whole	17,865,495 (100)	7.0	
Age (y)			<.001
<25	4,337,823 (24.3)	5.2	
25–29	5,157,537 (28.9)	5.1	
30–34	5,138,892 (28.8)	6.7	
35–39	2,645,174 (14.8)	12.3	
≥40	586,070 (3.3)	16.2	
Year			<.001[c]
2016	3,720,056 (20.8)	5.0	
2017	3,660,926 (20.5)	4.6	
2018	3,586,349 (20.1)	7.0	
2019	3,517,964 (19.7)	7.2	
2020	3,380,201 (18.9)	11.7	
Race/ethnicity			<.001
White	9,008,354 (50.4)	8.4	
Black	2,580,375 (14.4)	4.1	
Hispanic	3,575,629 (20.0)	5.7	
Asian	1,064,314 (6.0)	9.9	
Native American	125,870 (0.7)	0.0	
Other	784,695 (4.4)	8.3	
Unknown	726,259 (4.1)	2.8	
Primary payor			<.001
Medicaid	7,562,351 (42.3)	5.2	
Private including HMO	9,207,580 (51.5)	8.6	
Self-pay	453,190 (2.5)	3.3	
Other	621,555 (3.5)	8.8	
Unknown	20,820 (0.1)[d]		
Household income[b]			<.001
QT1 (lowest)	4,945,698 (27.7)	5.0	
QT2	4,496,707 (25.2)	6.0	
QT3	4,369,082 (24.5)	8.2	
QT4 (highest)	3,893,243 (21.8)	9.8	
Unknown	160,765 (0.9)	0.0	
Pregestational hypertension			<.001
No	17,361,325 (97.2)	6.9	
Yes	504,170 (2.8)	11.9	
Pregestational diabetes			.330
No	17,672,380 (98.9)	7.0	
Yes	193,115 (1.1)[d]		
Obesity			.039
No	15,826,116 (88.6)	7.2	
Yes	2,039,379 (11.4)	5.9	
Asthma			.002
No	16,925,291 (94.7)	6.9	
Yes	940,205 (5.3)	9.6	
Cigarette use			.972
No	16,936,206 (94.8)	7.0	
Yes	929,289 (5.2)	7.0	
Illicit drug use			.471
No	17,379,321 (97.3)	7.0	
Yes	486,175 (2.7)	6.2	
Alcohol use			.436
No	17,839,670 (99.9)	7.0	
Yes	25,825 (0.1)	0	
Anxiety disorder			<.001
No	17,040,776 (95.4)	6.7	
Yes	824,720 (4.6)	13.3	
Depressive disorder			<.001
No	17,217,646 (96.4)	6.9	
Yes	647,850 (3.6)	11.6	
Prior uterine scar			.404
No	14,642,977 (82.0)	7.1	
Yes	3,222,518 (18.0)	6.7	
Prior pregnancy loss			<.001
No	17,814,945 (99.7)	6.9	
Yes	50,550 (0.3)	39.6	
Grand multiparity			.039
No	17,809,045 (99.7)	7.0	
Yes	56,450 (0.3)	0	
Uterine anomaly			<.001
No	17,786,365 (99.6)	6.9	
Yes	79,130 (0.4)	25.3	
Uterine myoma			.438
No	17,605,085 (98.5)	7.0	
Yes	260,410 (1.5)	5.8	
Polycystic ovary syndrome			.027
No	17,743,510 (99.3)	7.0	
Yes	121,985 (0.7)	12.3	
Hospital region			.006
Northeast	2,853,469 (16.0)	6.7	
Midwest	3,758,403 (21.0)	6.8	
South	7,022,075 (39.3)	6.6	
West	4,231,548 (23.7)	8.3	
Hospital bed capacity			<.001
Small	3,460,213 (19.4)	5.5	
Mid	5,409,994 (30.3)	7.7	
Large	8,995,288 (50.4)	7.2	
Hospital setting			<.001
Rural	1,629,457 (9.1)	4.3	
Non-urban teaching	3,632,393 (20.3)	5.2	
Urban teaching	12,603,645 (70.5)	7.9	

QT = quartile.
[a] Prevalence rate of Turner syndrome per row level, expressed in 100,000 hospital deliveries. Proportional distributions per the exposure groups are shown in Supplemental Table 2.
[b] Census-level median household income.
[c] Cochran–Armitage trend test.
[d] Small number suppressed per HCUP guidelines.

Anderson. Turner syndrome pregnancy. Fertil Steril 2024.

with Turner syndrome being 7.0 (95% CI, 6.6–7.4) per 100,000 deliveries (Table 1). In other words, one in 14,235 hospital deliveries had a diagnosis of Turner syndrome.

Temporal trends of Turner syndrome

At the cohort level, the number of hospital deliveries with patients who have a diagnosis of Turner syndrome slightly increased during the study period: 5.0 in 2016, 7.0 in 2018, and 11.7 in 2020 per 100,000 deliveries (*P*-trend<.001; Table 1). The increasing trend of pregnant patients with Turner syndrome over time remained in a multivariable analysis (nonlinear association: aOR for 2018 vs. 2016 1.34; 95% CI, 1.11–1.62; and aOR for 2020 vs. 2016 2.18; 95% CI, 1.83–2.60) (Table 2).

Temporal trends of pregnant patients with a diagnosis of Turner syndrome were assessed per baseline demographic levels (Supplemental Table 2). Among race and ethnicity groups, increasing trends of Turner syndrome were larger among Black individuals compared with others (OR, 9.39 vs. 1.90–4.44). When assessed per the census-level median household income, the highest-quartile group had larger increasing trends compared with other groups (OR, 4.20 vs. 1.40–2.98). When examined per hospital region, the interval increase in Turner

TABLE 2

Nonobstetric characteristics associated with Turner syndrome.

	Turner syndrome		Multivariable model[a]	
Characteristic	**No**	**Yes**	**aOR (95% CI)**	***P* value**
Age (y)	29.0 (5.8)	30.9 (6.4)	1.04 (1.03–1.05)	<.001
Year				<.001[b]
2016	20.8	14.7	1.00 (reference)	
2017	20.5	13.5	0.92 (0.75–1.13)	.433
2018	20.1	19.9	1.34 (1.11–1.62)	.003
2019	19.7	20.3	1.36 (1.13–1.65)	.001
2020	18.9	31.5	2.18 (1.83–2.60)	<.001
Race/ethnicity				<.001[b]
White	50.4	60.2	1.00 (reference)	
Black	14.4	8.4	0.57 (0.46–0.70)	<.001
Hispanic	20.0	16.3	0.78 (0.66–0.92)	.003
Asian	6.0	8.4	1.01 (0.82–1.25)	.911
Native American	0.7	0	n/a	.917
Other	4.4	5.2	1.05 (0.81–1.35)	.717
Unknown	4.1	1.6	0.37 (0.23–0.57)	<.001
Primary payer				<.001[b]
Medicaid	42.3	31.1	1.00 (reference)	
Private including HMO	51.5	62.9	1.18 (1.03–1.35)	.019
Self-pay	2.5	1.2	0.57 (0.34–0.96)	.034
Other	3.5	4.4	1.40 (1.05–1.87)	.020
Unknown	0.1	[c]	4.38 (1.81–10.61)	.001
Census-level household income				.009[b]
QT1 (lowest)	27.7	19.5	1.00 (reference)	
QT2	25.2	21.5	1.05 (0.88–1.26)	.562
QT3	24.5	28.7	1.29 (1.08–1.53)	.004
QT4 (highest)	21.8	30.3	1.31 (1.09–1.56)	.004
Unknown	0.9	0	n/a	.908
Pregestational hypertension				
No	97.2	95.2	1.00 (reference)	
Yes	2.8	4.8	1.65 (1.26–2.15)	<.001
Obesity				
No	88.6	90.4	1.00 (reference)	
Yes	11.4	9.6	0.74 (0.61–0.90)	.002
Asthma				
No	94.7	92.8	1.00 (reference)	
Yes	5.3	7.2	1.32 (1.06–1.64)	.013
Anxiety disorder				
No	95.4	91.2	1.00 (reference)	
Yes	4.6	8.8	1.61 (1.32–1.97)	<.001
Prior pregnancy loss				
No	99.7	98.4	1.00 (reference)	
Yes	0.3	1.6	4.70 (3.01–7.32)	<.001
Uterine anomaly				
No	99.6	98.4	1.00 (reference)	
Yes	0.4	1.6	3.01 (1.93–4.69)	<.001
Hospital region				.001[b]
Northeast	16.0	15.1	0.82 (0.68–0.99)	.042
Midwest	21.0	20.3	1.00 (reference)	
South	39.3	36.7	1.03 (0.88–1.21)	.702
West	23.7	27.9	1.18 (0.99–1.39)	.059
Hospital bed capacity				<.001[b]
Small	19.4	15.1	1.00 (reference)	
Mid	30.3	33.1	1.45 (1.22–1.73)	<.001
Large	50.3	51.8	1.34 (1.14–1.58)	<.001
Hospital setting				<.001[b]
Rural	9.1	5.6	1.00 (reference)	
Nonurban teaching	20.3	15.1	1.02 (0.77–1.35)	.915
Urban teaching	70.5	79.3	1.50 (1.17–1.93)	.002

aOR = adjusted-odds ratio; CI = confidence interval; QT = quartile.
[a] Multivariable binary logistic regression model with conditional backward selection.
[b] Overall *P* value.
[c] Suppressed small number.

Anderson. Turner syndrome pregnancy. Fertil Steril 2024.

syndrome pregnancy was larger in the Midwest compared with other regions (OR, 3.51 vs. 1.83–2.41). At the hospital setting level, the increasing trends of pregnancy with Turner syndrome were larger in rural hospitals compared with nonurban or urban teaching hospitals (OR, 8.23 vs. 1.31–2.38).

Nonobstetric characteristics related to Turner syndrome

In univariable analysis (Table 1 and Supplemental Table 3), all the measured study covariates except for pregestational diabetes, substance use factors, prior uterine scar, and uterine myoma were statistically associated with Turner syndrome (all, $P < .05$).

The results of multivariable analysis are shown in Table 2. For baseline demographics, pregnant patients with Turner syndrome were older, more likely to be privately insured, and reside in higher census-level household income areas, but less likely to be Black or Hispanic (all, adjusted $P < .05$).

With regard to medical factors (Table 2), pregnant patients with Turner syndrome were more likely to have a diagnosis of pregestational hypertension (4.8% vs. 2.8%; aOR 1.65; 95% CI 1.26–2.15), anxiety disorder (8.8% vs. 4.6%; aOR 1.61; 95% CI 1.32–1.97), and asthma (7.2% vs. 5.3%; aOR 1.32; 95% CI 1.06–1.64), but less likely to be obese (9.6% vs. 11.4%; aOR 0.74; 95% CI 0.61–0.90) compared with those without Turner syndrome.

For gynecological factors (Table 2), pregnant patients with Turner syndrome were more likely to have a uterine anomaly (1.6% vs. 0.4%; aOR 3.01; 95% CI 1.93–4.69) and prior pregnancy losses (1.6% vs. 0.3%; aOR 4.70; 95% CI 3.01–7.32) compared with those without Turner syndrome. Notably, among the independent characteristics for Turner syndrome, these 2 factors had a marked association for Turner syndrome where the increased odds exceeded three-fold for both factors.

For hospital parameters (Table 2), pregnant patients with Turner syndrome were more likely to deliver at a larger bed capacity center within an urban teaching setting (both, adjusted $P < .05$).

Obstetric characteristics related to Turner syndrome

In a propensity score-weighted cohort (Supplemental Table 4), all the modeled baseline covariates shown in Table 2 were more balanced without clinical imbalance between the Turner syndrome group and the non-Turner syndrome group (all, standardized difference <0.20).

The results of multivariable analysis are shown in Table 3. Turner syndrome was associated with an increased risk of gestational diabetes (11.1% vs. 8.0%; aOR 1.45; 95% CI 1.07–1.96), intrauterine fetal demise (10.9% vs. 0.7%; aOR 8.40; 95% CI 5.30–13.30), intrauterine growth restriction (8.5% vs. 3.5%; aOR 2.11; 95% CI 1.48–2.99), and placenta accreta spectrum (aOR 3.63; 95% CI 1.20–10.97).

For delivery outcomes, pregnant patients with Turner syndrome were more likely to undergo cesarean delivery (41.6% vs. 32.3%; aOR 1.53; 95% CI 1.26–1.87). Moreover, the odds of periviable delivery (22–25 weeks: 6.1% vs. 0.4%; aOR 5.88; 95% CI 3.47–9.98) and previable delivery (<22 weeks: 3.3% vs. 0.3%; aOR 2.87; 95% CI 1.45–5.69) were increased compared with those without Turner syndrome. Overall, nearly three-quarters (74.1%) of deliveries among pregnant patients with Turner syndrome were term delivery (37 weeks gestation or greater).

Among the independent obstetric characteristics for Turner syndrome (Table 3), intrauterine fetal demise and periviable delivery at 22–25 weeks had a marked association that odds exceeded five-fold for both factors.

Secondary outcome

No case of aortic dissection was reported among pregnant patients with Turner syndrome who had hospital delivery during the study period.

DISCUSSION

Principal findings

This nationwide assessment suggests a gradually increasing number of pregnancies are occurring among individuals with Turner syndrome. These pregnancies are at increased risk for several adverse obstetrical outcomes such as intrauterine fetal growth restriction and fetal demise. Higher rates of periviable delivery and cesarean section were also observed among pregnant patients with Turner syndrome.

Insights for results

Prevalence and trends. Previously, the prevalence of maternal Turner syndrome in pregnancy was not well studied. We found that it is overall rare, impacting 7 per 100,000 deliveries in the United States, adding important information to the literature.

Although uncommon, the number of pregnant patients with Turner syndrome gradually increased over this 5-year study period. The exact cause of the increase was not assessed in this study but is likely multifactorial. One possible reason for this includes increasing utilization of noninvasive prenatal fetal chromosomal screening which may be secondarily detecting maternal chromosomal abnormalities (18).

Increased access to assisted reproductive technology and a more inclusive stance on pregnancy in appropriately screened patients may have also contributed to these findings. Primary ovarian failure and resultant infertility is a key feature of Turner syndrome and is reported to be highly distressing for patients (4). A survey of patients suggests they are highly interested in pregnancy. An improved understanding of the risk factors most associated with poor maternal outcomes, international guidelines detailing prepregnancy evaluation, and multidisciplinary care teams during pregnancy may be encouraging more people with Turner syndrome to attempt pregnancy (19, 20).

Assisted reproductive technology has increased access to pregnancy for many patients. This is largely accomplished via donor oocytes, but there is also an increased use of fertility preservation among patients with Turner syndrome (21–23). The data on reproductive outcomes after fertility preservation in those with Turner syndrome are still limited

TABLE 3

Obstetric characteristics related to Turner syndrome.

	Turner syndrome[a]		Multivariable model[b]	
	No	Yes	aOR (95% CI)	*P* value
Gestational diabetes				
No	92.0	88.9	1.00 (reference)	
Yes	8.0	11.1	1.45 (1.07–1.96)	.018
IUGR				
No	96.5	91.5	1.00 (reference)	
Yes	3.5	8.5	2.11 (1.48–2.99)	<.001
IUFD				
No	99.3	89.1	1.00 (reference)	
Yes	0.7	10.9	8.40 (5.30–13.30)	<.001
PAS				
No	99.9	99.3	1.00 (reference)	
Yes	0.1	[d]	3.63 (1.20–10.97)	.023
Gestational age (w)				<.001[c]
41-43	5.2	5.0	1.32 (0.83–2.08)	.238
39–40	56.6	42.2	1.00 (reference)	
37–38	26.9	26.9	1.23 (0.97–1.56)	.088
34–36	6.9	10.6	1.62 (1.16–2.26)	.005
26–33	2.3	4.0	1.27 (0.75–2.15)	.380
22–25	0.4	6.1	5.88 (3.47–9.98)	<.001
<22	0.3	3.3	2.87 (1.45–5.69)	.002
Unknown	1.4	1.9	1.68 (0.83–3.38)	.151
Cesarean delivery				
No	67.7	58.4	1.00 (reference)	
Yes	32.3	41.6	1.53 (1.26–1.87)	<.001

aOR = adjusted-odds ratio; CI = confidence interval; IUFD = intrauterine fetal demise including stillbirth; IUGR = intrauterine growth restriction; PAS = placenta accreta spectrum.
[a] Percentage per column is shown.
[b] Multivariable binary logistic regression model with conditional backward selection.
[c] Overall *P* value.
[d] Small number suppressed per HCUP guidelines.

Anderson. Turner syndrome pregnancy. Fertil Steril 2024.

(24, 25). Spontaneous pregnancy may still occur among 5%–8% of individuals with Turner syndrome, more often occurring among those with a mosaic karyotype (26).

Clinical characteristics of pregnant individuals with Turner syndrome. Individuals with Turner syndrome were more likely to have a history of pregnancy loss, compared with those without Turner syndrome. Among those conceiving spontaneously, this could be the result of aneuploidy from decreased oocyte quality. Oocyte donation improves the live birth rate, but the risk is still higher when compared with the general population (4, 26). Other etiologies considered include endometrial dysfunction, structural malformations of the uterus, and the chronic effects of auto-immune disease.

We found uterine anomalies to be associated with Turner syndrome pregnancies. A review of uterine size in 86 people with Turners syndrome aged 18–45 years found only 25% of those studies had normal size dimensions (27). In a comparison of uterine size at puberty induction, estrogen was initiated among 40 teenage girls with Turner syndrome and 40 healthy controls. Those with Turner syndrome started with a smaller uterine volume but this increased to a normal volume after 6–12 months of estrogen treatment (28). More recently, the association between Turner syndrome and uterine anomalies was called into question. The investigators argue that the absence of estrogen leads to the associated finding of hypoplasia (29). Additional anomalies, such as Mullerian agenesis have also been reported. In one study, several cases of bicornuate uterus were reported and associated with increased risk of miscarriage.

Obstetric outcomes related to Turner syndrome. We found a significant association between Turner syndrome and intrauterine growth restriction as well as intrauterine fetal demise. A retrospective analysis of pregnancy outcomes in 60 patients with Turner syndrome reported a fetal demise rate of 3%, both demises were the result of pre-eclampsia and oligohydramnios (30). Hypertension among women with Turner syndrome may contribute to these findings because it is a risk factor for both growth restriction and fetal demise. Additional risk factors associated with intrauterine fetal demise that may be more common among women with Turner syndrome include advanced maternal age, use of assisted reproductive technology, and nulliparity (31).

Odds of previable (<22 weeks) and periviable delivery (22–25 weeks) were both increased among patients with Turner syndrome. Preterm delivery is frequently reported as a complication of pregnancy in individuals with Turner syndrome. A French study examining 100 pregnant patients with Turner syndrome, excluding previable pregnancies, reported a preterm birth rate of 43% with just a 4% rate before 32 weeks (32). Similarly, a Canadian population-based study comparing delivery outcomes between pregnant patients with Turner syndrome (n = 44) and the general population found an increase in preterm delivery at <37 weeks but not in patients delivering at <32 weeks (33). The current findings

might be the result of increased granularity among gestational age groupings in preterm delivery (34–36, 26–33, 22–25, and <22 weeks). Increased odds of pre- and periviable deliveries were not previously reported, warranting further validation to confirm the findings.

Uterine anomalies, prior cesarean sections, and the use of assisted reproductive technology may explain the increased odds of placenta accreta spectrum in patients with Turner syndrome (34, 35). Overexpression of matrix metalloproteinases, enzymes involved in breaking down extracellular matrix, may contribute to both the pathogenesis of the placenta accreta spectrum and the aortopathy of Turner syndrome (36, 37). Future studies need to investigate the potential link between Turner syndrome and placenta accreta spectrum given the significant risks for an already high-risk population.

Cardiovascular outcomes. The increased risk of cardiovascular complications and mortality associated with Turner syndrome and pregnancy is of high concern. We found no cases of aortic dissection at delivery in this cohort of Turner syndrome from 2016–2020. No cases of mortality and no aortic dissections in our population of hospital-setting delivery could be the reflection of improved screening and the selection of healthier patients attempting pregnancy.

Strengths and limitations

Strengths of the current study include the use of a nationally representative database with a large sample size (1,255 vs. 8–131 cases in prior studies) (4, 26, 30, 32, 33, 38–46). Limitations include possible unmeasured confounders, such as karyotype and the method of conception. Mosaic karyotype and spontaneous conception have been linked to decreased pregnancy complications. Additionally, because our sample population included only pregnancies at the time of delivery, maternal deaths that occurred in a nondelivery setting may have not been captured in this analysis. A secondary entry for the same individual was not able to be captured. Postpartum complications after discharge following delivery are also not included in the current data set.

CONCLUSION

Although infrequent, pregnancies in individuals with Turner syndrome are gradually increasing in the United States. Awareness of the increasing number of pregnancies in patients with Turner syndrome is of value. It is reassuring that the majority of those who had a hospital delivery had a term delivery with no reports of aortic dissection at the time of delivery. Yet, given the high-risk pregnancy features of Turner syndrome, establishing society-based data and approach for preconception counseling and antenatal follow-up is timely and clinically compelling.

CRediT Authorship Contribution Statement

Zachary S. Anderson: Writing – original draft, Methodology, Investigation, Conceptualization. **Aaron D. Masjedi:** Writing – review & editing, Investigation. **Laurel S. Aberle:** Writing – review & editing, Investigation. **Rachel S. Mandelbaum:** Writing – review & editing, Supervision, Software, Resources, Methodology, Investigation, Data curation, Conceptualization. **Katherine V. Erickson:** Writing – review & editing, Investigation. **Shinya Matsuzaki:** Writing – review & editing, Resources, Investigation. **Doerthe Brueggmann:** Writing – review & editing, Investigation. **Richard J. Paulson:** Writing – review & editing, Supervision, Resources, Investigation. **Joseph G. Ouzounian:** Writing – review & editing, Supervision, Resources, Investigation. **Koji Matsuo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Interests

Z.S.A. has nothing to disclose. A.D.M. has nothing to disclose. L.S.A. has nothing to disclose. R.S.M. has nothing to disclose. K.V.E. has nothing to disclose. S.M. received research funding from Merck. D.B. has nothing to disclose. R.J.P. has nothing to disclose. J.G.O. has nothing to disclose. K.M. has nothing to disclose.

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Evaluación de las características obstétricas y de los resultados asociados al embarazo con síndrome de Turner

Objetivo: Evaluar las tendencias, características y resultados a nivel nacional de los embarazos con síndrome de Turner en Estados Unidos.

Diseño: Estudio transversal.

Lugar: Muestra nacional de pacientes hospitalizados en el Healthcare Cost and Utilization Project.

Sujetos: Un total de 17 865 495 partos hospitalarios entre 2016 y 2020.

Exposición: Un diagnóstico de síndrome de Turner, identificado según la Clasificación Internacional de Enfermedades de la Organización Mundial de la Salud (Código de la 10ª revisión de Q96).

Principales medidas de resultado: Resultados obstétricos relacionados con el síndrome de Turner, evaluados con probabilidad inversa de la cohorte de ponderación del tratamiento y modelos de regresión logística binaria multivariable.

Resultados: La prevalencia de pacientes embarazadas con síndrome de Turner fue de 7,0 por 100.000 partos (una de cada 14.235). El número de partos hospitalarios en pacientes con diagnóstico de síndrome de Turner aumentó de 5,0 a 11,7 por cada 100.000 partos durante el periodo de estudio (cociente de probabilidades ajustado [aOR] para 2020 frente a 2016; 2,18; intervalo de confianza [IC] del 95%: 1,83-2,60. Las pacientes embarazadas con síndrome de Turner tenían más probabilidades de tener un diagnóstico de hipertensión pregestacional (4,8% frente a 2,8%; aOR 1,65; IC 95%: 1,26-2,15), anomalías uterinas (1,6% frente a 0,4%; aOR, 3,01; IC 95%: 1,93-4,69) y abortos previos (1,6% frente a 0,3%; aOR 4,70; IC 95%: 3,01-7,32), en comparación con las mujeres sin síndrome de Turner. En cuanto al índice de las características obstétricas el síndrome de Turner se asoció con un mayor riesgo de muerte fetal intrauterina (10,9% frente a 0,7%; aOR 8,40; IC del 95%: 5,30-13,30), retraso del crecimiento intrauterino (8,5% frente a 3,5%; aOR 2,5; IC del 95%: 3,01-7,32) y cualquier espectro de placenta acreta (aOR (aOR 3.63; 95% CI 1.20–10.97). En cuanto a los resultados del parto, las pacientes embarazadas con síndrome de Turner tenían más probabilidades de sufrir un parto por cesárea (41,6% frente a 32,3%; aOR 1,53; IC del 95%: 1,26-1,87). Además, las probabilidades de parto periviable (22-25 semanas: 6,1% frente a 0,4%; aOR 5,88; IC 95%: 3,47-9,98) y parto prematuro (<22 semanas: 3,3% frente a 0,3%; aOR 2,87; IC 95%: 1,45-5,69) en comparación con las mujeres sin síndrome de Turner.

Conclusiones: Los resultados de una evaluación contemporánea a escala nacional en Estados Unidos sugieren que, aunque el embarazo con Turner es infrecuente, puede representar un grupo de alto riesgo, sobre todo de muerte fetal intrauterina y parto periviable. El establecimiento de un enfoque basado en la sociedad para el asesoramiento preconcepcional y el seguimiento prenatal sería clínicamente conveniente.

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The use of hormonal contraceptives in fertility treatments: a committee opinion

Practice Committee of the American Society for Reproductive Medicine

American Society for Reproductive Medicine, Washington, D.C.

The use of hormonal contraception can be considered to aid in the timing of assisted reproductive technology cycles, reduce the risk of ovarian cysts at in vitro fertilization cycle initiation, and optimize visualization before hysteroscopy. (Fertil Steril® 2024;122:243–50. ©2024 by American Society for Reproductive Medicine.)

El resumen está disponible en Español al final del artículo.

Key Words: Reproductive science, contraception, hormonal contraception, assisted reproduction, gynecology

Hormonal contraception has several indications in gynecology beyond the prevention of pregnancy. Hormonal contraception is frequently used in reproductive medicine for indications, such as ovarian cyst prevention, preoperative management, and hormone replacement. It is used in the treatment of reproductive disorders such as endometriosis, polycystic ovary syndrome (PCOS), and hirsutism. Noncontraceptive benefits also include menstrual management, such as the treatment of dysmenorrhea, menorrhagia, and menstrual irregularity (1). Many of these topics are covered more extensively in other American Society for Reproductive Medicine Practice Committee publications (2), and only indications related to fertility treatments will be covered here.

Although hormonal contraception is frequently used in the management of reproductive issues for women, it is also heavily relied on in the context of fertility treatments. A unique use of hormonal contraception within infertility treatment is hormonal pretreatment for in vitro fertilization (IVF). Hormonal contraceptive pretreatment includes menstrual cycle control, synchronization of the oocyte cohort, modification of the hormonal milieu before controlled ovarian stimulation (COS) for IVF, and suppression of ovarian cyst formation. Hormonal contraception can also be used as a pretreatment tool before hysteroscopy for fertility-related surgeries.

The impact of hormonal contraception on ovarian stimulation has been extensively studied, with investigation focusing on the type of hormonal contraception used, effect of the duration of hormonal contraception pretreatment, and concomitant use with different reproductive disorders. Studies have focused on outcomes, including oocyte yield, pregnancy, and live birth (LB) rates. Hormonal contraception may impact the markers of ovarian reserve. Therefore, these tests should be interpreted with caution while a woman is on hormonal contraception and counseling modified. Several factors should be considered before placing patients on hormonal contraception in the setting of fertility treatments and, in some cases, alternatives used.

HOW DOES HORMONAL CONTRACEPTION AFFECT TIMING OF ASSISTED REPRODUCTIVE TECHNOLOGY CYCLES?

Although COS for IVF classically begins with menses, hormonal contraception allows for the scheduling of an IVF cycle. This can be beneficial for both the patient and clinic. Some IVF clinics will use hormonal contraception to "batch" IVF cycles or have several patients complete their IVF cycles at the same time. Additionally, hormonal contraception can be used in third-party reproductive cycles to coordinate the oocyte donor with the oocyte recipient. Hormonal contraception also plays a pivotal role in reducing the risk of cancellation of cycles from unintended pregnancy in the donor (3).

The European Society of Human Reproduction guidelines on ovarian

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stimulation for IVF/intracytoplasmic sperm injection reinforce that the use of estrogen and progesterone for scheduling is probably acceptable on the basis of safety and efficacy data, although evidence surrounding the use of pretreatment hormonal contraception has been inconsistent (4).

DOES HORMONAL CONTRACEPTION IMPACT THE STIMULATION YIELD OF AN IVF CYCLE?

The suppressive nature of hormonal contraception on circulating follicle-stimulating hormone (FSH) and luteinizing hormone may be beneficial for synchronization of the oocyte cohort during COS; however, the suppression may also attenuate the ovarian response to gonadotropins (5). In young patients, high androgenic hormonal contraception exposure may suppress ovarian responsiveness and oocyte yield (6). In a prospective randomized study of hormonal treatment before IVF, women using hormonal contraception required more gonadotropins than women without hormonal pretreatment; however, there was no adverse effect on oocyte yield or pregnancy outcomes (5). Subsequently, a meta-analysis of 4 randomized controlled trials (RCTs) demonstrated that the ongoing pregnancy rate (odds ratio, 0.74) and oocyte yield were similar for women with pretreatment hormonal contraception vs. no pretreatment hormonal contraception in gonadotropin-releasing hormone (GnRH) antagonist cycles. The duration of treatment (weighted mean difference, +1.41 days; 95% confidence interval, +1.13 to +1.68) and total gonadotropin dose (weighted mean difference, +542 IU; 95% confidence interval, +127 to +956) were significantly higher in the hormonal contraception pretreatment group (7).

Overall, there is no overwhelming evidence to suggest that hormonal contraception significantly decreases ovarian stimulation response. When determining whether to pursue hormonal contraception pretreatment, the important factors to consider include patient age, ovarian reserve, and IVF protocol type. In addition, the duration of hormonal contraception may be considered; however, there is no evidence to suggest the timing and length of pretreatment with hormonal contraception influence assisted reproductive technology (ART) outcomes.

DOES THE TYPE OF HORMONAL CONTRACEPTION USED DIFFERENTIALLY AFFECT OVARIAN STIMULATION?

Some studies have suggested that any potential detrimental effect of hormonal contraception on oocyte yield is related to the progestin component (6). Specifically, progestins with greater androgenic properties, such as estrane- and gonane-derived progestins, may be associated with decreased oocyte yield and lower antimüllerian hormone (AMH) levels than those constituting antiandrogenic hormonal contraception, such as drospirenone, dienogest, and trimegestone (6). This may be because of androgens working synergistically with FSH in early follicular development and then inhibiting gonadotropin support of the growing follicles, followed by atresia of the growing follicles because of a lack of FSH (6). A small study has compared egg donors on "androgenic" hormonal contraception pretreatment before stimulation (containing norethindrone, norgestimate, and norgestrel) with those without hormonal contraception pretreatment. Donors on androgenic hormonal contraception demonstrated a significantly lower egg yield than those without hormonal contraception (hormonal contraception, 11.3 oocytes; no hormonal contraception, 16.6 oocytes; $P<.05$) (8). Nonetheless, a recently published retrospective analysis by Montoya-Botero et al. (9) found no significant difference in the clinical pregnancy or LB rate between women taking hormonal contraception containing the third-generation progestin desogestrel and those taking fourth-generation progestin drospirenone before COS for IVF.

There is a lack of consistent evidence to make a recommendation for a formulation of hormonal contraception being less suppressive for patients planning to undergo COS for ART.

DOES PRETREATMENT WITH HORMONAL CONTRACEPTION IMPACT THE LB RATE OF IVF CYCLES?

Pretreatment with hormonal contraception in antagonist and agonist cycles has been associated with a reduction in the LB rate in some studies. In a retrospective cohort study, pretreatment with hormonal contraception was associated with a reduction in the LB rate after fresh transfer (42.6% vs. 52.8%, $P<.001$), as well as the cumulative LB rate (62.8% vs. 67.6%, $P=.01$) (10). However, it has also been demonstrated that hormonal contraception administration for an interval of 12–30 days with a 5-day washout period does not affect clinical pregnancy, LB, or cumulative LB in patients undergoing COS for an IVF cycle (9). Additionally, a 2017 Cochrane Review of 29 RCTs in GnRH agonist and antagonist cycles found no clear evidence of a difference in the pregnancy or LB rates. In antagonist cycles, hormonal contraception was associated with a decreased risk of pregnancy loss (3).

WHAT ABOUT THE IMPACT OF OTHER TYPES OF HORMONAL CONTRACEPTION ON IVF SUCCESS?

A related issue is the impact of a levonorgestrel-releasing (LNG) intrauterine device (IUD) on ovarian stimulation. A patient may have an LNG-IUD for contraception and management of endometriosis or abnormal bleeding and be pursuing elective egg or embryo freezing. Providers also often face this question in the egg donor population. Thus, providers need to recognize the potential impact of the LNG-IUD on ovarian stimulation. Adeleye et al. (11) performed a retrospective cohort evaluating oocyte yield in women with a 52-mg LNG-IUD compared with that in subjects without an IUD. Subjects with an LNG-IUD had a lower peak estradiol level and required a higher FSH dose per cycle. No differences in the total or mature oocyte yield were noted in subjects with or without LNG-IUD. Furthermore, no differences in blastocyst progression or the fertilization, clinical pregnancy, or LB rates were observed in recipients of donor oocytes who

had a LNG-IUD in place during COS. Thus, egg donors or patients considering fertility preservation can retain their LNG-IUD before and during ovarian stimulation. However, providers should note a potential higher dose of FSH and, thus, cost. The impact of an etonogestrel implant during ovarian stimulation was similarly described in a case report. However, given the limited data and higher systemic progesterone dosing with an implant, a definitive recommendation cannot be made (12).

DOES PRETREATMENT HORMONAL CONTRACEPTION IMPACT THE LB RATE AMONG WOMEN IN SPECIAL POPULATIONS?

Diminished ovarian reserve

Data are limited regarding the potential impact of hormonal contraception pretreatment on IVF outcomes in women with diminished ovarian reserve (13). A retrospective analysis compared poor responder patients pretreated with hormonal contraception vs. natural cycle start in agonist-flare cycles and found no difference in the implantation or pregnancy rates. Randomized trials in this population are not available.

Polycystic ovary syndrome

Pretreatment hormonal contraception in women with PCOS may significantly aid in regulating menses and synchronizing follicular development; however, clinical trials have demonstrated mixed results surrounding ART outcomes (14, 15). This may be because of differing lengths of pretreatment hormonal contraception exposure. Pan et al. (14) studied the impact of 3 consecutive months of hormonal contraception before IVF in subjects with PCOS. They found improved implantation and clinical pregnancy rates in subjects using at least 3 months of hormonal contraception compared with those in non–oral contraceptive pill (OCP) users and those using <2 months of hormonal contraception. These subjects were also found to have a lower antral follicle count (AFC) and reduced symptoms of hyperandrogenism while on hormonal contraception. The benefit of a 3-month course of hormonal contraception may be, in part, related to the 70–90-day development of the ovarian secondary follicles to periovulation (16).

Conversely, other studies have demonstrated adverse effects of hormonal contraception pretreatment on the LB rate after fresh embryo transfer in women with PCOS. In a nested cohort study and secondary analysis of a multicenter randomized trial, Wei et al. (15) found that subjects with PCOS exposed to hormonal contraception for 21–25 days before GnRH antagonist protocol IVF had lower rates of clinical pregnancy (48.8% vs. 63.6%; relative risk [RR], 2.13) and LB (36.1% vs. 48.1%; RR, 0.75) after a fresh embryo transfer than those with spontaneous menses. Interestingly, they also found that women with hormonal contraception–induced menses in frozen embryo transfer cycles within this patient population had a similar pregnancy rate but a higher pregnancy loss rate (27.7% vs. 13%; RR, 2.13) than those with spontaneous menses.

It is important to consider the potential role for mitigating the risk of ovarian hyperstimulation syndrome in patients with PCOS because hormonal contraception pretreatment has been shown to moderately reduce ovarian high response without influencing the quality of oocytes (17).

Endometriosis

There is existing evidence that women with endometriosis may benefit from pretreatment hormonal contraception, specifically pertaining to improved pregnancy rates per retrieval (18, 19). This may be because of ovarian suppression inhibiting the production of inflammation-mediated aromatase expression and estradiol production, which may also have downstream effects on morphological and functional changes in the endometrium (20, 21).

One study by de Ziegler et al. (22) demonstrated that a 6–8-week course of hormonal contraception pretreatment in women with endometriosis resulted in higher pregnancy rates per retrieval and fresh embryo transfer than controls (35% vs. 12.9%) and that the effect was even more robust when endometriomas were present. More research is required to determine whether this effect is a result of endometrial receptivity, oocyte quality, or other effects and whether these results are consistent in multiple studies.

CAN HORMONAL CONTRACEPTION BE USED IN OVARIAN CYST MANAGEMENT BEFORE FERTILITY TREATMENTS?

Ovarian cyst formation is a common problem in reproductive-aged women. In a large cross-sectional study, ovarian cysts with a diameter of >30 mm were noted in 4%–7% of women during ultrasound evaluation before initiating an oral contraceptive (23). Functional ovarian cysts may be follicular or corpus luteum cysts and secrete estradiol or progesterone. These cysts may cause irregular menstrual bleeding, pain, inhibition of response to ovarian stimulation, and an increased risk of ovarian torsion. Nonfunctional ovarian cysts do not secrete hormones.

The impact of an ovarian cyst at the start of IVF stimulation is controversial. An early study suggested a negative impact of ovarian cyst formation on IVF outcomes (24). The investigators noted an increase in cycle cancellation likely because of premature luteinizing hormone surge with cysts 16–29 mm and poor response to stimulation with cysts 30–60 mm (24–26). Subsequent studies have suggested no impact of baseline ovarian cysts on the number of follicles aspirated or oocytes retrieved with IVF. Notably, patients were excluded if the cystic structure was >50 mm and/or if the cycle day 3 estradiol level was >50 pg/mL. Despite a lack of impact on mature oocytes retrieved, patients with nonfunctional ovarian cysts required increased gonadotropin dosing and had lower peak estradiol levels. Additionally, patients with unilateral cystic structures had significantly fewer follicles from the cystic ovary than from the contralateral ovary. These findings led the investigators to suggest that a nonfunctional ovarian cyst induces changes in the intraovarian endocrine environment to

interfere with follicular development and function. Additional studies have had inconsistent findings. Despite these conflicting findings, most providers will avoid or postpone a fertility treatment cycle if a large or functional ovarian cyst is present at baseline evaluation (27–31).

Christensen et al. (32) noted a lower prevalence of ovarian cysts in women using hormonal contraception than in those not using contraception or using a non–hormone-releasing IUD (RR, 0.22; 95% CI, 0.13–0.39). The incidence of ovarian cysts in women not on contraception was 9.5% (14/147) vs. 2.4% (5/211) in women who were on hormonal contraception for at least 3 months.

Because of the known decreased risk of ovarian cysts in women on hormonal contraception, they are often initiated to hasten the resolution of functional cysts to allow resumption of fertility treatment. However, a Cochrane Review (33) published in 2014 refuted this recommendation after evaluating 8 RCTs comparing hormonal contraception with expectant management for cyst treatment. To our knowledge, no study demonstrated a benefit of hormonal contraception over expectant management. Resolution occurred spontaneously within 4–6 weeks in the large majority of patients, whether treated with hormonal contraception or expectantly managed. Those that persisted were often pathologic (endometrioma, hydrosalpinx, dermoid cyst, and paraovarian cyst) and less likely a functional ovarian cyst.

On the basis of this available information, hormonal contraception should not be started to hasten the resolution of ovarian cysts but can be protective against the development of new ovarian cysts.

CAN HORMONAL CONTRACEPTION BE USED TO AID IN THE EVALUATION OF THE UTERINE CAVITY BEFORE FERTILITY TREATMENTS?

Hysteroscopy is frequently used in reproductive medicine to evaluate and treat uterine pathology for optimization before fertility treatments. Hysteroscopy is ideally performed when the endometrial lining is relatively thin, such as in the early follicular phase, just after the cessation of menses. A thickened endometrium can impair visualization of smaller lesions and easily breaks off or bleeds further diminishing visualization.

Scheduling of hysteroscopic procedures in the appropriate time frame can be difficult, particularly for patients with irregular menses or prolonged menstrual bleeding. Additional benefits of hormonal contraception use in this population include the prevention of pregnancy and ease of scheduling for surgery.

It is important to note that hormonal contraception most reliably prevents ovulation and, therefore, will more likely result in a thin endometrium when started early in the menstrual cycle. One study noted no ovulation when hormonal contraception was started when the maximum follicle diameter was 10 mm (mean cycle day 7.6) or lower or when the vaginal ring was started at a follicle diameter of 13 mm (median cycle day 11) or lower. Additionally, starting the menstrual cycle on day 1 vs. 5 led to fewer dominant follicles (34). In 2006, Grow and Iromloo (35) demonstrated that initiation of combination OCPs on menstrual cycle days 1–3 consistently maintained a thinner endometrium (4.1 mm) than starting in the late follicular phase (11 mm) or late luteal phase (12 mm).

Multiple other hormonal preparations, including nomegestrol acetate (36), oral desogestrel plus vaginal raloxifene (37), estradiol plus dienogest (38), gestrinone (39), and desogestrel alone (40), appear to demonstrate benefit for rapid endometrial preparation before operative hysteroscopy.

HOW DOES HORMONAL CONTRACEPTION AFFECT OVARIAN RESERVE MARKERS BEFORE INITIATING AN IVF CYCLE?

Ovarian reserve markers, such as AMH and the AFC, have been shown to be suppressed in response to prolonged hormonal contraception (41). One study has suggested a decrease of 30% in both the AMH level and AFC with long-term hormonal contraception (>6 months) (42). In a longitudinal study of >700 women, the AFC after cessation of hormonal contraception started to increase at 1 month and plateaued at 6 months, suggesting nearly 6 months for recovery of the AFC to prehormonal exposure (43). Eighty percent of women in this study had an increase in the AFC after stopping hormonal contraception, with 60% of women achieving normalization of the AFC. Subgroup analyses suggested that this was only observed in women with a low AFC on hormonal contraception. Women with a normal AFC on hormonal contraception were unlikely to observe an increase in the AFC when stopping hormonal contraception (43).

Cessation of hormonal contraception for 2–3 months may allow for a more accurate assessment of a patient's ovarian reserve, as exhibited by either the AMH level or AFC.

WHAT SHOULD PROVIDERS BE AWARE OF BEFORE USING HORMONAL CONTRACEPTION IN FERTILITY PATIENTS?

It is imperative that providers are aware of the eligibility criteria for combined hormonal contraceptive use outlined in the US Medical Eligibility Criteria, as well as the RRs of therapy in certain populations (44). Although most patients undergoing fertility treatment are at low risk of complications, certain medical comorbidities should be considered, including advanced age, smoking status, a history of migraine with aura, increased risks of venous thromboembolic events, and cardiovascular disease.

Migraines with aura are common in reproductive-aged women. Such a history, when present, needs to be elicited before initiating combination hormonal contraception (those containing both estrogen and progesterone). The World Health Organization Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception from 1999 (45) demonstrated that women with a history of migraine on combination hormonal contraception had 3 times higher odds of ischemic stroke than those with a history of migraine not on combined hormonal contraceptive. They noted a higher OR of 3.81 for migraine with aura and an OR of 2.97 for migraine without aura. The coexistent use of combined hormonal

contraception or history of hypertension or smoking had greater than multiplicative effects on the OR for ischemic stroke associated with migraine alone.

There is some thought that the short-term use of combined hormonal contraception (3–6 weeks) immediately before ovarian stimulation confers a decreased risk of complications (e.g., venous thromboembolism [VTE]) compared with the more long-term use for contraception. Although data for such short-term use are limited, there is evidence to support that the longer duration of use actually decreases some risks, such as VTE (46). This case-control study reported that the risk of VTE among current OCP users significantly decreased over time from an OR of 5.1 at <1 year to 2.1 after >5 years. There are data to support a significant impact of hormonal contraception on the coagulation system as early as 6 weeks of use. Low-dose hormonal contraception caused an increase in the levels of factors VII and X, plasminogen, fibrinogen, and D-dimer. Antithrombin II and protein C activities did not change over the study period. These investigators reported a reduced effect with a 20-μg pill compared with that with a 30-μg pill (47). Therefore, the short-term use does not appear to minimize the risks of hormonal contraception, although the periods of 1–2 weeks as often used in pretreatment for fertility treatments have not been specifically studied. However, it should be acknowledged that pregnancy itself is a significant risk factor for VTE and that the short-term use of OCPs before IVF stimulation would be expected to confer a lower risk than pregnancy itself.

Some patients may be concerned about adverse side effects noted with hormonal contraceptive use. These concerns have led to substantial changes in the makeup of hormonal contraception over time, such as a decrease in the estrogen dosage, new progestin components, and new sequences of administration (48). In a study from 2007, the most common symptoms in a population of French women using hormonal contraception were weight gain (25.2%), painful menses (20.7%), swollen legs (20.9%), and heavy menstrual bleeding (15.6%). Women using progestin-only pills and second-generation progestins were more likely to report irregular or breakthrough bleeding. The investigators found no evidence to support that decreasing the estrogen dosage decreases any symptom associated with the hormonal contraception. Although patients may have concerns about these side effects, it is worth noting that short-term use with fertility treatments will likely minimize potential side effects (48).

ARE THERE ALTERNATIVES TO HORMONAL CONTRACEPTION THAT CAN BE USED WITH FERTILITY TREATMENTS?

One alternative to combination hormonal contraception in those patients with risk factors for use is the progesterone-only pill (POP). Available evidence demonstrates that POP use does not increase the risk of ischemic stroke in patients with menstrual migraines (49) and, therefore, may be an acceptable alternative. However, data on the potential adverse outcomes with the POP are limited (Centers for Disease Control and Prevention). There are certain circumstances where POP use should also be approached cautiously. Women with hypertension using a POP have a slightly increased risk of cardiovascular events compared with those not using this method. It is also contraindicated in patients with active or recent (<5 years) breast cancer.

The disadvantages of the POP in reproductive medicine include a higher risk of irregular vaginal bleeding and functional ovarian cysts. Approximately 20%–30% of users experience intermenstrual bleeding or spotting, which is a common reason for discontinuation (50). This abnormal bleeding is most frequent on initiation and is likely because of incomplete ovarian suppression. In a study of 21 women who had been using a POP for at least 6 months, functional cysts were noted in 8 on initial examination. Four of the 13 women without cysts on baseline examination subsequently went on to develop new functional cysts associated with symptoms in 2 of these women (51). The use of POP before IVF is less likely to suppress cyst formation or premature follicular development.

SUMMARY

- Hormonal contraception pretreatment can be used for scheduling IVF treatments.
- There are inconsistent data that pretreatment with hormonal contraception impacts ovarian stimulation, although it may increase the amount of gonadotropins required.
- There is inconsistent evidence that the formulation of hormonal contraception impacts IVF outcomes.
- The use of hormonal contraception in the setting of PCOS or endometriosis is conflicting, and thus, definitive conclusions cannot be drawn.
- The use of hormonal contraception for the suppression of ovarian cyst has not been substantiated.
- Treatment with hormonal contraception can be used before hysteroscopy in the workup of infertility.
- The use of hormonal contraception may decrease markers of ovarian reserve, and those markers may improve after cessation of hormonal contraception for some patients, especially for those women with low AFCs.

When the use of hormonal contraception is contraindicated, POP may be used as an alternative.

CONCLUSIONS

- The use of hormonal contraception can be considered to aid in the timing of ART cycles, reduce the risk of ovarian cysts at IVF cycle initiation, and optimize visualization before hysteroscopy.
- Long-term hormonal contraception can falsely lower markers of ovarian reserve, and consideration should be given to stopping therapy to re-evaluate the baseline levels.

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This report was developed under the direction of the Practice Committee of the American Society for Reproductive Medicine (ASRM) as a service to its members and other practicing clinicians. Although this document reflects appropriate management of a problem encountered in the practice of

reproductive medicine, it is not intended to be the only approved standard of practice or to dictate an exclusive course of treatment. Other plans of management may be appropriate, taking into account the needs of the individual patient, available resources, and institutional or clinical practice limitations. The Practice Committee and Board of Directors of the ASRM have approved this report.

Declaration of Interests

This document was reviewed by the ASRM members, and their input was considered in the preparation of the final document. The following members of the ASRM Practice Committee participated in the development of this document: Clarisa Gracia, M.D., M.S.C.E.; Alan Penzias, M.D.; Paula Amato, M.D.; Jacob Anderson, M.B.A.; Kristin Bendikson, M.D.; Tommaso Falcone, M.D.; Rebeca Flyckt, M.D.; Jessica Goldstein, R.N.; Karl Hansen, M.D., Ph.D.; Micah Hill, D.O.; Sangita Jindal, Ph.D.; Suleena Kalra, M.D., M.S.C.E.; Tarun Jain, M.D.; Bruce Pier, M.D.; Michael Thomas, M.D.; Richard Reindollar, M.D.; Jared Robins, M.D.; Chevis N. Shannon, Dr.Ph., M.B.A., M.P.H.; Anne Steiner, M.D., M.P.H.; Cigdem Tanrikut, M.D.; and Belinda Yauger, M.D. The Practice Committee acknowledges the special contribution of Kristin Bendikson, M.D.; Belinda Yauger, M.D.; Megan Sax, M.D.; and Brooke Rossi, M.D., in the preparation of this document. All Committee members disclosed commercial and financial relationships with manufacturers or distributors of goods or services used to treat patients. The members of the Committee who were found to have conflicts of interest based on the relationships disclosed did not participate in the discussion or development of this document.

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Uso de anticonceptivos hormonales en los tratamientos de infertilidad: una opinión del comité

Se puede considerar el uso de anticonceptivos hormonales para asistir en el tiempo de inicio de los ciclos de reproducción asistida, reducir el riesgo de quistes ováricos al inicio del ciclo de fertilización in vitro y optimizar la visualización antes de la histeroscopia. (Fertil Steril 2024;122:243-50)

Palabras clave: Ciencia reproductiva, anticoncepción, anticoncepción hormonal, reproducción asistida, ginecología

Check for updates

Evidence-based diagnosis and treatment for uterine septum: a guideline

Practice Committee of the American Society for Reproductive Medicine

The American Society for Reproductive Medicine, Washington, D.C.

Objective: To provide evidence-based recommendations regarding the diagnosis and effectiveness of surgical treatment of a uterine septum.
Methods: This guideline provides evidence-based recommendations regarding the diagnosis and effectiveness of surgical treatment of a uterine septum. This replaces the last version of the same name (Fertil Steril. 2016 Sep 1;106(3):530-40).
Main Outcome Measure(s): Outcomes of interest included the impact of a septum on underlying fertility, live birth, clinical pregnancy, and obstetrical outcomes.
Result(s): The literature search identified relevant studies to inform the evidence for this guideline.
Conclusion(s): The treatment of uterine septa and subsequent outcomes associated with infertility, recurrent pregnancy loss, and adverse obstetrical outcomes are summarized. Resection of a septum has been shown to improve outcomes in patients with recurrent pregnancy loss and to decrease the likelihood of malpresentation. In the setting of infertility, it is recommended to use a shared decision-making model after appropriate counseling to determine whether or not to proceed with septum resection. (Fertil Steril® 2024;122: 251–65. ©2024 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Uterine septum, reproductive medicine, diagnosis, treatment

RECOMMENDATIONS

- It is recommended to use 3D transvaginal ultrasound with or without saline infusion as the first-line noninvasive diagnostic tool in uterine shape assessment. (Strength of Evidence: B; Strength of Recommendation: Moderate).
- No recommendation can be made regarding the association between a septate uterus and infertility due to insufficient evidence. (Strength of Evidence: C; Strength of recommendation: No recommendation).
- It is recommended to counsel patients that the presence of a septate uterus is associated with spontaneous abortion and obstetric complications. (Strength of Evidence: B; Strength of Recommendation: Moderate).
- Although septum incision in patients with infertility and/or undergoing fertility treatment is reasonable, a firm recommendation for this practice cannot be made on the basis of the current evidence. It is recommended to counsel patients with infertility and/or undergoing fertility treatment that resection of septum may or may not be associated with an increase in live births. Given limitations in the literature and the low risk of the procedure, septum incision may be offered to patients in a shared decision-making model. (Strength of Evidence: B; Strength of Recommendation: Moderate).
- It is recommended to offer hysteroscopic septum incision to patients with a septum and a history of recurrent miscarriage in a shared decision-making model. (Strength of Evidence: B; Strength of Recommendation: Moderate).
- It is recommended to counsel patients that septum incision may decrease the risk of adverse obstetric outcomes such as malpresentation and cesarean section, but there are no high-quality data to recommend this practice. (Strength of Evidence: B; Strength of Recommendation: Moderate).

It is not recommended to use septum characteristics such as size or shape to determine the impact on adverse reproductive outcomes. (Strength of Evidence: B/C; Strength of recommendation: Moderate/Weak).

- It is recommended, on the basis of expert committee opinion, to consider performing the procedure during the follicular phase or after progesterone withdrawal to help with visualization during surgery. However, there are no studies designed to prove or

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disprove this. (Strength of Evidence: Insufficient; Strength of recommendation: Weak).

- It is recommended to counsel patients that, on the basis of limited data, there is no evidence that resection of the unicollis cervical septum increases the risk of cervical insufficiency. (Strength of Evidence: C; Strength of recommendation: Weak).
- It is not recommended to perform another surgery for a residual septum under 1 cm. (Strength of Evidence: C; Strength of recommendation: Weak).
- There is insufficient evidence to recommend routine administration of oral estrogen, intrauterine balloons, and IUDs to decrease adhesion formation after resection of a septum. (Strength of Evidence: C; Strength of recommendation: Weak).
- It appears the rate of uterine rupture after resection of a septum is rare; however, this outcome is not often reported on in the current literature. (Strength of Evidence: B/C; Strength of recommendation: Moderate/Weak).
- It is recommended to counsel patients that they may proceed with fertility treatment in 1–2 months after resection of a septum. (Strength of Evidence: C; Strength of recommendation: Weak).
- There is insufficient evidence to recommend hysteroscopic resection of a septum in patients who have not yet attempted conception (Strength of Evidence: Insufficient Strength of recommendation: Insufficient evidence to make recommendation).

Müllerian anomalies are rare developmental anomalies of the reproductive tract. These anomalies are typically viewed as defects of fusion of the Müllerian (paramesonephric) ducts or canalization failures after fusion or both. A uterine septum occurs when the tissue connecting the 2 paramesonephric ducts fails to resorb before the 20th embryonic week. The presence of a uterine septum has been associated with infertility, recurrent miscarriage, and poor obstetrical outcomes such as preterm birth (1). The true prevalence of uterine septa is difficult to ascertain as uterine septa are often asymptomatic but appear to range between 1 and 2 per 1,000 to as frequent as 15 per 1,000 (2).

Initially, uterine septa were believed to be predominantly fibrous tissue covered by endometrium. However, biopsy specimens and magnetic resonance imaging (MRI) imaging suggest that septa are composed primarily of muscle fibers and less connective tissue (3–5).

Müllerian duct anomalies, such as unicornuate uterus and uterine didelphys, are associated with concurrent renal anomalies in approximately 11%–30% of individuals (6). However, data do not suggest an association between the septate uterus and renal anomalies, and, as such, it is not necessary to routinely evaluate the renal system in patients with a uterine septum.

Septate uteri have a spectrum of configurations ranging from an incomplete/partial septate to a complete septate uterus. A partial septate uterus refers to a single fundus and cervix with a uterine septum extending from the top of the endometrial cavity toward the cervix. The size and shape of the septum can vary by width, length, and vascularity. Although developmentally, the arcuate uterus may be considered as part of the spectrum of Müllerian anomalies, it is typically considered a normal variant and therefore functionally not part of the septate spectrum. The original American Fertility Society (AFS) classification system placed the arcuate uterus in its own category as, in contrast to other uterine malformations, it is not associated with adverse clinical outcomes (7). However, it is important to differentiate arcuate from septate uterus to better direct surgical intervention, when appropriate, for the septate uterus. In the revised American Society for Reproductive Medicine (ASRM) classification (Fig. 1), the arcuate uterus configuration is placed in the septate uterus box with a clear description.

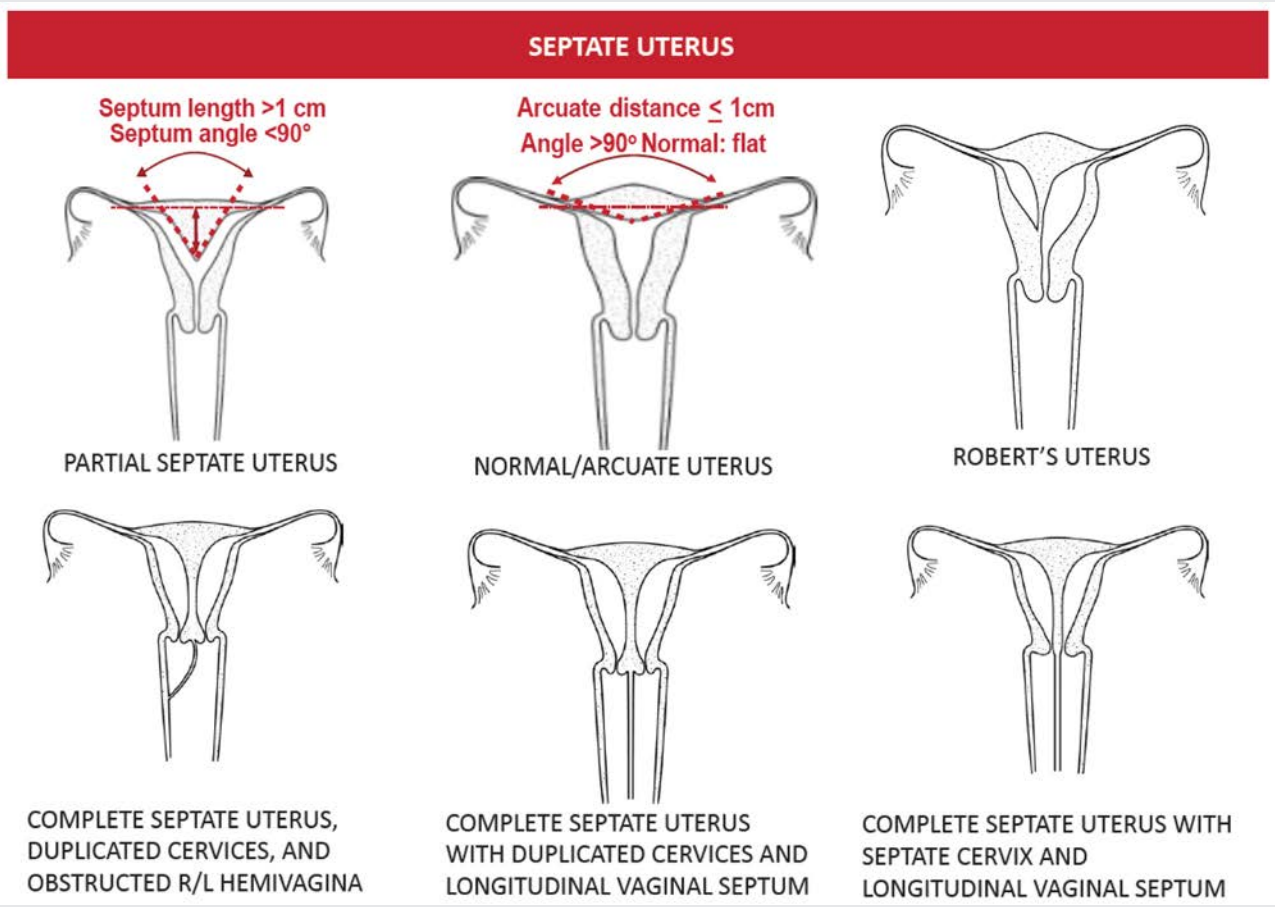
CLASSIFICATION

There are many proposed classification systems for Müllerian anomalies. The AFS classification from 1988 has been the most recognized and used (7). Many other classification systems have been developed to address limitations of the AFS classification such as exclusion of anomalies of the vagina and cervix, lack of clear diagnostic criteria, and inability to classify complex aberrations. The ASRM Task Force on Müllerian Anomalies Classification was formed and charged with designing a new classification to address the identified limitations. The Task Force set goals for a new classification and chose to base it on the iconic AFS classification from 1988 because of its simplicity and recognizability while expanding and updating it to include all categories of anomalies. The pictorial representation of this classification was published and shown in Figure 1. Literature searches were performed using all terms pertaining to uterine septum. The uterine septum may be associated with vaginal anomalies such as a longitudinal vaginal septum or obstructed hemivagina. This document will not cover the management of the vaginal anomalies. The management of cervical anomalies such as duplicated or septate cervix will be discussed.

LIMITATIONS OF THE LITERATURE

Multiple challenges exist in interpreting the literature related to the effectiveness and safety of the management of a uterine septum. Most studies compare outcomes pre- and postsurgery without comparison with an untreated control group, which is problematic given the significant rate of unassisted pregnancy with expectant management. Moreover, many studies are underpowered, and some report only surrogate outcomes such as clinical or ongoing pregnancy rather than live birth. In addition, the numerous and varied definitions and terminology used to describe the septate uterus make it challenging to interpret the data. Variable durations of infertility or the number of pregnancy losses before surgical intervention also makes comparisons between studies difficult, given the strong correlation between infertility and recurrent pregnancy loss duration and treatment outcomes. In addition, variations in surgical technique, experience, and approach are not well accounted for in the existing literature.

FIGURE 1



Diagrams of the ASRM definitions of normal/arcuate, septate, and bicornuate uterus on the basis of an assessment of available literature, understanding that these anomalies reflect points on a spectrum of development. Normal/arcuate: depth from the interstitial line to the apex of the indentation <1 cm and >90°. Septate: depth from the interstitial line to the apex of the indentation >1.0 cm and angle of the indentation <90°. External fundal contour is smooth with <1 cm indentation. ASRM. Müllerian Anomalies Classification. Fertil Steril 2021. ASRM = American Society for Reproductive Medicine.

Practice Committee of the American Society for Reproductive Medicine. Septate uterus. Fertil Steril 2024.

METHODS

This clinical practice guideline followed a methodological protocol established by ASRM staff and executive leadership, the ASRM Practice Committee, and an independent consulting epidemiologist. The ASRM Practice Committee identified the necessity to update the previously published guideline on uterine septum and empaneled a task force of experts to engage in its development. Members of the task force applied the Population, Interventions, Comparisons, and Outcomes framework to formulate focused questions related to clinical practice and evidence-based treatments for uterine septum, as well as preliminary inclusion/exclusion criteria.

This guideline provides evidence-based recommendations for surgical treatment in different clinical scenarios, such as infertility and recurrent pregnancy loss.

A comprehensive systematic review of the literature using the MEDLINE® database through PubMed® was conducted to identify peer-reviewed studies relevant to treatments for uterine septum. This document is an update to the previously published uterine septum guideline (2016). The searches were restricted to include papers published since the previous guideline with a date range of April 1, 2015, until November 14, 2022. No limit or filter was used for the time period covered or the English language, but articles were subsequently culled for the English language. Per inclusion/exclusion criteria that the task force agreed on (Table 1), studies included for assessment were randomized controlled trials (RCTs), systematic reviews or meta-analyses of RCTs; systematic reviews or meta-analyses of a combination of RCTs, controlled trials without randomization, and cohort studies; controlled trials without randomization; cohort studies; and case-control studies. Descriptive studies, case series, case reports, letters, nonsystematic reviews, opinions on the basis of clinical experience, and reports of expert committees were excluded from this guideline. Titles and abstracts of potentially relevant articles were screened and reviewed

TABLE 1

Inclusion and exclusion criteria.

Include	Exclude
Randomized controlled trials (RCTs); systematic reviews or meta-analyses of RCTs; systematic reviews or meta-analyses of a combination of RCTs, controlled trials without randomization, and cohort studies; controlled trials without RCTs, controlled trials without randomization, and cohort studies; controlled trials without randomization; cohort studies; and case-control studies	Descriptive studies, case series, case reports, letters, nonsystematic reviews, opinions on the basis of clinical experience, and reports of expert committees
Human studies	Animal studies
English	Non-English
Studies with a comparison group	Studies without a comparison group
Studies that report clinical (pregnancy, live birth, miscarriage, and/or obstetrical) outcomes	Studies that focus on prevalence with no fertility and/or obstetrical outcome measures
Studies that focus on septate uterus	Studies that do not focus on septate uterus, but focus on unicornuate or didelphic uteri, or fibroids and polyps, or cervix and vagina, OHVIRA or HWW syndrome, Asherman, Fryns, or MRKH syndrome
Studies that focus on imaging modalities including but not limited to MRI, 3D ultrasound, and sonohysterography	Studies with a focus on amenorrhea, blood flow, cancer, dysmenorrhea, endometriosis, hemodynamics, menorrhagia, ovarian maldescent, polycystic ovary syndrome, surgical technique only, uterine horn, uterine prolapse, and VEGF
	Studies with a focus on pediatric or postpartum population
	Studies with a focus on abdominal metroplasty
	Studies that focus on embryologic development

3D = 3-dimensional; HWW = Herlyn-Werner-Wunderlich; MRI = magnetic resonance imaging; MRKH = Mayer-Rokitansky-Küster-Hauser; OHVIRA = obstructed hemivagina and ipsilateral renal anomaly; VEGF = vascular endothelial growth factor.

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initially according to preliminary inclusion/exclusion criteria determined by members of the task force. All task force members reviewed the articles of all citations that potentially matched the predefined selection criteria. Final inclusion or exclusion decisions were made on examination of the articles in full. Disagreements about inclusion were discussed and resolved by consensus or arbitration after consultation with an independent reviewer/epidemiologist. The search yielded 323 studies, of which 49 studies met inclusion criteria.

Quality of evidence

A methodological specialist extracted data from included studies into an evidence table for outcomes identified by the task force, including live birth rate, clinical pregnancy rate, and surgical outcomes. Nonconflicted members of the task force critically assessed the strengths and limitations of available evidence that met inclusion/exclusion criteria to rate the quality of each study and assign a quality grade on the basis of the rating scale depicted in Table 2, which was recorded in the evidence table (Supplemental Table 1, available online).

The task force chair reviewed grades of quality assigned by members of the task force and provided oversight throughout the entire development process. If no grade was assigned, the task force chair determined a grade of quality on the basis of a study's strengths and limitations. The study design was evaluated, and the quality of the methodology was assessed on the basis of components including blinding, allocation concealment, appropriate control groups, intention-to-treat analysis, generalizability, and risk of bias.

The task force summarized data from the evidence table in narrative form to include the characteristics, quality, benefit, and conclusions of studies relevant to answering each treatment related to the question. The expert task force convened to review the literature and summarize findings. The task force chair presented these summaries of evidence and draft conclusions to the ASRM Practice Committee for deliberation of the strength of the evidence and the strength of the recommendations and approval of summary statements and recommendations. The quality of the evidence informed the strength of the guideline's evidence (Table 3). Patient perspective and feedback were elicited during the review and before the publication of the guideline.

HOW TO DIAGNOSE A UTERINE SEPTUM?

For accurate differentiation of Müllerian anomalies, it is essential to visualize both the external and internal contours of the uterus (Fig. 1). As such, the historical gold standard method for diagnosing and categorizing Müllerian anomalies employed concomitant laparoscopy and hysteroscopy. With radiologic advancements over the past 30 years, the diagnosis of a septate uterus has shifted from surgical to radiographic techniques. There are several nonsurgical techniques available, including hysterosalpingography (HSG), standard 2-dimensional transvaginal ultrasound (2D TVUS), 3-dimensional TVUS with or without saline infusion, and MRI.

Although HSG is often the initial test that provides evidence for a Müllerian anomaly in patients with infertility or recurrent pregnancy loss, without visualization of the external contour of the uterus, the diagnostic accuracy of the HSG is low for distinguishing septate and bicornuate uteri (8, 9). Similarly, hysteroscopy alone also cannot distinguish between these 2 anomalies. In addition, 2 studies that looked at the inter-observer

TABLE 2

Rating for quality of evidence.

Quality of evidence	Definition
High quality	Target population clearly identified Sufficient sample size for the study design Clear description of study design Appropriate control(s) Generalizable results Definitive conclusions Minimal risk of bias Limitations do not invalidate conclusions Evidence primarily on the basis of well-designed systematic reviews or meta-analyses of randomized controlled trials
Intermediate quality	Target population Sufficient sample size for the study design but could benefit from larger studies Control group identified Reasonably consistent results which limitations do not invalidate Fairly definitive conclusions Low risk of bias Evidence primarily on the basis of small randomized controlled trials; systematic reviews or meta-analyses of a combination of RCTs, controlled trials without randomization, and cohort studies; controlled trials without randomization; and/or well-designed observational studies
Low quality	Insufficient sample size for the study design Discrepancies among reported data Errors in study design or analysis Missing significant information Unclear or inconsistent results High risk of bias due to multiple flaws so that conclusions cannot be drawn High uncertainty about validity of conclusions

RCT = randomized controlled trial.

Practice Committee of the American Society for Reproductive Medicine. Septate uterus. Fertil Steril 2024.

diagnostic agreement of hysteroscopic videos found poor agreement among viewers (10) and only moderate improvement when standardized diagnostic criteria were employed (11).

A study of 117 female participants found that the use of 3D TVUS combined with saline infusion had 100% accuracy when compared with laparoscopy/hysteroscopy (12). In addition, 3D TVUS without saline infusion has been found to be over 88% accurate for diagnosing uterine septa in 3 studies compared with hysteroscopy/laparoscopy (12–14). Studies assessing concordance between 3D TVUS and hysteroscopy alone have shown high levels of agreement between the 2 when 3D TVUS was used first, and hysteroscopy was used as diagnostic confirmation (15, 16).

Magnetic resonance imaging is often used for the diagnosis of Müllerian anomalies. Studies have shown a high level of agreement between MRI and other radiologic techniques (4, 17); however, 1 study suggests that although MRI is an accurate method to diagnose Müllerian abnormalities overall, it is only 70% accurate for the diagnosis of uterine septum (18). A study divided 63 participants with suspected uterine anomalies into 3 groups of different imaging techniques. Accuracy of Group 1 (2D TVUS and MRI), Group 2 (2D and 3D TVUS and MRI), and Group 3 (only 3D TVUS) were compared. Three-dimensional transvaginal ultrasound diagnoses, as judged by intraoperative findings, were correct in 100% of cases, whereas the MRI diagnoses in the same group were correct in only 7 of 13 cases, and laparoscopies were needed less often once 3D TVUS was introduced (19).

It must be emphasized that studies to determine how to diagnose a septum best are limited by small sample sizes and are from select centers. Therefore, it is likely that the interpretation of radiologic studies depends on the interpreter's experience. When the diagnosis of a uterine septum is not clear, it may be helpful to seek consultation with a clinician with experience in diagnosing and managing Müllerian anomalies.

Summary

- Three-dimensional ultrasound with or without saline infusion has been shown to be an accurate nonsurgical method for diagnosing a uterine septum.
- Other methods including 2D US, MRI and hysteroscopy may be useful but are less accurate.

Recommendation

- It is recommended to use 3D TVUS with or without saline infusion as the first-line noninvasive diagnostic tool in uterine shape assessment (Strength of Evidence: B; Strength of Recommendation: Moderate).

DOES A SEPTUM IMPACT FERTILITY?

The true prevalence of infertility among patients with a septate uterus is difficult to determine because many of these anomalies remain undiagnosed, given that they often do not cause any specific symptoms. Because diagnosis requires evaluation of the uterine cavity and fundal contour, most

TABLE 3

Rating for strength of evidence.

Strength of evidence	Definition
Grade A	High confidence in evidence. A larger or further study very unlikely to change the reported effect. Most of the evidence is supported by well-constructed RCTs or extremely strong and consistent observational studies with generalizable results, sufficient sample sizes for the study design, adequate controls, definitive conclusions, and minimal risk of bias.
Grade B	Moderate confidence in evidence. Larger or further studies are not likely to change the reported effect but may more precisely identify the magnitude of the effect. Most of the evidence comprised RCTs with potential weaknesses including small sample size or generalizability or moderately strong and consistent observational studies with reasonably consistent results, sufficient sample sizes for the study designs, identified appropriate controls, fairly definitive conclusions, and low risk of bias.
Grade C	Low confidence in evidence. Evidence lacking to support the reported effect. Evidence comprised observational studies with significant methodological flaws and/or inconsistent findings on the basis of poor evidence, inconsistent results, insufficient sample size for study design, conclusions that cannot be drawn, and/or high risk of bias.

RCT = randomized controlled trial.

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patients with this anomaly are only diagnosed when they present with conditions that require evaluation of the uterine cavity, such as a history of infertility or adverse pregnancy outcome. As a result, many studies use these patient cohorts and are only able to evaluate reproductive outcomes among patients who have already been diagnosed with infertility. Thus, our current understanding of whether a septate uterus is associated with infertility comes from studies that are limited by selection bias.

One retrospective study of intermediate-quality evidence reported the incidence of septate uteri among patients with proven fertility compared with patients with infertility or recurrent pregnancy loss (20). A total of 3,181 patients who had a uterine cavity evaluation either at the time of sterilization surgery (n = 1,289) or during an evaluation for infertility or recurrent pregnancy loss (n = 1,892) were included. Among the sterilization group with proven fertility, the prevalence of a septate uterus was 1.6% (n = 20), which was not significantly different compared with the prevalence of 1.2% (n = 23) among patients with infertility or recurrent pregnancy loss (P= .43).

Summary

- There is insufficient evidence to conclude whether a septate uterus is associated with infertility.

Recommendation

- No recommendation can be made regarding the association between a septate uterus and infertility due to insufficient evidence (Strength of Evidence: C; Strength of recommendation: No recommendation).

DOES A SEPTUM CONTRIBUTE TO PREGNANCY LOSS OR ADVERSE PREGNANCY OUTCOME?

There are multiple observational studies examining the relationship between uterine septum and pregnancy loss. One intermediate-quality study prospectively screened patients who presented for uterine ultrasound assessment for gynecologic symptoms but with no history of infertility or recurrent miscarriage and recorded their reproductive history and the presence of uterine anomalies (21). Among 29 patients with a septate uterus, 42% of their reported pregnancies resulted in a first-trimester spontaneous abortion, which was significantly increased compared with 12% of patients without a uterine anomaly (P< .001). The incidence of second-trimester losses was similar between the groups (3.6% [n = 2] vs. 3.5% [n = 69]).

A similar association between septate uteri and first-trimester spontaneous abortion was observed in 2 intermediate-quality meta-analyses that evaluated the reproductive outcomes among patients with a septate uterus compared with those without a septate uterus (22, 23). The more recent meta-analysis included 6 studies and reported that patients with a septate uterus had a first-trimester spontaneous abortion relative risk (RR) of 2.65 (95% confidence interval [CI]: 1.39–5.06) compared with controls. In addition, a significant association was observed for second-trimester spontaneous abortion with a RR of 2.95 (95% CI: 1.51–5.77) compared with controls.

An association between septate uteri and adverse obstetric outcomes beyond the second trimester has also been reported by multiple studies (20, 24–26). In addition, 3 intermediate-quality meta-analyses have assessed these outcomes (22, 23, 27). The most recent meta-analysis reported that compared with controls, pregnant patients with a septate uterus have increased odds of preterm birth (odds ratio [OR] 4.06, 95% CI: 2.89–5.70), malpresentation (OR 13.76, 95% CI: 5.52–34.32), cesarean delivery (OR 5.19, 95% CI: 1.84–14.62), fetal growth restriction (OR 2.99, 95% CI: 1.19–7.51), and placental abruption (OR 10.70, 95% CI: 4.01–28.53) (24).

Summary

- There is good evidence that a septate uterus is associated with spontaneous abortion.

- There is good evidence that a septate uterus is associated with preterm birth, malpresentation, and cesarean delivery.
- There is fair evidence that a septate uterus is associated with placental abruption and fetal growth restriction.

Recommendation

- It is recommended to counsel patients that the presence of a septate uterus is associated with spontaneous abortion and obstetric complications (Strength of Evidence: B; Strength of Recommendation: Moderate).

DOES TREATING A SEPTUM IMPROVE FERTILITY IN INFERTILE PATIENTS?

Despite the absence of evidence linking the presence of a uterine septum with infertility, numerous studies have addressed the question of whether uterine septum incision has a beneficial effect on subsequent fertility and pregnancy outcomes. Until recently, all studies on this topic were observational (25). Most observational studies were case series, which reported on pregnancy rates among infertile patients after septoplasty (26–33). Such studies often contain methodological flaws and are prone to selection bias and regression to the mean.

In 1 such study, 33 of 72 participants (45.83%) with a septate uterus and otherwise unexplained primary infertility were conceived within 1 year of surgery (26). In another, 88 patients with primary unexplained infertility for over 2 years and a uterine septum were prospectively observed after hysteroscopic septoplasty (28), 41% of the patients conceived with a median time to conception of 7.5 ± 2.6 months.

There are a few cohort studies. In 1 prospective study, 44 participants with a septate uterus and no other causes of infertility were compared with 132 patients with unexplained infertility (34). The septum group was initially treated with hysteroscopic septum incision, and both groups were followed expectantly for 1 year. At 12 months, the pregnancy rate for the septum group was 38.6% compared with 20.4% in the unexplained infertility-only group, with live birth rates of 34.1% and 18.9%, respectively ($P<.05$). In another study involving 127 patients diagnosed with unexplained infertility and a uterine septum, 102 patients who chose to undergo hysteroscopic metroplasty were compared with 25 who chose not to undergo the operation (35). Pregnancy (43.1% vs. 20%) and live birth rates (35.3% vs. 8%) were significantly higher in the group choosing to undergo surgery ($P>.05$), despite no significant differences in age, body mass index, duration of infertility or septum classification.

Several studies attempted to answer the question of whether hysteroscopic septoplasty is indicated before in vitro fertilization (36–38). One such study evaluated embryo transfer outcomes in patients with an untreated uterine septum (n = 289), patients treated with hysteroscopic septum incision (n = 538), and matched controls without a history of a uterine anomaly (n = 1,654) (38). Pregnancy (12.4% vs. 29.2%) and live birth rates (2.7% vs. 21.7%) were significantly lower in patients with an untreated uterine septum compared with matched controls ($P<.05$). Pregnancy and live birth rates in patients who had undergone septoplasty were not significantly different compared with controls (22.9% vs. 26.0% and 15.6% vs. 20.9%, respectively; not significant). In a multivariate logistic regression analysis, septum incision before embryo transfer was an independent predictor of pregnancy (OR 2.507, 95% CI: 1.539–4.111, $P<.001$).

In the first RCT to assess reproductive outcomes related to a septate uterus, 80 participants with a septate uterus and a history of either infertility, pregnancy loss, or preterm birth were randomized to septum incision (n = 40) or expectant management (n = 40) and observed for the primary outcome of conception leading to live birth within 12 months after randomization (5). Live birth occurred in 12 of 39 participants in the septoplasty group (31%) and in 14 of 40 participants allocated to expectant management (35%) (RR 0.88, 95% CI: 0.47–1.65). There was 1 uterine perforation in a patient allocated to septum incision (1/39 = 2.6%). The recruitment period for this multicenter international trial of high quality was long, and the sample size was limited.

In the face of conflicting evidence from numerous lower quality studies demonstrating a benefit of septum incision and 1 RCT of limited sample size demonstrating no benefit, patients with infertility and a uterine septum should be counseled about the limitations of the literature and the option of undergoing septum incision in a shared decision-making model.

Summary

- Low-quality data suggest that surgical correction of a uterine septum may improve fertility in patients with unexplained infertility. One prospective RCT with a limited sample size did not demonstrate improvement in live birth rate.

Recommendation

- Although septum incision in patients with infertility and/or undergoing fertility treatment is reasonable, a firm recommendation for this practice cannot be made on the basis of the current evidence.
- It is recommended to counsel patients with infertility and/or undergoing fertility treatment that resection of the septum may or may not be associated with an increase in live births. Given limitations in the literature and low risk of the procedure, septum incision may be offered to patients in a shared decision-making model (Strength of Evidence: B; Strength of Recommendation: Moderate).

DOES TREATING A SEPTUM IMPROVE OBSTETRICAL OUTCOMES?

Numerous retrospective studies and 1 prospective randomized trial sought to evaluate pregnancy outcomes after septum incision. Significant heterogeneity exists between and within

the retrospective studies, with variable indications for surgery.

Many published studies follow a simple "before–after" design with reported pregnancy outcomes before and after the procedure and patients serving as their own controls. These low-quality studies have demonstrated an improvement in the assessed outcomes, including pregnancy loss and a variety of obstetric outcomes such as preterm delivery, fetal malpresentation, and cesarean section (30–32, 39–44).

The available retrospective studies with a comparison group have varied in the exact study question and design. Although some compared patients undergoing surgical correction of a septum with those without a history of uterine anomaly (38, 45, 46), others aimed to investigate differences in outcomes according to the type of uterine anomaly (arcuate, subseptate, and septate) and/or septum size (33, 47–49). In 1 study in the ART setting including 420 participants with an arcuate uterus (Group A) and 406 participants with a septate or subseptate uterus (Group B), the preterm birth rates before and after septum incision decreased similarly in both groups: 33.9% before and 7.2% after in Group A vs. 36.5% before and 8.0% after in Group B 50). One study including 73 patients with infertility undergoing hysteroscopic metroplasty found that compared with participants with an incomplete septum, those with a complete septum had a lower rate of miscarriage, but also a lower mean gestational age at delivery and infant birth weight after surgical correction (50). An international retrospective cohort study published in 2020 assessed 257 individuals with septate uterus in 21 centers in the Netherlands, the United States, and the United Kingdom. The participants were allocated to resection of septum vs. expectant management on the basis of reproductive history and severity of disease at the discretion of the treating physician. In total, 151 participants underwent septum resection, and 106 had expectant management; no significant difference in a live birth (53% vs. 71%, respectively, hazard ratio 0.71, 95% CI: 0.49–1.02), pregnancy loss (46.8% vs. 34.4%, respectively, OR 1.58, 95% CI: 0.81–3.09) or preterm birth (29.2% vs. 16.7%, respectively, OR 1.26, 95% CI: 0.52–3.04) was demonstrated. There was a significant decrease in malpresentation in patients who underwent septum resection compared with expectant management (19.1% vs. 34.6%, respectively, OR 0.56 95% CI: 0.24–1.33.) It should be noted that classification of septum changed over the study period ranging from 2000 to 2018 and patients with arcuate uterus included in the expectant management group which may have contributed to selection bias and contributed to improved outcomes reported in the expectant management group (51).

A variety of meta-analyses on this topic aimed to pool retrospective studies comparing patients undergoing surgical septum correction with a control group of patients with a uterine septum who were managed expectantly (2, 22, 50, 52). The most recent of these (50) also included the only prospective randomized trial on the topic (5), which demonstrated no difference in live birth in participants randomized to septum incision (n = 40) and those allocated to expectant management (n = 40) in a population with a septate uterus and a history of either infertility, pregnancy loss or preterm birth (live birth rates 31% vs. 35%; RR 0.88; 95% CI: 0.47–1.65). The study (5) was terminated early due to poor recruitment and was therefore underpowered to detect the prespecified endpoints.

In addition to the RCT, 10 observational studies met the inclusion criterion of comparing patients undergoing hysteroscopic septum incision to expectant management (50). For the 1,589 participants included in the meta-analysis, a statistically significant reduction in the rate of miscarriage in those undergoing septum correction was noted overall (pooled OR 0.45; 95% CI: 0.22–0.90); as well as in the subgroup analyses of those with a complete septum (pooled OR 0.16; 95% CI: 0.03–0.78) and those with a partial septum (pooled OR 0.36; 95% CI: 0.19–0.71). In addition, the risk of fetal malpresentation was significantly reduced (OR = 0.32, 95% CI: 0.16–0.65). For the subgroup of participants who underwent surgical correction of a partial septum, a significant decrease in the frequency of preterm birth was found compared with patients managed expectantly (OR = 0.30, 95% CI: 0.11–0.79). Overall, no significant differences were found between the 2 groups in the likelihood of clinical pregnancy, term live birth, or risk of cesarean delivery (50).

Summary

- Surgical correction of a uterine septum in patients with a history of poor reproductive outcomes appears to be associated with a lower rate of miscarriage.
- On the basis of limited observational data, surgical correction of a uterine septum appears to improve obstetric outcomes, including abnormal fetal presentation, preterm delivery, and the rate of cesarean section. However, no effect on the live birth rate has been demonstrated.

Recommendation

- It is recommended to offer hysteroscopic septum incision to patients with a septum and a history of recurrent miscarriage in a shared decision-making model (Strength of Evidence: B; Strength of Recommendation: Moderate).
- It is recommended to counsel patients that septum incision may decrease the risk of adverse obstetric outcomes such as malpresentation and cesarean section but there are no high-quality data to recommend this practice (Strength of Evidence: B; Strength of Recommendation: Moderate).

ARE SEPTUM CHARACTERISTICS ASSOCIATED WITH REPRODUCTIVE OUTCOMES?

Uterine septa comprise myometrium similar to the normal myometrium in the remainder of the uterus (53, 54), and the presence of a muscular septum is associated with an increased risk of recurrent miscarriage and poor pregnancy outcomes. Although the exact mechanism of these poor reproductive outcomes is unknown, it is logical to expect the larger complete septa to produce more adverse events than the smaller

partial septa. No prospective trials specifically address this question. All available data are in the form of retrospective case-controlled trials that examined the reproductive outcomes after metroplasty for complete and partial septa. Tomaževič et al. (38) retrospectively reviewed over 2,400 embryos transferred in patients with complete septa, partial septa, and arcuate uteri compared with normal controls and found a lower implantation rate and live birth rate in all 3 groups compared with controls. These differences from controls were eliminated in all 3 categories after metroplasty (52). Several smaller retrospective studies concluded an equal reduction in miscarriage rate after metroplasty of small and large septa (29, 33, 37). We conclusion is that there was no difference in outcomes after resection of a small vs. large septa.

Summary

- All available data are in the form of retrospective case-controlled trials. Most studies evaluated the early pregnancy loss incidence in patients before and after surgical correction.
- Patients with recurrent pregnancy loss demonstrated similar benefits after resection of small and large septa.

Recommendation

- It is not recommended to use the size or shape of a septum to determine the impact on adverse reproductive outcomes (Strength of Evidence: B/C; Strength of recommendation: Moderate/Weak).

SHOULD PREOPERATIVE MANAGEMENT TO THIN THE ENDOMETRIUM BE USED?

There are no high-quality data examining the benefits or risks of preoperative adjuvants such as oral contraceptive pills or gonadotropin releasing hormone agonists that may enhance intrauterine visualization but also disrupt the normal hormonal milieu, which can affect postsurgical healing. It is important to have adequate visualization to see both tubal ostia when transecting a septum. This can be achieved by operating in the early follicular phase or after progesterone withdrawal in patients with irregular ovulation or by placing patients on oral contraceptives to regulate the menstrual cycle and schedule the operative procedure.

Summary

- There are no high-quality studies designed to evaluate whether or not there is a benefit for preoperative hormonal suppression before incising a uterine septum.

Recommendation

- It is recommended, on the basis of expert committee opinion, to consider performing the procedure during the follicular phase or after progesterone withdrawal to help with visualization during surgery. However, there are no studies designed to prove or disprove this (Strength of Evidence: C; Strength of recommendation: Weak).

ARE THERE ANY RISKS OF CERVICAL INSUFFICENCY BY RESECTING THE CERVICAL PORTION OF THE SEPTUM?

A complete uterine septum extends from the fundus to the level of the external cervical os. Historically, it has been controversial as to whether the surgeon should incise the cervical portion of the septum or start the incision at the level of the internal cervical os and leave the cervical portion intact. Concerns for cervical septum removal include intraoperative bleeding and future cervical incompetence, with the potential benefit of more efficient, less complicated surgery. Three studies have evaluated these questions. One clinical trial randomized 28 participants with a complete uterine septum to septoplasty, including the unicollis cervical septum compared with septoplasty with cervical preservation. There were no differences in reproductive outcomes such as early and late abortion and preterm delivery between groups with significantly faster operative times when the unicollis cervical septum was removed. In addition, there were 2 cases of pulmonary edema and 3 cases of significant bleeding (>150 mL) in the cervical preservation group (55).

Two other small prospective studies of patients who underwent complete septum incision, including cervical septoplasty, found no significant bleeding and no evidence of cervical incompetence (56) and shorter operative times when compared with historical controls (56).

Summary

- Incision of a unicollis cervical septum leads to faster operative times and less fluid deficits.
- One RCT showed an improved safety and efficiency profile with resection of the unicollis cervical septum.
- No adverse reproductive outcomes were reported in these 3 studies.
- No cases of cervical insufficiency were reported in these studies.

Recommendation

- It is recommended to counsel patients that, on the basis of limited data, there is no evidence that resection of the unicollis cervical septum increases the risk of cervical insufficiency (Strength of Evidence: C; Strength of recommendation: Weak).

IS THERE A BENEFIT TO COMPLETE EXCISION OF RESIDUAL (< 10 MM) SEPTUM?

When transecting a uterine septum, the surgeon must decide if the goal is to create a flat fundus between the 2 tubal ostia, to transect only until what appears to be normal vasculature is identified, or to leave an "arcuate" shape that is not felt to be associated with poor reproductive outcomes. Although the data available are from 1 retrospective study with 72 patients, the results suggest that there is no difference in reproductive outcomes when a small residual septum is left in place vs. complete removal of the septum (57).

Reproductive outcome in 17 patients with a residual septum of between 0.5 and 1 cm after hysteroscopic metroplasty was compared with that in 51 patients with no residual septum or one of <0.5 cm. Septal surgery was performed with scissors or a resectoscope. The cumulative 18-month probability of becoming pregnant was 44.5% in the patients with a residual septum, and 52.7% in those with no residual septum (not significantly different), and the cumulative 18-month probability of giving birth to a child was 27.5% and 36%, respectively (not significant).

It is recommended that the uterine septum should be transected with the goal of restoring normal anatomy. However, leaving an arcuate shape due to observed normal muscular vasculature does not appear to reduce the benefit of the metroplasty.

Summary

- There is only 1 study on the impact of a residual septum. Limited data suggest that there is no difference in reproductive outcomes when a small residual septum (<1 cm) is left in place vs. complete removal of the septum.

Recommendation

- It is not recommended to perform another surgery for a residual septum under 1 cm (Strength of Evidence: C; Strength of recommendation: Weak).

IS ADHESION PREVENTION NEEDED?

Uterine septa arise from the incomplete resorption of uterine muscular tissue during the unification of the uterine horns in utero. Proper surgical correction of the congenital malformation involves incising the midline of the septa. Septal tissue should not be resected or removed. After the septum incision, there is natural tension to retract the tissue toward the anterior and posterior uterine walls. In theory, a septum incision with mechanical energy (cold scissors) should minimize the risk of damage to normal endometrial tissue compared with thermal energy with electrosurgery. However, there is no high-quality data to support one modality over another. There is a concern that the septum incision will lead to intrauterine scar tissue or septa reformation. The question is, what is the incidence of intrauterine adhesions after metroplasty, and if the use of adjuvants such as high doses of estrogen, intrauterine balloons, or intrauterine devices (IUDs) will reduce the risk of postmetroplasty adhesion formation? Prospective RCTs have shown no benefit to postoperative treatment with either an intrauterine balloon (58) or oral estrogen (59), whereas retrospective studies have shown no benefit of estrogen therapies or the placement of IUDs after septum incision (60–62). The use of auto-crosslinked polysaccharide gel has been shown in 1 study to reduce postseptum incision adhesion formation (63). This gel is currently unavailable in the US and warrants further investigation.

Summary

- Several studies were designed to evaluate the effectiveness of postprocedural therapy to reduce adhesion formation. The studies evaluated oral estrogen, intrauterine balloons and IUDs and 1 study evaluated a dissolvable gel that is not available in the US.
- There are no high-quality data to demonstrate the benefit of postoperative estrogen therapy, IUDs, or intrauterine balloon to prevent intrauterine adhesions postmetroplasty. The data on the value of intrauterine gels are too limited to draw conclusions.

Recommendation

- There is insufficient evidence to recommend routine administration of oral estrogen, intrauterine balloons and IUDs to decrease adhesion formation after septoplasty (Strength of Evidence: C; Strength of recommendation: Weak).

IS THERE AN INCREASED RISK OF UTERINE RUPTURE IN A PREGNANCY AFTER A HYSTEROSCOPIC RESECTION OF A SEPTUM?

There have been few case reports in the literature of uterine rupture during pregnancy or delivery after septum incision. According to a meta-analysis of reported ruptures, the risk of subsequent pregnancy-related uterine rupture is correlated with excessive septal excision, penetration of the myometrium, uterine wall perforation, and excessive use of cautery or laser energy during the initial septum incision procedure (2). A Belgium nationwide population-based cohort study of uterine rupture found only 2 of 90 ruptures occurred in patients who had undergone previous septoplasty (in comparison with 73 with a prior c-section), with an overall very low rupture rate in the population (64). Although uterine rupture is rarely reported in the available literature on septoplasty outcomes, in 1 study where it was a reported outcome, there were no reports of uterine rupture in the 75 patients who underwent septoplasty (65).

Summary

- There is a paucity of data limited to case reports and rare outcomes in population studies of uterine rupture after septoplasty.

Recommendation

- It appears the rate of uterine rupture after septoplasty is rare, however, this outcome is not often reported on in the current literature (Strength of Evidence: B/C; Strength of recommendation: Insufficient data to make a recommendation).

HOW LONG AFTER SURGICAL TREATMENT OF A UTERINE SEPTUM SHOULD A PATIENT WAIT TO CONCEIVE?

The time from septum incision to attempting pregnancy has not been evaluated in randomized controlled studies. However, A few studies address uterine healing after surgical treatment of a septum. One study assessed the postoperative appearance of the endometrium and correlated this with endometrial biopsy specimens in 19 participants who were randomized to follow-up hysteroscopy at 1, 2, 4, or 8 weeks after hysteroscopic septum incision (64). At 2 weeks postoperatively, the incised zone of the septum was depressed on both uterine walls and had wide areas lacking endometrial covering. By 8 weeks postoperatively, the uterine cavity was morphologically normal, and the covering endometrium was regular. Another prospective study evaluated 16 patients with office hysteroscopy every 2 weeks after hysteroscopic septum incision until wound healing was complete (65). After septum incision, 19% of patients at 1 month and 100% of patients by 2 months postoperatively demonstrated a healed uterine cavity.

A single retrospective cohort study evaluated pregnancy rates in 282 patients after in vitro fertilization/intracytoplasmic sperm injection when the embryo transfer was performed within 9 weeks, between 10 and 16 weeks, and 17+ weeks after uterine septum incision. Pregnancy rates and miscarriage rates were no different among the 3 groups (66).

Summary

- There are only a few low- to intermediate-quality studies addressing this question, but it appears that embryo transfer 1–2 months after septoplasty has similar reproductive outcomes to waiting >2 months.
- There are no data to indicate that patients should wait longer than 1–2 months to try to conceive after hysteroscopic septoplasty.

Recommendation

- It is recommended to counsel patients that they may proceed with fertility treatment in 1–2 months after septoplasty (Strength of Evidence: C; Strength of recommendation: Weak).

HOW SHOULD AN INCIDENTALLY DISCOVERED SEPTUM BE MANAGED IN A PATIENT WHO IS NOT (YET) TRYING TO CONCEIVE?

With improvements in noninvasive pelvic imaging and lower thresholds for obtaining imaging for complaints such as abdominal pain, there is a growing subset of patients with incidentally diagnosed uterine septum. There have been no randomized studies to date to evaluate if uterine septoplasty improves reproductive outcomes for patients before attempting conception.

Summary

- There are no data addressing this question.

Recommendation

- There is insufficient evidence to recommend hysteroscopic septoplasty in patients who have not yet attempted conception (Strength of Evidence: Insufficient Strength of recommendation: Insufficient evidence to make recommendation).

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Review process

This report was developed under the direction of the Practice Committee of the American Society for Reproductive Medicine as a service to its members and other practicing clinicians. Although this document reflects appropriate management of a problem encountered in the practice of reproductive medicine, it is not intended to be the only approved standard of practice or to dictate an exclusive course of treatment. Other plans of management may be appropriate, taking into account the needs of the individual patient, available resources, and institutional or clinical practice limitations. The Practice Committee and the Board of Directors of the American Society for Reproductive Medicine have approved this report.

This document was reviewed by ASRM members and their input was considered in the preparation of the final document. The following members of the ASRM Practice Committee participated in the development of this document: Alan Penzias, M.D.; Paula Amato, M.D.; Jacob Anderson; Kristin Bendikson, M.D.; Clarisa Gracia, M.D., M.S.C.E.; Tommaso Falcone, M.D.; Rebecca Flyckt, M.D.; Karl Hansen, M.D., Ph.D.; Micah Hill, D.O.; Sangita Jindal, Ph.D.; Suleena Kalra, M.D., M.S.C.E.; Tarun Jain, M.D.; Bruce Pier, M.D.; Michael Thomas, M.D.; Richard Reindollar, M.D.; Jared Robins, M.D.; Chevis N. Shannon, Dr.Ph., M.B.A., M.P.H.; Anne Steiner, M.D., M.P.H.: Cigdem Tanrikut, M.D.; and Belinda Yauger, M.D. The Practice Committee acknowledges the special contribution of Tommaso Falcone, M.D.; Jessica

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Patient/public perspective

To incorporate the perspectives of those who might be affected most by the recommendations in this guideline, a group of patient volunteers and lay stakeholders in reproductive medicine who were not involved in the scoping or development of this guideline reviewed the document. Their feedback was considered in the preparation of the final document.

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Updating policy

This document will be reviewed for currency within 5 years of publication.

Panel

This evidence-based guideline with recommendations for clinicians was developed by a multidisciplinary group, comprising the ASRM Practice Committee and a task force of medical experts, which included specialists in obstetrics and gynecology, reproductive endocrinology and infertility, fertility preservation, reproductive surgery, endometriosis, uterine anomalies, fibroids, assisted reproductive technology, in vitro fertilization, and epidemiology/biostatistics. Members of the task force for this clinical practice guideline consisted of medical professionals at various levels of training, including fellows and senior experts, as well as experts with $<$10 years of posttraining, CREST (Clinical Reproductive Scientist Training) Program scholars, a clinical epidemiologist who is also a reproductive medicine subspecialist, and a methodologic specialist. In addition, a select group of patients participated in document scoping and review.

Disclaimer

This report was developed under the direction of the Practice Committee of the American Society for Reproductive Medicine (ASRM) as a service to its members and other practicing clinicians. Although this document reflects appropriate management of a problem encountered in the practice of reproductive medicine, it is not intended to be the only approved standard of practice or to dictate an exclusive course of treatment. Other plans of management may be appropriate, taking into account the needs of the individual patient, available resources, and institutional or clinical practice limitations. The Practice Committee and the Board of Directors of the American Society for Reproductive Medicine have approved this report.

Declaration of Interests

Per ASRM policy, all members of ASRM task forces and the Practice Committee disclosed commercial and financial relationships with manufacturers or distributors of goods or services used to treat patients for the preceding 12 months. Committee members were reminded to update potential disclosures annually and if new potential conflicts arose during their appointments. Before live discussions or meetings, Committee members were reminded verbally and in writing to disclose any new or previously undisclosed relationships. Disclosures were reviewed for conflicts by the ASRM Chief Medical Officer and the Chair of the Practice Committee. Task force members for whom conflicts were identified were excused from this project. Members of the Practice Committee who were found to have conflicts of interest on the basis of the relationships disclosed did not participate in the discussion or development of the document.

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Diagnóstico y tratamiento basado en la evidencia para el septo uterino: una guía.

Objetivo: Proveer recomendaciones basadas en evidencia con respecto al diagnóstico y efectividad del tratamiento quirúrgico de un septo uterino.

Métodos: Está guía provee recomendaciones basadas en evidencia con respecto al diagnóstico y efectividad del tratamiento quirúrgico de un septo uterino. Esta reemplaza la última versión del mismo nombre. (Fertil Steril. 2016 Sep 1;106(3):530-40).

Medida principal de resultados: Los resultados de interés incluyeron el impacto del septo en la fertilidad subyacente, nacido vivo, embarazo clínico y resultados obstétricos.

Resultado(s): La búsqueda literaria identificó estudios relevantes para informar la evidencia de esta guía.

Conclusión (es): El tratamiento del septo uterino y los resultados asociados con la infertilidad, la pérdida recurrente de embarazo y los resultados obstétricos adversos son conocidos. La resección del septo ha demostrado mejorar los resultados en pacientes con pérdida de embarazo recurrente y disminuir la probabilidad de malas situaciones clínicas. En el escenario de la infertilidad, se recomiendo utilizar un modelo de toma de decisión compartida después del asesoramiento apropiado para determinar si proceder o no con la resección del septo.

Should we consider antimüllerian hormone a valid marker of semen quality?

Serum hormone evaluation is an integral part of the trinity of the workup for male factor fertility, the other two being a history and physical as well as semen analysis. With established reference points, these serum-level tests offer screening and indications of possible barriers to fertility. Moreover, it is crucial to have fully characterized hormonal reference ranges in the appropriate context. For instance, the distribution and positive predictive value of follicle-stimulating hormone levels in men with azoospermia have been shown to differ drastically from the previously proposed reference ranges often accompanying laboratory results (1). Despite falling within many laboratory "normal" reference ranges, follicle-stimulating hormone threshold levels >12.1 mIU/mL represent the upper 95th percentile of values.

Antimüllerian hormone (AMH) is not a routinely investigated serum hormone in a clinical setting. In their manuscript, Holt et al. (2) set forth a prospective evaluation of the association between AMH levels and semen quality in men in the recruitment for the "First In Treating Male Infertility," a double-blinded, placebo-controlled, single-center randomized clinical trial aimed to determine whether treatment with denosumab can improve semen quality in infertile men. The investigators report that a low serum AMH level is a marker of reduced sperm production and motility in infertile men, suggesting clinical relevance in the evaluation of male infertility.

Rarely do we see double-blinded, placebo-controlled studies in the sphere of male fertility. The larger collaborative study described invokes not just screening but possible intervention. Previous hormonal analyses, such as with 17-hydroxyprogesterone, have suggested nonconventional hormonal investigations as possible targets for stimulation therapy (3). Herein, the AMH level is currently discussed in a similar context.

Currently, no serum-level test remains a standalone test of fertility status. Overall, this study proposes an interesting premise with future applications. We are excited but will await the results of the larger collaborative study.

CRediT Authorship Contribution Statement

Kevin J. Campbell: Writing – original draft, Writing – review & editing.

Declaration of Interests

K.J.C. has nothing to disclose.

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The limitations of oocyte donor data for studying reproductive health inequities

In the present issue of *Fertility and Sterility*, Suresh et al. (1) estimated the extent to which markers of ovarian reserve (antral follicle count and antimüllerian hormone levels) and outcomes of controlled ovarian stimulation are affected by neighborhood disadvantage, a novel risk factor in the context of reproductive aging. They analyzed data from 547 young, healthy oocyte donors who underwent 905 oocyte retrieval cycles during 2008–2020 at a private fertility clinic in Georgia, United States. They found a weak association between greater neighborhood disadvantage (operationalized using the neighborhood deprivation index) and poorer outcomes of controlled ovarian stimulation, but little association with markers of ovarian reserve.

Given recent trends in delayed childbearing, it is important to understand the determinants of reproductive aging. Most epidemiologic research on ovarian reserve to date has focused on individual-level risk factors; however, the presence of racial and socioeconomic disparities in ovarian aging (2) demonstrates the importance of social and structural factors, including neighborhood context. The theory of "weathering," originally developed in the context of racial inequities in reproductive health, describes how the disproportionate accumulation of physical and social stressors because of racism and inequality may accelerate aging for minoritized populations (3). Neighborhoods shape exposure to environmental stressors and access to health-promoting resources, with implications for reproductive health. Previous studies have reported associations of neighborhood disadvantage with reduced fecundability and lower rates of clinical pregnancy and live births, yet mechanistic evidence is lacking. Suresh et al.'s (1) assessment of markers of ovarian function advances this line of inquiry.

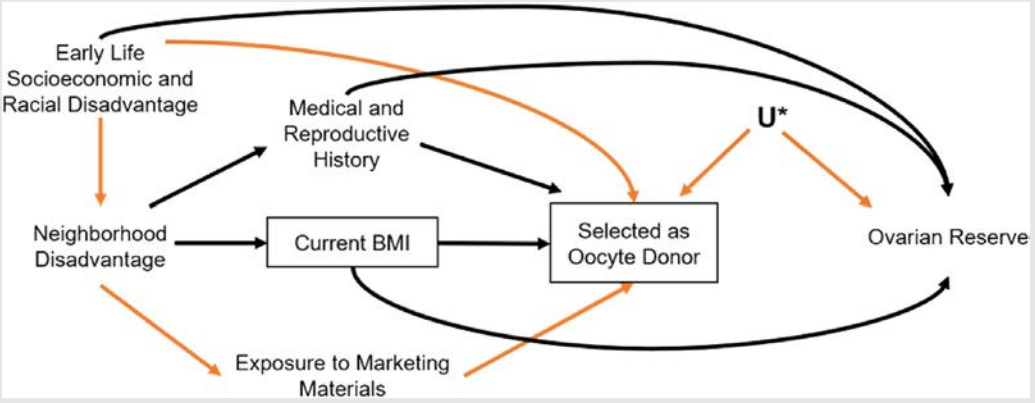
Previous epidemiologic research on ovarian function has been conducted primarily among patients undergoing fertility treatment, which limits generalizability to subfertile couples with access to fertility treatment. Suresh et al. (1) address this limitation by analyzing a study population of oocyte donors. However, the restriction to oocyte donors raises concerns not just about generalizability (i.e., external validity) but also about selection bias (i.e., internal validity). Selection bias would be present in this study if inclusion in the study population was influenced by both neighborhood deprivation and markers of ovarian function. Inclusion in the study population was clearly influenced by markers of ovarian function because potential donors were required to have a high ovarian reserve (suggested minimum antral follicle count of 20; suggested minimum antimüllerian hormone levels of 2 ng/mL). In terms of exposure, the investigators state that "our exposure, [neighborhood deprivation index], was not directly related to donor selection" (1); however, they note that the most-deprived Georgia Census tracts were underrepresented in the study population, indicating a link between exposure and study participation. This link need not be direct to create bias. Although the investigators mention "more broad and complex factors[,] including the populations targeted by oocyte donor marketing campaigns" (1), further interrogation of this topic is warranted to understand the potential for selection bias in this study.

A reproductive justice lens can help inform the assessment of potential selection bias. Throughout its history, the fertility industry has been deeply entwined with the eugenics movement (4). Potential oocyte donors generally undergo an extensive screening process involving a medical history questionnaire, a personal questionnaire, genetic testing, and psychological screening. Clinics and agencies often advertise their "selectivity" and "low donor-acceptance rates." Each stage of the screening process presents opportunities for eugenic impulses to inform decision-making about whose oocytes are most "fit," using criteria that often favor class-privileged, able-bodied, cisgender white women (4). Notwithstanding these privileges, oocyte donors may be vulnerable to ethical abuses because of the financial or other pressures that lead to donation. Because there is currently no regulatory mechanism to ensure the ethical selection and treatment of oocyte donors (rather, there are only ethical guidelines from the Society for Assisted Reproductive Technologies and the American Society for Reproductive Medicine), the potential for abuse is high. The lack of protections for oocyte donors is particularly salient for women of color, from whom the medical establishment has earned significant distrust because of the history of medical experimentation, forced sterilization, and other overt mechanisms of reproductive control in the US (4, 5). These considerations provide a critical context for research on social determinants of health in an oocyte donor population.

We can illustrate how socioeconomic targeting of oocyte donors on the geographic and individual levels could result in selection bias using a directed acyclic graph (DAG)-based approach, expanding on the DAG that Suresh et al. (1) present. Clinics and donor-matching agencies often target marketing materials to potential oocyte donors via university venues, creating a link between the areas where donors reside and their recruitment into oocyte donation. This is represented on the DAG as a bias path from neighborhood disadvantage to ovarian reserve because of conditioning on selection as an oocyte donor, resulting in a bias in the observed association (Fig. 1). Furthermore, as previously discussed, donor selection criteria often result in disproportionately rejecting potential donors from less privileged backgrounds. We represent this on the DAG by including "early life socioeconomic and racial disadvantage" as a common cause of current neighborhood disadvantage and selection as an oocyte donor (Fig. 1). Again, conditioning on selection as an oocyte donor opens a bias path from neighborhood disadvantage to ovarian reserve, resulting in a distortion of the effect estimate. The strength of the potential selection bias depends on how heavily selection was influenced by these factors and how they interact with one another to shape the selection process.

The potential for selection bias in the present study illustrates the importance of considering the social context in which data for reproductive health research are collected.

FIGURE 1



Directed acyclic graph (DAG) for the effect of neighborhood disadvantage on ovarian reserve. The DAG is modified from Supplemental Figure 1 (available online) in Suresh et al., with added nodes for "exposure to marketing materials" and "early life socioeconomic and racial disadvantage." We removed nodes for age and year for simplicity. Paths that represent potential selection bias are shown in *orange*.

Geller. Letter to the editor. Fertil Steril 2024.

Scholars of reproductive justice have pointed out the ways in which the fertility of white, high-income individuals is privileged, whereas that of low-income individuals of color is suppressed, including through the structuring of access to reproductive technologies (5). These biases inform not only who has the opportunity to become a parent using fertility treatment but also whose reproductive capacity is valued in the context of becoming an oocyte donor. Social determinants of oocyte donation create links between area- and individual-level socioeconomic status and representation in the data source, potentially leading to bias that renders results inaccurate. We highlight here that considerations of reproductive justice are not outside the purview of epidemiologists; rather, they are central to the internal validity of research, in addition to its external validity and policy implications.

We appreciate Suresh et al.'s (1) contribution to the literature and encourage continued inquiry into the influences of the neighborhood environment—physical, built, and social—on ovarian health across the life course. The observation of slightly worse ovarian stimulation outcomes among donors from the most compared with the least deprived quintiles of neighborhoods provides a signal for future research. The limitations of the present work highlight the challenges of conducting research on the social determinants of reproductive health amidst inequities in whose reproductive capacity is valued. Because of inequities in access to reproductive technologies, participants for whom invasively collected clinical data are available may disproportionately exclude those exposed to adverse social and environmental conditions. We encourage investigators to carefully consider the selection mechanisms inherent in clinical data sources and the trade-offs between analyzing highly selected clinical populations and community-based samples. The social context of reproductive health care access and equity can inform the potential for selection bias. A more complete understanding of the influence of the neighborhood environment on reproductive health will require insights from a range of study populations and approaches.

CRediT Authorship Contribution Statement

Ruth J. Geller: Conceptualization, Writing – original draft, Writing – review & editing. **Amelia K. Wesselink:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Interests

R.J.G. has nothing to disclose. A.K.W. has nothing to disclose.

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Rethinking the exclusion of obese women from infertility care

Obesity and its corollary such as metabolic syndrome, impair women's fecundity by interfering with normal ovulation, lowering the quality of oocytes, and disrupting uterine environment. Women with obesity therefore take a longer time to conceive spontaneously and have a higher miscarriage risk, even if they have a normal ovulation. Among those undergoing in vitro fertilization (IVF) treatment, women with obesity have a 15% lower relative risk for live birth (LB) compared with women of normal weight. When they become pregnant, they face an increased risk of a slew of pregnancy complications, including gestational diabetes, hypertensive disorders, preterm birth, and cesarean section. It is therefore logical to think that weight loss among overweight or obese women improves the chances of getting pregnant and mitigates pregnancy complications.

In this issue of *Fertility and Sterility*, Caldwell et al. (1) report a meta-analysis of randomized controlled trials that examined the impact of weight loss on pregnancy outcomes among obese and overweight women. They investigated the effectiveness of lifestyle interventions and oral medications on clinical pregnancy (CP), LBs, and miscarriage rates. Women seeking pregnancy, including those fertile and infertile, who undergo weight loss intervention have a higher chance of getting pregnant (42.1% vs. 36.8%, risk ratio [RR] 1.24, 95% confidence interval [CI], 1.07–1.44; 16 studies), achieving LB (34.8% vs. 32.0%, RR 1.19, 95% CI 0.97–1.45; 13 studies), but also a higher chance of experiencing miscarriage (16.0% vs. 12.4%, RR 1.17, 95% CI 0.79–1.74; 11 studies). Importantly, among women starting infertility treatment, those randomized to weight loss intervention have higher CP (39.1% vs. 34.3%; RR 1.28, 95% CI 1.03–1.60; 10 studies) and a higher risk of miscarriage (24.2% vs. 15.8%; RR 1.45, 95% CI 1.07–1.96; 8 studies), but differences in an LB were not statistically significant (30.1% vs. 28.7%; RR 1.27, 95% CI 0.92–1.75; 9 studies).

Thus, although the effect of lifestyle intervention on the chance of CP seems to be strong (absolute increase of 4.8%, numbers needed to treat 21), the higher miscarriage rate after lifestyle intervention reduces the magnitude of the effect on LB (absolute increase of 1.4%, numbers needed to treat 71). Of note, when calculating the relative risks for miscarriage, Caldwell et al. (1) use the number of pregnancies as the denominator. Use of the number of randomized women as the denominator (1,225 vs. 1,305) results in a miscarriage rate of 7.5% in the weight loss group and 4.7% in the control group (RR 1.51, 95% CI 1.10–2.08). The forest plot showing the risk of miscarriage using the number of randomized women as the denominator is provided in Supplemental Figure 1 (available online).

In summary, the review informs that although weight loss interventions, whether through diet, exercise, pharmacologic treatments, or a mix of thereof, enhance the chances of pregnancy, they are also associated with an increase in the risk of miscarriage, which limits the impact of LB rate.

Moreover, large and quick preconceptional weight loss might cause harm. Although patients losing weight through bariatric surgery shed 25%–35% of their body weight, this treatment is associated with an increased risk of small-for-gestational infants (odds ratio, 2.20; 95% CI, 1.64–2.95) and a shorter gestation (mean difference −4.5 days; 95% CI, −2.9–6.0) compared with women without surgery, as revealed by a large-scale prospective cohort study in Sweden (2). In addition, although the new class of weight loss drugs, glucagon-like peptide 1 agonist, capable of losing up to a quarter of body weight, has stirred a lot of excitement and many are using them off-prescription, it is worth noting that the safety data of taking these drugs among women seeking pregnancy is little to none.

Although Caldwell et al. (1) provide useful insight into the field, there remain several questions. Caldwell et al. (1) are not able to assess the impact of weight loss intervention relative to women's ovulatory status. Women with polycystic ovary syndrome are suggested to benefit most from weight loss (3). Second, because 4 out of 10 trials saw the control group undergo immediate infertility treatment although fertility treatment was delayed in the intervention, it remains unclear to which extent the differences are caused by the lifestyle intervention or by a difference in fertility treatments. For example, the higher LB rate in the control group of Mutsaerts et al. (4) 2016 might be because of immediate fertility (IVF) treatment with ovulation induction, intrauterine insemination, or IVF treatment, although such fertility treatments were delayed during the 26 weeks of weight loss program. Third, it is unclear which of the lifestyle interventions contribute to the treatment effect, ranging from diet, and physical activity to pharmacologic treatments, including orlistat, a lipase inhibitor licensed for weight loss, metformin, and liraglutide, a glucagon-like peptide-1 analogue for treating diabetes.

Although the answer to the above questions is to be awaited, the meta-analysis clearly shows the effectiveness of lifestyle interventions on the reproductive potential of infertile couples. Consequently, the implications of overweight and obesity on both mothers and infants should be thoroughly discussed with the patient. Clinicians should provide robust support and guidance to empower patients in their weight loss endeavors. By fostering a supportive approach, health care providers can play a pivotal role in optimizing the reproductive outcomes of individuals grappling with obesity.

Although losing weight should be encouraged and facilitated, some patients will not achieve weight loss. For example, in Mutsaerts et al. (4), one-fifth of the participants

in the lifestyle intervention discontinued the treatment, and only 40% of patients reached a weight loss of ≥5%.

We should be careful when denying fertility treatment to overweight or obese women when they are not able to reach the goal of weight loss. The first reason relates to maternal safety. Although obese women have an increased risk of pregnancy complications, these risks seem acceptable. For example, the risk of developing preeclampsia is nearly doubled in overweight women compared with normal-weight mothers. However, women with diabetes or previous preeclampsia face a doubled risk of hypertensive disorders but are not excluded from fertility treatment. The second argument often used is that children of obese women are at increased risk of, for example, macrosomia and shoulder dystocia, or an increased risk of long-term health issues. Although maybe true, these risks are, in absolute terms, not so high that pregnancy and thus fertility treatment should be denied. Third, obese women are excluded from publicly funded IVF treatment funding because their success chances are low; however, female age is the most important determinant of fertility treatment success. Denying a 30-year-old woman with a body mass index (BMI) of 35 fertility treatment for the reason of low success rates does not make sense when a 40-year-old woman with a normal BMI is allowed access to such treatment because her expected success rates are far lower.

Yet, the exclusion of overweight or obese women from accessing infertility treatment is widespread, despite the fact that nearly 40% of adults are currently overweight globally, a figure expected to rise to 50% by 2035. A survey of 347 IVF clinics in the United States—where 1 in 3 adults is overweight—revealed that one-third of the clinics use body weight, or BMI, to determine eligibility for IVF treatment. Similar stances on imposing BMI limits are also observed in expert opinions from medical societies and in the policies of countries that provide public funding for IVF treatment. To our knowledge, the American Society for Reproductive Medicine bucks the trend as the only medical society that explicitly pushes against obesity restrictions (Table 1).

In summary, overweight or obese women seeking pregnancy should be informed of the maternal and neonatal risks of fertility treatments and be supported rigorously during weight loss endeavors. Echoing the proposition of Legro et al. (5), we call for a thoughtful rethink when denying fertility treatment to overweight or obese women who have not achieved their weight loss goals despite their efforts.

CRediT Authorship Contribution Statement

Qian Feng: Validation, Data curation, Writing – original draft, Writing – review & editing, Visualisation, Project administration. **Ben W. Mol:** Conceptualization, Methodology, Investigation, Resources, Supervision, Writing – review & editing.

TABLE 1

Women's BMI restrictions before infertility treatment from medical societies and from countries offering public funding for IVF treatment.

Medical societies/countries	Stances on BMI restrictions
Medical societies	
British Fertility Society	Treatment should be deferred until the BMI <35 kg/m^2; among women aged younger than 37 years old, the cut-off is 30 kg/m^2
American Society for Reproductive Medicine	Obesity should not be the sole criterion for denying a patient or couple access to infertility treatment
Canadian Fertility and Andrology Society Clinical Practice Guideline Committee	No BMI cut-off was given. Programs that impose BMI cut-offs should offer resources for patients to help them lose weight
Chinese expert consensus	No BMI cut-off was given. Losing 5%–10% of body weight is highly recommended among all women seeking pregnancy
European Society of Human Reproduction and Embryology	No BMI cut-off was given. Fertility doctors should insist that a serious effort at achieving weight loss must be made before infertility treatment
The Royal Australian and New Zealand College of Obstetricians and Gynaecologists	BMI >35 kg/m^2 should be a contraindication to assisted fertility treatment such as IVF treatment
Countries	
New Zealand	BMI <30 kg/m^2
Sweden	Most clinics set at 35 kg/m^2; 2 clinics set at 30 kg/m^2
Spain	BMI <30 kg/m^2
United Kingdom	19 kg/m^2 <BMI <0 kg/m^2
Republic of Serbia	BMI <30 kg/m^2
Ireland	18.5 <BMI <30.0 kg/m^2

Note: BMI = body mass index; IVF = in vitro fertilization.

Feng. Reflections. Fertil Steril 2024.

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Advances in uterine fibroid research: linking progesterone and the transforming growth factor-β signaling pathway

The foundational role of sex steroid hormones, such as estrogen (E2) and progesterone (P4), in uterine fibroid growth is well established. This is also supported by the observation that fibroids typically arise during childbearing years and tend to regress after menopause. Although E2 has long been regarded as the primary driver of fibroid growth, mounting evidence suggests that P4 also plays an important role. This is particularly evident because enhanced mitotic activity within uterine fibroids is typically noted during the secretory phase of the menstrual cycle (marked by P4 dominance), compared with that during the proliferative phase. Clinical observations further support the involvement of P4 in fibroid growth, with P4 antagonists such as mifepristone and selective P4 receptor modulators including ulipristal acetate (UPA) effectively reducing fibroid size and related symptoms. However, magnetic resonance imaging observations have shown variability of fibroid growth within the same uterus, with some fibroids growing whereas others regressing, indicating the involvement of additional local factors in fibroid growth beyond sex steroids. Indeed, the role of several factors, including growth factors such as transforming growth factor-β (TGF-β), and alterations in the extracellular matrix (ECM) have been documented in fibroid pathogenesis.

Progesterone exerts its effects on target tissues through genomic and nongenomic actions, interacting with both nuclear P4 receptors (PGRs) and membrane-bound P4 receptors (mPRs), respectively. There are 2 isoforms of nuclear PGRs: PGR-B and PGR-A, with distinct functions. PGR-B represents a full-length protein with a weight of 116 kDa and comprises 933 amino acids. In contrast, PGR-A is truncated in the N-terminal region with a weight of 94 kDa and missing 164 amino acids found in PGR-B. PGR-B is typically known for its role as a potent activator in gene transcription, whereas PGR-A may exert suppressive effects on the function of PGR-B. Apart from genomic functions, P4 can induce rapid, nongenomic responses, happening within seconds to minutes, by activating either cytoplasmic PGRs or mPRs, also referred to as progestin and adipoQ receptors. Several mPR isoforms, including mPRα, mPRβ, and mPRγ, with mPRδ and mPRϵ are also proposed to respond to P4. Besides PGRs and mPRs, PR membrane component (PGRMC)1 and PGRMC2 represent novel membrane proteins categorized within the heme-binding protein family of membrane-associated PR proteins. PGRMC1 is characterized by a weight of 26–28 kDa and comprises 194 amino acids with distinct structural domains. It is predominantly located in the cell membrane while also being present in the endoplasmic reticulum and Golgi apparatus. Activation of PRs by P4 induces a conformational change, leading to the release of chaperones, formation of dimerization, and migration of the complex from the cytoplasm to the nucleus. Once in the nucleus, the complex interacts with specific P4 response elements located in the promoter region of target genes. Alternatively, P4 binding to PRs may activate various signaling pathways, such as AKT, MEK ½, and AKAP13/RhoA/ROCK, in fibroid cells.

Mechanistically, the fibroid structure is dynamic, characterized by a significant presence of ECM proteins such as collagens, fibronectin, and proteoglycans, alongside abnormal vasculature. Transforming growth factor-β signaling is markedly up-regulated in fibroids and plays a direct role in the induction of the fibrotic phenotype. Despite this, the specific contribution of P4 to ECM deposition and angiogenesis, as well as the underlying mechanisms, has not been extensively explored. A study conducted by Milewska et al. (1) revealed a potential link between P4 and TGF-β signaling pathways, implicating them in ECM deposition and angiogenesis. Although it is understood that "classical" nuclear P4 receptors, such as PGR-A and PGR-B, are the primary drivers of fibroid growth (2), Milewska et al. (1) highlighted the involvement of membrane P4 receptors such as mPRs and PGRMC in fibroid pathogenesis. These findings raise the possibilities of targeting the nongenomic actions of P4 and its downstream factors in the management of uterine fibroids.

In this study, the investigators conducted a comprehensive analysis of the expression profiles of nuclear PGRs and mPRs in uterine fibroids treated with UPA compared with those in untreated fibroids and normal myometrial tissues (1). They found that uterine fibroids exhibited higher messenger ribonucleic acid (mRNA) levels of PGR-A/B, PGR-B, mPRα, mPRβ, and PGRMC1, but not mPRγ and PGRMC2, than the normal myometrium (1). Treatment with UPA for 3 months at a dose of 5 mg/d led to a reduction in the mRNA and protein levels of mPRα, mPRβ, and PGRMC1 in fibroid tissues (1). However, UPA had no effect on the mPRγ and PGRMC2 levels. Interestingly, although the expression of PGR-B decreased in UPA-treated fibroids, the total expression levels of PGR-A/B increased (1), indicating a potential increase in the PGR-A isoform, which may inhibit the transcriptional activity of PGR-B. Similar observations were made in fibroid explant cultures, where P4 treatment increased the mRNA levels of various receptors, whereas UPA treatment reversed this induction and even increased the PGR-A/B levels (1). These findings suggest that both nuclear PGRs and membrane P4 receptors contribute to fibroid pathogenesis.

Next, this study focused on the impact of the TGF-β signaling. Transforming growth factor-β affects target tissues through SMAD-dependent and independent pathways. Uterine fibroids expressed many components of TGF-β signaling, including their receptors and downstream mediators. A previous report indicated that the transcript levels of TGF-β3 were higher in fibroid samples during the mid-secretory phase (P4 dominant) than during the proliferative phase (3), indicating potential regulation of P4 in TGF-β signaling in fibroid cells. Milewska et al. (1) established a potential link between P4 and TGF-β signaling pathways. The investigators found that the expression levels of TGF-β1, TGF-β3, SMAD2, and SMAD3

notably increased in uterine fibroids relative to normal myometrium. Ulipristal acetate treatment notably decreased the transcript levels of TGF-β components and levels of secreted TGF-β1 and TGF-β3 in fibroids (1, 4), indicating its suppression of these pathways. The involvement of TGF-βRs/SMAD3 signaling in P4 actions was further supported by the inhibition of P4-stimulated cell viability by inhibitors of TGF-βRI/II and SMAD3 (1). Additionally, UPA treatment led to a reduction in nuclear staining of phospho-SMAD3 in fibroid tissues (1), suggesting that the TGF-β-SMAD signaling is targeted by P4 and UPA in uterine fibroid cells.

Moreover, this study revealed the fibrotic role of P4 mediated by the TGF-β-SMAD signaling pathway. Progesterone treatment increased *COL1A1* expression and proCOLIA1 release, whereas UPA inhibited collagen type I expression and proCOLIA1 release in fibroid cells (1), indicating its antifibrotic effects. The involvement of SMAD3 in P4-mediated fibrotic effects was demonstrated, as an SMAD3 inhibitor reduced P4-stimulated *COL1A1* expression. Additionally, UPA treatment significantly reduced the expression of vascular endothelial growth factor and interleukin-6 in fibroid tissues, which are implicated in fibroid pathogenesis.

Furthermore, following recent findings (2, 5), the investigators evaluated the regulation of the RhoA pathway in uterine fibroids by P4 and UPA. In fibrotic conditions, factors such as TGF-β and vascular endothelial growth factor activate RhoA, a crucial regulator of ECM accumulation. They found that P4 increased the RhoA expression levels in uterine fibroid explants, whereas an SMAD3 inhibitor reduced these increased levels. Moreover, the combination of an SMAD3 inhibitor with UPA exhibited an additive effect on downregulating RhoA expression in uterine fibroid explants, suggesting an SMAD3-mediated regulation of RhoA by P4 (1).

In summary, this study is notable because it advances understanding of the TGF-β/SMAD signaling in fibroid growth, which is regulated by P4 and subsequently UPA, and the activation of RhoA and ECM deposition by TGF-β-SMAD and P4, suggesting a feed-forward loop of ECM dynamics in fibroids. Additionally, this study suggests the potential of targeting membrane P4 receptors and downstream factors in the management of uterine fibroids. The functional relevance of membrane P4 receptors in fibroid pathogenesis remains to be explored in future studies.

CRediT Authorship Contribution Statement

Md Soriful Islam: Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Interests

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Intrauterine adhesions. A sticky problem without a solution

Intrauterine adhesions (IUA) represent one of the most challenging conditions affecting women with the desire for future fertility. The evaluation and management of patients with IUA should include identification of risk factors, primary prevention, adequate treatment, and secondary prevention, along with early identification of potential obstetrical complications such as placenta accreta. In this issue of *Fertility and Sterility*, Zhang et al. (1) present their findings from a case-control study aimed at investigating factors affecting the prognosis of patients with IUA after transcervical resection of adhesions (TCRA). The study included 292 patients with a desire for future fertility who were diagnosed with moderate-to-severe IUA. All patients underwent TCRA, followed by a second-look office hysteroscopy approximately 2 months after the initial procedure. All patients received IUA barrier gel as well as an intrauterine (IU) balloon placement at the time of the index procedure, with removal of the balloon 5 days postoperatively. Patients were given postoperative sequential estrogen-progesterone treatment. Some patients received additional IUA barrier gel at the time of removal of the IU balloon. Intrauterine balloon dilation with a 16-Fr Foley balloon was performed 10 days postoperatively, and office hysteroscopy was performed 20 days after TCRA, and this order of interventions was repeated for 2–3 cycles after the index procedure per the Chinese Expert Consensus on Clinical Diagnosis and Treatment of Uterine Adhesions (2). Subsequent membranous adhesions and muscular adhesions involving <1/3 of the cavity were lysed immediately using the hysteroscope or hysteroscopic scissors.

Although 52 patients had adhesion recurrence, 240 had normal endometrial cavities on second-look hysteroscopy. The investigators report that IUA barrier gel reapplication 5 days after TCRA was associated with a lower risk of IUA recurrence, although the severity of adhesions on the American Fertility Society adhesion classification and the presence of chronic endometritis (CE) at baseline were risk factors for increased risk of adhesion recurrence.

As part of the exclusion criteria, women with infertility owing to uterine factors other than IUA were excluded from the study. However, the investigators included and analyzed those with associated fibroids and endometrial polyps. Although the article did not mention the location of the fibroids, it is known that submucous fibroids and endometrial polyps cause menstrual irregularity. This could affect the menstrual volume of the patients studied. In addition, fibroids have been reported to be solely responsible for infertility in 2%–3% of patients (3).

It is unclear if the initial TCRA was performed with hysteroscopic scissors or an energy device. A recent meta-analysis suggested that cold scissors were more efficient in preventing IUA recurrence, increasing menstrual flow, and reducing intraoperative blood loss (4). The study protocol included injecting a gel and placing a Foley catheter for 5 days after hysteroscopic adhesiolysis, followed by further injecting a gel in some women while inserting another size 16 Foley catheter on day 10 after hysteroscopic adhesiolysis. They did not specify the criteria for selecting who receives the gel application. Although no studies are available to guide practice, most gynecologists would consider using a pediatric-sized Foley catheter, such as a size 8, rather than a size 16, which might require cervical dilatation with the attendant discomfort, especially if the repeat insertion procedure is performed in the office setting.

An interesting finding in the study was the association between CE and the risk of IUA recurrence after TCRA. The investigators routinely collect endometrial samples to diagnose CE during hysteroscopic adhesiolysis.

Although the diagnostic accuracy of CE is increased with a histologic confirmation in addition to immunohistochemistry analysis, suggested hysteroscopy features include the presence of micro polyps, hyperemic regions with distinct related endometrial glands (strawberry sign), and endometrial interstitial edema. Using any of these hysteroscopic markers, Song et al. (5) found a diagnostic accuracy of 69.7% with a negative predictive value of 82.8%. These signs might serve as markers in determining whether a biopsy is needed to confirm CE during a hysteroscopic adhesiolysis.

Finally, considering the treatment regime applied by the investigators, it might be difficult to conclude that applying IU barrier gel 5 days after TCRA rather than the Foley catheter influenced postoperative adhesion formation in the patients studied. The challenge posed by the presence of the IUA continues. Further studies are needed to better understand this enigmatic pathology.

CRediT Authorship Contribution Statement

Jose Carugno: Conceptualization, Writing – original draft, Writing – review & editing. **Jude Okohue:** Conceptualization, Writing – original draft, Writing – review & editing. **Nash Moawad:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Interests

J.C. has nothing to disclose. J.O. has nothing to disclose. N.M. has nothing to disclose.

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Bilateral labiocrural fasciocutaneous flaps for vaginoplasty: when two become one

There are few gynecologic surgeries that have as many effective approaches as vaginoplasty. The main reason for this is that each option has specific advantages and disadvantages. These options can be divided into surgical and nonsurgical methods to create a neovagina (1). Nonsurgical management primarily involves vaginal dilation using graduated dilators to create a functional vagina. This is often first-line management because it is highly successful in compliant patients, uses native vaginal tissue, and prevents the inherent risks of surgery (2). For patients who do require a surgical approach, surgeons may recommend various options that, in addition to the vaginal surgery, may or may not require abdominal surgery (Vecchietti or Davydov) and may or may not require the use of an autologous or allogenic tissue graft (McIndoe, intestinal vaginoplasty, or Williams). After thorough counseling, patient preference and surgeon experience will ultimately determine that final recommended approach.

The fact that there are still multiple surgical approaches that are commonly used and accepted for vaginoplasty means that there is no single approach that has overwhelming advantages compared with other methods. This also means that there is still room for surgical innovation to develop a procedure that maximizes that chance of achieving a successful postoperative outcome and minimizes the risks associated with surgery and recovery. In the case of a vaginoplasty procedure, the most important treatment outcomes that should be optimized include the expected vaginal length and width and the sexual function of the neovagina. The risks of the surgery that should be considered include injury or fistula formation with the bladder or bowel, vaginal stenosis, vaginal prolapse, and risks related to wound healing from the autologous tissue graft site. These risks, in part, may be associated with the length of surgery, extent of the required dissection, and length of recovery.

Surgical innovation for vaginoplasty is, indeed, demonstrated in this month's issue of *Fertility and Sterility* in a video article by Uccella et al. (3) that describes a new surgical technique for vaginoplasty using bilateral labiocrural fasciocutaneous flaps as an autologous tissue graft. In this video, the investigators discuss this novel technique that was developed and performed by a multidisciplinary team of gynecologists and plastic surgeons. The investigators describe the dissection of a neovagina and use of bilateral 12 × 5–cm fasciocutaneous skin flaps from the labiocrural folds. The described case was successful in that it achieved a neovagina with a sufficient length and width, 9 cm in length at 2-year follow-up, and allowed for sexual intercourse. In addition, this patient had an uneventful postoperative course without the development of surgical complications.

Now that the investigators have demonstrated that this donor tissue site can be used to create a neovagina, the next step will be to evaluate how this graft site functions compared with other currently used tissue grafts. Certainly, the extent of the dissection required for this surgery is more significant than some other graft options. Thus, this surgery may have an increased risk of complications and morbidity that should carefully be evaluated in a larger patient cohort. Further, because of the extensive dissection involved and the reported operative time of 6 hours, there should be clearly identified advantages to this tissue graft before this should be routinely offered and used.

One notable advantage of this tissue graft is the preservation of blood supply (from the posterior labial artery), subcutaneous fat, and innervation. The investigators report that the patient maintained neovaginal sensation at 2-year follow-up. If these blood and nerve supplies result in a lower risk of graft failure and higher sexual function scores than other methods, then this could be a major advantage over other tissue graft options and could justify the extent of the surgery required to harvest this tissue. To understand this better, the surgeons should next perform nerve conduction studies to more objectively evaluate the nerve function of this tissue postoperatively. Beyond this, these patients should be evaluated with an objective measure of sexual function, such as the Female Sexual Function Index. These scores will be useful to not only demonstrate that this technique is effective in creating a functional neovagina, but will also allow for a postoperative comparison of sexual function between this and other commonly used surgical approaches (4).

An important consideration that the investigators highlight when using labiocrural tissue that is not encountered with most other tissue grafts used for vaginoplasty is the presence of terminal hair growth. The removal of unwanted hair follicles from donor tissue used for a neovagina is important because it relates to overall satisfaction. Unwanted hair growth after a tissue graft has more commonly been described after tissue graft placement in the oral cavity with multiple protocols that have been evaluated for preoperative use (5). Definitive epilation as described by the investigators is an important preoperative step in preparation for the use of this tissue.

In conclusion, this video describes an innovative surgical approach to the creation of a neovagina using an autologous fasciocutaneous graft that is vascular and nerve sparing and, in the presented case, resulted in an adequate vaginal length resulting in sexual function. The dissection required to obtain the labiocrural fasciocutaneous tissue was more extensive than that in other tissue graft sites, and both the length of surgery and postoperative hospital stay were longer than what is reported for more commonly used surgical approaches. However, the potential advantages of this tissue graft site could ultimately yield better functional postoperative outcomes than more commonly used tissue grafts. Objective measures of nerve function and sexual function are needed to determine whether the theoretical benefit of this surgical approach results in superior patient outcomes before this tissue graft site should be more widely considered by multidisciplinary surgical teams.

CRediT Authorship Contribution Statement

Phillip A. Romanski: Conceptualization, Writing – original draft, Writing – review & editing. **Pietro Bortoletto:** Conceptualization, Writing – original draft, Writing – review & editing. **Samantha M. Pfeifer:** Conceptualization, Writing – review & editing.

Declaration of Interests

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Low-serum antimüllerian hormone is linked with poor semen quality in infertile men screened for participation in a randomized controlled trial

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Objective: To investigate possible associations between serum antimüllerian hormone (AMH) concentration and semen quality in infertile men. Studies investigating the associations between serum AMH concentration and semen quality in infertile men have shown conflicting results.
Design: Infertile men were included during screening for participation in the First in Treating Male Infertility Study, a double-blinded, placebo-controlled, 1:1, single-center randomized controlled trial.
Setting: Not applicable.
Patients: At the screening visit, 400 participants produced a semen sample and had their serum analyzed for AMH concentration.
Intervention: Not applicable.
Main Outcome Measures: Serum AMH concentration and semen quality.
Results: All men were stratified according to serum AMH concentrations in quartiles (Q1–Q4). Men in the lowest quartile had a lower sperm concentration (1×10^6/mL) (Q1: 8.0 vs. Q2: 10.4 vs. Q3: 11.0 vs. Q4: 13.0), total sperm count (1×10^6) (Q1: 29.1 vs. Q2: 38.2 vs. Q3: 44.4 vs. Q4: 55.7), sperm motility (%) (Q1: 41 vs. Q2: 57 vs. Q3: 50 vs. Q4: 53), and progressive sperm motility (%) (Q1: 31 vs. Q2: 44 vs. Q3: 35 vs. Q4: 40) compared with the other quartiles. Moreover, men with a sperm concentration <2 million/mL had a lower serum AMH concentration compared with men having 2–16 $\times$ 10^6 /mL and >16 $\times$ 10^6/mL (31 pmol/L vs. 38 pmol/L vs. 43 pmol/L, respectively). In accordance, men with sperm motility <20% had a lower serum AMH concentration compared with men with sperm motility 20%–42%, and >42% (31 pmol/L vs. 43 pmol/L. vs. 39 pmol/L, respectively).
Conclusion: This study shows that low serum AMH concentration is associated with poor semen quality in infertile men, which implies that serum AMH concentration may have clinical value during the evaluation of male infertility.
Clinical Trial Registration Number: NCT05212337 (Fertil Steril® 2024;122:278–87. ©2024 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: AMH, male infertility, semen quality

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R.H. and K.Y. should be considered similar in author order.
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The trial was approved by the Danish Medicines Agency (EudraCT: 2021-003451-42), the Danish National Committee on Health Research Ethics (approval no. H-21040145), and the regional data protection agency, Privacy (approval no. P-2021-766) and monitored by the Good Clinical Practice (GCP) Unit, Copenhagen University Hospitals. Informed consent was obtained from all participants.
The data that support the findings of this study are available from the corresponding author on reasonable request.
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Infertility is a global health issue with a lifetime incidence of up to 17.5% (1). Male infertility contributes to approximately half of the cases (2); there are, however, no treatment options for most infertile men. Andrological evaluation of infertile men includes medical history, physical examination, including testicular examination and ultrasonography, semen analysis, and preferentially assessment of serum concentrations of follicle-stimulating hormone (FSH), inhibin B, luteinizing hormone (LH), and testosterone (3). Serum antimüllerian hormone (AMH) levels are rarely included in the andrological evaluation of infertile men. In contrast, it is measured routinely in women during their infertility workup and assists in the diagnosis of polycystic ovary syndrome and premature ovarian failure (4). In the male fetus, AMH is responsible for the regression of the müllerian duct during the first trimester and is therefore vital for normal sexual differentiation in males (5). Antimüllerian hormone is synthesized by immature Sertoli cells in fetal as well as postnatal life. Thus, AMH secretion from Sertoli cells increases markedly during minipuberty, remains high during childhood, and subsequently declines during the pubertal transition when Sertoli cells gain expression of the androgen receptor (6–8) that enables responsiveness to the high intratesticular testosterone concentrations (9, 10). In adult men, FSH stimulates the differentiated Sertoli cells to produce AMH (5), which during adulthood reaches a plateau corresponding to 3%–4% of the high levels during infancy and childhood (11, 12). In adult men, the role of AMH level is not well characterized, although in cases of differences in sex development, including persistent müllerian duct syndrome (13), serum AMH levels may be a useful diagnostic tool (10, 14). Serum AMH level has been associated with Sertoli cell function and other markers of spermatogenesis, such as serum FSH and inhibin B levels, as well as semen quality in both fertile and infertile men (15–18). Only a few studies have investigated the association between serum AMH concentrations and semen quality in infertile men, and most of these studies have reported conflicting results. In this study, we aimed to clarify the association between serum AMH concentrations and semen quality in a cohort of infertile men.

MATERIALS AND METHODS

Trial design and participants

The cohort was comprised of 400 infertile men invited to participate in a screening visit to assess eligibility for the First in Treating Male Infertility Study (19), a randomized controlled trial (RCT) conducted at the Department of Growth and Reproduction, Rigshospitalet, Denmark. Men with serious comorbidities, such as diabetes mellitus, cancer, or autoimmune diseases, were not invited to participate. At the screening visit, the participants had a venous blood sample taken and produced a semen sample. For this screening study, patients were screened from February 2022 to December 2023, and none of the men were excluded because of semen quality or reproductive hormones. Informed consent was obtained at the screening visit for all participants, and the study was conducted in accordance with the Helsinki Declaration. The trial protocol was published in 2022 (19).

Biochemical and semen analyses

Serum AMH concentration was measured using a sensitive immunoassay (Immunotech, Beckman Coulter Ltd., Marseilles, France) with an interassay coefficient of variation <5%. All semen samples were provided by masturbation on-site in a room close to the laboratory. The duration of abstinence, fever, and spillage were self-reported. Semen samples were analyzed, as described previously (20). In short, semen volume was determined by weighing, and assessment of sperm concentration was done using a Nucleocounter image cytometer NC-3000 (ChemoMetec, Denmark), although when sperm concentration was $<3 \times 10^6$/mL, the samples were reassessed using a Bürker-Türk hemocytometer. Sperm motility was classified as motile sperm (class ABC%) and progressive motile sperm (class AB%). Sperm morphology was evaluated according to stricter criteria. All semen samples analyzed at the laboratory within 2 years were included, and an average was calculated for each participant. Of all the participating men, 278 had one semen sample, 92 had 2 samples, 24 had 3 samples, and 6 had 4 samples. Testis size was evaluated using an orchidometer, and the average size of both testes was presented. Testis echo scores were evaluated using ultrasound, and in cases with a side difference, the highest score was presented. Cryptorchidism was defined as unilateral or bilateral undescended testicles in childhood.

Statistical analyses

Descriptive statistics were calculated for all variables and presented as medians with an interquartile range [IQR] for continuous variables because the data were not normally distributed (Table 1). Categorical variables were presented as numbers with percentages. When a participant delivered more than one semen sample, the average value of the samples for each semen variable was used. To explore potential subgroup differences, we conducted an additional analysis on the first semen sample only; however, the results of this analysis were consistent with the overall findings. In Table 2, variables were stratified in quartiles according to serum AMH concentration, and significant differences between groups were evaluated with Kruskal-Wallis tests. Categorical variables were analyzed using the Chi-square test. Variables achieving significant differences in Table 2 were ln-transformed when needed, secondarily evaluated with a univariant analysis, and adjusted for age. Differences in serum AMH concentrations between various groups were evaluated with Kruskal-Wallis (Figs. 1 and 2), although Mann-Whitney-U tests were used in Figure 1D and E. *P* values are shown in the figures. A significance level of $P < .05$ was considered statistically significant. In general, all statistical calculations were conducted using SPSS version 28.

RESULTS

Baseline characteristics

The study cohort consisted of a total of 400 infertile men, and their baseline characteristics are summarized in Table 1. The median age of the participants was 34.4 years (IQR 31.2, 38.4), although their body mass index (BMI) was 25.1 kg/m^2

TABLE 1

Characteristics of the study population.

Variable	n	Median	IQR
Age (y)	400	34.4	[31.2–38.4]
Height (cm)	395	183	[179–187]
Weight (kg)	395	85	[77–95]
BMI (kg/m^2)	395	25.1	[23.3–27.8]
Infertility history (mo)	227	18	[14–21]
Average testis size (mL)	228	17.5	[14.8–20.0]
Varicocele, no., (%)	228	51	(22.4%)
Cryptorchidism, no., (%)	228	20	(8.8%)
Echo score = 2, no., (%)	228	147	(64.5%)
AMH level (pmol/L)	400	38	[27–60]
Duration of abstinence (d)	400	3	[3–4]
Semen volume (mL)	400	3.8	[3.0–5.0]
Sperm concentration (10^6/mL)	400	11.0	[4.5–21.0]
Total sperm count (10^6)	400	42.0	[18.2–90.7]
Sperm motility (ABC%)	350	51	[34–65]
Sperm motility (10^6)	350	19.7	[6.5–46.6]
Progressive sperm motility (AB%)	350	36	[20–54]
Progressive sperm motility (10^6)	350	13.5	[3.9–33.4]
Sperm morphology (%)	350	3.4	[1.5–6.0]
Sperm morphology (10^6)	350	1.1	[0.4–3.4]

Note: Data are presented as median (IQR) unless otherwise indicated. Semen parameters are presented as an average of 1 to 4 semen samples. Testis size is evaluated using an orchidometer and presented as the average of both testes.
AMH = antimüllerian hormone; BMI = body mass index; IQR = interquartile range.

Holt. Low serum AMH is linked with poor semen. Fertil Steril 2024.

(IQR 23.3, 27.8) on the basis of an average height of 183 cm (IQR 179, 187) and weight of 85 kg (IQR 77, 95). Semen analysis showed that the cohort had a median sperm concentration of 11.0 × 10^6/mL (IQR 4.5, 21.0). The semen variables are presented in Table 1. Blood samples showed a median concentration of serum AMH of 38 pmol/L (IQR 27, 60). Among the 228 participants who had a full clinical examination performed, the average median testis size was 17.5 mL (IQR 14.8, 20.0), and 147 (64.5%) participants had an ultrasonic echo score of 2 (regular pattern) of their testicles. The remaining had a higher score, indicating a more heterogeneous testicular structure. Varicocele was detected in 51 (22.4%), although anamnestic cryptorchidism was reported in 20 (8.8%) individuals. The median infertility history was 18 months before inclusion [IQR 14, 21]. When grouping men according to age, serum AMH concentration was on average 13 pmol/L higher in men <30 years compared with men >40 years (44 pmol/L [IQR 34, 64] vs. 31 pmol/L [IQR 21, 45]; $P<.001$) (Fig. 1A). Men with an average testis size ≤15 mL had a 40% lower serum AMH concentration compared with men with a testis size ≥20 mL (38 pmol/L [IQR 27, 62] vs. 63 pmol/L [IQR 42, 80]; $P=.006$), even when adjusted for sperm count (data not shown) (Fig. 1C). There was no difference in serum AMH concentrations according to BMI, varicocele, cryptorchidism, or testicular echo scores (Fig. 1B to F).

Serum AMH concentration and semen quality

We stratified semen and testicular parameters according to quartiles (Q1–Q4) of serum AMH concentrations, as shown in Table 2. Participants in the lowest serum AMH quartile (Q1) were the oldest, with a median age of 36.7 (IQR 33.1, 40.7) years, compared with the 3 other quartiles (Q2: 33.9 years [IQR 31.7, 38.4] vs. Q3: 33.7 years [IQR 30.2, 36.4], and Q4: 34.0 years [IQR 31.0, 37.1]; $P<.001$). BMI did not differ between the quartile groups ($P=.121$). Similarly, infertility history and the prevalence of varicocele, cryptorchidism, and testicular echo score did not differ between the groups, however, men in Q1 tended to have a smaller average testis size (Q1: 15.0 mL [IQR 13.0, 20.0] vs. Q2: 16.0 mL [IQR 13.5, 20.0] vs. Q3: 17.5 mL [IQR 15.0, 20.0] and Q4: 18.0 mL [IQR 15.0, 22.5]; $P=.091$) (Table 2). Sperm concentration varied across serum AMH quartiles. In the lowest quartile (Q1), the median sperm concentration was 8.0 × 10^6/mL (IQR 2.7, 19.0), compared with 10.4 × 10^6/mL (IQR 5.7, 20.5), 11.0 × 10^6/mL (IQR 5.8, 20.8), and 13.0 × 10^6/mL (IQR 5.7, 29.4) in Q2, Q3, and Q4, respectively ($P=.006$). Likewise, total sperm count also differed between serum AMH quartiles, from 29.1 × 10^6 [10.8, 77.5] in the lowest quartile (Q1) to 55.7 × 10^6 (IQR 22.3, 107.4) in the highest quartile (Q4) ($P=.022$). Furthermore, the percentage of motile sperm (%) was different across serum AMH quartiles. In the lowest quartile (Q1), the median percentage of motile sperm was 41% (IQR 28, 63), in contrast to 53% (36, 65) in the group of the highest quartile (Q4) ($P=.042$). The percentage of progressive motile sperm (%) was also different across the groups because men in the lowest quartile (Q1) had a median percentage of progressive motile sperm of 31% (IQR 9, 52) compared with men in the highest quartile (Q4) who had 40% progressive motile sperm (IQR 24, 54) ($P=.031$). All significant findings were evaluated secondarily in a model adjusted for age, which had no effect on the results (data not shown). The duration of ejaculation abstinence, semen volume, and sperm morphology did not show any difference (Table 2).

World Health Organization reference ranges for semen quality and association with serum AMH concentration

In 2021, the World Health Organization (WHO) published its latest reference range for semen parameters (21). To investigate whether serum AMH concentrations differed in relation to semen parameters, we divided men into 2 groups according to the lower fifth percentile of semen parameters from men in the WHO reference population. To further explore a stepwise lower serum AMH concentration in infertile men with severely impaired semen quality, we added a third group of men who would be candidates for in vitro fertilization treatment and intracytoplasmic sperm injection treatment (Fig. 2). Men with sperm concentration <2 × 10^6/mL had lower serum AMH concentration (31 pmol/L [IQR 19, 53]) compared with men with 2–16 × 10^6/mL (38 pmol/L [IQR 27, 59], and >16 × 10^6/mL (43 pmol/L [IQR 29, 65]) ($P=.015$) (Fig. 2A). Similarly, men with a total sperm count <10 × 10^6 also had a lower serum AMH concentration (30 pmol/L [IQR 20, 53]) compared with men with 10–39 × 10^6 (37 pmol/L [IQR 27, 56]) and >39 × 10^6 (40 pmol/L [IQR 29, 65]) ($P=.004$) (Fig. 2E). Serum AMH concentrations were also different across the percentage (%) of motile sperm because men with motile sperm <20% had a lower serum AMH concentration (31 pmol/L [IQR 22, 44]) compared with men with motile

TABLE 2

Characteristics of the study population stratified according to serum antimüllerian hormone (AMH) levels in quartiles.

	1st quartile (Q1)			2nd quartile (Q2)			3rd quartile (Q3)			4th quartile (Q4)			
Variable	**n**	**Median**	**IQR**	**n**	**Median**	**IQR**	**n**	**Median**	**IQR**	**n**	**Median**	**IQR**	***P* value**
AMH level (pmol/L)	100	20	[17–23]	100	33	[29–35]	100	46	[42–53]	100	77	[68–94]	-
Age (y)	100	36.7	[33.1–40.7]	100	33.9	[31.7–38.4]	100	33.7	[30.2–36.4]	100	34.0	[31.0–37.1]	**<.001**
BMI (kg/m^2)	98	25.8	[24.1–28.2]	98	25.1	[23.5–27.7]	99	24.6	[22.8–27.0]	100	24.9	[23.3–27.8]	.121
Infertility history (mo)	42	20	[15–24]	46	18	[14–24]	73	18	[12–24]	66	21	[17–29]	.167
Average. testis size (mL)	43	15.0	[13.0–20.0]	45	16.0	[13.5–20.0]	73	17.5	[15.0–20.0]	67	18.0	[15.0–22.5]	.091
Varicocele, no, (%)	43	7	(16.3%)	45	12	(26.7%)	73	12	(16.4%)	67	20	(29.9%)	.169
Cryptorchidism, no, (%)	43	4	(9.3%)	45	5	(11.1%)	73	6	(8.2%)	67	5	(7.5%)	.920
Echo score = 2, no, (%)	43	32	(74%)	45	30	(67%)	73	49	(67%)	67	36	54 (%)	.249
Duration of abstinence (d)	100	3	[3–4]	100	3	[3–4]	100	3	[3–4]	100	3	[3–3]	.486
Semen volume (mL)	100	3.6	[2.9–4.6]	100	4.0	[3.0–4.6]	100	4.1	[3.3–5.2]	100	3.9	[2.9–5.0]	.317
Sperm concentration (10^6/mL)	100	8.0	[2.7–19.0]	100	10.4	[5.7–20.5]	100	11.0	[5.8–20.8]	100	13.0	[5.7–29.4]	**.006**
Total sperm count (10^6)	100	29.1	[10.8–77.5]	100	38.2	[17.6–79.2]	100	44.4	[22.7–85.8]	100	55.7	[22.3–107.4]	**.022**
Sperm motility (ABC%)	81	40	[28–63]	91	57	[35–68]	91	50	[37–62]	87	53	[36–65]	**.042**
Sperm motility (10^6)	81	11.1	[2.6–39.3]	91	21.1	[6.9–42.9]	91	20.6	[8.1–48.4]	87	25.7	[7.8–54.0]	**.020**
Prog. sperm motility (AB%)	81	31	[9–52]	91	44	[23–57]	91	35	[20–51]	87	40	[24–55]	**.031**
Prog. sperm motility (10^6)	81	6.1	[1.2–27.4]	91	16.3	[4.9–35.0]	91	14.0	[5.7–28.7]	87	18.7	[5.2–39.9]	**.012**
Sperm morphology (%)	81	3.0	[1.5–5.0]	91	3.0	[1.9–6.0]	91	3.5	[1.5–6.5]	87	4.0	[2.0–6.1]	.672
Sperm morphology (10^6)	81	0.8	[0.2–2.2]	91	1.1	[0.5–2.9]	91	1.2	[0.3–5.2]	87	1.3	[0.5–3.8]	.335

Note: Data are presented as median (IQR) unless otherwise indicated. Semen parameters are presented as an average of 1 to 4 semen samples. Testis size is evaluated using an orchidometer and presented as the average of both testes. *P* value: Kruskal-Wallis test for all, except for varicocele, cryptorchidism, and echo score where the chi-squared test was used.

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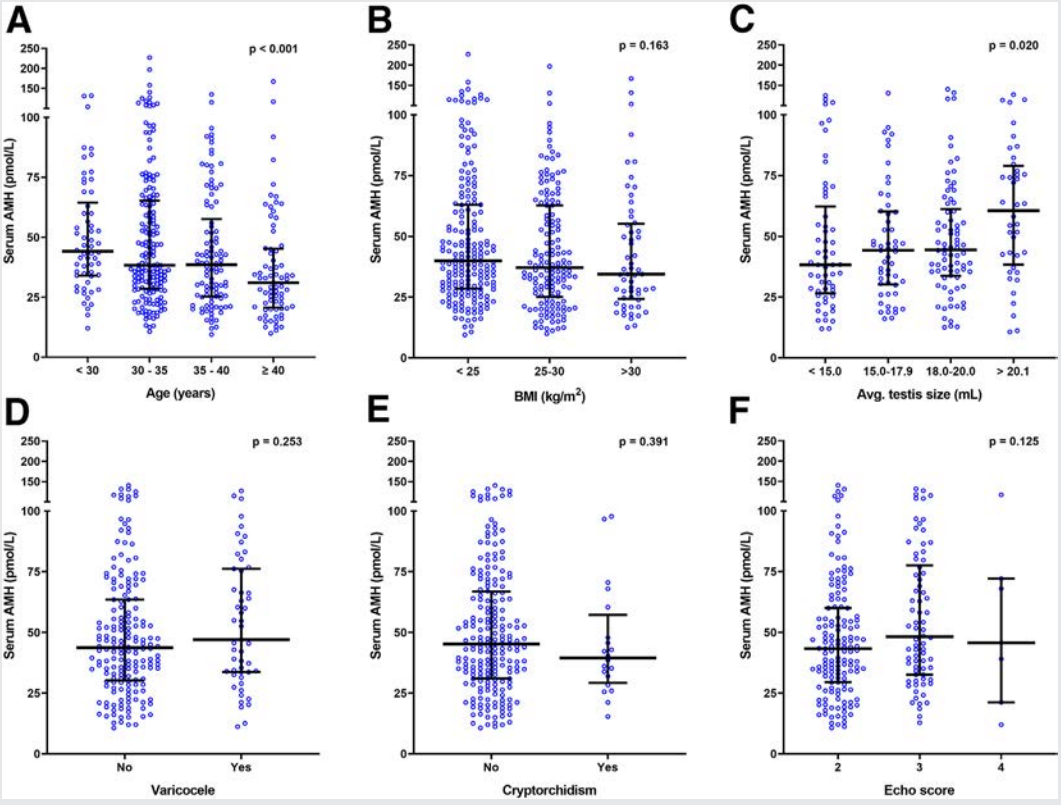
FIGURE 1



Anthropometrics and serum antimüllerian hormone (AMH) concentration. Serum AMH concentrations according to different anthropometric variables. (**A**) Age (years) and serum AMH concentrations ($P<.001$). (**B**) BMI (kg/m^2) and serum AMH concentrations ($P<.163$). (**C**) Average testis size (mL) and serum AMH concentrations ($P<.020$). (**D**) The presence of varicocele (y/n) and serum AMH concentrations ($P<.253$). (**E**) The presence of anamnestic cryptorchidism (y/n) and serum AMH concentrations ($P<.391$). (**F**) Echo score and serum AMH concentrations ($P<.125$). *Note:* Blue Circles represent each individual and black lines represent the median ± IQR. *P* value for differences between the groups: Kruskal-Wallis or Mann-Whitney U test. BMI = body mass index; IQR = interquartile range; y/n = yes and no.

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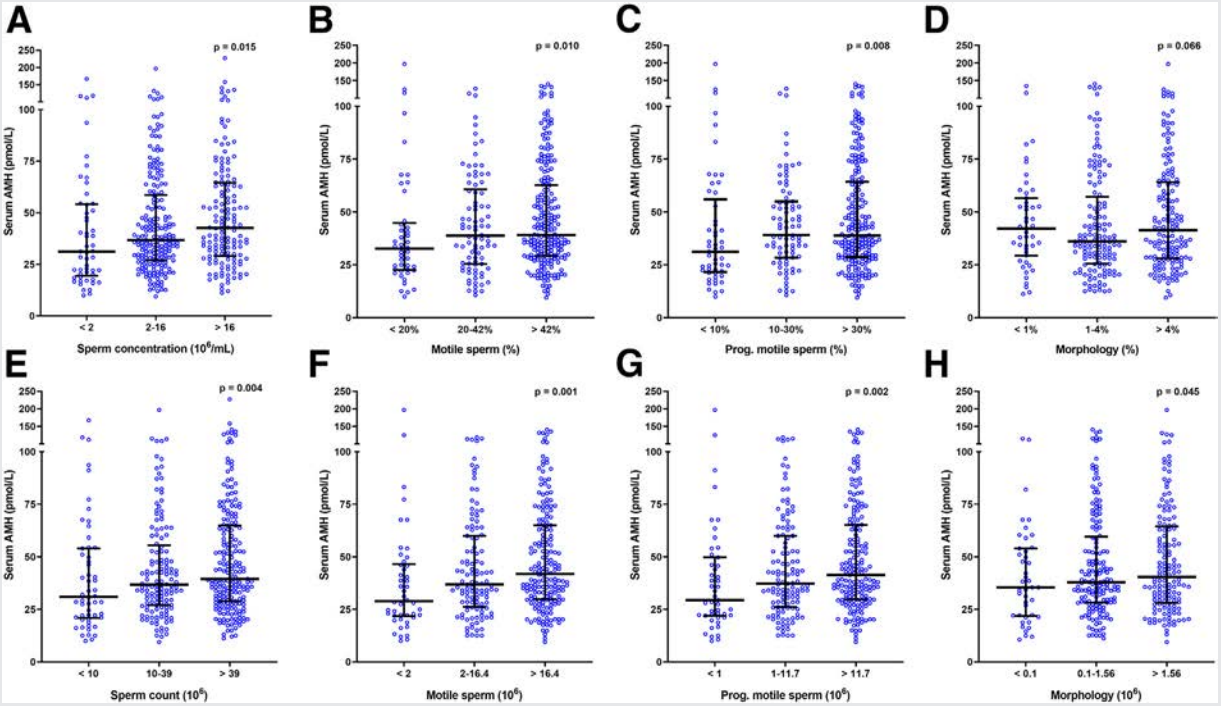
sperm 20%–42% (38 pmol/L [IQR 26, 60]) and >42% (39 pmol/L [IQR 29, 63]) ($P=.010$). Likewise, men with progressive motile sperm <10% had a lower serum AMH concentration (31 pmol/L [IQR 21, 53]) compared with men with progressive motile sperm 10%–30% (43 pmol/L [IQR 29, 58]) and >39% (39 pmol/L [IQR 29, 64]) ($P=.008$) (Fig. 2B and C). To define 3 groups according to the number of motile sperm (n), progressive motile sperm (n), and morphologically normal sperm (n), the lower fifth percentile of data from men in the current WHO 2021 reference population was used. Total sperm count (39×10^6) was multiplied by the percentage of motile sperm (42%), progressively motile sperm (30%), and morphologically normal sperm (4%), respectively, resulting in upper cut-offs of 16.4×10^6 motile, 11.7×10^6 progressive motile, and 1.56×10^6 morphologically normal spermatozoa. Serum AMH concentration was lower in men with the lowest number of motile sperm ($<2 \times 10^6$) and progressive motile sperm ($<1 \times 10^6$) compared with men with a higher number of motile sperm ($2.0–16.4 \times 10^6$ and $>16.4 \times 10^6$) and progressive motile sperm ($1.0–11.7 \times 10^6$ and $>11.7 \times 10^6$), respectively (number of motile sperm: 28 pmol/L [IQR 21, 47] vs. 36 pmol/L [IQR 26, 60] vs. 42 pmol/L [IQR 30, 65]; $P=.001$ and number of progressive motile sperm: 29 pmol/L [IQR 21, 50] vs. 37 pmol/L [IQR 26, 59] vs. 41 pmol/L [IQR 30, 65]; $P=.002$) (Fig. 2F and G). There was no difference in serum AMH concentrations in men according to sperm morphology (%), although men with $<0.1 \times 10^6$ morphologically normal sperm had a lower serum AMH concentration compared with men with $0.1–1.56 \times 10^6$ and $>1.56 \times 10^6$ number of morphologically normal sperm (34 pmol/L [IQR 21, 53] vs. 38 pmol/L [28, 59] vs. 40 pmol/L [IQR 28, 65], respectively; $P=.045$) (Fig. 2D and H).

DISCUSSION

This study suggests that low serum AMH concentrations in infertile men increase the likelihood of poor semen quality. Infertile men with the lowest serum AMH concentration had a lower sperm concentration, total sperm count, and fewer motile sperm compared with infertile men with a higher serum AMH concentration. The link between serum AMH concentration and sperm count suggests that AMH level is a

FIGURE 2



Semen variables and serum antimüllerian hormone (AMH) concentration. Serum AMH concentration and different semen parameters are grouped according to the WHO reference range for semen parameters. (**A**) Sperm concentration (10^6/mL) and serum AMH concentrations ($P<.015$). (**B**) The percentage of motile sperm (%) and serum AMH concentration ($P<.010$). (**C**) The percentage of progressive motile sperm (%) and serum AMH concentrations ($P<.008$). (**D**) The percentage of morphologically normal sperm and serum AMH concentrations ($P<.066$). (**E**) sperm count (10^6) and serum AMH concentrations ($P<.004$). (**F**) number of motile sperm (10^6) and serum AMH concentrations ($P<.001$). (**G**) The number of progressive motile sperm (10^6) and serum AMH concentrations ($P<.002$). (**H**) The number of morphologically normal sperm (10^6) and serum AMH concentrations ($P<.045$). WHO = World Health Organization. *Note: Blue circles* represent each individual and black lines represent the median ± IQR. *P* value for differences between the groups: Kruskal-Wallis for all.

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marker of Sertoli cell function, whereas the link with sperm motility is more surprising and may suggest a yet unknown role of AMH concentration on spermatozoa or the male reproductive tract unless the proposed impact on sperm count also contributes to improved sperm function and a higher probability of maturing properly during epididymal transit. Previously, a study found expression of the AMH receptor in ejaculated human spermatozoa, which is a prerequisite for a direct effect on sperm motility, unless the effect is on the organs in the male reproductive tract involved in sperm maturation and storage (16). In our cohort of infertiles, we detected a positive association between AMH concentrations in serum and the percentage of motile and progressively motile spermatozoa. Previous studies did not find any association between serum AMH concentrations and progressive motile sperm (15, 22), although others found a negative association (16). In seminal plasma, a positive link between seminal AMH concentrations and progressively motile sperm (%) has been described (22, 23), which is compatible with the positive association between serum AMH concentrations and sperm motility reported here. Noteworthy, the link between serum AMH concentrations and semen quality was found also when the infertile men were stratified according to the WHO-defined threshold for sperm concentration at 16×10^6/mL and the setpoint for intracytoplasmic sperm injection of 2×10^6/mL (24). Infertile men with the lowest sperm concentration, total sperm count, sperm motility, progressive sperm motility, and the total number of morphologically normal sperm had significantly lower serum AMH concentrations compared with men with higher sperm parameters, indicating low serum AMH concentration is a prognostic marker of poor semen quality in infertile men. Considerable overlap exists between the groups, so serum AMH concentrations cannot be used to diagnose patients with more severely impaired semen quality, although we believe that they can be used to identify men better suited for stimulatory agents to improve spermatogenesis.

Our findings are in line with a recent publication showing low serum AMH concentration as a marker of impaired semen quality and Sertoli cell function in infertile men (18). The association between serum AMH and Sertoli cell function is supported by studies in men with normal reproductive health (16, 17). Moreover, in a large cohort of 970 young men from the general population, serum AMH concentration was

associated with serum FSH and inhibin B concentrations but not sperm production (15), which may be explained by the very few men with severely impaired semen quality in this cohort and no analysis of men with very low serum AMH concentrations. The role of AMH concentration in adulthood and its potential effects are largely unknown (14). In the prepubertal testis, Sertoli cells account for the largest proportion of testicular cell types, unlike in the adult testis, where germ cells account for the largest proportion (11). In our cohort, we also found that serum AMH concentration was declining with age (16, 18, 25, 26), although not as steep as in women. Multiple studies have suggested an association between serum AMH concentrations and risk factors for cardiovascular disease (CVD) in men (17, 25–27) and CVD, type 2 diabetes, and atherosclerosis in women (28–30). Because serum AMH concentration is a marker of ovarian reserve, which declines with age, it has been postulated that serum AMH concentrations may reflect the status of arterial aging, thereby linking serum AMH concentrations with atherosclerosis and CVD in women (28, 29). In our cohort, we found no association between BMI and serum AMH concentrations, but the link with age is in line with the association between low serum AMH concentration and impaired Leydig cell function, illustrated by lower serum testosterone concentrations and testosterone: LH ratio (15, 17, 18). Low-serum testosterone concentrations in men are a known risk factor for metabolic syndrome, type 2 diabetes, and CVD (31–33), and future studies may investigate whether serum AMH concentrations may directly or indirectly be linked with cardiovascular health.

In our study, infertile men with an average testis size of $\leq$15 mL had significantly lower serum AMH concentrations compared with men with testicles $\geq$20 mL, indicating that infertile men with low serum AMH concentrations may have a lower proportion of mature Sertoli cells. Although men with high serum AMH concentrations have higher sperm counts, it is unlikely that the high serum AMH concentrations are because of more abundant immature Sertoli cells, and it would require testicular biopsies and histologic examinations to clarify this. In men with obstructive azoospermia, AMH concentration is undetectable in the seminal fluid (34, 35), thereby supporting its testicular origin. However, seminal AMH concentration may be also undetectable in men with normal sperm production (22, 35) and therefore cannot be used as a single marker of obstructive azoospermia. In men with nonobstructive azoospermia, seminal AMH concentrations have failed as a predictive marker for positive sperm retrieval (36, 37), in contrast to serum AMH concentrations, which have shown promising results (38, 39). In our study, we have not measured the seminal AMH concentration. Idiopathic infertile men have a vast interindividual and intraindividual variation in semen quality, and we suggest that reproductive hormone concentrations (40), and particularly serum AMH concentrations in combination with serum FSH, LH, inhibin B, testosterone concentrations, and semen analyses, may be used to differentiate the pathophysiology of idiopathic male infertility. Hereby, specified future treatments, e.g., those designed to support impaired Sertoli cell, Leydig cell, germ cell, spermatozoa, or reproductive epithelial cell function, would be an option. Not all infertile men with severely impaired semen quality have low serum AMH concentrations because 5%–10% of participating men with severely impaired semen quality have a high serum AMH concentration. Unfortunately, one limitation of our study is that we have not investigated other markers of Sertoli cell and Leydig cell function such as serum FSH, inhibin B, LH, testosterone, and insulin-like peptide 3 concentrations in all 400 infertile men or the men with severely impaired semen quality, which would be rewarding to distinguish the endocrine phenotype in low- vs. high-serum AMH concentration.

In most men where there is no obvious cause of infertility (i.e., hypogonadotropic hypogonadism, varicocele, or duct obstruction), there is no medical treatment today and consequently, female partners need to undergo fertility treatment despite having normal reproductive health. Potential treatments such as clomiphene citrate (41) and vitamin D supplementation (42) have been investigated with conflicting results, and currently, an ongoing trial explores the effects of daily subcutaneous injection with an FSH analogue on spontaneous pregnancies (clinicaltrails.gov: NCT05403476). Foolicle-stimulating hormone is a potent regulator of AMH production through activation of nuclear factor kappa-B (43). Interestingly, a novel study has shown that receptor activators of nuclear factor kappa-B ligand (RANKL) signaling regulate male reproductive function through Sertoli–germ cell interaction (44). This study showed positive effects on semen quality of inhibition of RANKL with denosumab, a drug used to treat osteoporosis, in a subgroup of infertile men with higher levels of serum AMH concentrations, indicating serum AMH concentrations may be used to select infertile men who would benefit from treatment with an inhibitor of RANKL signaling. All participating men in our study have been screened for eligibility for the ongoing First in Treating Male Infertility Study, an RCT designed to investigate the effects of denosumab on sperm concentration in infertile men, where serum AMH concentration is used as a marker to select the men with a higher likelihood of a positive response to the treatment and as a marker of treatment outcome (19).

CONCLUSION

In conclusion, we show that a low-serum AMH concentration is a marker of impaired sperm production in a cohort of infertile men, which may have clinical relevance. Serum AMH concentrations may provide insight into gonadal function and Sertoli cell capacity in infertile men, and future studies are warranted to investigate this further.

CrediT Authorship Contribution Statement

Rune Holt: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. Sam Kafai Yahyavi: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. Gustav Wall-Gremstrup: Investigation. Mads Joon Jorsal: Writing – review & editing. Frederikke Bay Toft: Writing – review &

editing. Niels Jørgensen: Writing – review & editing. Anders Juul: Writing – review & editing. Martin Blomberg Jensen, Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing.

Declaration of Interests

R.H. has nothing to disclose. S.K.Y. has nothing to disclose. G.W.G. has nothing to disclose. M.J. has nothing to disclose. F.T. has nothing to disclose. N.J. has nothing to disclose. A.J. has nothing to disclose. M.B.J. reports funding from Innovationsfonden, Novo Nordisk, and XY Therapeutics; consulting fees from Novo Nordisk, Gedeon Richter, Merck, XY Therapeutics, Amgen; Three patents on RANKL and fertility and one on using AMH as a marker to select infertile men for stimulatory treatments have been granted; the spin-out company XY Therapeutics is trying to develop RANKL inhibitors as a novel treatment option; M.B.J. is the inventor of the patent, which is outlicensed to the Company XY therapeutics where he is CEO. M.B.J. has also been on advisory boards of Novo Nordisk, FAES Pharma and Vertex.

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La hormona antimülleriana sérica baja está relacionada con mala calidad del semen en hombres infértiles seleccionados para participar en un ensayo aleatorizado controlado.

Objetivo: Investigar las posibles asociaciones entre la concentración sérica de la hormona antimüulleriana (AMH) y la calidad del semen en hombres infértiles. Los estudios que investigan las asociaciones entre la concentración sérica de la AMH y la calidad del semen en hombres infértiles han mostrado resultados contradictorios.

Diseño: Se incluyeron hombres infértiles durante la selección para participar en el First in Treating Male Infertility Study, un ensayo controlado aleatorizado, doble ciego, controlado con placebo, 1:1, en un solo centro.

Entorno: No aplicable.

Pacientes: En la visita de cribado, 400 participantes produjeron una muestra de semen y se les analizó el suero para determinar la concentración de AMH.

Intervención: No procede.

Principales medidas de resultado: Concentración sérica de AMH y calidad del semen.

Resultados: Todos los hombres fueron estratificados según las concentraciones séricas de AMH en cuartiles (Q1-Q4). Los hombres del cuartil más bajo tenían una concentración de espermatozoides (1 x 10^6/mL) más baja (Q1: 8,0 frente a Q2: 10,4 frente a Q3: 11,0 frente a Q4: 13,0), un recuento total de espermatozoides (1 x 10^6) (Q1: 29,1 frente a Q2: 38,2 frente a Q3: 44,4 frente a Q4: 13,0), una motilidad espermática (%) (Q4: 41 frente a Q4: 55,7) y una concentración de espermatozoides (1 x 10^6/mL) más baja (Q1: 8,0 frente a Q2: 10,4). Q4: 55,7), motilidad espermática (%) (Q1: 41 vs. Q2: 57 vs. Q3: 50 vs. Q4: 53) y motilidad espermática progresiva (%) (Q1: 31 vs. Q2: 44 vs. Q3: 35 vs. Q4: 40) en comparación con los demás cuartiles. Además, los hombres con una concentración de espermatozoides <2 millones/mL tenían una concentración sérica de AMH inferior en comparación con los hombres que tenían 2-16 x 10^6/mL y >16 10^6/mL (31 pmol/L vs. 38 pmol/L vs. 43 pmol/L, respectivamente). Asimismo, los hombres con motilidad espermática <20% tenían una concentración sérica de AMH inferior en comparación con los hombres con motilidad espermática 20%-42% y >42% (31 pmol/L vs. 43 pmol/L. vs. 39 pmol/L, respectivamente).

Conclusiones: Este estudio muestra que la baja concentración sérica de AMH se asocia con mala calidad del semen en hombres infértiles, lo que implica que la concentración sérica de AMH puede tener valor clínico durante la evaluación de la infertilidad masculina.

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Effect of postthaw change in embryo score on single euploid embryo transfer success rates

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Objective: To assess whether the change in embryo morphology from precryopreservation to postthaw is associated with the embryo transfer success rates in single euploid embryo transfer cycles.
Design: Retrospective cohort study.
Setting: Academic affiliated fertility clinic.
Patient(s): Patients who underwent a single euploid embryo transfer cycle from September 2016 to April 2022 were included. A decision support tool was used to assign each embryo a reproductive potential score on the basis of the day of biopsy, expansion, and grade of trophectoderm and inner cell mass at the time of cryopreservation and after thaw. Embryos were divided into 4 groups: group 1 included embryos with the same score after thaw (reference); group 2 included those with a higher score; group 3 included those with a lower score; and group 4 included those that did not re-expand after thaw.
Intervention(s): No interventions administered.
Main Outcome Measure(s): The primary outcome was the live birth rates (LBRs) per embryo transfer. The secondary outcomes included the chemical pregnancy, clinical pregnancy, and clinical pregnancy loss rates. Comparative statistics and univariate analyses were performed using the Kruskal-Wallis and χ^2tests. Multivariate logistic regression fitted with generalized estimating equation was performed to compare the odds of live birth between groups.
Result(s): A total of 7,750 embryo transfers performed for 4,613 patients met inclusion criteria: 5,331 in group 1; 486 in group 2; 1,726 in group 3; and 207 in group 4. In the univariate analysis, there was a statistically significant difference in the LBR between groups 1, 2, 3, and 4 (55.8% vs. 51.4%, 47.5%, and 26.6%). Logistic regression controlling for oocyte age, antimüllerian hormone, body mass index, endometrial thickness, year of embryo transfer, time from thaw to final grading, and embryo score before cryopreservation showed significantly lower odds of live birth when the embryo was downgraded (odds ratio [OR], 0.70; confidence interval [CI], 0.62–0.79) or did not re-expand (OR, 0.36; CI, 0.26–0.51) than those with no change in score. When controlling for all variables, there was a significant increase in the odds of live birth between embryos that had a higher score after thaw and those without a change (OR, 1.42; CI, 1.14–1.76). There was no significant difference in the clinical pregnancy loss rate among the 4 groups.
Conclusion(s): The change in the quality of the embryo after thaw is an important factor in embryo transfer success. In an adjusted analysis, the chemical and clinical pregnancy rates and LBR per embryo transfer all significantly decrease in embryos that were downgraded or did not expand on the day of single euploid embryo transfer. Embryos that re-expand and have improved quality after thaw have the highest odds of live birth. (Fertil Steril® 2024;122:288–96. ©2024 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Embryo vitrification, embryo grading, single euploid embryo transfer

The first successful live birth from embryo cryopreservation, the process of freezing embryos in liquid nitrogen, resulted in 1983 (1). Although cryopreservation initially employed a slow-freeze technique, the development of vitrification increased the cooling rate, reduced the volume of cryoprotectant, and minimized the production of ice crystals by solidifying the sample into a noncrystalline phase. By eliminating the crystalline phase, embryos avoid osmotic changes that can cause ice crystal formation and cellular damage (2). Vitrification has been shown to be superior to slow freeze with regard to the thaw survival and clinical pregnancy rates per cycle (3).

Before an embryo being vitrified, morphological analysis is conducted to evaluate the embryo. The Gardner grading system evaluates embryos on the basis of blastocele expansion and hatching status, size and compactness of inner cell mass (ICM), and cohesiveness and number of trophectoderm cells (4). This widely used system is based on

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visual information obtained by an embryologist, making it subject to variability (5). Each parameter is scored independently according to the following modified Gardner criteria. Expansion is graded from 1–6, with 1 being early blastocyst development, 4 being <50% hatched from the zona pellucida, and 6 being hatched out of the zona pellucida. The ICM and trophectoderm are graded from A to D. For blastocysts graded as 3–6 (i.e., full blastocysts onward), the development of the ICM was assessed on the following scale: A, is tightly packed and has many cells, to D, has very few cells. The trophectoderm was assessed on the following scale: A, has many cells forming a cohesive epithelium, to D, has very few large cells. The final alphanumeric score for each embryo is composed of the score assigned from each parameter (6). The use of the Gardner scoring system has revealed a strong correlation between the morphology of blastocysts and implantation and pregnancy rates (7). It also assists in selecting the single highest scoring embryo for transfer to reduce the number of in vitro fertilization pregnancies resulting in multiple gestations.

At our institution, we use a modified Gardner grading system, as previously described, in addition to an internal scoring system on the basis of the day of embryo cryopreservation, expansion, ICM, and trophectoderm grade as an embryo selection support tool (8). This tool, using a composite score of these factors, was created on the basis of internal data on the implantation, ongoing pregnancy, and live birth rates (LBRs) (9). The tool supports the embryologist's decision making by providing a total score and rank of a patient's embryos to choose the best embryo for transfer.

Both the Gardner scoring system and our internal scoring system evaluate embryos' reproductive potential on the basis of characteristics at the time of cryopreservation. When thawed, embryos use their energy to re-expand and should resume the stage of cell division they were in before cryopreservation. However, the extent of re-expansion and grading may change after embryo thaw (10–19). The objective of this study was to assess whether the change in overall embryo morphology after thaw, as indicated by a change in score, is associated with the embryo transfer success rates in single euploid embryo transfer cycles.

MATERIALS AND METHODS

Participants and study design

This was a retrospective, single-academic-center study that included single euploid embryo transfer cycles from September 2016 to April 2022. Cycles were included if a single, euploid, autologous frozen embryo transfer in a synthetic endometrial preparation cycle was performed. Patients using donor oocytes, gestational carriers, rebiopsied embryos, or mosaic embryos or with a diagnosis of intrauterine synechiae (Asherman syndrome), uterine malformations, uterine fibroids, and recurrent pregnancy loss were excluded. Multiple cycles for individual patients were included.

Demographic and cycle information included age, body mass index (BMI), antimüllerian hormone (AMH), endometrial thickness before progesterone start, and year of embryo transfer. Embryo information included the day of blastocyst biopsy and cryopreservation, extent of embryo expansion, quality of the ICM and trophectoderm before cryopreservation and after thaw, and time from embryo thaw to final grading at the time of embryo transfer.

All embryos were routinely given an alphanumeric grade before embryo cryopreservation. At our institution, a decision support tool on the basis of previous published data is used to rank a patient's embryos to determine the embryo with the highest reproductive potential for transfer (9). This tool is based on the modified Gardner grading. To create the initial algorithm, as described by Friedenthal et al. (9), a mixed-effect logistic model for the outcome of implantation was created by analyzing single euploid embryo transfer cycles on the basis of embryo grading before cryopreservation. The embryo day of biopsy/cryopreservation, expansion, morphology of ICM, and morphology of trophectoderm were the parameters used to predict the probability of implantation. Odds ratios (ORs) from these models were then used as weighted multipliers to create a composite score on the basis of the parameters for each embryo (9).

In this study, the decision support tool was used to assign each individual embryo a score at the time of cryopreservation and a score on the basis of grading after thaw. These scores were compared with determine whether the postthaw embryo was graded the same as it had been before cryopreservation or whether it was given a higher or lower overall grade. This change was determined by comparing the scores generated by the decision support tool. Embryos that did not re-expand after thaw were categorized separately. These embryos were determined to have not re-expanded on the basis of the lack of blastocoel cavity. Therefore, they could not be assigned a morphological grade.

Embryos in group 1 had the same score before cryopreservation and after thaw. Group 2 included embryos that had a higher (improved) score after thaw, group 3 included embryos that had a lower (poorer) score after thaw, and group 4 included embryos that did not re-expand after thaw and, thus, were not given grades for ICM or trophectoderm and, as a result, could not be scored.

The chemical pregnancy, clinical pregnancy, and clinical pregnancy loss rates and LBRs were calculated and compared between groups. This study was approved by the Institutional Review Board at Icahn School of Medicine at Mount Sinai, with a waiver of consent for retrospective analysis of deidentified data.

Procedures

All patients underwent in vitro fertilization stimulation cycles with treatment protocols at the discretion of their physician. Patients underwent controlled ovarian hyperstimulation, as previously described (18). Final trigger shot of compounded human chorionic gonadotropin or gonadotropin-releasing hormone agonist or both were administered for final oocyte maturation when patients met criteria on the basis of ultrasound findings and estradiol levels (20). The vaginal oocyte retrieval was performed 36 hours after trigger administration (20). Oocytes were stripped of cumulus cells and fertilized using intracytoplasmic sperm injection. Injected oocytes were

checked 1 day after vaginal oocyte retrieval for fertilization, and embryos were cultured out to the blastocyst stage up to day 7, as needed. Laboratory procedures regarding embryo culture and biopsy techniques were previously described by Hernandez-Nieto et al (8). When the embryos reached blastocyst stage appropriate for biopsy and cryopreservation, they were given a morphological modified Gardner system grade on the basis of the extent of embryo expansion and quality of ICM and trophectoderm (8). Day 5 embryos in our laboratory are routinely graded the afternoon of biopsy, and day 6 and 7 embryos are graded the morning of biopsy. Embryos are re-examined at the time of biopsy and given a final grade before biopsy and cryopreservation. Embryos then typically undergo vitrification within 1 hour after biopsy. Our institution began routinely collapsing any embryos that re-expanded before vitrification on January 1, 2021.

Biopsied samples were sent out for preimplantation genetic testing for aneuploidy (PGT-A) using next-generation sequencing. Multiple PGT-A reference laboratory test results were used over this time period. The results on the presence of aneuploidy, euploidy, or mosaicism or an indeterminate result was reported by the PGT-A laboratory.

For standardization and per typical clinical practice, single euploid embryo transfers in this study were performed in a synthetic preparation cycle. The uterine cavity was prepared with micronized oral estradiol (Estrace; Teva Pharmaceuticals, Parsippany, NJ) 2 mg twice daily for 4 days and then 2 mg 3 times daily, with additional dosing regimens per physician discretion. After a minimum of 9 days of estradiol administration, transvaginal ultrasonography was performed to assess endometrial thickness. When an adequate thickness was achieved, with a goal of at least 8 mm, 50 mg of intramuscular progesterone in oil (Watson Pharma, Inc., Parsippany, NJ) or a combination of 100 mg of vaginal progesterone twice daily and 200 mg of oral progesterone 3 times daily was administered. After starting the progesterone, patients were brought in 1–3 days before embryo transfer for a final ultrasound to ensure no contraindication for embryo transfer, such as fluid in the cavity, and evaluate progesterone levels. For all cases, thawing and transfer of the embryos were performed on the sixth day of progesterone supplementation regardless of the day of embryo development at the time of cryopreservation (21).

When more than 1 embryo was available for thaw, the embryo chosen for transfer is determined by the embryologist with the use of the scoring support tool. The score is generated on the basis of the embryo's reproductive potential, as determined in previously published work on our center's experience with embryos of similar age, expansion, and grades. The scoring model is a composite score and may reflect a change in the expansion, ICM, or trophectoderm after thawing. The individual parameter that changed was not the focus of this study because the combination of factors allows for a more complete assessment on embryo reproductive potential. This scoring system was described in more detail by Friedenthal et al. (9), and the heat map from that study is shown in Supplemental Figure 1 (available online) for further understanding of the comparative reproductive potential of embryos at our clinic.

The embryo chosen for thaw underwent a standard warming process (22). After thaw, embryologists are assessing for blastocoel re-expansion and embryo morphology and ensuring that the embryo survived the thawing process. They are evaluating the quality of the cells in the embryo—the size, cellular membranes, and signs of necrosis and degeneration. Those that do not survive typically have lysed cells with degenerate cytoplasm. In such cases, the patient would be made aware, and a second embryo would be thawed, or the transfer would be cancelled. The embryo thaw survival rate in our laboratory between 2016 and 2022 was 97.5%. Embryos were given a final expansion and morphology grade at the time of transfer. This is the grade that is placed in the electronic medical record and used in this study for comparison. The time of embryo thaw and embryo transfer was documented, and time lapse between thaw and final grading was calculated. The embryologists who provided the grade before cryopreservation and after thaw were also documented.

The embryo transfer procedure itself was performed in the operating room, without anesthesia and under transabdominal ultrasound guidance. The typical protocol is to perform trial transfer followed by direct transfer using the Wallace 18 catheter; however, physicians can use their discretion for the use of more rigid catheters or after-load technique (8).

Outcome measures

The primary goal was to determine the association between a change in embryo score after thaw and LBR per embryo transfer.

The secondary outcomes analyzed were the chemical pregnancy rate (positive β-human chorionic gonadotropin per embryo transfer), clinical pregnancy rate (presence of gestational sac(s) on ultrasound per embryo transfer), and clinical pregnancy loss rate (pregnancy loss after visualization of a gestational sac on ultrasound). Embryos with the same score before cryopreservation and after thaw were considered the reference group (group 1). All outcomes for embryos with a higher score after thaw (group 2), embryos that were downgraded after thaw (group 3), and embryos that did not re-expand after thaw (group 4) were all compared with those for group 1.

Statistical analysis

Statistical analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC). Patient, cycle, and embryo specific data were compared between the groups. Continuous data were reported as means $\pm$ standard deviations with the Clopper-Pearson binomial 95% confidence intervals (CIs). Comparative statistics were performed using the Kruskal-Wallis test for continuous data. Multiple comparison analysis on the basis of the post hoc Dwall-Steel-Critchlow-Fligner method pairwise 2-sample Wilcoxon comparisons was also performed, using the pairwise comparison to evaluate where significant differences between specific groups were.

Univariate analysis was performed using the χ^2test to compare the chemical pregnancy, clinical pregnancy, and clinical pregnancy loss rates and LBR between all 4 groups.

Bonferroni correction was used for categorical outcomes, adjusting the *P* value to $<.016$ (for 3 comparisons).

A multivariate logistic regression analysis fitted with a generalized estimating equation (GEE) was performed on the primary outcome of live birth per embryo transfer and secondary outcomes of chemical pregnancy and clinical pregnancy per embryo transfer and clinical pregnancy loss per pregnancy. Analysis was conducted controlling for oocyte age, AMH, BMI, endometrial thickness at the time of progesterone initiation, year of embryo transfer, time from embryo thaw to embryo transfer, and embryo score at the time of cryopreservation. The GEE was used to account for the presence of individual patients with multiple cycles. Adjusted ORs for all cycle outcomes were calculated with group 1 being the reference group.

RESULTS

After applying the inclusion and exclusion criteria, a total of 4,613 unique patients who underwent a total of 7,750 single euploid embryo transfer cycles were included: 5,331 cycles in group 1 (no change in score, 68.7%); 486 in group 2 (higher score, 6.3%); 1,726 in group 3 (downgraded score, 22.3%); and 207 in group 4 (lack of re-expansion, 2.7%). Demographic and cycle data are presented in Table 1. There was a significant difference in oocyte age and AMH level between groups. There was no significant difference in the time from embryo thaw to embryo transfer when the final grade was given. The mean times from thaw to final embryo grading in groups 1–4 were 4 hours and 34 minutes, 4 hours and 32 minutes, 4 hours and 37 minutes, and 4 hours and 30 minutes, respectively ($P=.410$).

The embryologists who graded the embryo before cryopreservation and after thaw were recorded. The embryologist who graded the embryo after thaw was different from the embryologist who graded the embryo before cryopreservation 98.2% of the time. The likelihood of a different embryologist performing grading was not different between groups ($P=.781$). When the same embryologist graded the embryo before cryopreservation and after thaw, there was a change in embryo score 28.1% of the time, which was similar to when a different embryologist graded after thaw, with a change in score 31.3% of the time ($P=.438$).

Embryo scores before cryopreservation were significantly different among groups. The mean embryo scores in groups 1–4 were 3.57 ± 1.17, 2.29 ± 0.89, 3.62 ± 1.12, and 2.37 ± 1.37, respectively ($P\leq.0001$). Pairwise analysis showed that a significant difference was found between groups 1 and 2 and groups 1 and 4. There was no significant difference in embryo score before cryopreservation between groups 1 and 3 ($P=.683$) or groups 2 and 4 ($P=.998$). Thus, groups 2 and 4 had similarly low scores before cryopreservation yet were in different groups because of the different direction of change.

Cycle outcomes were calculated for each group individually. In the univariate analysis, there was a statistically significant difference in the LBR between the groups. The LBRs in groups 1–4 were 55.8%, 51.4%, 47.5%, and 26.6%, respectively ($P\leq.00001$). Similarly, there was a significant difference between the chemical pregnancy rate and clinical

TABLE 1

Patient, cycle, and embryo characteristics.

Variables	Group 1 (n = 5,331, 68.7%)	Group 2 (n = 486, 6.3%)	Group 3 (n = 1,726, 22.3%)	Group 4 (n = 207, 2.7%)	*P* value
Oocyte age (y)	35.4 ± 3.9	35.6 ± 4.0	35.6 ± 3.9	36.1 ± 3.8	.009
BMI (kg/m^2)	24.2 ± 4.5	24.5 ±4.9	24.2 ± 4.6	24.1 ± 4.4	.65
AMH (ng/mL)	3.5 ± 3.4	3.0 ± 2.8	3.4 ± 3.6	3.3 ± 4.3	.003
Endometrial thickness (mm)	9.2 ± 1.6	9.2 ± 1.5	9.2 ± 1.5	9.1 ± 1.5	.555
Time from embryo thaw to embryo transfer (hours and minutes)	4 h and 34 min ± 58 min	4 h and 32 min ± 52 min	4 h and 37 min ± 58 min	4 h and 30 min ± 1 h and 2 min	.410
Embryo score at cryopreservation	3.5 ± 1.1	2.2 ± 0.8	3.6 ± 1.1	2.3 ± 1.3	<.0001
Embryo score at ET	3.57 ± 1.1	3.0 ± 1.1	2.9 ± 0.9	—	<.0001

Note: Group 1, no change in score from precryopreservation to postthaw; group 2, improved score after thaw; group 3, downgraded score after thaw; and group 4, lack of re-expansion resulting in no score after thaw. AMH = antimüllerian hormone; BMI = body mass index; ET = embryo transfer.

Bergin. Postthaw embryo score changes. Fertil Steril 2024.

TABLE 2

Embryo transfer outcomes.

Outcomes	Group 1 (n = 5,331)	Group 2 (n = 486)	Group 3 (n = 1,726)	Group 4 (n = 207)	*P* value
Chemical pregnancy rate (%) (n)	78.8% (4,202)	73.8% (359)	68.9% (1,190)	43.0% (89)	<.0001
Clinical pregnancy rate (%) (n)	66.0% (3,519)	62.3% (303)	57.9% (1,000)	34.3% (71)	<.0001
Clinical pregnancy loss rate (%) (n)	15.4% (542)	17.5% (53)	18.1% (181)	22.5% (16)	.079
Live birth rate (%) (n)	55.8% (2,977)	51.4% (250)	47.4% (819)	26.5% (55)	<.0001

Note: The pregnancy, clinical pregnancy, and live birth rates were calculated per embryo transfer. The clinical pregnancy loss rate was calculated per clinical pregnancy, defined as visualization of gestational sac on ultrasound.

Bergin. Postthaw embryo score changes. Fertil Steril 2024.

pregnancy rate per embryo transfer between the 4 groups, as shown in Table 2. For all patients with clinical pregnancies, clinical pregnancy loss was not statistically significantly different between groups. The clinical pregnancy loss rates in groups 1–4 were 15.4%, 17.5%, 18.1%, and 22.5%, respectively (P=.079). All outcomes are represented in Table 2 and Figure 1.

Multivariate logistic regression fitted with GEE and controlling for oocyte age, AMH, BMI, endometrial thickness, year of embryo transfer, time from thaw to final embryo grading, and embryo score at the time of cryopreservation showed significantly lower odds of live birth when the embryo was downgraded (OR, 0.70; CI, 0.62–0.79; $P\leq$.0001) or did not re-expand (OR, 0.36; CI, 0.26–0.51; $P\leq$.0001) than those with no change in score. There was a significant improvement in the odds of live birth between embryos that had an improved score and those without a change (OR, 1.42; CI, 1.14–1.76; P=.002).

Groups 2 and 1 showed similar odds of chemical pregnancy (OR, 1.17; CI, 0.91–1.51; P=.22). However, embryos in group 2 with an improved score after thaw had higher clinical pregnancy rates per embryo transfer (OR, 1.43; CI, 1.13–1.79; P=.002). Clinical pregnancy loss was not different between these groups (OR, 0.98; CI, 0.68–1.40; P=.891).

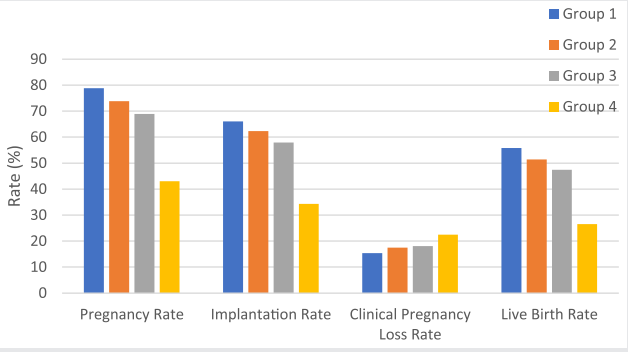
Group 3 showed significantly lower odds of chemical pregnancy (OR, 0.57; CI, 0.50–0.66; $P\leq$.0001) and clinical pregnancy (OR, 0.71; CI, 0.63–0.80; $P\leq$.0001) per embryo transfer than group 1. Clinical pregnancy loss was not different between these groups (OR, 1.09; CI, 0.90–1.31; P=.376).

Group 4 showed significantly lower odds of chemical pregnancy (OR, 0.26; CI, 0.20–0.36; $P\leq$.0001) and clinical pregnancy (OR, 0.35; CI, 0.25–0.48; $P\leq$.0001) per embryo transfer than group 1. Clinical pregnancy loss was not different between these groups (OR, 0.75; CI, 0.42–1.34; P=.339).

The multivariate regression effect size and CIs for each outcome are shown in Figure 2.

Although our protocol on routinely recollapsing any embryos that re-expand between biopsy and cryopreservation changed during this study (2021), we did include year in our logistic regression to attempt to account for changes in the laboratory over time. Additionally, a subanalysis was performed on the outcome of live birth by group for the years before routine collapsing (2016–2020) and the years after routine collapsing (2021–2022), and the findings were unchanged.

FIGURE 1



Univariate analysis on embryo transfer outcomes by group.

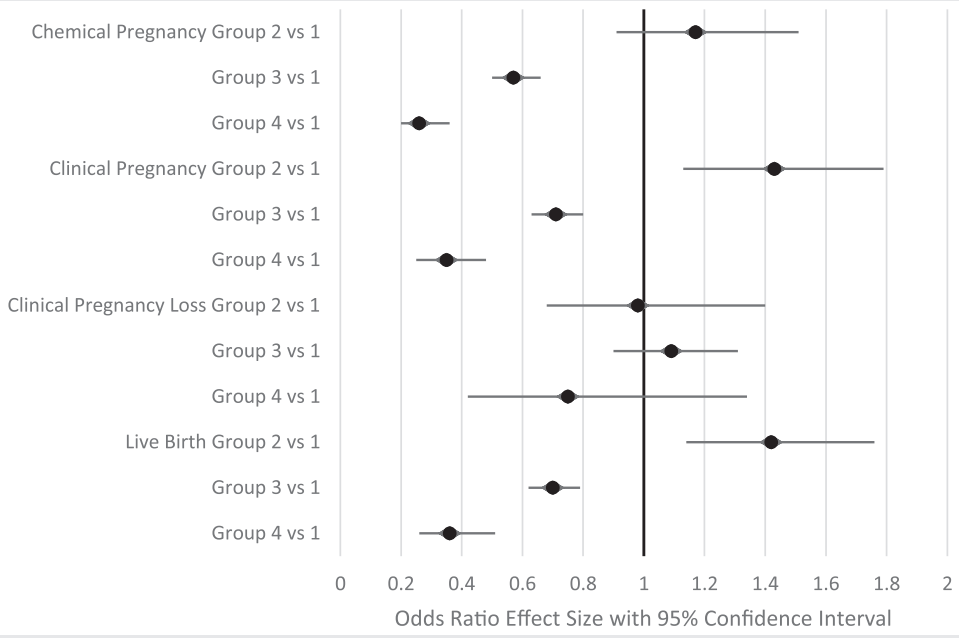
Bergin. Postthaw embryo score changes. Fertil Steril 2024.

DISCUSSION

In the retrospective study by Gardner et al. (7) that validated the Gardner Schoolcraft scoring system, embryo transfers involving high-scoring embryos were shown to result in the highest pregnancy rates. Since then, numerous studies have been published showing that the embryo scores on the basis of morphology are associated with embryo transfer outcomes (21, 23–25). Ultimately, selecting the most favorable embryo increases the chance of live birth per embryo transfer, decreasing the number of transfers required for successful live birth, and saving patients' time and money, as well as physical and emotional energy that assistive reproductive technology requires. With the development and utilization of PGT, most embryos in our practice undergo the cryopreservation and thawing process. Data on how morphology and expansion may be affected by the cryopreservation and thawing process are limited. Postthaw embryo quality assessment may provide additional information that may be useful for clinicians and their patients in predicting success of embryo transfer after thaw and understanding outcomes.

Our institution's scoring system includes parameters that are commonly used: day of blastocyst vitrification; degree of

FIGURE 2



Odds of chemical pregnancy, clinical pregnancy, clinical pregnancy loss, and live birth between groups using multivariate regression analysis fitted with a generalized estimating equation and adjusted for oocyte age, body mass index, antimüllerian hormone, endometrial thickness, year of embryo transfer, time from embryo thaw to grading, and embryo score before cryopreservation.

Bergin. Postthaw embryo score changes. Fertil Steril 2024.

expansion; ICM; and trophectoderm quality. The parameters are combined into a score that has been previously studied and supported (9). The benefits of using a composite score allows the embryo to be graded as a whole on the basis of known embryo transfer success rates for that combination of parameters, rather than relying on a change in 1 aspect of the embryo. This support tool allows for distinction between embryos with similar grading, e.g., when some parameters are the same and others are different between embryos and it is not clear which parameter to prioritize. The tool takes away subjectivity in prioritization of a single component with the use of a score on the basis of the combination of parameters.

In this large cohort study, we examined the association of change in postthaw euploid embryo parameters and assistive reproductive technology outcomes. We used the described scoring system to facilitate comparability between all embryo parameters as a whole before cryopreservation and after thaw. Previous studies examining postthaw embryo parameters have examined independent grades and re-expansion as indicators of embryo potential (10, 11). Studies have found that the degree of re-expansion was correlated with the clinical pregnancy rates and LBRs, indicating that blastocyst re-expansion after thaw is an important factor (12–14). Coello et al. (19) more specifically found that the initial and minimum blastocele areas were the most predictive of implantation of those studied. Day of blastocyst vitrification has consistently been shown to be associated with outcomes, with slower growing embryos resulting in lower success rates (8, 15–18). Sekhon et al. (10) showed that a downgrade in ICM specifically was associated with lower odds of implantation.

In the current study, when controlling for variables including embryo score before cryopreservation, embryos that had an improved score after thaw had higher odds of clinical pregnancy and live birth. Although those embryos with an improved, upgraded score had a lower overall score than those with no change, there were higher odds of clinical pregnancy and live birth in the adjusted analysis. This suggests that the absolute score of the embryo after thaw may not be as predictive of embryo transfer outcome as how the score changed from the time of cryopreservation. It is possible that expectations for embryos that may be considered poor quality either before cryopreservation or after thaw could be adjusted when evaluating the change in embryo morphology after thaw.

Embryos that were downgraded overall in the scoring model had significantly lower odds of pregnancy, clinical pregnancy, and live birth. Despite beginning as high-quality embryos, the decrease in score after thaw was associated with lower LBRs compared with embryos that stayed the same or were upgraded. Although they had a similar absolute postthaw grade as the embryos that improved, their pregnancy outcomes were significantly lower. This, again, provides insight on how the change in score is meaningful. Embryos that were not given a grade because of their lack of re-expansion had the lowest pregnancy rates and LBRs among all groups.

Despite the differences in the pregnancy and clinical pregnancy rates and LBR, physicians and patients can be reassured that there was no difference in the clinical pregnancy loss rates between the groups. When a gestational sac is identified on ultrasound, the change in embryo grading after thaw did not appear to impact the clinical pregnancy loss rates.

Overall, the change in quality after thaw may reflect the intrinsic ability of the embryo to implant. The vitrification and thawing process may act as a "stress test" for embryos. Embryos that maintain their quality through the cryopreservation process and continue to improve after thaw appear to have a higher chance of pregnancy and live birth. Embryos that begin as high-quality embryos before cryopreservation but then have lower quality after thaw have lower pregnancy rates and LBRs than those with the same initial score that maintain their grading after thaw. Embryos that lack the ability to re-expand enough to receive an ICM or trophectoderm grade after thaw have the lowest odds of resulting in pregnancy and live birth. This situation may occur because of inherent quality issues of the embryo; however, the laboratory technique cannot be ruled out.

Embryos that are downgraded or do not re-expand after thaw still have clinically significant LBRs and should continue to be used with an understanding of these findings. Although at this time it is not possible to predict how an embryo will perform after thaw, exploration of modifiable and nonmodifiable factors associated with a change in embryo score is an area of future research. This would allow patients to be counseled more thoroughly and provide opportunity for more personalized care.

The strengths of our study include the number of cycles and use of an internally validated scoring system to quantify the overall quality of an embryo at different times. The inclusion of the time of embryo thaw to time of final embryo grading strengthens our study by showing that there was no difference in the time that the embryos had opportunity to re-expand and be regraded. The limitation of our study include its retrospective nature and the inherent subjectiveness of the embryo grading process that leads to the embryo score, which is encountered in any study using the Gardner scoring system. In our institution, embryos were often graded by a different embryologist before cryopreservation and after thaw; however, our embryologists undergo extensive internal training on the embryo grading process for consistency. In addition, we analyzed the data on the embryologist performing the grading before cryopreservation and after thaw, and there were similar rates of embryo grade change whether the embryo was graded by the same embryologist or a different one after thaw. The frequency of having a different embryologist perform the grading after thaw was also similar between groups. Further studies may include the use of captured images before cryopreservation and after thaw for consistent grading purposes.

CONCLUSION

Grading embryos after thawing provides clinically useful information on embryo potential. Embryos that retain the same score or are upgraded are more likely to result in live births than those that are downgraded or do not re-expand. Directionality of the change in the score after thaw may be helpful in anticipating likelihood of embryo transfer success on the day of the procedure or retrospectively understanding outcomes.

Scores assigned after thawing may provide useful insights into a patient's fertility because recurrence of downgraded or collapsed embryos may be reflective of an intrinsic process that is contributing to pregnancy success. Further studies are needed to confirm this relationship. Sibling embryo comparisons may allow for distinguishing whether the change in score after thaw is entirely embryo specific or persists in a cohort of embryos. As artificial intelligence becomes more widely used, computers may be able to assist with more consistent and objective scores for embryos, reducing interobserver and intraobserver biases.

CRediT Authorship Contribution Statement

Keri Bergin: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft; **William Borenzweig:** Data curation, Writing – original draft; **Sarah Roger:** Writing – original draft; **Richard Slifkin:** Methodology, Writing – review & editing; **Morgan Baird:** Data curation; **Joseph Lee:** Writing – review & editing; **Alan B. Copperman:** Methodology, Writing – review & editing; **Erkan Buyuk:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of Interests

K.B. has nothing to disclose. W.B. has nothing to disclose. S.R. has nothing to disclose. R.S. has nothing to disclose. M.B. has nothing to disclose. J.L. has nothing to disclose. A.B.C. reports advisory board for Progyny. E.B. has nothing to disclose.

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Efecto del cambio en la puntuación del embrión después de la descongelación en las tasas de éxito de la transferencia de embrión único euploide.

Objetivo: Evaluar si el cambio en la morfología embrionaria desde antes de la criopreservación hasta después de la descongelación se asocia con las tasas de éxito de la transferencia embrionaria en ciclos de transferencia de un solo embrión euploide.

Diseño: Estudio de cohorte retrospectivo.

Entorno: Clínica de fertilidad afiliada a una institución académica.

Paciente(s): Se incluyeron pacientes que se sometieron a un ciclo de transferencia de un solo embrión euploide desde septiembre de 2016 hasta abril de 2022. Se utilizó una herramienta de apoyo en la toma de decisiones para asignar a cada embrión una puntuación de potencial reproductivo en función del día de la biopsia, la expansión y las puntuaciones del trofoectodermo y de la masa celular interna en el momento de la criopreservación y después de la descongelación. Los embriones se dividieron en 4 grupos: el grupo 1 incluyó embriones con la misma puntuación después de la descongelación (referencia); el grupo 2 incluyó aquellos con una puntuación más alta; el grupo 3 incluyó aquellos con una puntuación más baja; y el grupo 4 incluyó aquellos que no volvieron a expandirse después de la descongelación.

Intervención(es): No se realizaron intervenciones.

Medida(s) de Resultado Principal(es): El resultado principal fue la tasa de nacidos vivos (LBR) por transferencia de embriones. Los resultados secundarios incluyeron las tasas de embarazo químico, de embarazo clínico y de aborto clínico. Se realizaron estadísticas comparativas y análisis univariantes utilizando las pruebas de Kruskal-Wallis y χ^2. Se realizó una regresión logística multivariante ajustada con un modelo de ecuaciones de estimación generalizada para comparar las probabilidades de nacido vivo entre los grupos.

Resultado(s): Un total de 7.750 transferencias de embriones realizadas en 4.613 pacientes cumplieron con los criterios de inclusión: 5.331 en el grupo 1; 486 en el grupo 2; 1.726 en el grupo 3; y 207 en el grupo 4. En el análisis univariante, hubo una diferencia estadísticamente significativa en la LBR entre los grupos 1, 2, 3 y 4 (55.8% vs. 51.4%, 47.5% y 26.6%). La regresión logística controlando por edad del ovocito, hormona antimulleriana, índice de masa corporal, grosor endometrial, año de transferencia del embrión, tiempo desde la descongelación hasta la calificación final y puntuación del embrión antes de la criopreservación mostró probabilidades significativamente menores de nacido vivo cuando el embrión bajo de grado (razón de probabilidades [OR], 0.70; intervalo de confianza [CI], 0.62–0.79) o no se re-expandió (OR, 0.36; CI, 0.26–0.51) en comparación con aquellos sin cambio en la puntuación. Al controlar todas las variables, hubo un aumento significativo en las probabilidades de nacido vivo entre los embriones que tuvieron una puntuación más alta después de la descongelación en comparación con aquellos sin cambio (OR, 1.42; CI, 1.14–1.76). No hubo una diferencia significativa en la tasa de aborto clínico entre los 4 grupos.

Conclusión(es): El cambio en la calidad del embrión después de la descongelación es un factor importante en el éxito de la transferencia de embriones. En un análisis ajustado, las tasas de embarazo químico y clínico y la LBR por transferencia embrionaria de embrión único euploide disminuyen significativamente en los embriones que se redujo de grado de calidad morfológica o no se expandieron el día de la transferencia. Los embriones que vuelven a expandirse y mejoran su calidad después de la descongelación tienen las mayores probabilidades de nacido vivo.

Check for updates

Development and validation of an automated robotic system for preparation of embryo culture dishes

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Objective: To study the development and clinical validation of the ART Pipetting Robot for the IVF Laboratory (APRIL), a liquid-handling robot customized for the precise preparation of microdroplet culture dishes in the field of in vitro fertilization (IVF).
Design: A prospective randomized study conducted at an academic IVF center comparing mouse and human embryo outcomes and quantitative measures of accuracy in embryo dishes prepared using APRIL compared with standard manual preparation.
Setting: Academic IVF center.
Subjects: The study involved the assessment of the automated culture dish preparation system, APRIL, compared with manual preparation methods in the context of IVF treatment.
Intervention: ART Pipetting Robot for the IVF Laboratory is an enclosed liquid-handling robot equipped with custom three-dimensional–printed adapters and designed to dispense embryo culture media and mineral oil into microdroplet culture dishes.
Main Outcome Measures: The study evaluated the precision and consistency of APRIL in culture dish preparation by looking at droplet mass, pH of prepared media droplets, and mouse and human embryo development rates. Clinical implementation was assessed by comparing embryo development and outcomes in dishes prepared by APRIL and human embryologists.
Results: Compared with embryo culture dishes prepared using standard manual procedures, embryo culture dishes prepared using APRIL demonstrated a greater than 10-fold improvement in consistency (coefficient of variation, 0.46% vs. 6%–7%), maintained optimal pH levels (pH range, 7.281–7.33 vs. 7.275–7.311), and had a greater mouse embryo blastocyst rate (100% vs. 90%–91%). Human embryos cultured in dishes prepared by APRIL had a higher rate of development on days 3 (92.4% vs. 82.6%) and 5 (19.75% vs. 15.57%), and a total number of usable embryos (50.3% vs. 46.1%) compared with manually prepared dishes, although the last two outcomes did not reach statistical significance.
Conclusion: The results suggest that the use of an automated robotic system for preparation of embryo culture dishes may improve accuracy and outcome measures while reducing the need for trained laboratory personnel to prepare the dishes manually. (Fertil Steril® 2024;122:297–303. ©2024 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: IVF, robotics, culture dish preparation, automated system, embryo development

In vitro fertilization (IVF) treatment is a complex laboratory practice that requires the expertise and precision of highly trained and skilled embryologists and technicians performing meticulous tasks that are critical for achieving successful patient outcomes. The global rise in the use of assisted reproductive technologies (ARTs) and shortage of trained embryologists and laboratory personnel increase the work burden on laboratory staff. This consequently limits the number of patients that can be treated, increases the treatment cost, and increases the potential for human error.

One routine but essential component of IVF treatment practice is the preparation of specialized dishes for embryo and oocyte culture. The manual preparation of these dishes is time-consuming and repetitive as well as requires a high level of consistency, accuracy, and precision to standardize

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culture conditions and optimize embryonic development (1). However, the manual preparation of these dishes is tedious and introduces the possibility of human error and variability, which could negatively impact culture conditions and result in suboptimal outcomes (2).

The introduction of robotic automation has transformed industries (3), including health care, and has consistently shown benefits in areas where highly repetitive tasks are required (4). Requiring a high degree of precision and accuracy, tasks such as pipetting can become tedious over time, resulting in human errors. In contrast, robots and automation systems can perform these tasks with optimal consistency and increase productivity by freeing up laboratory personnel to work on other important tasks (5). Robotics and automation are now being developed to assist in a range of IVF laboratory procedures. including performing intracytoplasmic sperm injection (6).

We identified embryo dish preparation as a favorable opportunity for introducing robotics into the IVF laboratory because of its repetitive nature, making it well-suited for a robot. Additionally, dish preparation does not involve the manipulation of human gametes or embryos and solely involves culture media, mineral oil, and dishes, making it a safe avenue to introduce robotics to the laboratory. Because the automated plate-preparation robot does not handle human samples (eggs, sperm, or embryos) directly, it represents a safer platform to introduce robotics and automation into the IVF laboratory.

Here we report the development and clinical validation of the ART Pipetting Robot for the IVF Laboratory (APRIL), a liquid-handling robot customized to prepare microdroplet culture dishes. We evaluated the efficacy of APRIL in the preparation of microdroplet culture dishes for IVF. We compared the blastocyst rates and other relevant outcomes of dishes prepared by APRIL with those prepared manually by embryologists. We determined the feasibility and potential benefits of introducing robots in the IVF laboratory setting.

MATERIALS AND METHODS

APRIL

An enclosed liquid-handling robot (Opentrons OT-2, Opentrons Labworks, Inc., New York, NY) was outfitted with custom three-dimensional–printed adapters to hold up to 20 microdroplet culture dishes (Vitrolife, Cat No. 16003), two 30 mL bottles of culture media (Global Total, Cat No. LGGT-030), and one 100 mL bottle of mineral oil (Cooper Surgical LifeGuard Oil, Cat No. AMLG-100). The adapters were printed by PrintAWorld and fabricated from PC-ABS using STL files created on the basis of the dish specifications. Two Opentrons robot arms were installed: a P300 single-channel pipette to dispense the media and a P1000 single-channel pipette to dispense the oil. All contact materials underwent toxicity testing using a mouse embryo assay (MEA), and they were found to be nonembryotoxic (7). A dedicated high-efficiency particulate air filtration module was placed on top of the robot to optimize air quality within the robot. The robot was programmed to dispense 25 μL of embryo culture media into each of the 12 wells on the microdroplet dish, followed by a 5 mL oil overlay. Unique aspects of the computer code include allowing the robot to track which pipette tips were used in previous runs to proceed to the next available tip. Additionally, the robot calculates the cumulative oil dispensed to submerge the pipette into the oil bottle at progressively increasing depths to avoid air being dispensed or excess oil from the outside of the tip dripping onto the robot deck. The computer code also includes a field to specify the number of dishes desired for each preparation run.

Ethics

The study was reviewed by the Columbia University Institutional Review Board and was determined to be exempt from requiring approval.

Droplet mass calculations

Three embryologists (A, B, and C) each prepared 10 embryo culture dishes on 3 separate days by using a glass Pasteur pipet (Cooper Surgical IVF Pasteur Pipet, Cat No. PP-5.75-1000) to place 25 μL of media in each well followed by a 5 mL mineral oil overlay. The total mass of the prepared droplets for each dish was measured using an analytic scale (Mettler ToledoStandard ME Analytical Lab Balance) after taking into account the dish mass. In addition, 10 dishes prepared by APRIL were measured on 3 separate days. The mean, coefficient of variation (CV), and SD were calculated for each run of 10 dishes.

pH Testing

To ensure that APRIL did not irreversibly impact the pH of the culture media in the prepared dishes, we conducted pH quality checks in line with the protocol of the embryology laboratory where validation was run. We compared the pH of dishes prepared by APRIL to dishes made manually by an embryologist. On 3 separate days, two microdroplet dishes were prepared by an embryologist, and two dishes were prepared by APRIL. The two manually made dishes and the two robot-made dishes were placed in a K-Systems G210 InviCell incubator to equilibrate to culture conditions overnight (6.9% CO_2, 5.0% O_2).

After overnight incubation, pH testing was conducted individually on each dish. Dishes were removed individually from the incubator, and media was aspirated using a 1 mL syringe fitted with an 18-gauge needle. Given the small volume of each droplet, media across droplets within a dish were pooled for testing. Between droplets, the aspiration needle was wiped down with a Kimwipe, and a small portion of the media was expelled to remove any residual oil from the needle.

Once all the media droplets were aspirated and pooled together, the media was loaded onto an i-STAT CG4+ Cartridge (Blue; Cat No. 03P85-50) until the fill line of the cartridge was reached. The cartridge was then loaded into an i-STAT 1 analyzer (Cat No. 04P75-03) following the manufacturer's protocol. pH values were recorded.

MEA

The MEA was used to ensure that the preparation of dishes by APRIL did not introduce toxicity to the culture system (7). Two culture dishes were prepared manually and two were prepared by APRIL. After the dishes were incubated overnight, 10–12 single-cell mouse embryos (Embryotech B1–20) were cultured in each dish, with only one embryo cultured in each microdroplet. The dishes were placed in a K-Systems G210 InviCell incubator and left undisturbed for 96 hours for culture.

After 96 hours of incubation, each dish was observed by an embryologist, and the number of embryos that had reached the blastocyst stage was recorded. Early blastocyst-stage embryos were included in this number.

Clinical implementation

We used a parallel split study to evaluate embryonic development in dishes prepared using APRIL compared with manually made dishes. At the time of egg retrieval, patients with ≥ 10 eggs retrieved were identified as potential candidates, and sufficient manually made and robot-made dishes were prepared and incubated overnight. Eggs were fertilized, and on day 1, when ≥ 10 two pronuclear (2PN) embryos were observed, 2PN embryos were split randomly by an embryologist between the two groups. When there was an odd number, the extra 2PN embryo was placed in the manually prepared dish. Embryos were observed and graded by an embryologist on day 3, and the number of embryos with ≥ 6 cells was recorded. All embryos in both conditions were transferred into a new dish of the same type that had been prepared the day before. Embryos were cultured to the blastocyst stage for cryopreservation for up to 7 days. During culture, embryos were assessed for developmental stage and grade. The number of embryos that developed to the point of cryopreservation was recorded on each day and is referred to as the number of usable embryos.

RESULTS

Droplet mass

Dishes (n = 30) were prepared by APRIL and three embryologists (n = 30 each, 90 total) and weighed. The mean, CV, and SD were calculated. The average CV between dishes prepared by APRIL mass was 0.46%, compared with 7.11%, 5.98%, and 6.96% for embryologists, respectively. The droplet mass in the dishes produced by the robot showed a range of 0.2931–0.298 with one outlier at 0.3004. For embryologist 1, the range was 0.2157–0.2934 with two outliers at 0.304 and 0.3165, embryologist 2 ranged from 0.2344 to 0.3088, and embryologist 3 ranged from 0.2006 to 0.3177 (Fig. 1; Supplemental Table 1, available online).

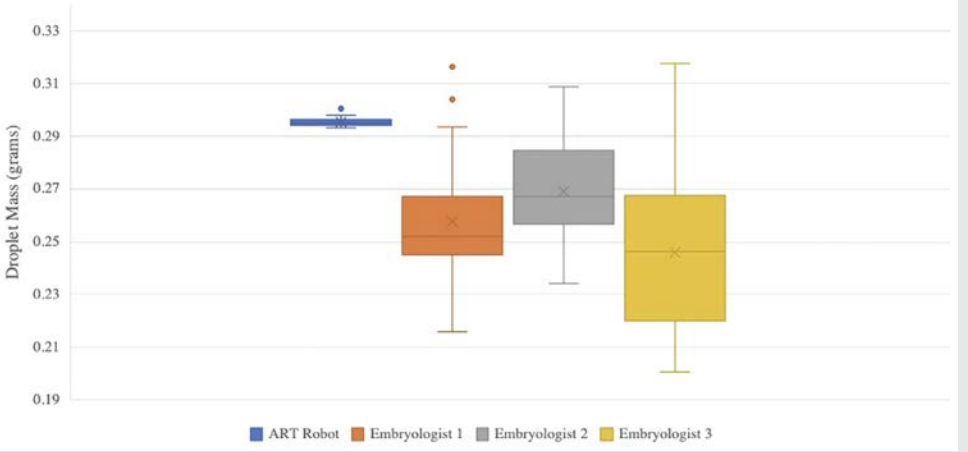
pH Test

Embryo culture dishes (n = 6) were prepared by APRIL and embryologists (n = 6). The pH range of the dishes made by APRIL after overnight incubation was 7.281–7.33, and the range of those made by an embryologist was 7.275–7.311 (Fig. 2; Supplemental Table 2, available online). The optimal pH for Global Total embryo culture media after overnight incubation is 7.3, with an acceptable range of 7.27–7.33 (8, 9).

MEA

A total of 21 mouse embryos were cultured in 2 dishes prepared by an embryologist, and 23 in the 2 dishes prepared by APRIL. Blastocyst formation rates (blastocyst rates) were 100%, 100%, 90%, and 91%, respectively (Fig. 3; Supplemental Table 3, available online). According to Food and Drug Administration (FDA) specifications, a blastocyst formation rate of >80% is required to pass the MEA assay (7).

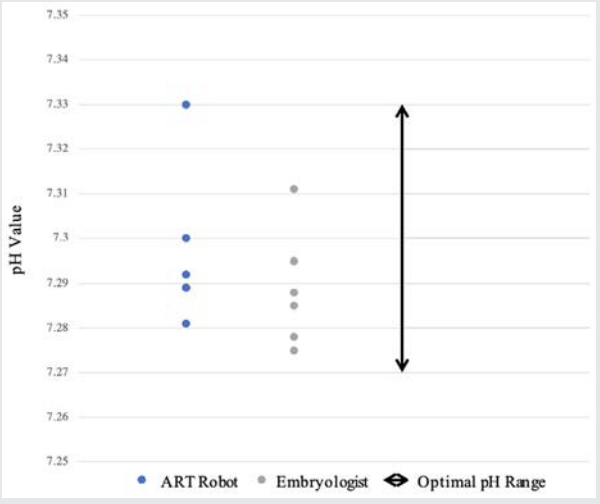
FIGURE 1



Distribution of droplet mass in dishes prepared using an assisted reproductive technology (ART) robot vs. manually. Distribution of droplet mass in dishes prepared using an ART robot vs. manually by three different embryologists. The bars indicate the minimum and maximum values observed, whereas the individual dots represent outliers beyond this range.

Lattin. Robot optimizes IVF dish preparation. Fertil Steril 2024.

FIGURE 2



pH of media after overnight incubation in dishes prepared by an assisted reproductive technology (ART) robot vs. manually. The dots show the pH values after incubation, whereas the arrow shows the optimal pH range for embryo culture dishes.

Lattin. Robot optimizes IVF dish preparation. Fertil Steril 2024.

Clinical implementation

Embryos (n = 324) were cultured in APRIL-prepared dishes (n = 157) and embryologist-prepared dishes (n = 167). Compared with embryologist-prepared dishes, embryos cultured in APRIL-prepared dishes had a higher rate of development to day 3 (92.4% vs. 82.6%, $P<.05$) and day 5 (19.75% vs. 15.57%) and a total number of usable embryos (50.3% vs. 46.1%), although the last two outcomes did not reach statistical significance (Fig. 4; Supplemental Tables 4 and 5, available online).

DISCUSSION

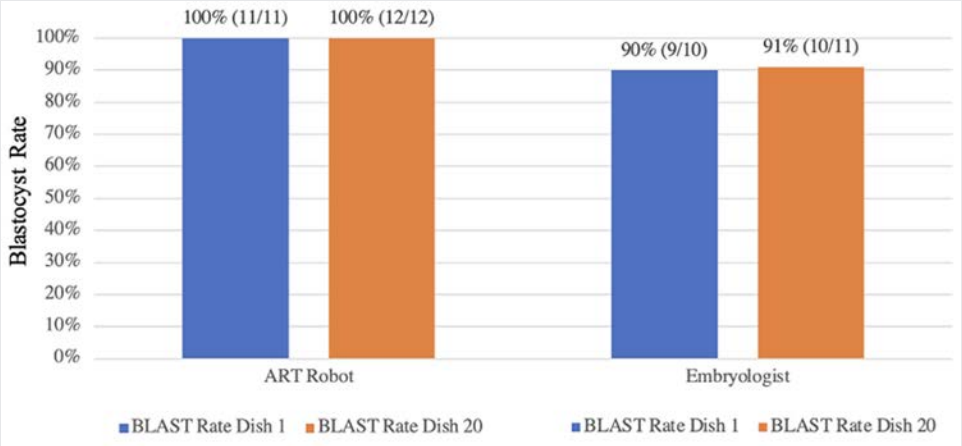
In this study, we demonstrated that the preparation of embryo culture dishes using an automated robot has advantages over preparation by human technicians. The droplet volume added to the dishes prepared by the robot was more consistent compared with those added manually, with an average CV approximately 10-fold lower (0.46% vs. 6.68%). The increased precision was true both within a single session of dish preparation and even more significantly between different days of preparation. These preliminary data suggest that the use of robots in the preparation of dishes may result in more consistent and reproducible results because the potential for variability because of human error is eliminated.

The pH of the media prepared by the robot was within the acceptable range for embryo culture. This indicated that the use of APRIL in the preparation of dishes for embryo culture does not significantly impact the pH of the resulting dishes, which is important because pH plays a crucial role in embryo development and small variations can have detrimental effects (2, 10).

In addition, we found that the MEA showed a comparable blastocyst rate for both the robot-prepared and human-prepared culture dishes. The MEA is a test designed by the FDA to evaluate the potential toxicity of a culture system or novel device to embryos. Its primary purpose here is to assess whether the introduction of an automated dish preparation system poses any harm to the embryo culture environment. The 90%–100% blastocyst rate range of all the dishes indicates that the automated dish prep is safe and complies with the FDA's standards for embryotoxicity, making it a valuable finding for ensuring the safety and acceptability of such systems in research and medical applications (7).

Finally, we found that there was a statistically significant difference in the number of embryos that had ≥6 cells on day 3 in the robot dishes compared with those prepared by human

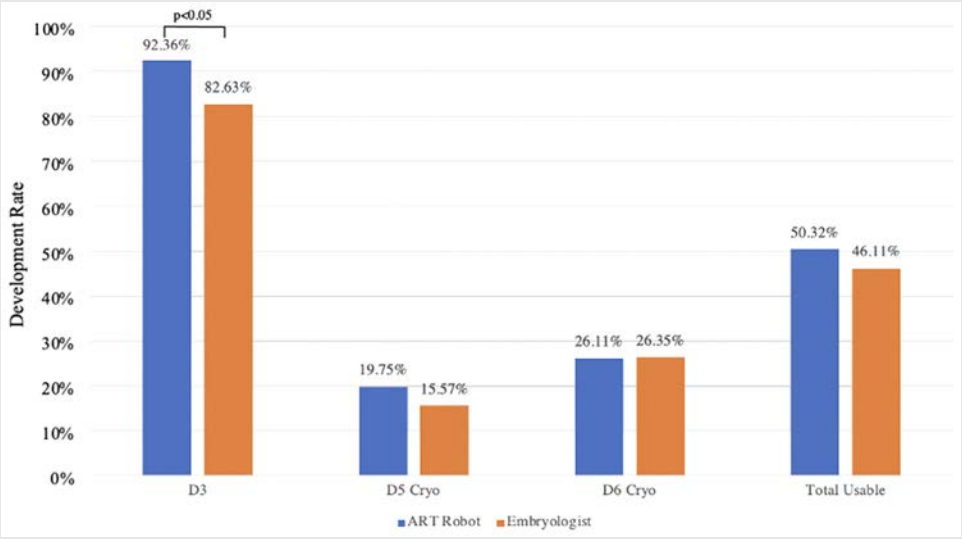
FIGURE 3



Mouse embryo assay blastocyst rate in dishes prepared using the assisted reproductive technology (ART) robot vs. manually. BLAST rate is the blastocyst formation rate. Dish 1 is the first dish prepared by the ART robot or manually by an embryologist and dish 20 is the last dish prepared by the ART robot or embryologist.

Lattin. Robot optimizes IVF dish preparation. Fertil Steril 2024.

FIGURE 4



Human embryo development in dishes prepared by an assisted reproductive technology (ART) robot vs. manually. Total usable is all embryos eligible for transfer or vitrification on the basis of development.

Lattin. Robot optimizes IVF dish preparation. Fertil Steril 2024.

embryologists, with more embryos having ≥6 cells on day 3 in the robot dishes. There was also a greater percentage of total usable embryos in the robot-prepared dishes vs. the embryologist-prepared dishes, although not statistically significant. A larger study will be necessary to test this. Additionally, there was a trend toward more day 5 blastocyst embryos in the robot dishes, which has been reported to have significantly higher implantation, pregnancy, and live birth rates compared with day 6, but may vary across laboratories (11, 12). The comparable blastocyst development rate suggests that automation matches current manual techniques, and the statistically significant difference in results in day 3 embryos suggests clinical potential in future iterations of an automated platform.

Using APRIL can free up embryologists' time and attention to focus on other functions for which robots are not yet ideally suited. With the current global shortage of trained embryologists, the need to optimize their time is an apparent necessity. When considering the introduction of automation and robotics into the IVF laboratory, embryo culture dish preparation is a good initial step because the robot is not handling human gametes or samples; there is no risk of injury or damage to precious human samples.

Despite the advantages demonstrated in this study, there are certain limitations associated with the use of APRIL. First, the robot's applicability is limited currently to the specific type of dishware used in the study (Vitrolife, Cat No. 16003). To use APRIL with different types of culture dishes, modifications to the computer code and custom three-dimensional–printed adapters would be required. Second, although APRIL can perform most of the dish preparation process autonomously, it still requires some human oversight. Human technicians need to ensure that there are no errors in the setup, such as incorrect loading of the culture media, mineral oil bottles, dishes, and pipette tips.

Overall, these findings demonstrate that the use of APRIL to prepare embryo culture dishes provides a more consistent and reproducible method for producing high-quality dishes for embryo culture. ART Pipetting Robot for the IVF Laboratory can be a valuable tool in the field of ART, providing greater precision, optimal pH values for media culture, comparable MEA blastocyst rates, and better outcomes in human embryo development. Although automated dish preparation has been used for animal systems (13), this is the first human application.

CONCLUSION

This study's preliminary data suggest that the use of a robot to prepare embryo culture dishes for IVF is a viable alternative to manual preparation by a human embryologist. Further research is needed to assess the long-term benefits of using a robot in this capacity. It will be important to continue to monitor the performance of the robot and to conduct additional studies to assess its impact on other aspects of embryo culture and development in the context of IVF treatment.

CRediT Authorship Contribution Statement

Miriam T. Lattin: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alexandre S. Djandji:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Matan T. Kronfeld:** Data curation, Conceptualization. **Tara Samsel:** Writing – review & editing, Data curation, Conceptualization. **Ruifeng Ling:** Writing –

review & editing, Investigation, Formal analysis, Data curation. **Martin Ciskanik:** Writing – review & editing, Investigation, Data curation. **Sasha Sadowy:** Writing – review & editing, Supervision, Project administration, Investigation. **Eric J. Forman:** Writing – review & editing, Project administration, Funding acquisition. **Zev Williams:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of Interests

M.T.L. has nothing to disclose. A.S.D. has nothing to disclose. M.T.K. has nothing to disclose. T.S. has nothing to disclose. R.L. has nothing to disclose. M.C. has nothing to disclose. S.S. has nothing to disclose. E.J.F. is on the Advisory Board for ALIFE outside the submitted work. Z.W. has nothing to disclose.

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Desarrollo y validación de un sistema robótico automatizado para la preparación de placas de cultivo de embriones.

Objetivo: Estudiar el desarrollo y la validación clínica del ART Pipetting Robot for the IVF Laboratory (APRIL), un robot de manipulación de líquidos personalizado para la preparación exacta de placas de cultivo de microgotas en el área de la fecundación in vitro (FIV).

Diseño: Estudio prospectivo aleatorizado realizado en un centro académico de FIV que compara tanto los resultados en embriones humanos como de ratón con las medidas cuantitativas de precisión en placas de embriones preparadas utilizando APRIL en comparación con la preparación manual estándar.

Entorno: Centro académico de FIV: El estudio consistió en la evaluación del sistema automatizado de preparación de placas de cultivo, APRIL, en comparación con los métodos de preparación manual en el contexto del tratamiento de FIV.

Intervención: ART Pipetting Robot for the IVF Laboratory es un robot cerrado de manipulación de líquidos equipado con adaptadores impresos tridimensionalmente a medida y diseñado para dispensar medios de cultivo de embriones y aceite mineral en placas de cultivo de microgotas.

Principales medidas de resultado: El estudio evaluó la precisión y consistencia de APRIL en la preparación de placas de cultivo mediante el análisis de la masa de las gotas, el pH de las gotas de medio preparadas y las tasas de desarrollo de embriones humanos y de ratón. La aplicación clínica se evaluó comparando el desarrollo embrionario y los resultados en placas preparadas por APRIL y embriólogos humanos.

Resultados: En comparación con las placas de cultivo de embriones preparadas mediante procedimientos manuales estándar, las placas de cultivo de embriones preparadas con APRIL demostraron una mejora de más de 10 veces en la consistencia (coeficiente de variación, 0,46% frente a 6%-7%), mantuvieron niveles óptimos de pH (intervalo de pH, 7,281-7,33 frente a 7,275-7,311) y tuvieron una mayor tasa de blastocistos de embriones de ratón (100% frente a 90%-91%). Los embriones humanos cultivados en placas preparadas con APRIL presentaron una mayor tasa de desarrollo en los días 3 (92,4% frente a 82,6%) y 5 (19,75% frente a 15,57%), y un número total de embriones utilizables (50,3% frente a 46,1%) en comparación con las placas preparadas manualmente, aunque los dos últimos resultados no alcanzaron significación estadística.

Conclusiones: Los resultados sugieren que el uso de un sistema robótico automatizado para la preparación de placas de cultivo de embriones puede aumentar la precisión y los resultados, al tiempo que reduce la necesidad de personal de laboratorio cualificado para preparar placas manualmente.

Check for updates

World Endometriosis Research Foundation Endometriosis Phenome and Biobanking Harmonization Project: V. Physical examination standards in endometriosis research

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Objective: The World Endometriosis Research Foundation established the Endometriosis Phenome and Biobanking Harmonisation Project (EPHect) to create standardized documentation tools (with common data elements) to facilitate the comparison and combination of data across different research sites and studies. In 2014, 4 data research standards were published: clinician-reported surgical data, patient-reported clinical data, and fluid and tissue biospecimen collection. Our current objective is to create an EPHect standard for the clinician-reported physical examination (EPHect-PE) for research studies.

Design: An international consortium involving 26 clinical and academic experts and patient partners from 11 countries representing 25 institutions and organizations. Two virtual workshops, followed by the development of the physical examination standards underwent multiple rounds of iterations and revisions.

Subjects: N/A

Main Outcome Measure(s): N/A

Result(s): The EPHect-PE tool provides standardized assessment of physical examination characteristics and pain phenotyping. Data elements involve examination of back and pelvic girdle; abdomen including allodynia and trigger points; vulva including provoked vestibulodynia; pelvic floor muscle tone and tenderness; tenderness on unidigital pelvic examination; presence of pelvic nodularity; uterine size and mobility; presence of adnexal masses; presence of incisional masses; speculum examination; tenderness and allodynia at an extra-pelvic site (e.g., forearm); and recording of anthropometrics.

Conclusion(s): The EPHect-PE standards will facilitate the standardized documentation of the physical examination, including the assessment and documentation of examination phenotyping of endometriosis-associated pelvic pain. (Fertil Steril® 2024;122: 304–15. ©2024 by American Society for Reproductive Medicine.)

El resumen está disponible en Español al final del artículo.

Key Words: Endometriosis, standardization, harmonization, phenotyping, physical examination, EPHect

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Endometriosis is estimated to affect 10% of reproductive-aged women (and those assigned female at birth), with diverse signs and symptoms ranging from infertility, dysmenorrhea, dyspareunia, dyschezia, dysuria, fatigue, and chronic pelvic pain (1). Pelvic endometriosis has 3 anatomic subtypes (peritoneal, deep, and ovarian endometrioma) that are captured in a proposed International Classification of Diseases-11 coding classification standard (2). Furthermore, endometriosis has been identified as an underlying factor that can give rise to chronic secondary visceral pain (3). Endometriosis-associated pain can have devastating impacts on patients due to its complex pathophysiology and heterogenous presentations, from cyclical pain to daily pain, accompanied by systemic symptoms such as fatigue, and a consequent significant impact on mental health (4). The heterogeneity of the disease with respect to its natural history, clinical presentation, and treatment response creates significant challenges when comparing research findings or conducting large-scale, multicentered research when no consensus exists regarding minimum standards for research data collection. As in other diseases, there has been a need for standardized research data collection tools in endometriosis to compare and combine data from different sites to facilitate collaborative research.

Targeting this goal, the World Endometriosis Research Foundation (WERF) established the Endometriosis Phenome and Biobanking Harmonisation Project (EPHect), a global consensus for the standardization and harmonization of endometriosis research data. In 2014, the first 4 EPHect data research tools were published for the collection of clinician-reported surgical data, patient-reported clinical data, and fluid and tissue biospecimens (5–8). Each tool consists of a "minimum" and a "standard" version, which encompass standard operating procedures for rigorous uniform data and biospecimen collection methods in endometriosis research. The tools are available open access (5–8) and also at ephect.org.

As of August 2023, 58 sites from 24 countries are conducting research adhering to the EPHect standards. The EPHect patient-reported clinical data tool has been translated with cultural adaptation into 18 languages (9–11), and implemented in different ethnic (12), geographical (13), and age (14) populations. The standards have also been used for numerous discoveries, for example, to quantify clinical symptomatology (15, 16), and associate biomarkers (17–19), genomic loci (20, 21) and epigenetics (22), mental health (23), and early life events (24) with endometriosis.

Despite these advances, there remains a gap in the rigorous, systematic, documentation of research data derived from the physical examination of patients with endometriosis. A comprehensive, standardized, physical examination can provide insight into clinically detectable endometriosis and potential pain mechanisms in patients with endometriosis (25). For example, even in patients who undergo surgery for endometriosis (and thus are surgically phenotyped), there are highly relevant findings in patients with endometriosis that cannot be assessed through surgical visualization (26). These include other pain-generating and maintaining factors (e.g., bladder, bowel, and musculoskeletal) and psychological comorbidities, which can be related to underlying peripheral and central nervous system sensitization which can be seen in those with endometriosis (27). These central nervous system mechanisms can give rise to what is now termed nociplastic pain (28–30). The physical examination can help identify these other pain generators to increase understanding of these clinical findings and, therefore, improve methods of phenotyping on the basis of factors related to pain mechanisms in combination with anatomical findings – essential to advance personalized treatment. In addition, with the move toward nonsurgical clinical diagnosis and empiric medical treatment of symptoms in patients with a working diagnosis of endometriosis (4, 26, 31, 32), systematic physical assessment and pain-focused phenotyping can enhance early clinical diagnosis and monitor signs and symptoms during follow-up. This includes clinical findings consistent with palpable disease (e.g., deep endometriosis), which can be identified and assessed without surgery via physical examination.

Therefore, there is a need to collect harmonized physical examination data to supplement the other EPHect research standards. In this article, we propose the EPHect physical examination (EPHect-PE) research data standards with common data elements, and the methods leading to their development. Resources are also provided to standardize physical examination procedures for the EPHect-PE.

METHODS

The process for establishing the WERF EPHect-PE data research tool is illustrated in Figure 1. Institutional Review Board status was not required for this article. A proposal was developed, presented to, and approved by the WERF Board. For the EPHect-PE Working Group, a core group was created representing 5 gynecologist clinician-researchers (P.J.Y., C.A., K.V., P.S., and S.A.-S.) who have published original research on the topic of the physical examination in endometriosis and endometriosis-associated pain (27, 33–38) as well as 2 representatives from WERF (L.H. and S.A.M.). A broader group within the EPHect-PE Working Group was created to include a clinical fellow who performed literature reviews (J.L.), individuals representing patient and advocacy groups (D.B., F.J., and A.T.), 2 additional WERF representatives (G.D.A. and L.R.), a physiatrist (physical medicine) clinician–researcher collaborator with one of the core group gynecologists (J.S.) (35, 36), and an international expert in pain science who has published extensively on pain assessment including nervous system sensitization testing in endometriosis and pelvic pain (L.A.-N.) (39–44). The process was coordinated by a clinical fellow (T.L.) with the support of WERF (L.H.).

The proposed scope of the EPHect-PE tool was for a standardized approach to the examination of both clinical evidence of endometriosis lesions and pain phenotyping to provide insight into the underlying pain mechanisms in each patient. As with previous EPHect tools, a "standard" version to document the complete recommended physical

examination findings was created alongside a "minimum" version with the essential core components to be used in circumstances of logistical or time constraints.

Virtual workshops were held with the core and broader group. The workshops consisted of the following activities: agreement on the goals of the workshop, a summary of the literature review results, and identification of the current knowledge gaps for physical examination in endometriosis; presentation of the state-of-the-art of pain assessment with a focus on pelvic pain and endometriosis (L.A.-N.); and sessions on the pelvic examination for endometriosis (C.A.), pelvic floor and vulvar assessment (P.J.Y.), neurologic examination including for allodynia (S.A.-S.), myofascial/musculoskeletal examination (P.S. and J.S.), and quantitative sensory testing (QST) (K.V.). The core group members also shared physical examination forms that they currently used in their clinical and research activities (e.g., on the basis of those of the Multidisciplinary Approach to the Study of Chronic Pelvic Pain network (45)). During each session, potential items for the EPHect-PE standard were identified and discussed which involved core common data elements for a pain- and endometriosis-focused physical examination.

The initial EPHect-PE "standard" and "minimum" tool was developed, along with accompanying images and videos. The initial drafts of the EPHect-PE went through iterative rounds of feedback and revisions with the core and broader members of the EPHect-PE Working Group. Stakeholder consultation was then conducted with international pain associations and additional national patient organizations. Leadership in these organizations were contacted, who identified individuals to provide further feedback on the draft standards and were included as members of the WERF Physical Examination Working Group (see Acknowledgment section: E.A., J.C., E.C., H.G.C., A.W.H., A.J., G.L., D.C.M., O.C.N., and F.F.T.).

After the stakeholder consultations, the draft EPHect-PE tool was further revised and then approved by the core and broader group members. The accompanying manuscript was written, revised, and approved by the full EPHect-PE Working Group comprising a total of 26 clinical and academic experts and patient partners from 11 countries representing 25 institutions and organizations.

RESULTS

The EPHect-PE tool in its standard form is provided in Appendix 1 (available online) and in its minimum form in Appendix 2, with the rationale behind each examination item described below. In addition, videos and photos of the myofascial/musculoskeletal examination are available online (Appendix 3). These standards can be applied to patients with both confirmed endometriosis (e.g., previous visualizing surgery or radiologic imaging) or with suspected endometriosis (as part of a clinical diagnosis) (4), in addition to comparison or control groups (46) such as those with pelvic pain without endometriosis, chronic pain at sites other than the pelvis, endometriosis without pelvic pain, or healthy persons with no signs of symptoms of gynecologic conditions.

Patient-Reported Information (A1–A5)

Before the physical examination, it is recommended that patients complete the EPHect Endometriosis Patient Questionnaire (EPHect-EPQ) for clinical and covariate phenotype data collection, which focuses on the symptoms and characteristics of pelvic pain, fertility, menstrual and reproductive history, hormonal/pain medication use, medical history, comorbidities, and personal information across the life course of that person (6). However, in circumstances where the EPHect-EPQ was completed >3 months before the physical examination, items selected from the EPHect-EPQ merit repeating at the time of examination (see the supplementary section of the EPHect-PE in Appendices 1 and 2). These items may influence physical examination findings and also include the last menstrual period, any hormonal therapy, as well as the use of analgesics and neuromodulatory pain medications.

Before the physical examination, the patient is also asked to rate their overall pain severity and pelvic pain severity on the day of the examination on an 11-point numeric rating scale (NRS) from 0 to 10, with 0 representing no pain and 10 representing worst imaginable pain. The NRS score is then compared with the average overall or pelvic pain severity over the last 4 weeks as a reference pain level. Included in the standard physical examination assessment is the use of a body map (47–50) to allow quantification of a widespread pain index. Both forms use the Michigan Body Map (49, 50), which can be collapsed to the Fibromyalgia Body Map (47, 48), both being widely used in chronic pain clinical care and scientific discovery. The widespread pain index has confirmed clinically translational validity in associations with more opioid use and persistent pain after hysterectomy (51, 52).

A detailed explanation for the purpose and step-by-step process of the examination should be provided and consent for the physical examination should be obtained from the patient before beginning the examination. Examiners (i.e., individuals performing the examination) should have a trauma-informed approach to care and create opportunities for the patient to have input, choice, and control over components of the physical examination (53).

Anthropometrics (B1–B3)

With patient permission, the examiner measures height and weight from which body mass index can be subsequently calculated.

Reproduction of Pain

For each physical examination component, the examiner asks whether each maneuver reproduces at least some aspect of the patient's pain (25). This information aims to isolate which examined organ or structure can reproduce the pain specific to the individual being examined. For example, a patient with the primary complaint of left lower quadrant (LLQ) pain is found to have an LLQ abdominal wall trigger point and left uterosacral nodule that reproduces the LLQ pain,

suggesting both myofascial pain and deep endometriosis in the etiology of this pain.

Pelvic Girdle and Back (C1–C11)

Items C1–C9 represent tests of pelvic girdle pain (PGP), defined as pain between the upper iliac crests and gluteal folds in the region of the sacroiliac joint (54). Pelvic girdle pain is known to be important in pregnancy-associated pain but may also play a role in pelvic pain outside pregnancy. Five examination maneuvers were adapted from Tu et al. (54): long dorsal sacroiliac ligament tenderness, active straight leg raise, Faber test, posterior pelvic pain provocation test (P4), and symphysis pubis tenderness. Detailed instructions, pictures, and videos are provided in Appendix 3 to facilitate consistency in examination techniques. In addition to the PGP tests, the examiner palpates the lumbar paraspinal musculature for tenderness (L1–L5) as there can be lumbar components of both back and pelvic pain (54).

Abdomen (D1–D6)

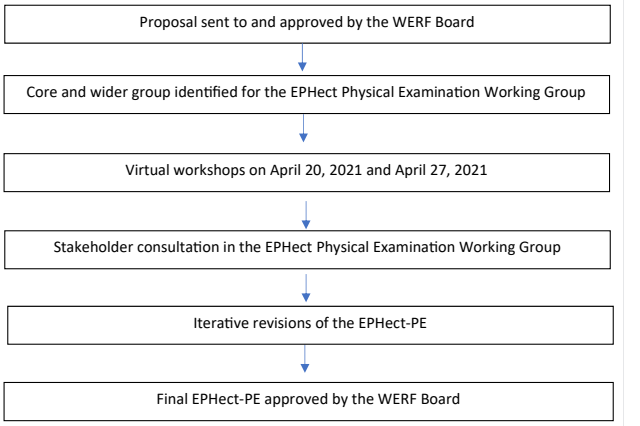
The abdomen is divided into 5 regions: left upper, right upper, left lower, right lower, and suprapubic region. Allodynia is tested by brushing a Q-tip or cotton swab from cranial to caudal on the right and left abdomen, and then from lateral to medial. Abdominal wall cutaneous allodynia has been shown to be able to discriminate between those with continuous pelvic pain, compared with those with cyclical pain or controls (55–57).

Light palpation is done in each region for tenderness and, if present, the Carnett's test is performed to differentiate the abdominal wall from visceral sources of pain (58). In this maneuver, the abdominal wall musculature is contracted, and the test is positive if tenderness remains the same or worsens, which suggests an abdominal wall source of pain (58). A positive Carnett's test was associated with greater severity of chronic pelvic pain in a cohort consisting primarily of patients with suspected or diagnosed endometriosis (27, 59). Furthermore, in each region, the examiner also palpates for myofascial trigger points, defined as "a hyperirritable spot in a taut band of a skeletal muscle that is painful on compression, stretch, overload, or contraction of the tissue which usually responds with a referred pain that is perceived distant from the spot" (60). Myofascial trigger points were associated with other signs of central sensitization in patients with endometriosis (35, 36). Similarly, all previous surgical incisions on the abdomen are examined for allodynia, tenderness, and masses. The differential diagnosis of an incisional mass includes but is not limited to a hernia, abdominal wall endometriosis, and desmoid tumors. Detailed instructions, pictures, and videos are provided in Appendix 3.

Pelvic Examination: Consent

The decision to proceed with the pelvic examination involves shared decision-making between the examiner and the patient, with the goal of having each patient believe prepared for the procedure and in control. A pelvic examination only occurs if the patient declaratively consents. If an individual does not consent to the pelvic examination, they may still choose to consent to the external (abdominal and back) examination. This discussion and consent may occur at the beginning of the consultation or after the external examination. Opportunities to review the examination and its rationale should be offered in a patient-centered approach in each case. Frequent "checking-in" during the examination is important to enable the patient to ask questions or to pause or stop the pelvic examination at any time. There are circumstances where a pelvic examination may not be appropriate, such as age, cultural sensitivities, and patient choice. Patient groups that merit particular consideration, and where a pelvic examination may be omitted, include adolescents, those with a history of trauma, and individuals with vaginismus, where a pelvic examination may not be possible or may cause significant pain or distress. In addition, certain pelvic examination components are difficult for some patients (e.g., deeper pelvic examination and speculum examination), and thus these may be omitted or modified. The consent dialogue should also include a discussion of the presence of a chaperone during the examination (61). Examiners should also follow local guidelines for consent.

FIGURE 1



Flow diagram depicting the WERF EPHect development and consensus process for physical examination standards.

Lin. WERF EPHect physical exam standards. Fertil Steril 2024.

Vulva (E1–E4)

Although a detailed vulvar examination is beyond the scope of the EPHect-PE, allodynia of the labia and Q-tip palpation of the vulvar vestibule to assess for provoked vestibulodynia are included, as it is a potential source of dyspareunia and a common comorbid condition in individuals with endometriosis-related pain (62). For consistency of assessment between examiners, the vulvar vestibule is palpated in one direction, clockwise, beginning at 12 o'clock above the distal urethra. Assessment of the anocutaneous reflex is incorporated into the standard tool only for confirmation of

an intact sacral reflex. A schematic of the vulva is provided within the tool (Fig. 2).

Pelvic Floor (F1–F15)

Pelvic floor myofascial pain syndrome, characterized by hypertonicity, tenderness with palpation, and a decreased ability of the pelvic floor muscles to contract and relax, can occur in isolation but frequently co-exists with other pelvic pain conditions including endometriosis (63). Furthermore, the presence of pelvic floor tenderness itself is associated with a greater severity of chronic pelvic pain in a cohort consisting primarily of patients with suspected or diagnosed endometriosis (27). In pelvic pain patients, quantitative sensory testing pain-pressure thresholds for the palpation of pelvic floor muscles were lower (indicative of pain with lower pressure applied) compared with controls (64), and pelvic floor tenderness was associated with a higher score on the McGill Pain Inventory (65). Pelvic floor tenderness has also been found to correlate with reduced pressure pain thresholds at the thumbnail, reflecting central sensitization (38). It should be noted that pelvic floor pain could arise outside the context of central sensitization, such as from inflammation, trauma, or sporting/physical activities, and could be the source of symptoms such as dyspareunia and dyschezia.

Given the critical importance of pelvic floor myofascial pain in the pathophysiology of pelvic pain, an efficient, selective, approach for phenotyping a pelvic floor contribution to pain in those with endometriosis is incorporated into the EPHect-PE. The standard form includes a sequential examination of pelvic floor muscles from superficial to deep, integrating components described by Gyang et al. (63) and Meister et al. (66) to assess for tenderness. The superficial muscles are palpated first: bulbospongiosus, ischiocavernosus, and transverse perineal muscles. Subsequent deeper muscles palpated are the pubococcygeus, iliococcygeus, coccygeus, and obturator internus. Palpation for bands of the deeper muscles is also done. Detailed descriptions, images, and videos are provided online (Appendix 3).

For simplicity, the minimum form only consists of the palpation of iliococcygeus, which involves the further insertion of a single digit just beyond approximately 2 cm past the introitus, and palpation on the right (8 o'clock) and left (4 o'clock) (Fig. 3) (63, 66). Moreover, in the minimum tool, tenderness of the right and left iliococcygeus is assessed as present or absent.

There are no universally accepted standards for the amount of pressure to apply during pelvic floor muscle palpation for tenderness. In the study of Meister et al. (67), examiners had an average of 0.225 kg (0.5 pounds) of pressure which is approximated by tissue depression of 5.5 mm on palpation of the mid-thigh. Tu et al. (68) used 0.4–0.6 kg/cm^2 for the pelvic floor examination. Gyang et al. (63) suggested the pressure should be <2 kg/cm^2 of pressure (i.e., the pressure to induce blanching of the nail bed), whereas Shafrir et al. (38) used approximately 2 kg/cm^2 pressure for the palpation of the abdominal wall and pelvic floor muscles (38). For EPHect-PE, we recommend using an approximate pressure similar to an indentation of the mid-thigh by 5.5 mm (67), which ensures a pressure significantly <2 kg/cm^2.

After the examination for tenderness, an examiner provides an overall assessment of the pelvic floor tone graded into 3 categories: hypertonic, normotonic, and hypotonic. A clinically useful comparison is the masseter muscle contracted (hypertonic), the masseter at rest (normotonic), and the cheek (hypotonic). The patient is then asked to contract and then relax their pelvic floor muscles. The examiner assesses global pelvic floor relaxation graded into 3 categories: full relaxation (return fully to resting state), some relaxation (partially contracted), or no relaxation (remains fully contracted).

Bladder (G1 and G2)

Anterior vaginal wall palpation (extending to the vaginal wall anterior to the cervix) by a single-digit internal pelvic examination is also included to assess visceral bladder tenderness which, in some cases, may be related to painful bladder syndrome (33). Bladder and pelvic floor tenderness have been associated with a higher score on the central sensitization inventory in those with endometriosis (69). Urethra palpation, involving transvaginal palpation closer to the introitus, is also performed in the standard tool.

Deeper Pelvic Tenderness (H1 and H2)

The deeper pelvic examination is structured on the basis of whether a uterus is present. Again, a single digit (index finger) is used, without an accompanying abdominal hand as used in the bimanual examination, since the latter may activate abdominal wall myofascial trigger points and/or cause simultaneous bladder tenderness, limiting the specificity of interpretation. For patients with a uterus, a suggested order of examination would include the cervix, right paracervical (i.e., 1–3 cm lateral to the cervix, approximating the region of the right adnexa), right uterosacral ligament, cul-de-sac (including retrocervical), left uterosacral ligament, and left paracervical (i.e., 1–3 cm lateral to the cervix, approximating the region of the left adnexa). Posthysterectomy, these anatomic regions are replaced by palpation of 3 separate locations in the vaginal vault (right, central, and left) and a Q-tip palpation of the vaginal vault for focal tenderness. Similar to the pelvic floor examination, there is no universal standard for the amount of applied pressure. However, it would be reasonable to apply the approximate pressure as noted for the pelvic floor muscles.

The presence of nodularity suggestive of deep endometriosis should be formally assessed. Most published papers on the physical examination in endometriosis focus on the accuracy of palpable nodularity in predicting deep endometriosis at the time of surgery, as reviewed recently (31, 70). In these studies, there is significant variability in its sensitivity and specificity to the location of deep endometriosis (71–76). Hudelist et al. (75) found that palpable nodularity had a sensitivity for deep disease of 50% for the uterosacral ligaments, 73%–78% for the pouch of Douglas, vagina, and rectovaginal space, 25% for the bladder, and 39% for the

rectosigmoid. Moreover, palpable nodularity by itself cannot accurately diagnose the pouch of Douglas obliteration in the absence of some assessment of mobility (77). Although tenderness of these structures is common in those with endometriosis, palpation for tenderness alone has a low specificity (high false-positive rate) for observing abnormalities at surgery, including endometriosis (78), likely because the occurrence of central sensitization can result in multiple tender sites on pelvic examination regardless of structural findings.

Bimanual Examination (I1–I3)

A bimanual examination is then performed to assess uterine size, orientation, and mobility. Enlargement of the uterus can suggest concurrent adenomyosis, fibroids, or pregnancy. Uterine size is classified as either below the symphysis or above the symphysis measured in centimeters (to avoid the language of "gestational weeks," which may cause distress for patients with infertility). Mobility (or lack thereof) can be useful in assessing deep endometriosis associated with decreased mobility or fixation. Uterine tenderness may be present in patients with adenomyosis, fibroids, and/or a clinical diagnosis of "chronic uterine pain" (79). Both adnexa are palpated during the bimanual examination for the presence of tenderness, masses, and decreased mobility, suggestive of ovarian endometriomas and/or deep endometriosis. During this evaluation, the abdominal hand may activate abdominal wall myofascial trigger points and/or bladder tenderness, and thus tenderness may not necessarily indicate uterine or ovarian tenderness.

Optional Examinations (J1–J5)

If indicated (e.g., suspicion of vaginal deep disease), a speculum examination enables direct visualization of transmural invasive deep endometriosis of the vagina or endometriosis of the cervix. Similarly, if indicated (e.g., suspicion of parametrial or rectal disease) and with patient consent, a pelvirectal examination can be done to palpate nodularity (but only documented in the standard form). Otherwise, these examination items can be omitted.

Pain during and after the Examination (K1 and K2)

This assessment is performed after completing the above components of the abdominal, back, and pelvic examination. First, the patient is asked whether there was complete, partial, or no reproduction of their pelvic pain during the physical examination. Then, the patient is asked whether new pelvic pain has developed (80).

Extra-pelvic Site (L1 and L2)

The core working group extensively discussed the utility of examining extra-pelvic sites and performing QST in clinical and research settings. However, the tools or expertise to perform QST may not be universally available and has the further issue of time limitations. Thus, comprehensive QST is not included in these recommendations, but a single extra-pelvic site (a volar aspect of the distal third of the forearm (81)) was chosen to assess allodynia and tenderness (hyperalgesia).

DISCUSSION

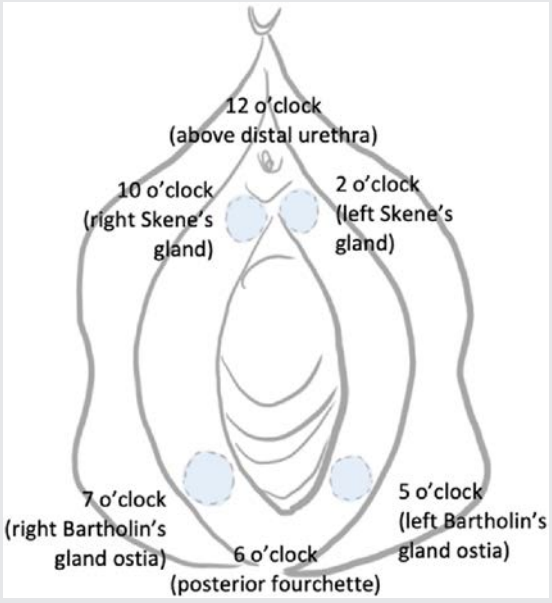
In this article, the development of physical examination data research tool of the WERF EPHect is described. Both minimum and standard forms for physical examination in research settings (WERF EPHect-PE) are provided, with common data elements consisting of patient-reported data relevant to the physical examination (e.g., last menstrual period, medication use, etc.), anthropometrics, and examination of the abdomen, vulva, pelvic floor musculature, deep pelvic region, and any relevant extra-pelvic locations. This examination encompasses physical findings related to endometriosis lesions (e.g., nodularity) and is focused on the phenotyping of pain mechanisms (e.g., features suggesting central sensitization, comorbid bladder pain, and myofascial trigger points). This phenotyping can be used alongside patient-reported outcomes of the NRS for pain and the body map for a widespread pain index, to enable diagnosis of nociplastic pain (28–30).

Because numerous professional societies increasingly support a working diagnosis of endometriosis on the basis of history and physical examination, with or without imaging, followed by initiation of empiric medical treatment of endometriosis in many clinical scenarios (4, 26, 31), this scenario may become increasingly common for research studies. The pelvic examination component of the EPHect-PE allows for the assessment of nodularity as a sign of deep endometriosis, adnexal masses that may reflect ovarian endometriomas, and uterine fixation in cases of the pouch of Douglas obliteration. As such, on the basis of physical examination, clinically diagnosed patients with endometriosis could be phenotyped into those with and without signs of deep or ovarian endometriosis. Ideally, the EPHect-PE would be combined with imaging data, whether currently published standards (82) or a future EPHect research standardized tool on imaging, to provide a relevant, noninvasive phenotype for these patients. Furthermore, biomarker data from the EPHect standards for biospecimen collection (7) could be analyzed on the basis of physical examination or imaging characteristics of endometriosis in patients with a nonsurgical diagnosis.

Although endometriosis lesion characteristics are important for nociplastic pain mechanisms, there is limited correlation between these surgical or histologic findings and pain symptoms in those with endometriosis (83). Therefore, we sought to include a physical examination assessment that potentially provides insight into other pain mechanisms, such as signs suggestive of central nervous system sensitization or involvement of the musculoskeletal system. This focus on physical examination findings alongside patient-reported symptoms such as the body map facilitates a better understanding of pain generators and the development of nociplastic pain.

Importantly, these clinical observations contribute to a better understanding of the varied etiological pathways for pain. For example, pelvic floor dysfunction could arise from trauma, inflammation due to endometriosis lesions in the

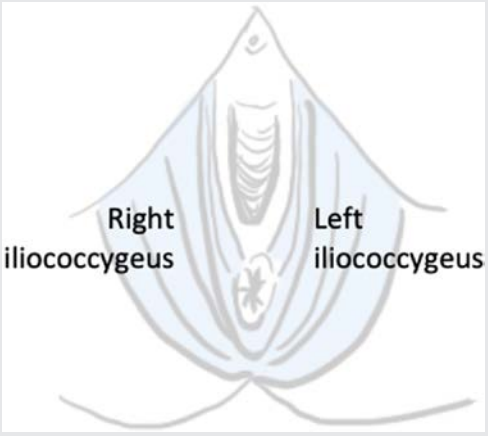
FIGURE 2



Schematic of the vulva as described in the EPHect minimum and standard forms for physical examination.

Lin. WERF EPHect physical exam standards. Fertil Steril 2024.

FIGURE 3



Examination of the iliococcygeus muscle is in both the EPHect minimum and standard forms for physical examination.

Lin. WERF EPHect physical exam standards. Fertil Steril 2024.

pelvis, or central nervous system sensitization arising from endometriosis-associated pain. Physical examination for pain phenotyping of the endometriosis patient is important to measuring pain as a clinical outcome that in and of itself must be addressed, whether surgery is performed or not. For example, a patient with endometriosis on the EPHect surgical phenotype data form (5) could have additional clinical diagnoses from the EPHect-PE, such as PGP, abdominal wall myofascial trigger points, or pelvic floor tenderness. Similarly, patients with a clinical diagnosis of endometriosis could be classified phenotypically using the EPHect-PE into those with or without provoked vestibulodynia, which would be relevant to the symptom of dyspareunia (62). These patients would be phenotypically distinct from those with endometriosis alone without other pain generators. Moreover, biomarker data from EPHect standards for biospecimen collection (8) could be analyzed on the basis of different patterns of comorbid pain generators.

The detailed instructions in this manuscript and supplementary online images/videos (Appendix 3) facilitate the consistency of the physical examination for harmonized research data collection and subsequent comparability across sites and studies. However, areas remain for further validation studies (e.g., pressure applied during palpation), and there may be inherent subtle examination differences between clinicians and within a clinician over time such as with increasing examiner experience (70). For specific studies incorporating the EPHect-PE with multiple examiners, we recommend including a process for quality assurance to better ensure consistency over the course of the study. The EPHect-PE could also be repeated over time to enable consideration of longitudinal changes in pain findings after interventions.

Notably, the EPHect-PE can be combined with patient-reported questionnaires that contribute to pain phenotyping. The Pain Catastrophizing Scale (84) is already included in the EPHect-EPQ for clinical and covariate phenotype data standards. In addition, diagnostic criteria can be incorporated on the basis of history for other chronic pain conditions, such as irritable bowel syndrome (85) and painful bladder syndrome (86, 87). Together, these assessments would enhance pain phenotyping and provide a more complete description of pain comorbidities associated with endometriosis beyond the physical examination findings. Patient heterogeneity is driven, in part, by characteristics that can only be documented by an intentional phenotypic assessment such as that described herein, without which the true biologic underpinnings of clinically translational endometriosis pain phenotypes will remain unknowable.

Strengths and Limitations

Strengths of this latest EPHect tool include the standardization of documentation of the physical examination findings and pain-focused phenotyping in individuals with endometriosis. By specifically gathering standardized clinical and patient information related to the outcome of pain, our patients with endometriosis-associated pain have the potential to be compared with other pain patient cohorts. This overarching goal has been put forth by the Innovative Medicine Intiative-National Institutes of Health Transatlantic Emphasis Group on Research and Translation-to-care Efforts for Pain consortium, a worldwide effort to advance the development of effective pain management (88). Another strength is the involvement of multiple interdisciplinary and international professionals, including patient partners and organizations for endometriosis. The engagement of patients in research is consistent with recommendations for patient-oriented, community-engaged, health research (89, 90). Patient partners

are important for developing the physical examination tool that is relevant to patient outcomes of interest; these partners guide how to consider the discussion leading to pelvic examination, the consent process, and the use of a chaperone. Additional strengths include the detailed reporting tools and visual and video descriptions. Although the EPHect tools are primarily designed for research, they may also have application in the clinical setting by standardizing the description of clinical findings and thus enhancing meaningful clinical communication about endometriosis (91).

Limitations include the inability to include recognized measures of the neurophysiologic process of central sensitization such as QST, as its inclusion requires equipment and training outside the capacity of most clinicians. Another limitation is that many physical examination components are based on expert opinion due to a limited evidence base. The physical examination standards are also predominately focused on pelvic endometriosis and are less applicable to extra-pelvic disease that is less amenable to physical examination (e.g., thoracic endometriosis). There is also a need for inter- and intra-rater reliability and reproducibility studies, and the time to complete the examination may vary on the basis of experience. Moreover, future work will determine whether patterns or aspects of physical examination findings are associated with clinically relevant outcomes (e.g., patient-reported outcomes and treatment response).

Future Directions

World Endometriosis Research Foundation will continue to document the utilization of each EPHect tool over time to evaluate the impact of these standards in enabling the comparison of studies and populations to achieve larger sample sizes pertaining to key outcomes to advance research. We see a strong need to facilitate the adoption of the pain-focused EPHect-PE in clinical drug trials for endometriosis-associated pain. The EPHect-PE could be used to phenotype study patients into subgroups, for example, based on different characteristics such as the number(s) of comorbid pain generators, reflecting the relative contribution of nociplastic pain. One might hypothesize that medical and surgical treatments that focus only on endometriosis lesions would be less effective in those patients with high nociplastic burden. Alternatively, with novel treatments, it may be possible to examine whether nociplastic pain is modifiable with effective treatment and how long it takes to observe any change. It should be noted that with future research, there may be selected items that become most important for phenotyping in endometriosis, whereas there may be other items with less utility. Similarly, subsequent studies may demonstrate that some items are overlapping in terms of the underlying factor being assessed. Therefore, there may be a refinement process of the EPHect-PE components over time.

Furthermore, additional EPHect research tools will be developed over time. As the EPHect tools are increasingly adopted worldwide, this will continue to promote collaborative studies among geographically disparate sites, maximizing the diversity of persons with endometriosis included in scientific discovery and thus clinically translational validity, ensuring sample sizes necessary for power to detect rarer but nonetheless true endometriosis-related factors, and exponentially increase our collective understanding of this complex disease.

CONCLUSION

In conclusion, we present a novel research data tool, EPHect-PE, to standardize the physical examination. This includes physical examination of signs of endometriosis lesions and the phenotyping of other pain-generating and/or maintaining factors that provide insight into potential pain mechanisms.

CRediT Authorship Contribution Statement

Tinya Lin: Writing – review & editing, Writing – original draft. **Catherine Allaire:** Writing – review & editing, Conceptualization. **Sawsan As-Sanie:** Writing – review & editing, Conceptualization. **Pamela Stratton:** Writing – review & editing, Conceptualization. **Katy Vincent:** Writing – review & editing, Conceptualization. **G. David Adamson:** Writing – review & editing, Conceptualization. **Lars Arendt-Nielsen:** Writing – review & editing. **Deborah Bush:** Writing – review & editing. **Femke Jansen:** Writing – review & editing. **Jennifer Longpre:** Writing – review & editing. **Luk Rombauts:** Writing – review & editing. **Jay Shah:** Writing – review & editing. **Abeesha Toussaint:** Writing – review & editing. **Lone Hummelshoj:** Writing – review & editing, Project administration, Conceptualization. **Stacey A. Missmer:** Writing – review & editing, Conceptualization. **Paul J. Yong:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization.

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Declaration of Interests

T.L. has nothing to disclose. C.A. reports consultancy fees from AbbVie and Pfizer. S.A.-S reports consultancy fees from Myovant-Pfizer, Organon, and Bayer. P.S. has received royalties from UpToDate for a section about acute pelvic pain and from *Frontiers in Reproductive Health* as Specialty Editor for Gynecology, and participated in an AbbVie advisory board and is part of a team that received botulinum toxin and funds for monitoring a clinical trial that were provided by Allergan, Inc. through a Clinical Trials Agreement with the National Institutes of Health (NIH). K.V. has received research funding from Bayer AG and honoraria for consultancy from Bayer AG, Eli Lilly, AbbVie, and Reckitts. G.D.A. reports consultancy fees from Organon, Labcorp, and Cooper, and is the CEO of and has equity in ARC Fertility. L.A.-N. has nothing to disclose. D.B. is the owner of EPP Coaching and Consulting and has received travel expenses and speaking fees from Myovant and Guerbet. F.J., J.L and L.R., have nothing to disclose. J.S. is part of a team that received botulinum toxin and funds for monitoring a clinical trial that was provided by Allergan, Inc. through a Clinical Trials Agreement with the NIH. A.T. has nothing to disclose. L.H. is remunerated by WERF as the EPHect-PE project manager. S.A.M. reports consultancy and grant funding from AbbVie for population-based research unrelated to this project and from *Frontiers in Reproductive Health* as Field Chief Editor. P.J.Y. has nothing to disclose.

The WERF EPHect Working Group (not listed in the author list): E.A. has nothing to disclose. J.C. is the IPPS vice-president and a consultant for SoLa Therapy, AbbVie, and Myovant; E.C. is an employee of Endometriosis UK; H.C.G has nothing to disclose. A.W.H.'s institution (University of Edinburgh) has received payment for consultancy and grant funding from Roche Diagnostics to assist in the early development of a possible blood diagnostic biomarker for endometriosis, consultancy fees from Gesynta and Joii, and grant funding from the UKRI, NIHR, CSO, and Wellbeing of Women for endometriosis research. A.W.H. has received payment for a lecture from Theramex, is president-elect of the World Endometriosis Society, co-editor in chief of *Reproduction and Fertility*, was a member of the NICE and ESHRE Endometriosis Guideline Groups, and is a trustee and medical advisor to Endometriosis UK; A.J. has nothing to disclose. G.L. is an employee of the US Veterans Health Administration, reports research funding from the NIH and Department of Defence, and has served as a consultant for Myovant, AbbVie, and Pelvic Sola Therapy. D.C.M. and O.C.N. have nothing to disclose. F.F.T. has royalties from Wolters Kluwer, has consulted for Bayer and Tremeau Pharmaceuticals, and received research support from Dot Laboratories.

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Proyecto de la Fundación Mundial para la investigación del fenómeno de la endometriosis y la harmonización del biobanqueo. Estándares del examen físico en investigación de endometriosis.

Objetivo: La Fundación Mundial para la Investigación en Endometriosis estableció el proyecto del fenómeno de la endometriosis y la harmonización del biobanqueo (EPHect), para crear herramientas estandarizadas de documentación (con elementos comunes de datos) para facilitar la comparación y combinación de datos entre diferentes estudios y sitios de investigación. En el 2014, se publicaron 4 estándares de datos de investigación: datos quirúrgicos reportados por los clínicos, datos clínicos reportados por las pacientes y recolección de bioespecímenes líquidos y de tejidos. Nuestro objetivo actual es crear un estándar EPHect para el examen físico reportado por el clínico (EPHect-PE) para estudios de investigación.

Diseño: Se conformó un consorcio internacional compuesto por 26 expertos clínicos y académicos y pacientes socios de 11 países representando 25 instituciones y organizaciones. Se llevaron a cabo dos talleres de trabajo virtuales, seguidos por el desarrollo de estándares para el examen físico que fueron revisados e iterados en múltiples rondas.

Sujetos: N/A

Medida(s) de desenlace principal(es): N/A

Resultado(s): La herramienta EPHect-PE provee una evaluación estandarizada de las características del examen físico y del fenotipado del dolor. Datos de la espalda y la cintura pélvica; el abdomen, incluida la alodinia y los puntos gatillo; la vulva, incluida la vestibulodinia provocada; tono y sensibilidad de los músculos del piso pélvico, sensibilidad en el examen pélvico unidigital, presencia de nodularidad pélvica, tamaño y movilidad uterina, presencia de masas anexiales; presencia de masas incisionales, examen con espéculo; sensibilidad y alodinia en un lugar extrapélvico (por ejemplo, el antebrazo), y registro de datos antropométricos.

Conclusiones: Las normas EPHect-PE facilitarán la documentación estandarizada de la exploración física, incluyendo la evaluación y documentación del fenotipo de exploración del dolor pélvico asociado a la endometriosis. (Fertil Steril 2024: Sociedad Americana de Medicina Reproductiva).

Palabras clave: Endometriosis, estandarización, armonización, fenotipificación, exploración física, EPHect.

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Neighborhood deprivation in relation to ovarian reserve and outcomes of ovarian stimulation among oocyte donors

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Objective: To study the relationship between neighborhood deprivation index (NDI) and markers of ovarian reserve and outcomes of controlled ovarian stimulation among young, healthy oocyte donors.
Design: Retrospective cohort study.
Patients: A total of 547 oocyte donors who underwent 905 oocyte retrieval cycles (2008–2020) at a private fertility center in Sandy Springs, Georgia, United States.
Interventions: Neighborhood deprivation index was calculated using principal component analysis applied to census-level measures of poverty, employment, household composition, and public assistance, which was then standardized and linked to donor information on the basis of donor residence.
Main Outcome Measures: Markers of ovarian reserve, including antral follicle count (AFC) and antimüllerian hormone (AMH) levels, and outcomes of controlled ovarian stimulation including number of total and mature oocytes retrieved and ovarian sensitivity index (OSI) (defined as the number of oocytes retrieved/total gonadotropin dose × 1,000). Multivariable generalized estimating equations with Poisson and normal distribution were used to model the relationship between NDI and outcome measures adjusting for age, body mass index, and year of retrieval.
Results: The mean (SD) age of donors was 25.0 (2.8) years and 29% of the donors were racial or ethnic minorities. There were no associations between donor NDI and ovarian reserve markers. For every interquartile range increase in NDI, there was a reduction of −1.5% (95% confidence interval: −5.3% to 2.4%) in total oocytes retrieved although the effect estimate was imprecise. Associations of NDI with a number of mature oocytes retrieved and OSI were in a similar direction. We observed evidence for effect modification of the NDI and OSI association by donor race. There was a suggestive positive association between NDI and OSI in Black donors but no association in White donors.
Conclusion: In this cohort of young, healthy, racially diverse oocyte donors, we found little evidence of associations between NDI and markers of ovarian reserve or outcomes of ovarian stimulation. (Fertil Steril® 2024;122:316–25. ©2024 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Neighborhood deprivation, ovarian reserve, ovarian stimulation, socioeconomic status, oocyte donor

In the United States, the use of assisted reproductive technology (ART), most commonly in-vitro fertilization (IVF), has increased from approximately 100,000 cycles in 2000 to >300,000 in 2020 (1). These increasing trends in utilization of ART may signal concerning declines in fertility (2), but may also reflect intentional delays in childbearing among women in recent decades (3–5). Nonetheless, an increasing percentage of family-planning couples are seeking out IVF for reproduction. However, as of 2020, <50% of intended egg retrievals resulted in a live birth delivery (6).

Increasing maternal age is not only a predictor of infertility but also a predictor of lower IVF success after embryo transfer. With regard to clinical factors, low ovarian reserve is associated inversely with live birth, whereas a

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Data will be shared on reasonable request.
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higher number of mature oocytes retrieved, a higher number of high-quality embryos, and a greater number of embryo transfers are all associated with increased cumulative live birth success (7, 8). These clinical predictors are promising, but reflect only a small fraction of the patient experience. Thus, it is important clinically to identify modifiable factors that contribute to ART treatment success, such as environmental milieu.

Neighborhood disadvantage, characterized broadly by a higher level of poverty, unemployment, use of public assistance, woman-headed households, and low educational attainment (9), has been associated with poorer health outcomes (10). Neighborhood disadvantage has also been associated with adverse perinatal outcomes including preterm birth, low birth weight, and still birth (11–14), in addition to poor maternal health outcomes (15, 16). Among pregnancy planners, residing in a more disadvantaged neighborhood was associated with reduced fecundability compared with those in less disadvantaged settings in the United States (17), suggesting an inverse association between neighborhood deprivation with reproductive health and fertility. Possible underlying mechanisms of these associations between neighborhood deprivation and reproductive health include lack of access to health-promoting resources such as green space, healthy food, healthcare, and social services in concert with increased exposure to environmental pollutants and higher levels of chronic stress that can impact reproduction (18, 19). Additionally, neighborhood deprivation may precipitate limited access to and success of fertility treatment via these potential mechanisms. However, there is less, and conflicting, evidence on the associations between neighborhood deprivation and fertility outcomes for couples seeking infertility treatment. Greater neighborhood median annual household income was associated with an increased likelihood of live birth after IVF among women undergoing infertility treatment (20). Another study conducted among women in New York found no association between live birth rate and household income or IVF insurance coverage (21). With regard to ovarian reserve, a study conducted among women in St. Louis, Missouri, found that neighborhood deprivation was associated with reduced antimüllerian hormone (AMH) concentrations, but not antral follicle count (AFC), among women who were obese or overweight (22). Thus, it remains unclear how neighborhood deprivation might affect fertility, and more specifically ovarian reserve, in women.

The objective of this study was to examine the relation of neighborhood deprivation, using the neighborhood deprivation index (NDI) (23), with ovarian reserve and outcomes of controlled ovarian stimulation among a population of young, healthy, racially diverse oocyte donors. Furthermore, we aimed to explore donor race or ethnicity and body mass index (BMI) as potential effect modifiers of these associations as these factors have been shown previously to modify associations of neighborhood deprivation and reproductive outcomes (13, 22).

MATERIALS AND METHODS

Study design and population

This retrospective study use data from nonidentified vitrified oocyte donors undergoing controlled ovarian stimulation cycles at a private fertility clinic in Sandy Springs, Georgia, from 2008 to 2020. The data collection project was approved through the institutional review board of Emory University (IRB00080463). Oocyte donors were screened before donation according to the American Society for Reproductive Medicine recommendations (24). At initial screening, donors at reproductive biology associates had to be aged 21–31 years, have a BMI between 18 and 27 kg/m^2, have regular menstrual cycles, and have no medical contraindications to elective surgery. All donor candidates also had carrier screening and infectious disease testing, answered a 300-question survey regarding their family history, educational attainment, occupation, medical history, exercise habits, tobacco use, and diet, and had a general physical examination including a pap smear and cultures. Oocyte donor candidates were also screened for ovarian reserve via AFC (suggested minimum: 20) and, starting in 2012, via AMH (suggested minimum: 2 ng/mL). Donor candidates also underwent substantial psychological evaluation and counseling. In most cases, donor candidates were determined to be ineligible on the basis of out-of-range psychological evaluations, a current history or medication use for psychological disorders, and a family history of early heart disease or psychiatric illness. In some cases, oocyte donation was allowed for candidates with out-of-range values at the discretion of the physician.

Our initial database contained information on 662 donors. From there we excluded donors who did not reside in Georgia (n = 80) and donors with P.O. boxes listed for their residential address (n = 3). From the sample containing 579 donors and 966 oocyte retrieval cycles, we further excluded 32 donors and 61 cycles who completed their retrieval before 2008, who were missing information on retrieval year, who underwent gonadotropin hormone-releasing hormone (GnRH) agonist downregulation protocol, or who did not have information on number of oocytes retrieved. After all exclusions, our analytic sample included 547 unique donors who underwent 905 oocyte retrieval cycles.

At the time of the first oocyte retrieval, donors provided information on date of birth, race, education level, parity, and smoking status using a standardized questionnaire. Height and weight were measured using standardized procedures to calculate BMI. Cycle characteristics were collected via medical chart abstraction.

Exposure assessment

Donors' residential addresses were collected from their medical records and geocoded using ArcGIS. If the address changed over time, this was noted, along with the year of move. Each oocyte retrieval cycle was linked to the address that was before and in closest proximity to the date of oocyte retrieval. To estimate place-based socioeconomic status of a donor's residential neighborhood environment, we used NDI, a validated, composite measure that has previously been used in studies of reproductive and perinatal health (23, 25, 26). The NDI was calculated using principal component analysis applied to federal and state data and included eight subcomponents: percent of households in poverty, percent of woman-headed households with dependents,

percent of households with an annual income <$35,000, percent of households using public assistance, percent of men in management occupations, percent of crowded housing, percent residential turnover, and percent with less than a high school education. The loading of each score was then used to weigh each factor's contribution to the summary NDI score, which was then standardized using the NDI values across all years in all census tracts of Georgia to have a mean of 0 and an SD of 1 (27). Neighborhood deprivation index values were linked to census tracts on the basis of donor's residential address. NDI was then categorized into quintiles (on the basis of the distribution of NDI in our cohort) for analysis with higher NDI scores and quintiles indicative of greater disadvantage.

Outcome assessment

Primary outcomes of interest were measures of ovarian reserve, AFC and AMH levels, and outcomes of controlled ovarian stimulation including a number of total and mature oocytes retrieved, all of which were abstracted from the medical records of donors. During the donor screening process, a blood sample was taken and sent for the analysis of AMH by Quest Diagnostics. Antral follicle count refers to the sum of antral follicles in both ovaries measured by transvaginal ultrasonography in the follicular phase of the menstrual cycle at baseline, before any ovarian stimulation. An antagonist protocol was employed for ovarian stimulation. After ovarian stimulation with gonadotropins and maturation trigger, oocyte retrieval was performed using a transvaginal ultrasound-guided aspiration. Total oocyte count was defined as the sum of all oocytes retrieved. Mature oocyte count was the sum of all metaphase II oocytes retrieved. Ovarian sensitivity index (OSI) was calculated as the total number of oocytes divided by the gonadotropin dose, multiplied by 1,000 (28).

Covariate assessment

Confounding was evaluated using a priori knowledge and a directed acyclic graph (Supplemental Fig. 1, available online). Adjusted models included donor age at retrieval (continuous), donor BMI (continuous), and year of retrieval (categorical). Both donor age and BMI were updated if a donor contributed >1 retrieval cycle. Because missing covariate data were rare (<5%), we performed a single imputation for covariates with missing data using the median value for continuous variables and the most common level for categorical variables.

Statistical analysis

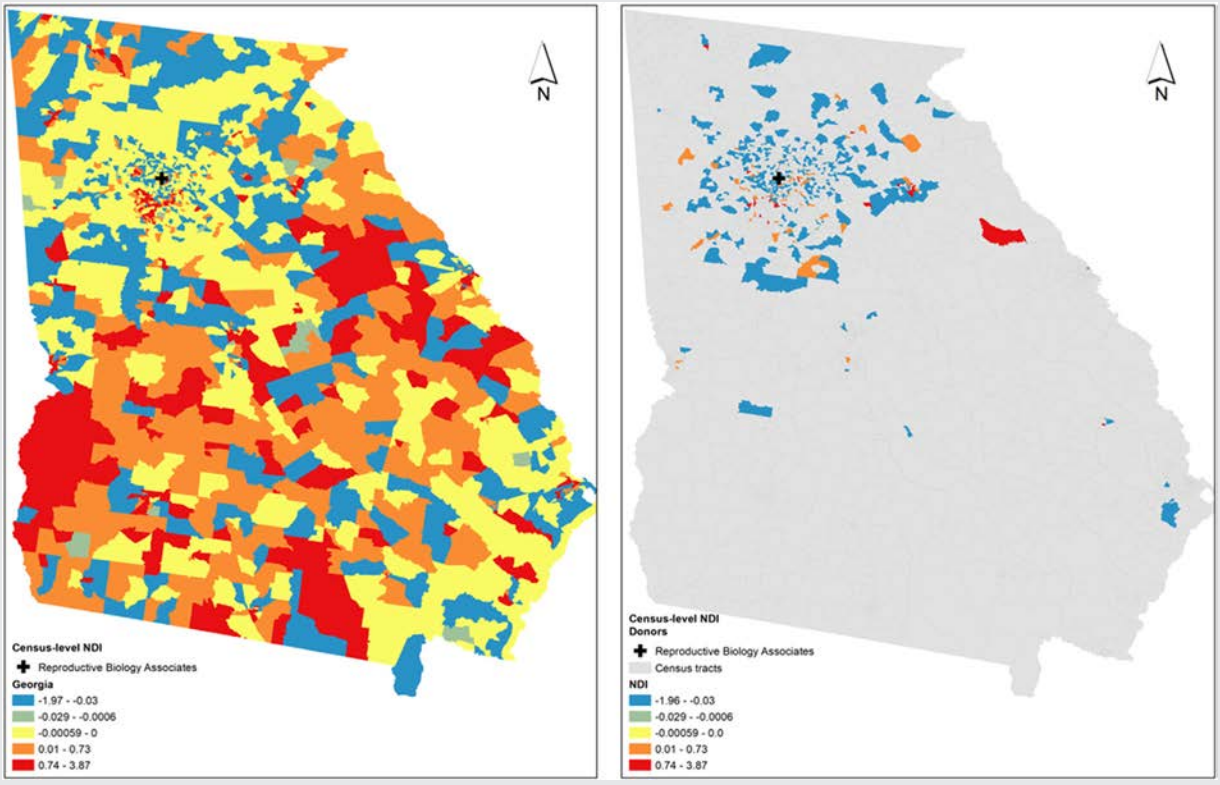
We present counts and percentages of demographic, reproductive, and ovarian stimulation parameters at the donor's first oocyte retrieval by quintile of NDI, defined on the basis of the distribution of NDI in our study population. We used unadjusted and adjusted generalized estimating equations with Poisson distribution to estimate associations between NDI (measured both continuously per interquartile range [IQR] increase and across quintiles of NDI on the basis of our study population) and AFC, number of total oocytes retrieved, and number of mature oocytes retrieved. These models accounted for the repeated observations that some donors contributed over multiple retrievals. Robust standard errors were applied in all models to account for overdispersion. After natural log transformation, AMH values were roughly normally distributed, and thus we used generalized estimating equations with normal distribution to evaluate NDI in relation to AMH and OSI values. For all outcomes, nonlinearity was assessed with restricted cubic splines, which used the likelihood ratio test comparing the model with the linear term to the model with the linear and the cubic spline terms. We also stratified our models by race (Black vs. White) and BMI (<25 vs. $\geq$25 kg/m^2) to evaluate differential associations between NDI and ovarian reserve and ovarian stimulation outcomes by race and overweight or obese status. Additionally, we included an interaction term between the dichotomized measures of race and BMI and NDI in the adjusted models to estimate a *P*-for-interaction. In sensitivity analyses, we examined the association between NDI quintile and AMH, AFC, total and mature oocytes retrieved, and OSI including only the first ovarian stimulation cycle for donors and accounting for potential autocorrelation between donors residing in the same census tract using a multilevel model which included donor id and census tract of residence. These models included the same covariates and distribution specifications as the main analyses. Data cleaning was performed in R 4.2.2 (29). All statistical analyses were performed using SAS version 9.4 (30).

RESULTS

Our final analytic sample included 547 oocyte donors who lived in Georgia. The state of Georgia includes 1,949 census tracts and 382 (19%) were represented by donors in our study (Fig. 1). The NDI distribution of census tracts in our sample ranged from −1.81 to 2.16, whereas the NDI distribution for all census tracts in the state of Georgia was −1.97 to 3.87. Donors in our study sample were young (mean age: 25 years, range: 20–32 years) and racially diverse: 71.1% White, 11.6% Black, 5.7% Asian, 5.2% Hispanic, and 6.5% other races. Forty-two (7.6%) donors had BMI values outside of the range defined for donor selection (18–27 kg/m^2), likely because of weight gain or loss between donor screening and oocyte retrieval and physician discretion. Most (64.2%) donors underwent only 1 retrieval (range: 1–6 retrievals). The median (IQR) AMH was 4.7 (3.4) ng/mL, AFC was 36 (17) and the median number of total and mature oocytes retrieved was 32 (17) and 24 (13), respectively.

Generally, demographic and reproductive characteristics were similar across NDI quintiles (Table 1). Donor characteristics such as age, BMI, education and smoking status and ovarian stimulation characteristics, including gonadotropin total dose, number of follicles >14 mm at trigger, peak estradiol, and maturation trigger type, were comparable across NDI quintiles. Days of stimulation differed by NDI quintile, with 82% of donors living in neighborhoods in the fourth quintile of NDI (higher deprivation) receiving 10–11 days of stimulation compared with 56% of donors in the lowest quintile (low deprivation). Year of retrieval also differed by NDI quintile with a larger proportion of donors residing in

FIGURE 1



Distribution of neighborhood deprivation index (NDI), by quintiles, for all Georgia census tracts (left panel) and for tracts where oocyte donors in our study reside (right panel), 2008–2020.

Suresh. Neighborhood deprivation and ovarian reserve. Fertil Steril 2024.

neighborhoods in the lowest quintile of NDI in earlier years (2008–2011). There was a higher percentage of Black and Hispanic donors in the highest quintile of NDI (22.0% and 6.0%) compared with the lowest quintile (5.2% and 1.7%), respectively, whereas White and Asian donors were more likely to reside in low NDI census tracts compared with high NDI tracts.

Overall, we observed no strong associations between donor NDI and ovarian reserve outcomes. The adjusted mean AMH and AFC among donors in the lowest quintile of neighborhood deprivation were 4.7 ng/mL (95% confidence interval [CI]: 4.1–5.5) and 38.9 (95% CI: 36.9–41.1) compared with 4.9 ng/mL (95% CI: 4.4–5.5) and 39.5 (95% CI: 36.9–42.4) among donors in the highest quintile of NDI, respectively (Table 2). When comparing donors in the highest quintile of NDI to the lowest quintile of NDI, we generally observed lower or worse ovarian stimulation outcomes although differences were small and imprecise (Table 3). Donors living in neighborhoods in the highest quintile of NDI had an adjusted mean count of 32.9 (95% CI: 31.0–34.9) total oocytes retrieved and 24.9 (95% CI: 23.4–26.6) mature oocytes retrieved compared with an adjusted mean count of 34.7 (95% CI: 32.2–37.5) total oocytes retrieved and 26.1 (95% CI: 24.1–28.2) mature oocytes retrieved among donors from neighborhoods in the lowest quintile of NDI. Adjusted average OSI was 13.2 (95% CI: 12.0–14.6) for donors living in neighborhoods in the lowest NDI quintile and 12.8 (95% CI: 11.9–14.6) in the highest quintile.

When modeled as a continuous variable using restricted cubic splines, there was no evidence of a non-linear association between total oocyte count and NDI (P for nonlinearity = 0.90). In the adjusted linear model, for every IQR increase in NDI (i.e., increasing deprivation), there was a −1.5% (95% CI: −5.3% to 2.4%) reduction in total oocytes retrieved (Supplemental Table 1, available online). Associations of NDI with a number of mature oocytes retrieved and OSI were of similar magnitude and direction, although all estimates were imprecise. When we evaluated the specific subcomponents of NDI, none were associated with total number of oocytes retrieved, although the strongest negative association was observed for neighborhoods with a higher percent of woman-headed households with dependents (−2.6% decrease per IQR increase) followed by percent of men in management positions (−1.8% per IQR increase), percent of households with annual income below $35,000 (−1.7% per IQR increase), and percent residential turnover (−1.5% per IQR increase) (Supplemental Table 1). Associations with a number of mature oocytes retrieved and OSI were similar in direction and magnitude for each NDI subcomponent.

When stratified by race, we did not observe effect modification for the association between NDI and markers of

TABLE 1

Characteristics of oocyte donors by neighborhood deprivation index at first cycle, 2008–2020.

		Quintile of neighborhood deprivation index				
	Total	<20th	20th–39th	40th–59th	60th–79th	≥80th
No. of donors		118	106	101	104	118
Age at first retrieval (y)	547					
20–22		34 (29%)	23 (22%)	36 (36%)	31 (30%)	53 (45%)
23–26		37 (31%)	41 (39%)	28 (28%)	36 (35%)	30 (25%)
27–29		37 (31%)	33 (31%)	29 (29%)	29 (28%)	29 (25%)
30–32		10 (8.5%)	9 (8.5%)	8 (7.9%)	8 (7.7%)	6 (5.1%)
Year of retrieval	547					
2008–2011		49 (42%)	34 (32%)	39 (39%)	30 (29%)	30 (25%)
2012–2014		34 (29%)	33 (31%)	15 (15%)	27 (26%)	29 (25%)
2015–2017		19 (16%)	25 (24%)	35 (35%)	28 (27%)	37 (31%)
2018–2020		16 (14%)	14 (13%)	12 (12%)	19 (18%)	22 (19%)
Race or ethnicity	542					
Asian		9 (7.8%)	5 (4.7%)	5 (5.0%)	6 (5.8%)	6 (5.2%)
Black		6 (5.2%)	7 (6.6%)	15 (15%)	15 (14%)	25 (22%)
Hispanic		2 (1.7%)	6 (5.7%)	5 (5.0%)	5 (4.8%)	7 (6.0%)
Other		7 (6.0%)	5 (4.7%)	4 (4.0%)	9 (8.7%)	8 (6.9%)
White		92 (79%)	83 (78%)	71 (71%)	69 (66%)	70 (60%)
BMI (kg/m^2)	545					
14.5–21.0		31 (26%)	29 (27%)	26 (26%)	26 (25%)	40 (34%)
21.1–24.9		72 (61%)	56 (53%)	54 (54%)	50 (49%)	51 (43%)
25.0–33.5		15 (13%)	21 (20%)	20 (20%)	27 (26%)	27 (23%)
Education	537					
≤High school		1 (0.8%)	2 (1.9%)	0 (0%)	3 (3.0%)	1 (0.9%)
Some college		98 (83%)	78 (76%)	81 (82%)	75 (74%)	84 (72%)
Advanced degree		19 (16%)	23 (22%)	18 (18%)	23 (23%)	31 (27%)
Smoking status	536					
Never smoker		106 (91%)	94 (91%)	90 (90%)	94 (91%)	108 (95%)
Ever smoker		10 (8.6%)	9 (8.7%)	10 (10%)	9 (8.7%)	6 (5.3%)
No. of previous births	547					
0		87 (79%)	86 (79%)	82 (75%)	87 (79%)	86 (79%)
1		10 (9%)	14 (13%)	10 (9%)	13 (12%)	11 (10%)
2 or more		13 (12%)	9 (8%)	17 (16%)	10 (9%)	12 (11%)
Gonadotropin dose (IU)	546					
≤1,500		5 (4.2%)	1 (1.0%)	1 (1.0%)	7 (6.7%)	3 (2.5%)
1,501–2,500		59 (50%)	55 (52%)	53 (52%)	46 (44%)	70 (59%)
2,501–3,500		51 (43%)	43 (41%)	42 (42%)	50 (48%)	43 (36%)
3,501–5,000		3 (2.5%)	6 (5.7%)	5 (5.0%)	1 (1.0%)	2 (1.7%)
Days of stimulation	546					
8–9		33 (28%)	21 (20%)	16 (16%)	10 (9.6%)	20 (17%)
10–11		66 (56%)	62 (59%)	63 (62%)	85 (82%)	75 (64%)
12–13		19 (16%)	22 (21%)	22 (22%)	9 (8.7%)	23 (19%)
No. of follicles >14 mm at trigger	540					
≤12		8 (6.8%)	8 (7.8%)	2 (2.0%)	5 (4.9%)	5 (4.3%)
13–24		75 (64%)	59 (57%)	65 (64%)	60 (59%)	64 (55%)
25–40		30 (26%)	32 (31%)	32 (32%)	33 (32%)	41 (35%)
41–55		4 (3.4%)	4 (3.9%)	2 (2.0%)	4 (3.9%)	7 (6.0%)
Peak estradiol (pg/mL)	539					
<2,000		28 (24%)	21 (20%)	25 (25%)	20 (20%)	15 (13%)
>6,000		45 (38%)	48 (47%)	37 (37%)	39 (39%)	41 (35%)
2,001–4,500		20 (17%)	16 (16%)	16 (16%)	21 (21%)	28 (24%)
4,501–6,000		24 (21%)	18 (17%)	23 (23%)	21 (21%)	33 (28%)
Maturation trigger type	541					
GnRH Agonist (Lupron)		86 (73%)	80 (75%)	71 (72%)	84 (81%)	97 (84%)
hCG		32 (27%)	26 (25%)	27 (28%)	20 (19%)	18 (16%)

Data are presented as number of donors (%).
GnRH = gonadotropin hormone-releasing hormone; hCG = human chorionic gonadotropin.

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ovarian reserve (Supplemental Table 2). However, we observed marginal effect modification of the association between NDI and OSI by race whereby there was a suggestive positive association between NDI and OSI in Black donors (1.9 per IQR increase [95% CI: −0.0 to 3.7]) but no association in White donors (−0.5 per IQR increase [95% CI: −1.3 to 0.4]). When models were stratified by donor BMI, we also did not observe differences in the associations between NDI and markers of ovarian reserve or outcomes of ovarian stimulation (Supplemental Table 2). Sensitivity analyses on donors'

TABLE 2

Association between donor neighborhood deprivation index quintile and markers of ovarian reserve.

	Antimüllerian hormone (ng/mL) (AMH)[a]				Antral follicle count (AFC)[b]			
	No. of donors	No. of cycles	Unadjusted mean (95% CI)	Adjusted mean (95% CI)[c]	No. of donors	No. of cycles	Unadjusted mean (95% CI)	Adjusted mean (95% CI)[c]
Donor NDI								
Q1 (lowest deprivation)	68	113	4.7 (4.0–5.5)	4.7 (4.1–5.5)	112	172	38.6 (36.6–40.8)	38.9 (36.9–41.1)
Q2	67	121	4.3 (3.8–4.9)	4.4 (3.9–4.9)	101	171	36.5 (34.5–38.7)	36.9 (35.0–38.9)
Q3	58	116	4.8 (4.3–5.4)	4.8 (4.2–5.4)	92	169	37.8 (35.5–40.2)	37.7 (35.5–40.0)
Q4	71	128	4.7 (4.2–5.3)	4.7 (4.2–5.3)	99	172	39.5 (36.9–42.2)	38.6 (36.4–41.1)
Q5 (highest deprivation)	83	129	4.9 (4.4–5.5)	4.9 (4.4–5.5)	110	169	40.1 (37.5–43.0)	39.5 (36.9–42.4)

AFC = antral follicle count; BMI = body mass index; CI = confidence interval; NDI = neighborhood deprivation index.
[a] Generalized estimating equations with normal distribution and log link were used to evaluate the association between NDI and AMH. The total sample size for this model was 347 donors and 607 cycles because of missing data on AMH.
[b] Generalized estimating equations with Poisson distribution and robust standard errors were used to evaluate the association between NDI and AFC. The total sample size for this model was 514 donors and 853 cycles because of missing data on AFC.
[c] Multivariable models adjusted for age, BMI, and year of retrieval.

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first ovarian stimulation cycle and accounting for autocorrelation were similar to the main results (Supplemental Tables 3 and 4).

DISCUSSION

Among this racially diverse cohort of nonidentified vitrified oocyte donors in Georgia, we found no strong associations between NDI and markers of ovarian reserve or ovarian stimulation outcomes. When analyses were stratified by race, no significant differences were observed; however, there was marginal evidence of effect modification by race for the association between NDI and OSI. Associations between NDI subcomponents, total and mature oocytes retrieved, and OSI were also modest and imprecise.

To date, only one other study has examined the relation of neighborhood deprivation with ovarian reserve, but not ovarian stimulation outcomes. Using a cross-sectional study of 193 healthy, regularly menstruating women from St. Louis, Missouri, Komorowski et al. (22) did not find an association between neighborhood deprivation (defined using the area deprivation index [ADI]), AMH, and AFC; however, similar to our study findings, a small reduction in both measures was observed among women with overweight or obesity. Thus, the null findings of our study agree with the lack of association among women with BMI of <25 kg/m^2 in the study by Komorowski et al. (22) because the mean BMI of our donor sample was 22.6 kg/m^2 and 20% were overweight or obese. Despite the different constructs in NDI, which uses eight neighborhood factors, and ADI, which uses 17 neighborhood factors, these measures are relatively comparable because the factor loadings for NDI and ADI were greatest for poverty and income. However, these metrics are not entirely comparable because ADI is constructed on the basis of national-level percentiles, whereas our NDI score was based on the state of Georgia.

Although the existing literature on NDI and markers of ovarian reserve is sparse, there is a growing body of literature showing significant associations between NDI and couple-based fertility outcomes including time-to-pregnancy and live birth after IVF. Among a large prospective cohort of North American pregnancy planners, Willis et al. (17) observed that neighborhood disadvantage and fecundability were associated negatively, particularly among participants with lower

TABLE 3

Association between donor NDI quintile and outcomes of controlled ovarian stimulation.

			Total oocytes retrieved[a]		Mature oocytes retrieved[a]		Ovarian sensitivity index (OSI)[b]	
	No. of donors	No. of cycles	Unadjusted mean (95% CI)	Adjusted mean (95% CI)[c]	Unadjusted mean (95% CI)	Adjusted mean (95% CI)[c]	Unadjusted mean (95% CI)	Adjusted mean (95% CI)[c]
Donor NDI								
Q1	118	181	34.7 (32.0–37.6)	34.7 (32.2–37.5)	26.0 (24.0–28.1)	26.1 (24.1–28.2)	13.0 (11.8–14.4)	13.2 (12.0–14.6)
Q2	106	181	33.4 (31.0–36.0)	33.6 (31.4–36.1)	24.6 (22.8–26.5)	24.7 (23.0–26.5)	12.0 (10.9–13.3)	12.2 (11.1–13.5)
Q3	101	181	33.2 (30.9–35.7)	33.1 (30.9–35.5)	24.9 (23.1–26.8)	24.8 (23.1–26.7)	12.6 (11.4–13.9)	12.6 (11.4–13.9)
Q4	104	181	35.1 (32.4–38.0)	34.3 (31.9–37.0)	26.0 (23.9–28.3)	25.6 (23.5–27.8)	13.2 (12.0–14.5)	13.0 (11.9–14.3)
Q5	118	181	33.2 (31.3–35.3)	32.9 (31.0–34.9)	25.2 (23.7–26.9)	24.9 (23.4–26.6)	13.1 (12.1–14.1)	12.8 (11.9–14.6)

BMI = body mass index; CI = confidence interval; NDI = neighborhood deprivation index.
[a] Generalized estimating equations with Poisson distribution and robust standard errors were used to evaluate the association between NDI and total and mature oocytes retrieved.
[b] Generalized estimating equations with normal distribution and identity link were used to evaluate the association between NDI and OSI. The total sample size for this model was 546 donors and 903 cycles because of missing FSH.
[c] Multivariable models adjusted for age, BMI, and year of retrieval.

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annual incomes (<$50,000). Similarly, Richardson et al. (31) showed that, among a retrospective cohort of 3,901 women undergoing their first fresh single-embryo transfer in the United Kingdom, the rate of clinical pregnancy and live birth was higher among women from the least deprived areas compared with the most deprived areas. Horns et al. (32) also observed that couples from more deprived areas (defined using ADI) were less likely to experience a live birth than couples from less deprived areas among a large cohort of 13,873 subfertile men undergoing semen analysis in Utah. At the individual level, two small studies from India and Turkey have found that higher socioeconomic status (as measured by a composite score on the basis of education, occupation of the head of the family, and monthly household income) has been associated with improved ovarian reserve (33, 34), suggesting that individual-level socioeconomic factors may also influence reproductive outcomes.

The biological mechanisms that potentially underlie the associations between neighborhood deprivation with ovarian reserve and response to ovarian stimulation are not entirely clear. Neighborhood deprivation impacts adversely myriad health outcomes, which may subsequently influence reproductive health. Neighborhood deprivation is associated with the severity of depression (35), telomere length (36), child physical activity (37), prenatal smoke exposure (38), and weight gain (39) (among others), which may mediate the effects of NDI on fertility. Moreover, the differential influence of structural racism, as proxied by race or ethnicity, on the associations between neighborhood deprivation and health outcomes is of increasing scientific interest and importance for informing social and health policy. O'Campo et al. (25) found that neighborhood deprivation had a stronger association with preterm birth among non-Hispanic White women (odds ratio: 1.57, 95% CI: 1.41–1.74) comparing the highest to lowest deprivation quartiles) compared with non-Hispanic Black women (odds ratio: 1.15, 95% CI: 1.08–1.23). On the other hand, Martenies et al. (13) found that the joint effects of social and environmental factors at a neighborhood level were associated with increased risk of low birthweight among Black women, but not White women, indicating significant effect measure modification by race. However, our current study only observed slight differences in the associations between NDI and OSI by race, with positive associations observed among Black donors and negative associations among White donors. This may be related to the extensive screening that donors undergo, which provides this study with a selectively healthy and relatively less deprived population of donors. The NDI distribution of the 382 census tracts represented in this study is lower than that of all Georgia census tracts, again indicating potential selection bias that may hinder our ability to detect these differences. More research is needed to better understand how racism, neighborhood environment, and disparate exposures of weathering impact markers of ovarian reserve and controlled ovarian stimulation, at individual and community levels (40).

A primary limitation of our study is the extensive screening to exclude any donors—most notably those with low ovarian reserve, >32 years of age, with high BMIs, current mental health conditions, sexually transmitted infections, or reporting high-risk sexual behaviors. This screening might have implications for several types of biases in our study. First, it is highly possible that many of these selection criteria are also potential pathways through which NDI can affect ovarian reserve. Therefore, by conditioning on these intermediate factors, we might have biased our results toward the null. Second, by applying these extensive screening criteria we may have limited the generalizability of our findings. Relative to the state of Georgia, our sample of donors overrepresented census tracts with the lowest NDI (e.g., least disadvantage) and underrepresented tracts with the highest NDI (e.g., most disadvantage). This may be because of the location of the fertility clinic in metropolitan Atlanta, which is surrounded by census tracts with low NDI, whereas most highest NDI census tracts are situated in middle and southern Georgia. This could also be because of the intersections of more broad and complex factors including the populations targeted by oocyte donor marketing campaigns, the eligibility criteria, and distrust of reproductive health technologies by certain disadvantaged communities. This reduced variability in NDI may have impacted our ability to detect a meaningful difference in ovarian outcomes because our range of NDI was skewed toward lower disadvantage. Similarly, our distribution of ovarian reserve skewed to higher values because of selection criteria. Third, there is the possibility that our selection criteria may have induced selection bias, which can occur when selection into a study is associated with both the exposure and outcome. We believe this to be unlikely because our exposure, NDI, was not directly related to donor selection and we adjusted for the key variables that might link the two, namely age and BMI. Although selection into the cohort was clearly tied to our outcome, this alone should not cause bias (just reduced generalizability as acknowledged above).

Another potential limitation is using census tracts as the unit of exposure. Because we measured NDI at the census tract level, we are unable to observe within-tract differences in NDI because some donors might experience more or less deprivation than the assigned score, suggesting potential exposure misclassification. Moreover, bias in our study results may arise from using the census tract, an arbitrary administrative unit, to define donor neighborhoods (41), resulting in the modifiable areal unit problem (42). In addition, although we do have a much higher proportion of minority racial or ethnic groups represented in our sample population compared with other fertility clinic populations, we still had a limited number of Black donors (and notably lower percentages than those existing in the metropolitan Atlanta area) in our sample, which might have affected our power to observe a difference in the effect of NDI on ovarian reserve and ovarian stimulation outcomes in White and Black donors. It is also possible that our generalizability to donor populations in other states in the United States may be limited because our measure of NDI was created specific to Georgia and states in the Southeast have some of the highest levels of NDI compared with other regions (43). Finally, lack of information on the specific assay used to measure AMH limited our ability to consider the

potential misclassification and limitations arising from changing AMH measurements over time. However, we adjusted for calendar year in our analytical models to account for the likely changes in the AMH assay used over the study period.

Strengths of our study include the retrospective design, diverse cohort and including objective measures of markers of ovarian reserve and outcomes of controlled ovarian stimulation. Outcomes of ovarian stimulation provide clearer insights into the health and function of the ovary than ovarian reserve alone and thus contribute more to the understanding of influences of neighborhood deprivation and ovarian health than currently exist in the literature. Additionally, the similarity in results between our main analyses and our sensitivity analyses conducted on the first cycles of donors and accounting for potential autocorrelation within census tracts strengthens our results. In addition, this study used the NDI, which was calculated on the basis of the distribution of values across the state of Georgia, thus providing a more localized reference for calculating deprivation, although the ADI ranks census groups on the basis of national-level percentiles that are less reflective of local trends.

CONCLUSION

In conclusion, we did not observe strong associations between NDI and markers of ovarian reserve and ovarian stimulation among our large, diverse cohort of young, healthy oocyte donors residing in Georgia. Future research on this topic should consider including additional measures of neighborhood deprivation, such as environmental barriers, healthcare access, and social inequities, among unselected populations, which might help better elucidate this relationship. It may also be important to collect information on residence during key periods of reproductive development—such as in utero, childhood, and adolescence—to better capture potential cumulative effects of neighborhood deprivation on adult fertility.

CRediT Authorship Contribution Statement

Tanvi Suresh: Writing – original draft, Formal analysis. **Sarah LaPointe:** Writing – review & editing, Formal analysis. **Jaqueline C. Lee:** Writing – review & editing, Data curation. **Zsolt P. Nagy:** Writing – review & editing, Project administration, Data curation. **Daniel B. Shapiro:** Writing – review & editing, Project administration, Data curation. **Michael R. Kramer:** Writing – review & editing, Methodology, Formal analysis. **Heather S. Hipp:** Writing – review & editing, Supervision, Project administration, Data curation. **Audrey J. Gaskins:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of Interests

T.S. has nothing to disclose. S.L.P. has nothing to disclose. J.C.L. has nothing to disclose. Z.P.N. has nothing to disclose. D.B.S. has nothing to disclose. M.R.K. has nothing to disclose. H.S.H. has nothing to disclose. A.J.G. reports funding from R01 ES032446 from the National Institute of Environmental Health Sciences (NIEHS) for the submitted work; funding from NIEHS R21ES034130, NICHD R01HD096033, and an ASRM Research Grant; and stock options in Doveras Fertility Inc. outside the submitted work.

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Vecindario deprimido en relación con la reserva ovárica y los resultados de la estimulación ovárica entre donantes de ovocitos

Objetivo: Estudiar la relación entre el índice de privación de vecindario (IPV), los marcadores de reserva ovárica y los resultados de la estimulación ovárica controlada, entre donantes de ovocitos jóvenes y sanos.

Diseño: Estudio retrospectivo de cohortes.

Pacientes: Un total de 547 donantes de ovocitos que se sometieron a 905 ciclos de recuperación de ovocitos (2008-2020) en un centro privado de fertilidad en Sandy Springs, Georgia, Estados Unidos.

Intervenciones: El índice de privación del vecindario se calculó utilizando un análisis de componentes principales aplicado en medidas a nivel censal de pobreza, empleo, composición de los hogares y asistencia pública, los cuales se estandarizaron y vincularon a toda la información sobre la base de la residencia de las donantes.

Principales medidas de resultados: Marcadores de reserva ovárica, incluidos el recuento de folículos antrales (RFA) y los niveles de hormona antimülleriana (HAM), y resultados de la estimulación ovárica controlada, incluido el número de ovocitos totales y de los maduros recuperados y el índice de sensibilidad ovárico (ISO) (definido como el número de ovocitos recuperados/dosis total de gonadotropina por 1.000). Se utilizaron ecuaciones multivariables generalizadas de estimación con distribución normal y Poisson para modelar la relación entre el IPV y las medidas de resultado ajustadas por edad, el índice de masa corporal y el año de la recuperación.

Resultados: La edad media (DE) de las donantes fue de 25,0 (2,8) años y el 29% de las donantes pertenecían a minorías raciales o étnicas. No hubo asociaciones entre IPV de las donantes y los marcadores de reserva ovárica. A medida que aumentaba el rango intercuartílico en el IPV, hubo una reducción de -1,5% (intervalo de confianza del 95%: 5,3% a 2,4%) en el total de ovocitos recuperados, a pesar que la estimación del efecto era imprecisa. Las asociaciones del IPV con un número de ovocitos recuperados maduros y el ISO fueron en una dirección similar. Hubo una asociación positiva sugestiva entre el IPV y el ISO en donantes negras, pero ninguna asociación en donantes blancas.

Conclusión: En esta cohorte de donantes de ovocitos jóvenes, sanas y racialmente diversas, encontramos poca evidencia de asociaciones entre el IPV y los marcadores de reserva ovárica o entre los resultados de la estimulación ovárica.
(FertilSteril 2024;-:---. 2024porAmericanSocietyforReproductiveMedicine.)

Effectiveness of preconception weight loss interventions on fertility in women: a systematic review and meta-analysis

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Importance: Weight loss before conception is recommended for women with overweight or obesity to improve fertility outcomes, but evidence supporting this recommendation is mixed.
Objective: To examine the effectiveness of weight loss interventions using lifestyle modification and/or medication in women with overweight or obesity on pregnancy, live birth, and miscarriage.
Data Sources: An electronic search of MEDLINE, Embase, Cochrane Library, including Cochrane Database of Systematic Reviews and Cochrane Central Register of Controlled Trials, and Cumulative Index to Nursing and Allied Health Literature was conducted through July 6, 2022, via Wiley.
Study Selection and Synthesis: Randomized controlled trials examining weight loss interventions through lifestyle and/or medication in women with overweight or obesity planning pregnancy were included. Random-effects meta-analysis was conducted, reporting the risk ratio (RR) for each outcome. Subgroup analyses were conducted by intervention type, type of control group, fertility treatment, intervention length, and body mass index (BMI).
Main Outcome(s): Clinical pregnancy, live birth, and miscarriage events.
Result(s): A narrative review and meta-analysis were possible for 16 studies for pregnancy (n = 3,588), 13 for live birth (n = 3,329), and 11 for miscarriage (n = 3,248). Women randomized and exposed to a weight loss intervention were more likely to become pregnant (RR = 1.24, 95% CI 1.07–1.44; $I^2 = 59\%$) but not to have live birth (RR = 1.19, 95% CI 0.97–1.45; $I^2 = 69\%$) or miscarriage (RR = 1.17, 95% CI 0.79–1.74; $I^2 = 31\%$) compared with women in control groups. Subgroup analyses revealed women randomized to weight loss interventions lasting 12 weeks or fewer (n = 9, RR = 1.43; 95% CI 1.13–1.83) and women with a BMI $\geq$ 35 kg/m^2 (n = 7, RR = 1.54; 95% CI, 1.18–2.02) were more likely to become pregnant compared with women in the control groups. Miscarriage was higher in intervention groups who underwent fertility treatment (n = 8, RR 1.45; 95% CI 1.07–1.96).
Conclusion(s): Pregnancy rates were higher in women undergoing preconception weight loss interventions with no impact on live birth or miscarriage rates. Findings do not support one-size-fits-all recommendation for weight loss through lifestyle modification and/or medication in women with overweight or obesity immediately before conception to improve live birth or miscarriage outcomes. (Fertil Steril® 2024;122:326–40. ©2024 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Lifestyle intervention, antiobesity medication, pregnancy, preconception weight loss, overweight/obesity

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The prevalence of obesity (body mass index [BMI] $\geq$ 30 kg/m^2) among women aged 20–39 in the United States has more than tripled and severe obesity has increased 10-fold between 1960 and 2014 (1). Obesity increases risk of cardiometabolic diseases and pregnancy complications, impairs fertility, and influences the next generation through increased risk of poor fetal outcomes and obesity in offspring (2–4). Therefore, weight loss before pregnancy or fertility treatment is routinely recommended (5).

Weight loss through lifestyle modification, including caloric restriction and increased physical activity, is considered first-line treatment for obesity, but lifestyle interventions are intensive, tend to be less successful in women (6), have limited long-term efficacy (7, 8), and lack convincing evidence supporting benefits for fecundity, pregnancy, birth, or fetal outcomes (9, 10). Pharmaceutical interventions utilizing antiobesity medications (AOMs) produce superior weight loss compared with lifestyle modification (11–15), but less is known about their impact on reproductive health outcomes (16). Six large-scale, randomized controlled trials (RCTs) (17–21) have been published since the most recent data synthesis, and prior reviews had methodological limitations that preclude definitive conclusions on the effects of weight loss through lifestyle modification and/or medication on fertility and pregnancy outcomes.

The first systematic review only included studies of lifestyle interventions for weight loss in women before assisted reproductive technology (ART) (22), with conclusions supporting recommendations for weight loss before ART. A similar review and meta-analysis of weight loss on fertility/birth outcomes in women undergoing in vitro fertilization (IVF) was published in 2017 and echoed the narrative review's findings that weight loss improved pregnancy (RR: 1.61, 95% CI 1.15–2.27), live birth (RR: 1.86, 95% CI: 1.41–2.45), and miscarriage (RR: 0.56, 95% CI: 0.34–0.93) (23). However, studies among women seeking infertility treatments are not necessarily generalizable to all women of reproductive age desiring pregnancy.

A 2019 systematic review was not limited to women undergoing ART and included studies using lifestyle modification, pharmaceutical, and bariatric surgery interventions for weight loss (24). However, it only included a quantitative synthesis of weight outcomes, rather than pregnancy or live birth outcomes. A recent meta-analysis found interventions combining diet and exercise led to higher pregnancy (RR: 1.87, CI: 95% 1.20–2.93) and live birth rates (RR: 2.20, CI: 95% 1.23–3.94) compared with women in control groups (10). In another, lifestyle interventions led to higher pregnancy rates (RR: 1.43, CI: 95% 1.02–2.01), had no impact on live birth but were associated with a higher miscarriage rate (RR: 1.50, CI: 95% 1.04–2.16) (9). These meta-analyses were used per protocol analyses to calculate pregnancy and live birth rates rather than using intent-to-treat (ITT).

Findings from recent large-scale RCTs question the efficacy of weight loss interventions for improving fertility and live birth outcomes and raise concerns about increased risks for women from weight loss attempts before fertility treatment (9, 19–21, 25). An up-to-date and comprehensive quantitative synthesis that includes these RCTs and pharmaceutical interventions is needed to replace blanketed guidelines for preconception weight loss in women with obesity.

To that end, we conducted a systematic review and meta-analysis that includes studies of women with overweight or obesity seeking pregnancy and the use of a weight loss intervention (lifestyle modification and/or medication) on subsequent pregnancy, live birth, and miscarriage. To overcome methodological limitations of previous reviews, we used ITT principles to provide a more pragmatic, "real-world" analysis of the extent to which being randomized and exposed to a weight loss intervention improves outcomes rather than successfully completing the weight loss intervention, appreciating that lack of compliance is common in weight management interventions, particularly in reproductive-aged women (26–28).

MATERIALS AND METHODS

The 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines were followed and registered on PROSPERO (CRD42021269138) (29). Inclusion/exclusion criteria, search criteria, search strategy, process used to evaluate evidence quality, and meta-analysis methods are detailed below. Institutional Review Board approval was not required because of the public availability of the data.

Search strategy

The search was conducted on July 6, 2022. See Supplemental Table 1 (available online) for database search strategies and Figure 1 for the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 diagram.

Population and condition

Randomized controlled weight loss interventions in women with overweight (BMI $\geq$ 25 kg/m^2 and $<$ 30 kg/m^2) or obesity (BMI $\geq$ 30 kg/m^2) planning pregnancy were included. Studies that required pregnancy as inclusion criteria were excluded since pregnancy rate could not be determined. Cross-sectional, observational studies, conference papers, abstracts, dissertations, and reviews were excluded. We did not include non-English articles.

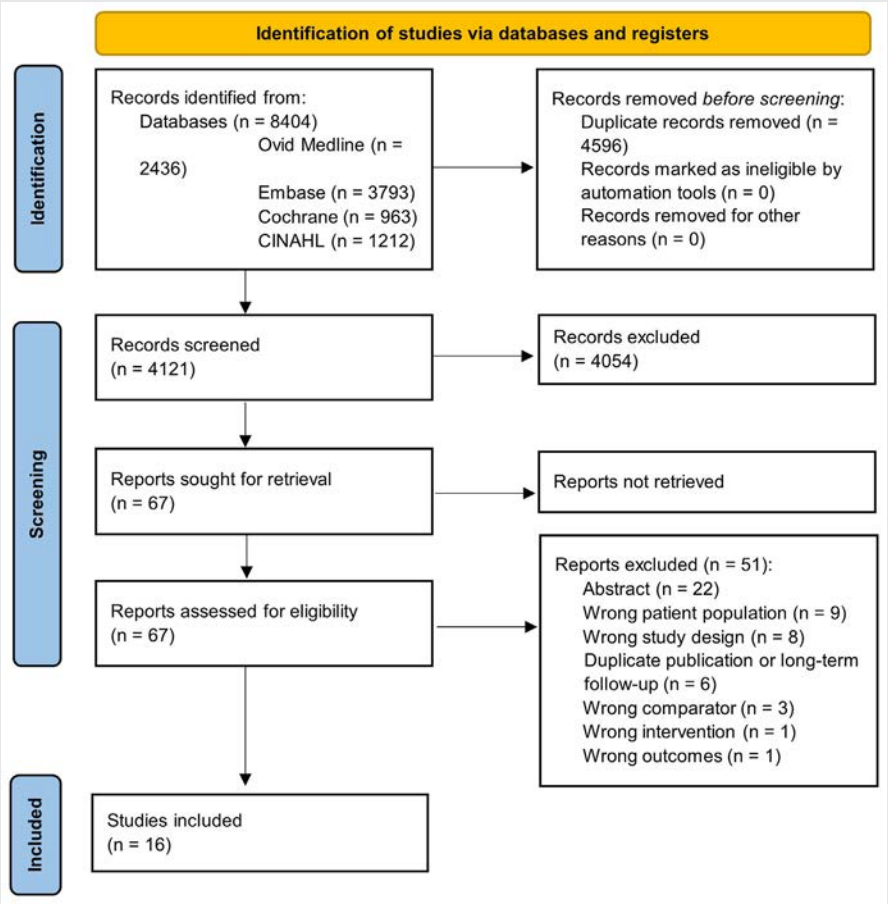
Intervention

Weight loss could be achieved by any intervention, including lifestyle modifications (diet and/or physical activity/exercise) and/or pharmacological treatment. We excluded studies where the intervention was solely exercise as exercise has many beneficial effects independent of weight loss, and this review focused on the outcomes of interest as they relate to weight loss. Included studies required a control group of any form to compare rates of primary and secondary outcomes.

Outcomes

The primary outcome was clinical pregnancy, defined as either pregnancy visualized by ultrasonography of 1 or more gestational sacs or definitive clinical proof of pregnancy

FIGURE 1



PRISMA diagram of included studies.

Caldwell. Preconception weight loss and fertility. Fertil Steril 2024.

(positive blood or urine test for human chorionic gonadotropin). Secondary outcomes were live birth and miscarriage rates.

Study selection

Review and selection of potential manuscripts were facilitated by Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia). Identified records were imported, and duplicates were removed. Titles and abstracts were screened in duplicate by independent reviewers (A.E.C., A.M.G., A.P.B., J.M.N., S.P., C.E., T.N.). Manuscripts meeting inclusion criteria on the basis of title/abstract were retrieved for full-text review and screened in duplicate by independent reviewers (A.E.C., A.M.G., A.P.B., J.M.N.). Discrepancies in inclusion/exclusion were resolved by a third reviewer or group discussion.

Data extraction

The following data were extracted by 2 independent reviewers (A.E.C., A.M.G., A.P.B., J.M.N., S.P., C.E., T.N.) from each manuscript meeting eligibility criteria (author, type of study, country of origin, etc.), procedural (participant number, population characteristics, protocol for inclusion, randomization, number randomized, etc.) and outcome data (pregnancy, live birth, miscarriage events) using a specifically designed form.

Risk of bias assessment

Risk of bias was assessed using the Cochrane Collaboration Handbook tool (30). Bias related to the randomization process, deviations from intended intervention delivery, missing outcome data, measurement of the outcome, and selective reporting were categorized as either low, high, or unclear levels of bias. Studies were assessed independently by 2 reviewers, with a third reviewer as needed in the event of disagreement.

Statistical analysis

Statistical analyses were performed using R. Binary data were assessed using the log-risk-ratio (LRR), and random-effects models (REM) were used to estimate the average LRR given the heterogeneity of studies. The LRR was used for modeling

TABLE 1

Descriptions of included studies.

Publication	Country Setting	Intervention duration	ITT sample size (N)	Group size (n)	Age (y)	BMI (kg/m^2)	Intervention Details	Retention (%)	Weight change (kg)	Fertility treatment
Becker et al., 2015 (39)	Brazil	12 weeks	35	Intervention: 16	31.4	28.7	CR + Low Glycemic	88	−4.51	No
				Control: 19	31.3	28.8	NWL; UC	63	0.72	
Einarsson et al., 2017[a] (42)	Sweden, Denmark, Iceland	12 weeks	314	Intervention: 159	31.5	33.1	VLCD+MR, RD	96	−9.1	Yes
				Control: 155	31.7	33.0	NWL; IVF	99	1.19	
Espinós et al., 2017[b] (40)	Spain	12 weeks	41	Intervention: 21	32.0	34.6	CR, PA	95	−5.4	Yes
				Control: 20	32.9	34.0	NWL; UC	95	NR	
Jiskoot et al., 2021[c] (26)	Netherlands	52 weeks	183	Intervention: 123	29.0	33.5	CBT, DA, PA	59	−6.3	No
				Control: 60	28.0	30.6	NWL; UC	57	−2.32	
LeBlanc et al., 2021[d] (17)	United States	26 weeks	326	Intervention: 164	31.3	34.8	CR+DASH, PA	99	−3.7	No
				Control: 162	31.6	34.9	NWL; UC	99	0.6	
Moran et al. 2011 (35)	Australia	5–9 weeks	46	Intervention: 21	33.8	34.0	CR + MR, PA	86	−3.8	Yes
				Control: 25	32.5	33.9	NWL; UC	80	−0.5	
Muirhead et al., 2021 (36)	Australia	10 weeks	48	Intervention: 24	33.7	34.7	CR + MR + RD, PA	88	−5.3	No
				Control: 24	31.5	32.9	WL; RD	75	−2.8	
Mutsaerts et al., 2016[e] (38)	Netherlands	26 weeks	574	Intervention: 289	29.7	36.0	CR, PA	77	−4.4	Yes
				Control: 285	29.8	36.0	NWL; Infertility tx.	100	−1.1	
Price et al., 2020, 2021[f] (18, 44)	Australia	12 weeks	164	Intervention: 85	32.6	37.9	VLCD+MR, PA, RD	84	−11.2	No
				Control: 79	32.1	39.5	WL; CR, PA, RD	67	−2.1	
Rönö et al., 2018 (19)	Finland	up to 39 weeks	228	Intervention: 116	33.0	30.4	CR, PA	100	NR	No
				Control: 112	32.0	29.4	NWL; UC	100	NR	
Rothberg et al., 2018 (33)	United States	16 weeks	14	Intervention: 7	32	40.0	VLCD + MR	86	−14	Yes
				Control: 7	31	41.0	WL; CR	71	−5.0	
Sim et al., 2014 (37)	Australia	12 weeks	49	Intervention: 27	32.9	35.1	VLCD+MR, RD, PA	85	−6.6	Yes
				Control: 22	32.8	38.0	NWL; UC	77	−1.6	
Legro et al., 2015 (43), 2016[g] (34)	United States	16 weeks	282	Intervention: 95	28.6	35.2	CR+MR, PA, Orlistat	100	−6.1	Yes
				Control: 187	28.9	34.8	NWL; Infertility tx.	100	NR	
Legro et al., 2022 (20)	United States	16 weeks	379	Intervention: 188	32.4	39.4	CR+MR, PA, Orlistat	84	−7.3	Yes
				Control: 191	32.1	39.2	NWL; PA	79	−0.3	
Salamun et al., 2018 (41)	Slovenia	12 weeks	28	Intervention: 14	31.1	35.5	CR, PA, Metformin+ Liraglutide	86	−7.6	Yes
				Control: 14	30.1	37.8	WL; CR, PA, Metformin	79	−7.0	
Wang et al., 2021[h] (21)	China	4–12 weeks	877	Intervention: 439	30.5	29.3	Orlistat	100	−7.3	Yes
				Control: 438	31.0	29.5	NWL; Placebo	100	−0.3	

Note: BMI = body mass index (kg/m^2); CBT = cognitive behavioral therapy; CR = calorie restriction central to dietary advice; DA = dietary advice not centering around calorie restriction; DASH = diet approaches to stop hypertension; ITT = intent-to-treat; IVF = In vitro fertilization; NR = not reported; NWL = nonweight loss;, PA = physical activity prescription; UC = usual care; VLCD = very low-calorie diet; MR = meal replacements; RD = registered dietician; Tx = treatment; WL = weight loss.

[a] Pregnancy rates from one cycle of IVF only.

[b] Only includes outcomes from 1st embryo cryotransfer.

[c] SMS + and SMS- combined to form intervention group.

[d] Including rates and statistics for singleton pregnancies ≥14 weeks because full results only reported in this group. ITT denominators for meta-analysis came from consort because investigators excluded ppl who were lost to follow-up.

[e] Those lost to follow-up are included in our ITT analysis – presumed not to have become pregnant.

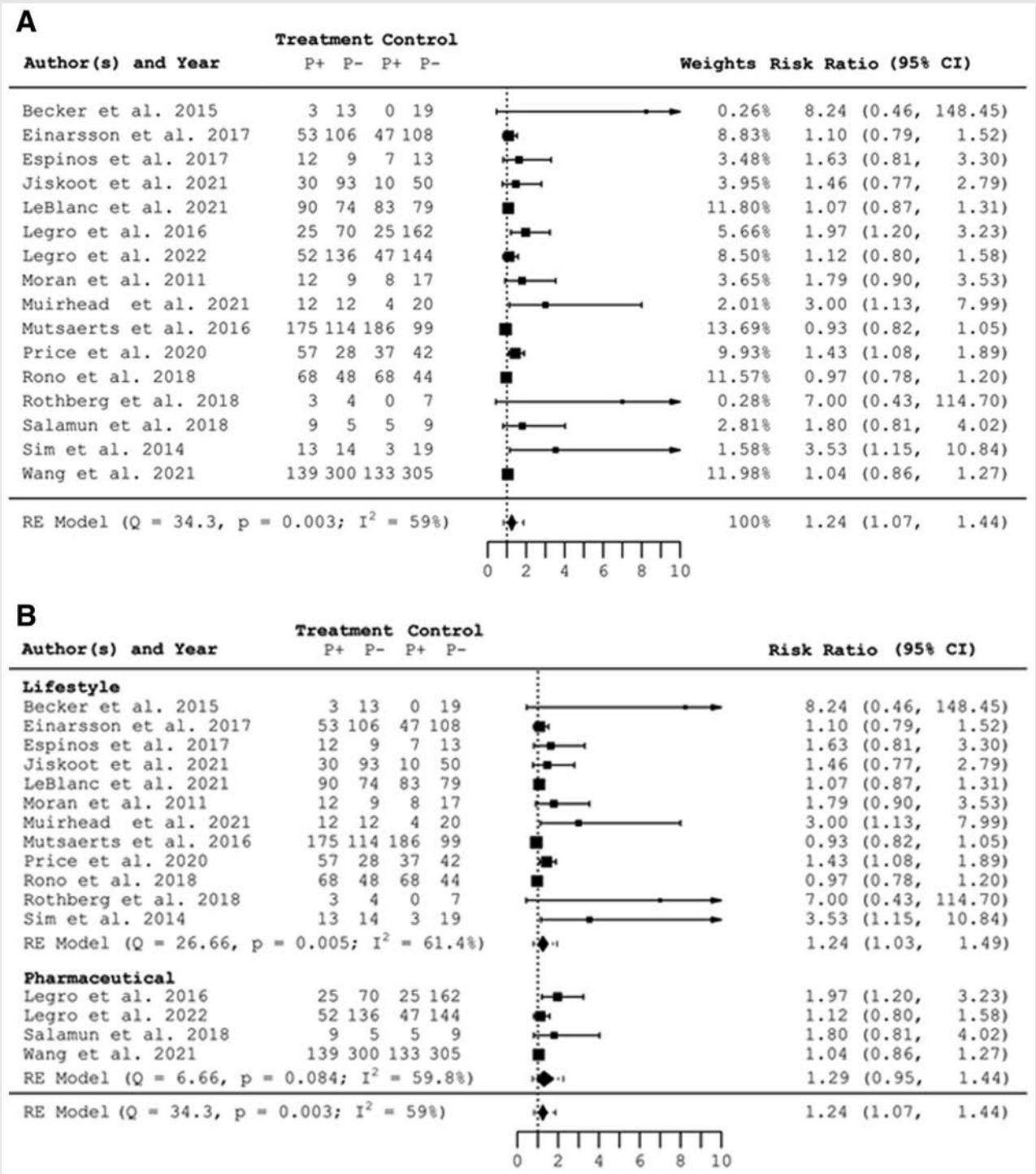
[f] Only including singleton pregnancies because live birth and miscarriage rates are not reported for multiple pregnancies but do include spontaneous pregnancies during the intervention (investigators exclude as noncompleters).

[g] Both intensive lifestyle intervention groups from 2015 were subsequently published in 2016 and compared with immediate fertility treatment in 2016. Meta-analysis combined these treatment groups and excluded the oral contraceptive group from 2015.

[h] Meta-analysis includes clinical pregnancy rate and clinical pregnancy loss from all pregnancies (not just singletons because live births and miscarriages numbers reported for singleton and multiple pregnancies).

Caldwell. Preconception weight loss and fertility. Fertil Steril 2024.

FIGURE 2



Forest plots showing risk ratios for pregnancy. (**A**) Impact of treatment on pregnancy by study. (**B**) Impact of treatment on pregnancy by intervention type. (**C**) Impact of treatment on pregnancy by control type. (**D**) Impact of treatment on pregnancy by fertility treatment. (**E**) Impact of treatment on pregnancy by intervention length. (**F**) Impact of treatment on pregnancy by baseline BMI.

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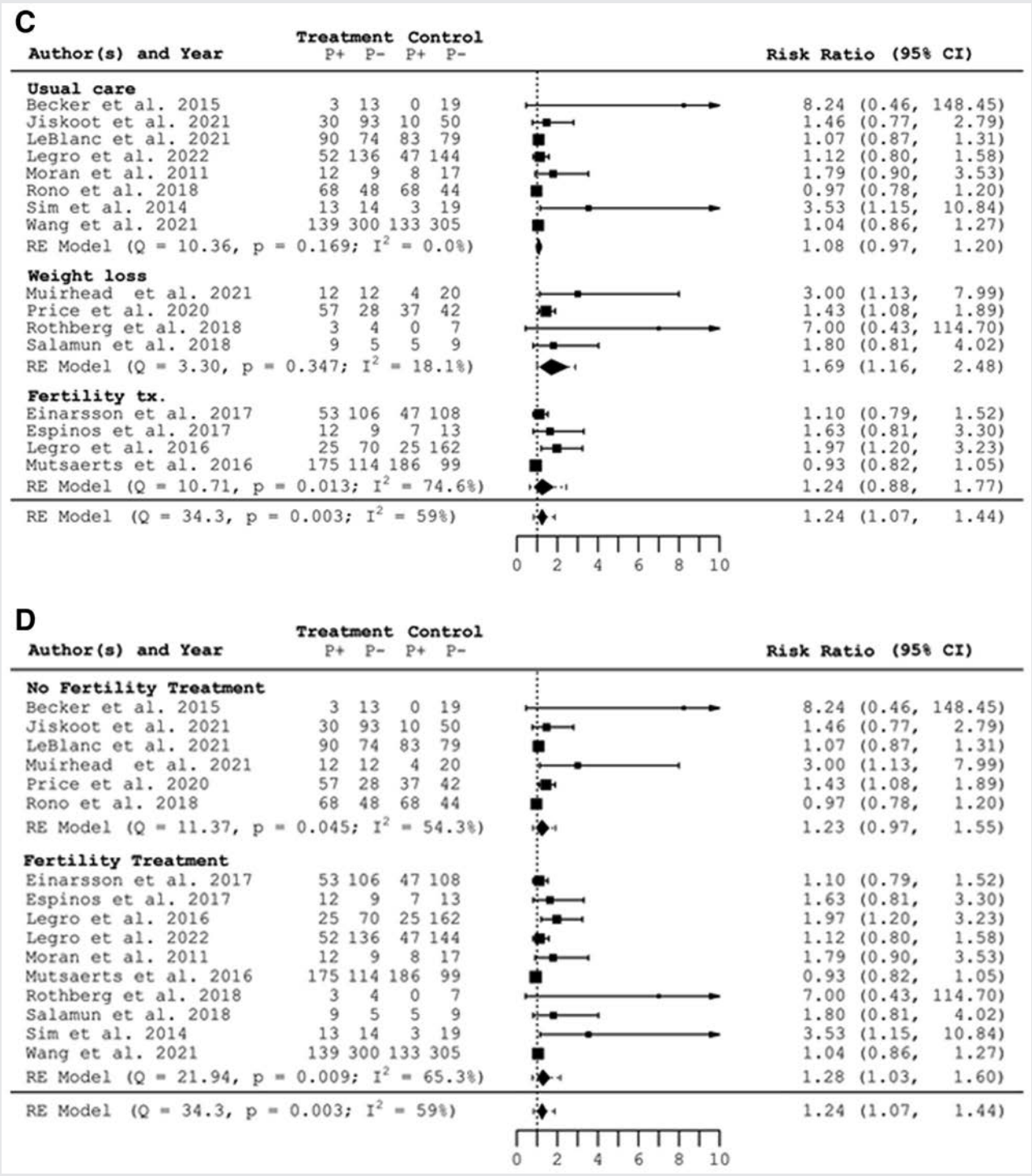
FIGURE 2



continued

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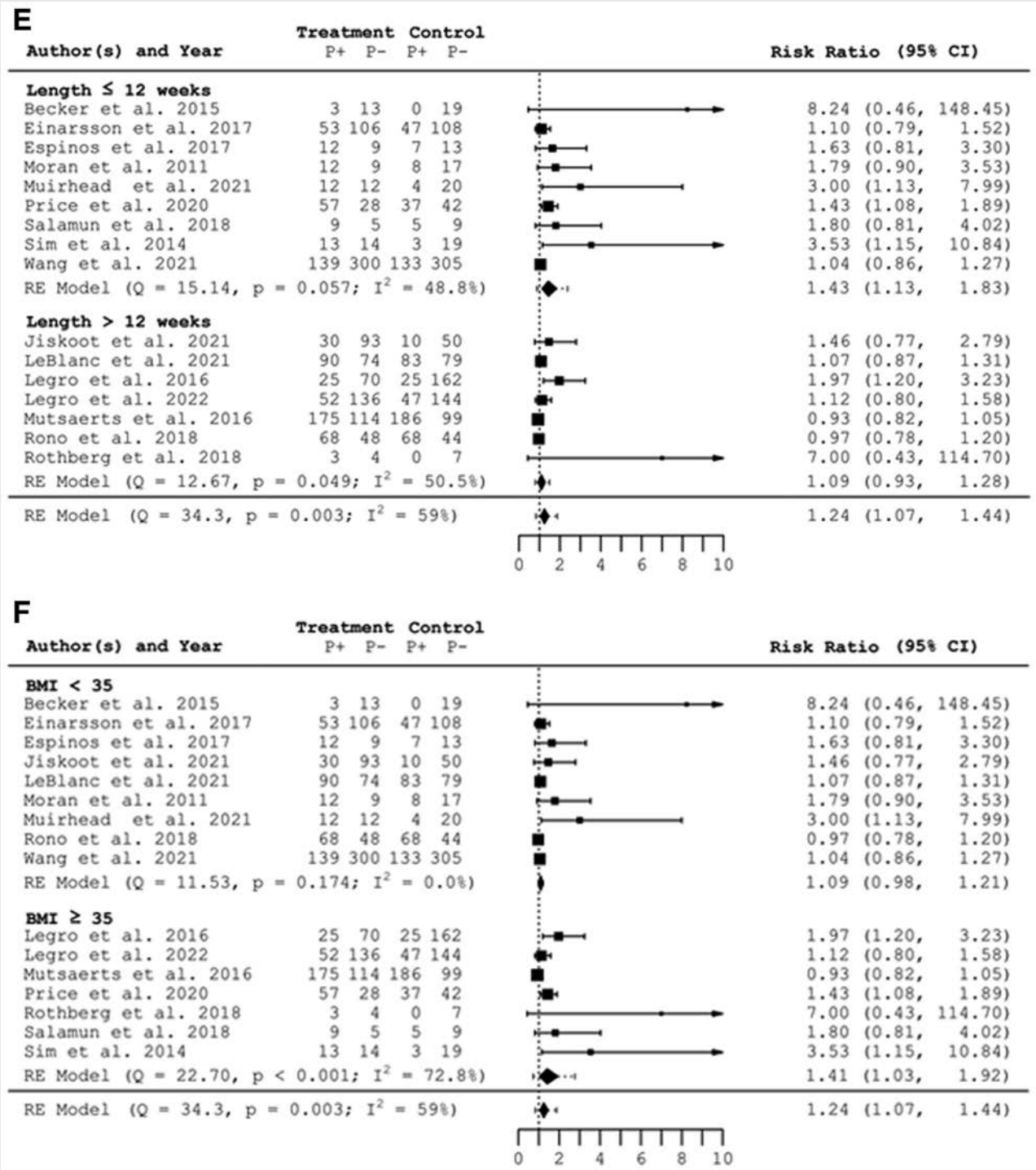
because it was distributed closer to normal than the risk ratio (RR) (31); however, for ease of interpretation, the model-estimated LRRs were then transformed into the more widely used risk ratios (RRs). Heterogeneity was evaluated using Chi-square tests with P values and I^2 index values for each outcome. To explore heterogeneity, we conducted subgroup analyses by study design characteristics including intervention type (lifestyle only or AOMs+/−lifestyle), control group

FIGURE 2



continued

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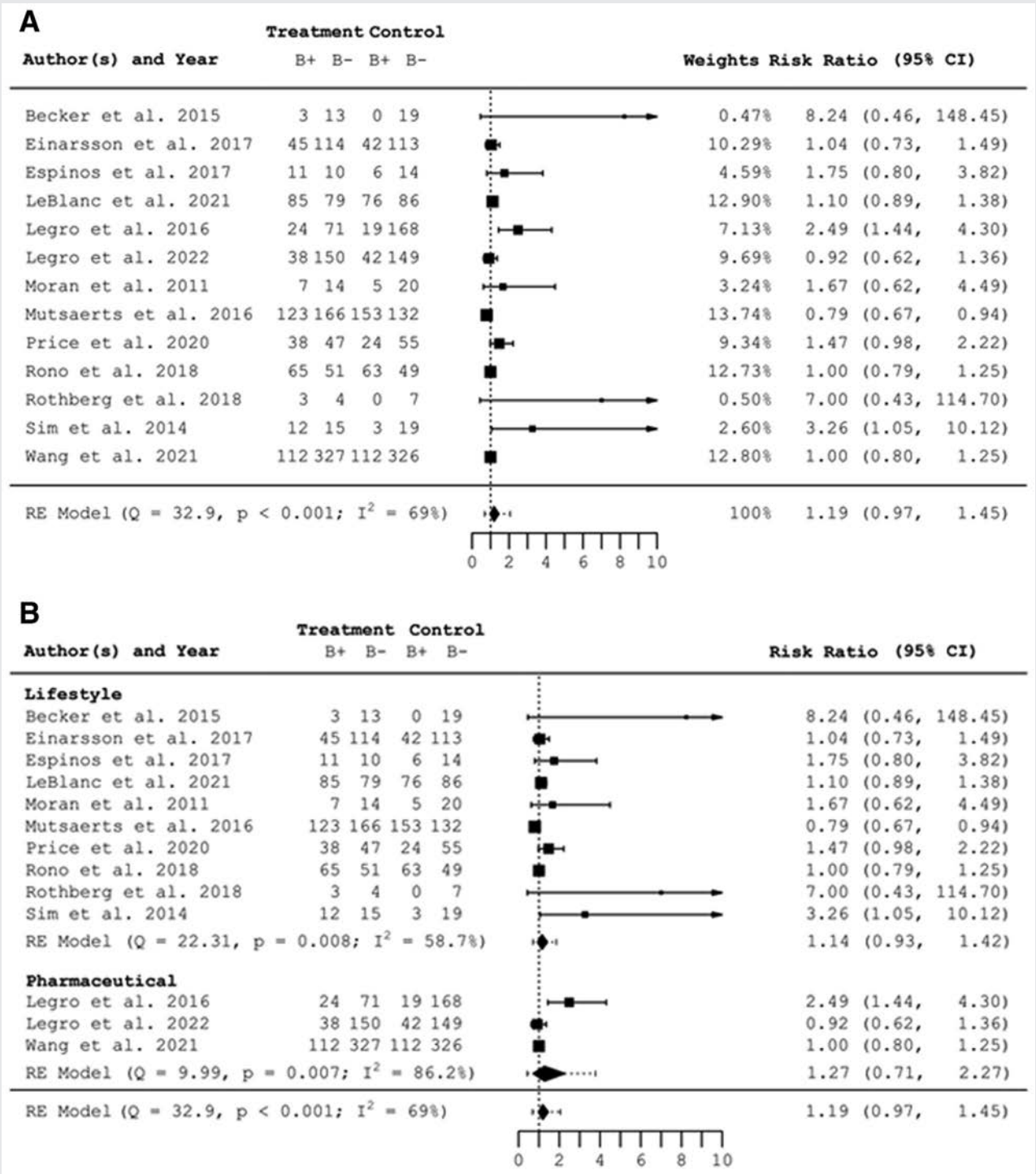
(usual care, immediate fertility treatment or active weight loss), intervention length ($\leq$ 12 weeks or $>$ 12 weeks) fertility treatment (yes or no), and baseline BMI ($<$35 kg/m^2 or $\geq$35 kg/m^2). Further, given the heterogeneity among studies, we calculated a 95% prediction interval, which can be interpreted as an interval that would contain approximately 95% RRs calculated in similar future studies (32). All presented RRs are estimated from the REM as opposed to the raw data. The

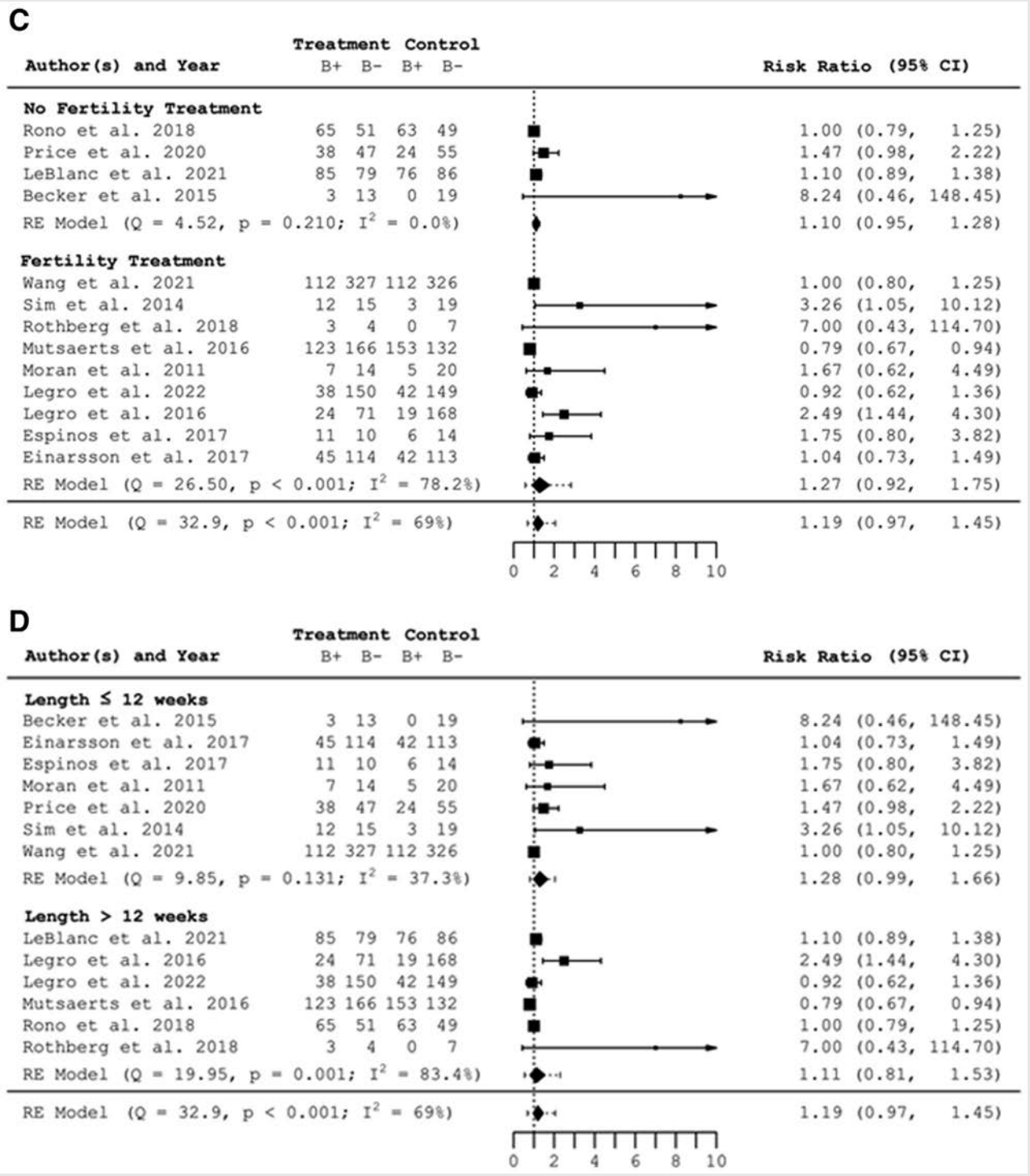
FIGURE 3



Forest plot showing risk ratios for live birth. (**A**) Impact of treatment on live birth by study. (**B**) Impact of treatment on live birth by intervention type. (**C**) Impact of treatment on live birth by fertility treatment. (**D**) Impact of treatment on live birth by intervention length.

Caldwell. Preconception weight loss and fertility. Fertil Steril 2024.

FIGURE 3



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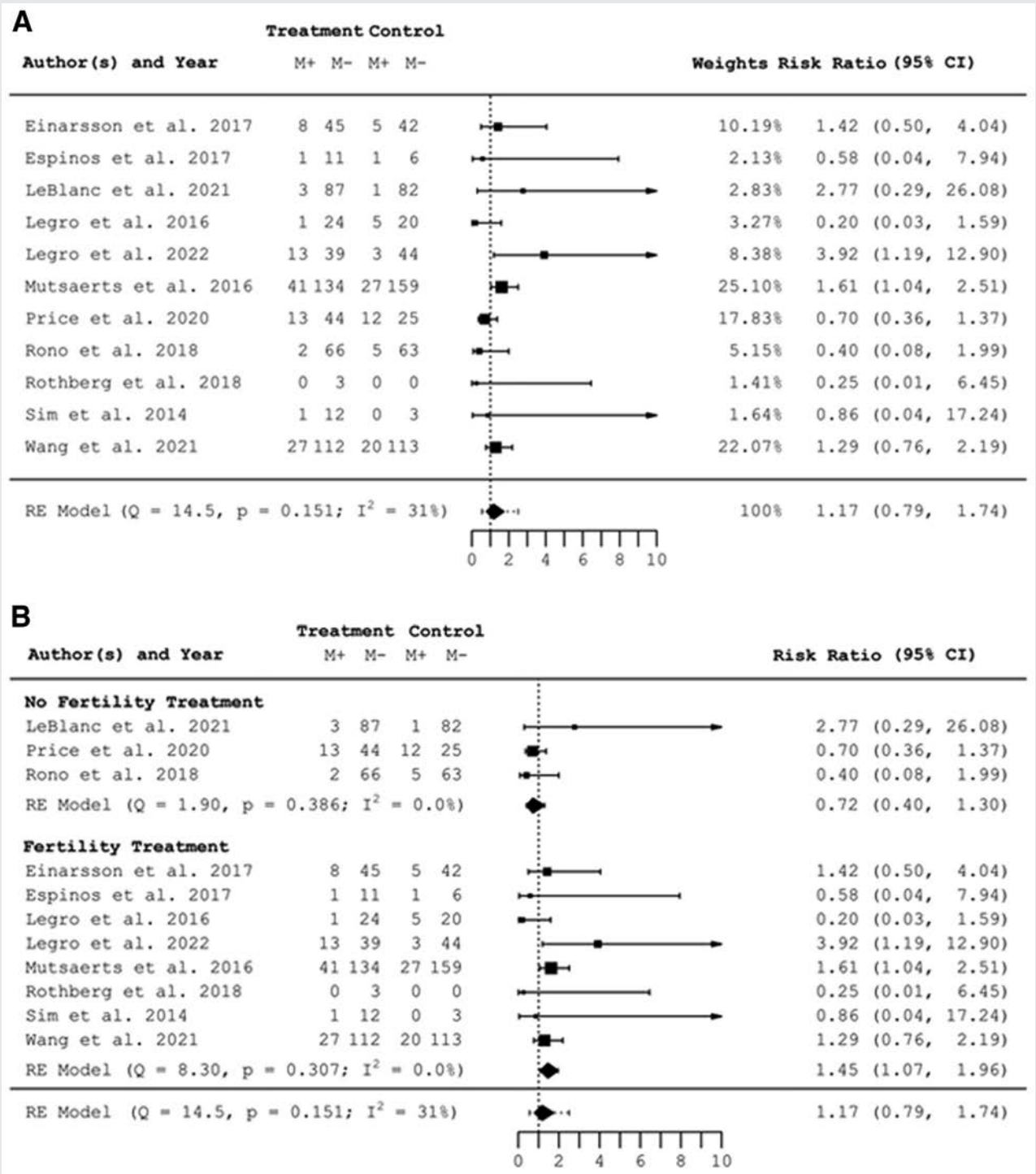
Caldwell. Preconception weight loss and fertility. Fertil Steril 2024.

FIGURE 4



Forest plot showing risk ratios for miscarriage. (**A**) Impact of treatment on miscarriage by study. (**B**) Impact of treatment on miscarriage by fertility treatment.

Caldwell. Preconception weight loss and fertility. Fertil Steril 2024.

primary outcome was pregnancy, and all other analyses, both secondary and subgroup, were unadjusted for multiplicity and should be interpreted as such.

RESULTS

Study selection

The preliminary literature search yielded 4,121 records that were screened for inclusion (Fig. 1). After screening 67 full-text records for eligibility, 16 studies met criteria for inclusion.

Study characteristics

Of the 16 studies, 4 took place in the United States (17, 20, 33, 34) or Australia (18, 35–37), 2 took place in the Netherlands (26, 38), and the others in Brazil (39), Spain (40), Finland (19), Slovenia (41), China (21), and Sweden, Denmark, and Iceland (42). Eight studies were multisite RCTs (17–20, 34, 38, 42, 43), and 8 were single site, including data on 3,588 participants for pregnancy, 3,329 for live birth, and 3,248 for miscarriage. Sample sizes ranged from 14 (33) to 877 (21), and studies were published between 2011 and 2022. Weight loss intervention length ranged from 5 weeks up to 1 year. Twelve trials evaluated weight loss through lifestyle modification (17–19, 26, 33, 35–40, 42), 3 through lifestyle plus AOMs (20, 34, 41, 43), and 1 using an AOM only (21). Retention in the weight loss interventions ranged from 59%–100% and 57%–100% in the control groups. Characteristics of the studies are displayed in Table 1 (17, 18, 19–21, 26, 34, 36, 38–43).

Ten studies included ovulation induction (OI), intrauterine insemination (IUI), or IVF (20, 21, 33–35, 37, 38, 40–42) after weight loss whereas the others did not include fertility treatment (17–19, 26, 36, 39). The mean age of women at baseline ranged from 29 to 33 years old, and BMI was 28.7 to 39.4 kg/m^2. Nine studies enrolled women with a BMI $<$ 35 kg/m^2 and 7 with a BMI $\geq$ 35 kg/m^2 (18, 20, 33, 37, 38, 41, 43). Weight loss ranged from 3.7 kg (17) to 14 kg (33) in the intervention groups and 5 kg (33) to 1.2 kg (42) in the control groups.

Risk of bias assessment results

All the included trials implemented random sequence generation and were assessed a low risk of selection bias (Fig. 2) (45). Due to the nature of weight loss lifestyle interventions, blinding was not possible for participants except in Wang et al. (21), where Orlistat was compared with a placebo. Thus, 15 studies were identified as high risk of performance bias. High risk of attrition bias was noted in 3 trials (26, 33, 35). Finally, 1 trial had high risk of selective reporting bias because the outcomes included on ClinicalTrials.gov did not match the results reported in the published manuscript (33).

Intervention type

Of the 12 lifestyle modification only trials (17–19, 26, 33, 35–40, 42), several different dietary approaches were included, such as caloric restriction with and without meal replacements, very low-calorie diets (VLCD) with meal replacements or low glycemic index diet. All but 2 trials included a physical activity component (39, 42). The physical activity component of most interventions included a gradual progression to meet the Physical Activity Guidelines for Americans recommendation of 150 min/week of moderate intensity physical activity (46). Two studies included lifestyle plus pharmaceutical interventions (20, 34, 43) utilizing caloric restriction through meal replacements, increasing physical activity, and an AOM administration (Orlistat). Another study included caloric restriction, increasing physical activity, Metformin, and Liraglutide administration (41). One study used an AOM (Orlistat) administration alone (21).

Control group type

Women in the control group in the 4 trials received some type of weight loss intervention, including caloric restriction and at least 1 meeting with a registered dietitian (18, 33) health coach (36), or Metformin (41). Two studies had active control groups that were not designed to support clinically meaningful weight loss, including accumulating 10,000 steps per day (20) or recommendations to consult general practitioner for weight loss and received printed intervention material (37); therefore, we classified both as usual care. One study utilized a placebo for the control group (21). The remaining 5 studies consisted of usual care (17, 19, 26, 35, 39). In 4 studies, participants received immediate fertility treatment (38, 40, 42, 43). The 2 lifestyle and pharmaceutical treatment groups in Legro et al. (34, 43) were combined and compared with the immediate fertility treatment group from the 2016 publication to avoid nonindependence in the control groups in the meta-analytic comparisons (47).

Primary and secondary outcomes

Pregnancy. All 16 studies reported pregnancy by group, with 7 reporting no difference in pregnancy rates (19–21, 35, 38, 40, 42) and 5 reporting statistically significantly higher pregnancy rates in the intervention group relative to controls (18, 34, 36, 37, 41). Aggregated data from 16 RCTs show that women randomized and exposed to a weight loss intervention had higher pregnancy rates 753/1788 (42.1%) compared with women in the control group 663/1800 (36.8%) (RR = 1.24; 95% CI 1.07–1.44; I^2 = 59%, Fig. 2A). The 95% prediction interval was (0.82, 1.88), which indicates that although the average estimated effect was for a higher RR in weight loss intervention compared with controls, there may be situations where this does not hold. When analyzed by type of weight loss intervention, significantly higher pregnancy rates were observed in lifestyle modification only intervention 528/1052 (50.2%) compared with control groups 453/970 (46.7%) (n = 12; RR = 1.24, 95% CI 1.03–1.49), but not in studies including AOMs (n = 4) (Fig. 2B). Results showed higher pregnancy rates from weight loss intervention 81/130 (62.3%) compared with an active weight loss control group 46/124 (37.1%) (n = 4, RR = 1.69, 95% CI 1.16–2.48) but not usual care (n = 8, RR = 1.08, 95% CI 0.97–1.20) or immediate access to ART (n = 4, RR = 1.24, 95% CI 0.88–1.77, Fig. 2C). Fertility treatment was associated with higher pregnancy rates (n = 10, RR 1.28, 95% CI 1.03–1.60], Fig. 2D). Weight loss

interventions that were 12 weeks or fewer were associated with higher rates of pregnancy 310/806 (38.5%) compared with the control group 266/796 (30.7%) (n = 9, RR = 1.43, 95% CI 1.13–0.83, I^2 = 49%, Fig. 2E). Interventions where the study population of women had an average baseline BMI ≥ 35 kg/m^2 had higher rates of pregnancy in the intervention 159/416 (38.2%) compared with the control (23.4%) (n = 7, RR = 1.41, 95% CI 1.03–1.92, I^2 = 73%, Fig. 2F).

Live birth. Overall, live births were higher, but not statistically significantly so, in the intervention group 566/1627 (34.8%) compared with control 545/1702 (32.0%) (n = 13, RR = 1.19, 95% CI 0.97–1.45, I^2 =69%, Fig. 3A). There were no differences in live birth rates by intervention type in lifestyle only (n = 10, RR = 1.14, 95% CI 0.93–1.42) or those including medications (n = 3, RR = 1.27, 95% CI 0.71–2.27, Fig. 3B). Nor were there differences by control group type in usual care (n = 7, RR = 1.05, 95% CI 0.93–1.18), active weight loss control (n = 2, RR = 1.69, 95% CI 0.71–4.07), or those receiving immediate fertility treatment (n = 4, RR = 1.29, 95% CI 0.77–2.18, Fig. 3C). Women randomized to weight loss interventions ≤ 12 weeks had a nonsignificant trend toward more live births 228/768 (29.7%) compared with the control group 192/758 (25.3%) (n = 7, RR = 1.28, 95% CI 0.99 to 1.66, Fig. 3D). Subgroup analyses by baseline BMI or fertility treatment had no impact on live births.

Miscarriage. Pooling results from the 11 studies reporting miscarriage rates, there was no difference between the intervention 110/687 (16.0%) compared with the control group 79/636 (12.4%) (n = 11, RR = 1.17, 95% CI 0.79–1.74, I^2 = 31%, Fig. 4A). There was no impact of intervention characteristics (intervention type, control group type, or intervention length) or baseline BMI on miscarriage rates per participant. However, in studies providing fertility treatment, a higher miscarriage rate was observed in intervention groups 92/472 (19.5%) relative to control 61/488 (12.5%) (n = 8, RR 1.45 95% CI 1.07–1.96; Fig. 4B).

DISCUSSION

This meta-analysis focuses on randomized studies for preconception weight reduction in women with overweight or obesity planning pregnancy. Weight loss interventions included those using lifestyle modification only and the addition or sole use of AOMs compared with different types of control conditions. Overall, results suggest a beneficial effect in women randomized to lifestyle interventions without AOMs on clinical pregnancy rates. There was no impact of weight loss interventions on overall live birth or miscarriage compared with control. However, women randomized to weight loss interventions and undergoing fertility treatment had significantly higher rates of miscarriage.

Lifestyle interventions only (n = 12) were associated with higher pregnancy rates but not the use of AOMs either solo or in conjunction with lifestyle interventions (n = 4). This review is notable for the addition of weight loss interventions, including AOMs. Little is known about the use of AOMs to achieve a healthy weight before pregnancy; thus, this is a timely addition to the literature. New trials will need to examine the impact of more effective AOMs, as well as the benefits and risks of using these newer AOMs for preconception weight loss. Clinicians should provide appropriate contraceptive counseling to reproductive-aged women taking AOMs to decrease potential maternal and fetal risk (16).

Strengths of this review include subgroup analyses conducted by study and population characteristics. Enrollment into preconception weight loss interventions is complicated because of the competing demands of advancing maternal age and delaying infertility treatment, in addition to barriers noted in the general population, such as availability of evidence-based programs, time, cost, motivation, perceived effectiveness, and social support. Thus, the finding that weight loss interventions ≤ 12 weeks (n = 7) resulted in higher pregnancy rates is something to explore in future trials. Although the result was not quite significant, there was a trend toward more live births for women in weight loss interventions ≤ 12 weeks (n = 7) compared with the control. This amount of time may be more palatable for women of reproductive age wishing to conceive and is in line with the view by Hoek et al. (48), noting that different phases (preconception, active fertility treatment, pregnancy) require strategic approaches for weight management.

Furthermore, study populations where the baseline BMI of women was ≥ 35 kg/m^2 were associated with higher pregnancy rates in the intervention compared with the control. Although there are no available data on a weight loss threshold for improvement in fertility outcomes in women, our findings add to the literature the importance of identifying population characteristics in which weight loss may be most beneficial. In future individual participant data meta-analyses, exploring the treatment response by baseline BMI will provide crucial information for clinical practice (49).

There are several limitations of the current synthesis. First, we did not limit our population of interest to women undergoing infertility treatment or those not undergoing fertility treatment. Identifying a representative population of women seeking pregnancy is an inherent difficulty that must be overcome in future studies as rates of overweight and obesity among reproductive-aged women continues to increase. Although the percent of variability due to heterogeneity in this meta-analysis was lower than that observed in 2 recent meta-analyses (9, 10) (59% vs. 65% and 92%), the narrative synthesis details the wide variety of study characteristics, including the target study population, weight loss duration, and weight loss intervention details. Given the heterogeneity among studies, we calculated a 95% prediction interval, which can be interpreted as an interval that would contain approximately 95% RRs calculated in similar future studies. This indicates that whereas the average estimated effect was for a higher RR in weight loss intervention compared with controls for pregnancy, there may be situations where this does not hold.

Additional unexplored variables may influence the extent to which weight loss improves conception, live birth, and miscarriage. We were unable to conduct subgroup analyses by polycystic ovary syndrome or other causes of infertility, which may affect the extent to which weight loss interventions improve conception rates, particularly when participants with male factor infertility are included, as in

several studies in this review. Finally, we were unable to parse out unassisted or natural conceptions with subsequent live birth and miscarriage rates compared with OI, IUI, or IVF pregnancies and subsequent live birth and miscarriage rates as these were not provided in the large-scale trials. This highlights a need to further explore our finding that miscarriage rates were higher in women undergoing fertility treatment.

CONCLUSIONS

Our results suggest that there is no one-size-fits-all recommendation for preconception weight loss in women with overweight or obesity on fertility outcomes. A more nuanced and personalized approach should be taken when recommending weight loss through lifestyle modification to improve fertility outcomes. At the same time, adverse pregnancy and perinatal outcomes associated with obesity pose serious risks for women and their offspring. Preconception weight loss should be supervised by clinicians with specific expertise in obesity treatment, especially with the increased availability of AOMs. Weight loss may need to precede attempting conception for a longer interval than that used in existing studies, which may not be feasible in women with lower ovarian reserve or advancing maternal age. Clinicians in primary care and obstetrics and gynecology should provide patients the opportunity to thoroughly discuss preconception health to weigh the potential costs and benefits of attempting weight loss through lifestyle modifications or AOMs before attempting pregnancy (50). Improving the preconception health of reproductive-aged women with overweight or obesity will entail conducting adequately powered, interdisciplinary, well-designed RCTs to generate more robust evidence.

CRediT Authorship Contribution Statement

Ann E. Caldwell: Writing – review & editing, Writing – original draft, Validation, Project administration, Investigation, Data curation, Conceptualization. **Anna M. Gorczyca:** Writing – review & editing, Visualization, Methodology, Data curation. **Andrew P. Bradford:** Writing – review & editing, Methodology, Data curation. **Jacinda M. Nicklas:** Writing – review & editing, Investigation, Data curation. **Robert N. Montgomery:** Writing – review & editing, Visualization, Methodology, Formal analysis, Data curation. **Heather Smyth:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Shannon Pretzel:** Methodology, Data curation. **Thy Nguyen:** Writing – review & editing, Methodology, Data curation. **Kristen DeSanto:** Writing – review & editing, Validation, Software, Data curation. **Celia Ernstrom:** Writing – review & editing, Methodology, Data curation. **Nanette Santoro:** Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization.

Declaration of Interests

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Efectividad de las intervenciones de pérdida de peso antes de la concepción sobre la fertilidad en mujeres: una revisión sistemática y metaanálisis.

Importancia: Se recomienda la pérdida de peso antes de la concepción a las mujeres con sobrepeso u obesidad para mejorar los resultados de fertilidad, pero la evidencia que respalda esta recomendación es mixta.

Objetivo: Examinar la efectividad de las intervenciones de pérdida de peso mediante modificación del estilo de vida y/o medicación en mujeres con sobrepeso y obesidad en términos de embarazo, recién nacido vivo y de abortos espontáneos.

Fuentes de datos: Se realizó una búsqueda electrónica en MEDLINE, Embase, Cochrane Librery, incluida Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, y Cumulative Index to Nursing and Allied Health Literature hasta el 6 de julio de 2022, a través de Wiley.

Selección y síntesis de estudios: Se incluyeron ensayos controlados aleatorios que examinaron intervenciones de pérdida de peso a través del estilo de vida y/o medicación en mujeres con sobrepeso u obesidad que planeaban un embarazo. Se realizó un metaanálisis de efectos aleatorios, reportando el cociente de riesgos (RR) para cada resultado. Los análisis de subgrupos se realizaron por tipo de intervención, tipo de grupo de control, tratamiento de fertilidad, duración de la intervención e índice de masa corporal (IMC).

Resultados principales: embarazo clínico, nacidos vivos y eventos de aborto espontáneo.

Resultados: Fue posible realizar una revisión narrativa y un metaanálisis de 16 estudios sobre embarazo (n = 3,588), 13 sobre nacidos vivos (n = 3,329) y 11 sobre aborto espontáneo (n = 3,248). Las mujeres asignadas al azar y expuestas a una intervención de pérdida de peso tenían más probabilidades de quedar embarazadas (RR = 1.24; IC del 95 %: 1.07–1.44; I^2 = 59 %) pero no de tener nacidos vivos (RR = 1.19, IC del 95 %: 0.97–1.45; I^2 = 69%) o aborto espontáneo (RR = 1.17; IC del 95%: 0.79 a 1.74; I2 = 31%) en comparación con las mujeres de los grupos control. Los análisis de subgrupos revelaron que las mujeres asignadas al azar a intervenciones de pérdida de peso que duraron 12 semanas o menos (n = 9, RR = 1.43; IC del 95%: 1.13 a 1.83) y mujeres con IMC $\geq$ 35 kg/m^2 (n = 7, RR = 1.54; IC del 95%, 1.18 a 2.02) tuvieron más probabilidad de quedarse embarazadas en comparación con las mujeres de los grupos control. El aborto espontáneo fue mayor en los grupos de intervención que se sometieron a tratamiento de fertilidad (n = 8, RR 1.45; IC del 95%: 1.07–1.96).

Conclusiones: Las tasas de embarazo fueron mayores en mujeres sometidas a intervenciones de pérdida de peso antes de la concepción, sin impacto en las tasas de nacidos vivos o abortos espontáneos. Los hallazgos no respaldan una recomendación única para la pérdida de peso mediante la modificación del estilo de vida y/o la medicación en mujeres con sobrepeso u obesidad inmediatamente antes de la concepción para mejorar los resultados de los nacidos vivos o los abortos espontáneos.

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Functional evidence for two distinct mechanisms of action of progesterone and selective progesterone receptor modulator on uterine leiomyomas

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Objective: To study the specific mechanisms through which progesterone and selective progesterone receptor modulators impact the growth, synthesis, and accumulation of the extracellular matrix in uterine leiomyomas.
Design: Laboratory study.
Setting: Academic Research Institutions.
Patients (s): This study involved reproductive-age women diagnosed with infertility associated uterine leiomyomas who underwent myomectomy either after selective progesterone receptor modulator ulipristal acetate (UA) treatment or without any pharmacological pretreatment. Control samples included healthy myometrium tissue (n = 100). Specimens were obtained from the Department of Reproduction and Gynecological Endocrinology and Biobank, Medical University of Bialystok, Poland.
Interventions: Daily (5 mg/d) UA treated for 2 months (n = 100) and untreated (n = 150) patients with uterine leiomyomas or normal healthy myometrium (n = 100) tissue samples immediately after surgery were collected for transcriptional analysis and assessments.
Main Outcome Measures: Progesterone-induced activation of the signaling pathways related to uterine leiomyomas extracellular matrix synthesis, deposition, and growth, as well as the expression profile of progesterone receptors in uterine leiomyomas, were assessed.
Results: The results indicated that progesterone activated the transforming growth factor-β and SMAD3 signaling pathways and promoted proliferation, growth, and extracellular matrix remodeling in uterine leiomyomas by up-regulating SMAD3, transforming growth factor-β (TGF-β) receptor type 1 and II, Ras homolog A, vascular endothelial growth factor, or increasing the fibrosis-related gene collagen, type I, α-1, and procollagen, type I, α-1 production. In contrast, UA had inhibitory effects on these processes. The study also showed that both nuclear and membrane progesterone receptors play distinct roles in uterine leiomyoma pathobiology.
Conclusions: We showed that both nuclear and membrane progesterone receptors were relevant in the treatment of uterine leiomyomas, especially when combined with selective progesterone receptor modulators. Novel therapeutic approaches combining selective progesterone receptor modulators with or without direct and indirect extracellular matrix targeting through selected specifically

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TGF-β and SMAD3 (SMAD3, TGF-β receptor types 1 and II, Ras homolog A, vascular endothelial growth factor, collagen, type I, α-1) signaling pathways could therefore be a treatment option for uterine leiomyomas. (Fertil Steril® 2024;122:341–51. ©2024 by American Society for Reproductive Medicine.)

El resumen está disponible en Español al final del artículo.

Key Words: Leiomyoma, progesterone, selective progesterone receptor modulator, extracellular matrix, growth factors

Uterine leiomyomas (ULs) are common gynecological tumors in reproductive-age women, with a range of occurrence from 217–3,745 cases per 100 000 women per year, and they are a major indication for hysterectomy (1, 2). Uterine leiomyomas may negatively affect embryo implantation in the uterus and increase the risk of miscarriage (3, 4) or cause heavy menstrual bleeding, anemia, and pelvic pain, thus decreasing the quality of life of women (2). Somatic mutations are thought to underlie the primary cause of ULs (5). Uterine leiomyomas' development and growth are highly dependent on ovarian steroid hormones, growth factors, and cytokines (5), which further influence smooth muscle cell proliferation, followed by excessive production of extracellular matrix (ECM) (5). Although estrogen has long been considered the most important initiating factor, recent research indicates that progesterone (P4) plays a key role in UL growth and development (6), as suggested by the increased expression of nuclear progesterone receptors (PRs) in ULs (7). Progesterone may also play a role in the epigenetic changes that lead to mutations in normal uterine muscle cells (3). There is still a data gap in the characterization and profiling of all the PRs in ULs.

Transforming growth factor β (TGF-β) regulates the expression of genes involved in tissue remodeling and ECM regulation (8). Up-regulated TGF-β signaling in ULs has been suggested as a mechanism for the development of their fibrotic phenotype (9). It has also been shown that P4 upregulates TGF-β expression in ULs (10). The TGF-β signaling complex is usually composed of the canonical SMAD 2, 3, and 4 transcription factor-dependent pathway or noncanonical pathway activating the small Ras homolog GTPase Ras homolog family member A (RhoA) (8). Ras homolog family member A may induce the growth of ULs via interactions with cytoskeleton proteins (11). Transforming growth factor β and SMAD3 pathways have been also shown to upregulate vascular endothelial growth factor (VEGF) expression, and SMAD3 mediates TGF-β1 induction of VEGF production in several tissues (12, 13). However, there exists inadequate functional data on the interactions of the above-mentioned factors with P4, as well as more generally on the mechanisms underlying P4 action in the regulation of UL proliferation, apoptosis, and ECM synthesis (14).

Among the selective P4 receptor modulators (SPRMs), ulipristal acetate (UA) successfully modulates PRs' action through its combined agonistic and antagonistic activity (15). Ulipristal acetate decreases UL cell viability, suppresses their expression of growth factors, and induces apoptosis, but does not affect the function of normal myometrial cells (15). Clinical studies have shown that treatment with UA significantly shrinks ULs and controls the frequency and abundance of uterine bleeding (16). Functional data on the precise mechanisms underlying the interference of UA with P4 action in the regulation of UL proliferation, apoptosis, and ECM synthesis and remodeling remain obscure. Our present study was therefore conducted to characterize the PR profile in ULs and to investigate the molecular mechanisms underlying the action of SPRM UA and its putative differential P4-induced activation of the signaling cascades involved in UL in the deposition and growth of ECM. Finally, to explore the novel molecular mechanisms and signaling pathways that may enhance the therapeutic effects on ULs.

MATERIALS AND METHODS

Study design

This was a controlled laboratory experiment to investigate the molecular mechanisms underlying the action of the SPRM UA and how it differs from the P4-induced activation of the signaling cascades involved in UL in the deposition and growth of the ECM. We wanted to investigate additionally the mechanisms that explain the therapeutic effects of UA in the control of UL growth and progression.

For the assessment and transcriptional analysis of human ULs, we used patient-derived UL tissue samples, primary UL cells, and UL explant cultures. To study the UA and P4 molecular mechanisms of action, we assessed the PR profile in UA-treated and UA-untreated (control) ULs in vivo as well as in explant culture in vitro. After establishing the PR profile in ULs, we tested the impact of P4 and UA on the activation of the TGF-β signaling pathway and SMAD3 signaling. For this, patient-derived UL explants were treated without or with inhibitors of TGF-β type I and II receptors (TGF-βRI and II) and SMAD3. To investigate the SMAD3 translocation, UL primary cell cultures were treated with UA. The therapeutic effects of UA on UL cell proliferation, growth, and ECM accumulations were investigated in vitro and in vivo. The sample numbers are stated in the figure legends, and replicates are described below. The statistical methods are described in separate sections and indicated in the figure legends.

Uterine leiomyoma tissue samples

Fresh ULs (n = 250) or healthy myometrium (n = 100) tissue samples were collected immediately after surgery at the Department of Reproduction and Gynecological Endocrinology, Medical University of Bialystok, Poland, during the years 2017–2021. The Local Human Investigation Ethics Committee approved the study (R-I-002/482/2017,

APK.002.4.2021). Written informed consent was obtained from all the patients (before inclusion) before the surgery. Uterine leiomyomas were collected from patients (aged 25–45 years) who underwent myomectomy because of infertility associated with ULs or had serious clinical symptoms like heavy menstrual bleeding and severe intermenstrual bleeding associated with lower abdominal pain. Patients were divided into two groups: the UA-treated (UA-L) group (n = 100) and the UA-untreated (NT-L) group (n = 150) before the surgery. Preoperative UA therapy consisted of the administration of UA (5 mg/d) for 3 months. A myomectomy was performed in the first phase of the menstrual cycle, 1 month after the end of the therapy. The type, size, and location of ULs were different. In patients with multiple myomas, tumors (10–40 mm in diameter) from all (UA-L and NT-L) groups were randomly selected. Myomas (>40 mm) were not selected to avoid any potential degeneration and necrosis that may have occurred in them.

Qualification criteria for the study, drugs, and inhibitors, ULs and myometrium explants and their primary cell culture, cell viability, real-time quantitative polymerase chain reaction (qPCR), cytokines measurement, and immunohistochemical and immunocytochemistry analyses have been shown in the Supplemental Materials (available online).

Study approval

The Local Human Investigation Ethics Committee approved the study (R-I-002/482/2017, APK.002.4.2021). The research was carried out according to the Code of Ethics of the World Medical Association (Declaration of Helsinki). Written informed consent was obtained before the surgery from all patients' prior inclusion.

Statistical analysis

Results were expressed as mean ± SEM. Statistical significance was assessed using one-way ANOVA with the post hoc Bonferroni test and two-way ANOVA with the post hoc Bonferroni test using GraphPad PRISM v. 7.0 (GraphPad Software, Inc). $P \leq .05$ was considered statistically significant.

RESULTS

Progesterone and UA modulate the PRs subtype expression profile in ULs

As P4 is pivotal for UL development and growth (1, 8), we characterized the expression profile of the nuclear progesterone receptors (PGRs) and all membrane P4 subtypes (membrane P4 receptor α [mPRα], mPRβ, mPRγ, P4 receptor membrane component 1 [PGRMC1], and P4 receptor membrane component 2 [PGRMC2]) in UA-treated (n = 100) and nontreated control (n = 150) UL samples, as well as in normal myometrium (n = 100) tissue samples. The expression levels of PGRA and B, PGRB, mPRα, mPRβ, and PGRMC1 were significantly higher in the nonUA-treated UL tissue samples compared with normal myometrium (Fig. 1A). In contrast, only PGRA and B expression remained up-regulated in the UA-treated UL samples, whereas those of PGRB, mPRα, mPRβ, and PGRMC1 were similar to those in normal myometrium. The expression levels of mPRγ and PGRMC2 were similar in all three types of samples (Fig. 1A). The expression level of PGRA and B was significantly up-regulated after P4 and UA treatments of leiomyoma explants, but P4 showed no additive effects to the UA action (Supplemental Fig. 1A, available online). In contrast, PGRB expression was up-regulated by P4 but down-regulated by UA treatment. Progesterone did not abolish this UA-induced effect (Supplemental Fig. 1B, available online). Similarly, the expression levels of mPRα, mPRβ, mPRγ, and PGRMC1 were significantly up-regulated after P4 but down-regulated after UA treatments. Progesterone did not reverse this UA action (Supplemental Fig.1 C to F, available online). Progesterone and UA treatments did not affect PGRMC2 expression level in leiomyoma explants (Fig. 1G, available online).

Immunohistochemical studies demonstrated abundant cytoplasmic staining for mPRα, mPRβ, mPRγ, and PGRMC1 in the nontreated control ULs tissues (NT-L) group (Fig. 1B to D and Supplemental Fig. 2D and E, available online), whereas traceable or weak staining was found in the UA-treated ULs tissues (UA-L) group (Fig. 1E to G). Densitometric quantification and optical density (OD) evaluation revealed a significantly decreased percentage ratio of mPRα, mPRβ, mPRγ and PGRMC1 expression in the UA-L group compared with the NT-L group (Fig. 1H to J and Supplemental Fig. 2F, available online). Ulipristal acetate treatment did not affect the strong PGR nuclear (Supplemental Fig. 2A and B, available online) and PGRMC2 staining of ULs (UL-L vs. NT-L groups) (Supplemental Fig. 2G, H, available online).

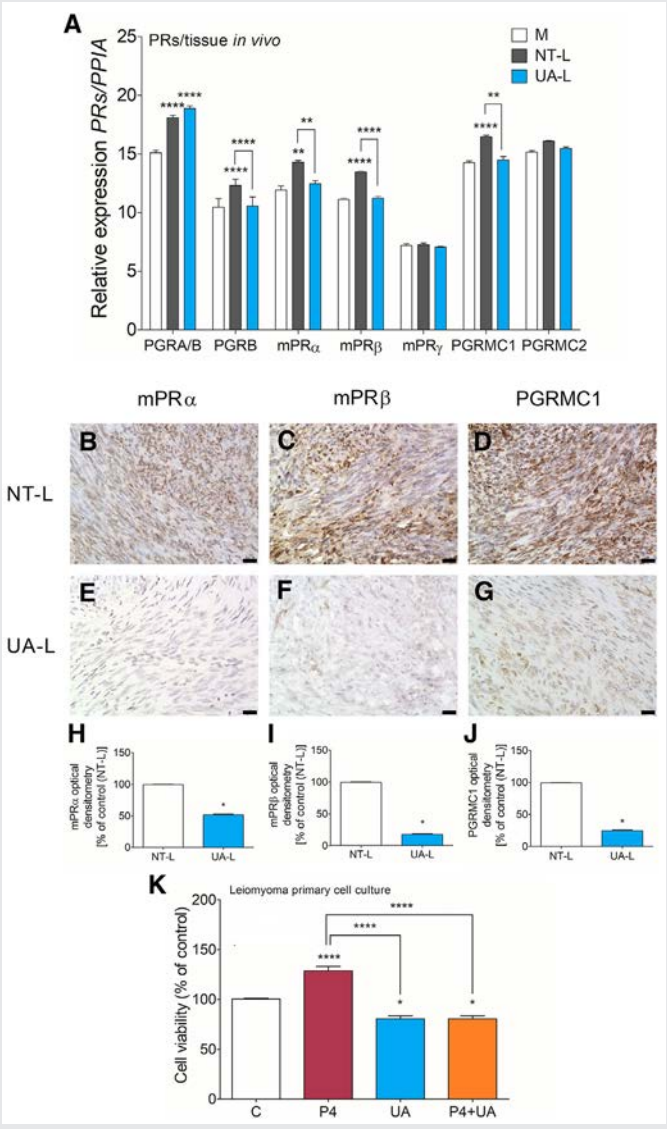
Next, we analyzed the effects of P4 (0–1 μM) and UA (0–100 μM) on the viability of primary UL cells in vitro. Progesterone (0.01–1 μM) significantly and dose-dependently stimulated (Fig. 1K and Supplemental Fig. 3A, available online), whereas UA (1 μM) or higher doses inhibited UL cell viability (Fig. 1K and Supplemental Fig. 3B, available online). We also found that UA itself had no effect on primary UL cell viability but inhibited the stimulatory effects of P4 (Fig. 1K).

Progesterone and UA activate differently the TGF-β signaling pathway in ULs

The TGF-β superfamily pathways are regarded as a potential stimulus for tumor progression (9, 17). Therefore, we characterized the expression profiles of the TGF-β family members in UL tissues. Transforming growth factor β1, *TGF-β3*, *SMAD2*, and *SMAD3* expression levels in ULs were significantly up-regulated compared with normal myometrium (Fig. 2A to D). This response was abolished by UA treatment in TGF-β1, TGF-β3, TGF-βR2, and SMAD3 in UL tissues and explants (Fig. 2A to D). In accordance, the secretion levels of TGF-β1 and TGF-β3 were significantly higher in ULs compared with normal myometrium, whereas UA treatment significantly suppressed these levels in ULs but not in normal myometrium (Fig. 2E and F).

To investigate further the TGF-β signaling pathway involvement affected by the UA or P4 actions in ULs, a

FIGURE 1

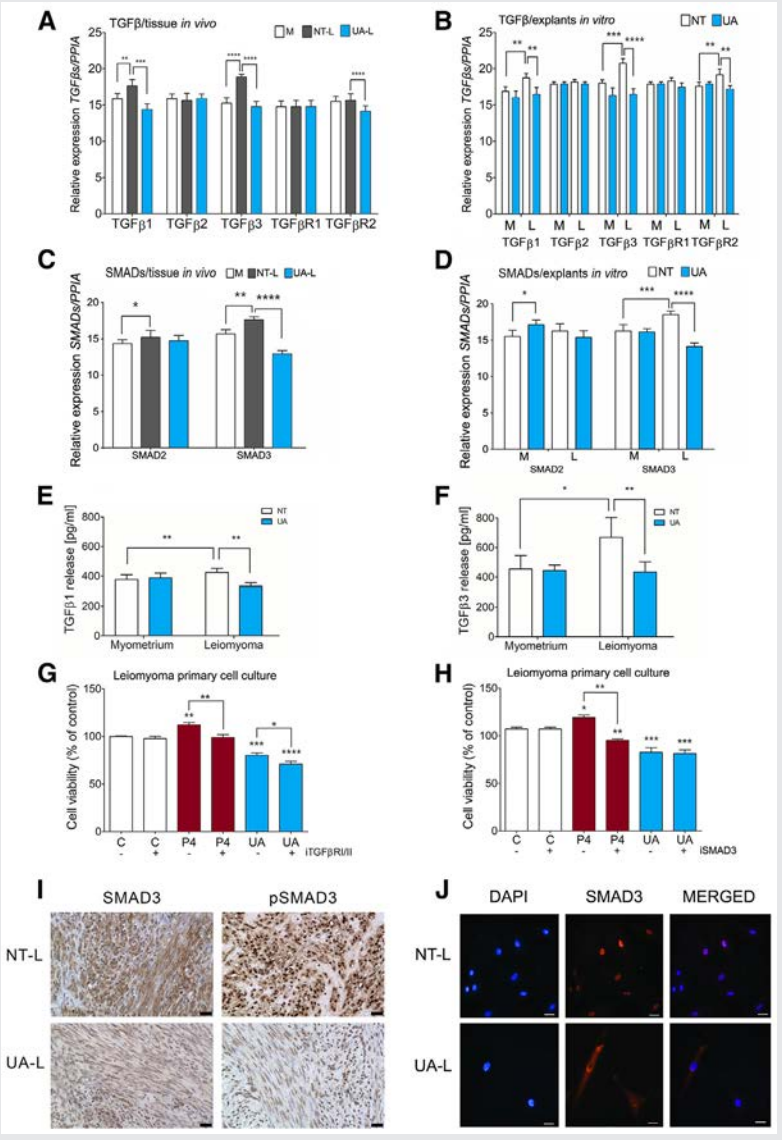


Characterization of the progesterone receptors (PRs) expression profile in uterine leiomyomas (ULs) after ulipristal acetate (UA) and progesterone (P4) treatment. The quantitative polymerase chain reaction gene expression profile of nuclear progesterone receptor isoforms (PGRA and PGRA/B) and membrane progesterone receptors (mPRα, mPRβ, PGRMC1, and PGRMC2) in normal myometrium (M) (n = 100) and ULs tissues of nontreated (NT-L) (n = 150) and UA-treated (UA-L) women (n = 100) (**A**). Immunohistochemical (IHC) staining for mPRα, mPRβ, and PGRMC1 in nontreated UL tissues (NT-L) (n = 150) (**B** to **D**) and UA-treated UL tissues (UA-L) (n = 100) (**E** to **G**). Quantification of UL tissue IHC staining of mPRα (**H**), mPRβ (**I**), and PGRMC1 (**J**) using Fiji (Image J). The results are presented as percentages of the controls compared with the nontreated control. Scale bar, 200 μm. The viability of cells after UA, P4, and UA + P4 treatments of ULs primary cell culture (n = 30) was measured using the MTT assay (**K**). The cell viability level of the treated groups is presented as a percentage of the control group, considered to be 100%.
Each bar represents the ratio of the investigated gene to the housekeeping gene (*PPIA* ± SEM). *Statistically significant differences between nontreated (NT-L) and treated (UA-L) groups (*, $P<.05$; ****, $P<.0001$) (one-way ANOVA with the post hoc Bonferroni's test).
C/NT-L = control/nontreated ULs; M = normal myometrium; mPRα = membrane progesterone receptor α; mPRβ = membrane progesterone receptor β; mPRγ = membrane progesterone receptor γ; MTT assay = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay; NT-L = nontreated ULs; P4 = progesterone treated; PGRA = nuclear progesterone receptor isoform A; PGRA/B = nuclear progesterone receptor isoform A/B; PGRMC1 = progesterone receptor membrane component 1; PGRMC2 = progesterone receptor membrane component 2; UA = ulipristal acetate treated; UA-L, ulipristal acetate treated ULs; ULs = uterine leiomyomas.

Milewska. P4/SPRM actions on uterine leiomyoma. Fertil Steril 2024.

TGF-βRI and II inhibitor (iTGF-βRI and II) was used (Fig. 2G). Coculture of iTGF-βRI and II with UA or P4 significantly enhanced the inhibitory effects of UA on the UL primary cell viability and inhibited P4-stimulated leiomyoma cell viability (Fig. 2G). Furthermore, iTGF-βRI and II alone did not affect UL cell viability (Fig. 2G). When the SMAD3 inhibitor (iSMAD3) was added to P4 or UA, it abolished the P4-dependent stimulation of the primary UL cell viability but showed no additive effects on the UA action (Fig. 2H).

FIGURE 2



Ulipristal acetate (UA) and progesterone (P4) treatment effects on the transforming growth factor (TGF-β) superfamily signaling pathway in uterine leiomyomas (ULs). The quantitative polymerase chain reaction (qPCR) gene expression profile of TGF-β isoforms (TGF-β1, TGF-β2, and TGF-β3) and their receptors (TGF-βR1, TGF-βR2) in normal myometrium (M) (n =100) and ULs tissues of nontreated (NT-L) (n = 150) and UA-treated (UA-L) (n = 100) women (**A**), as well as in normal myometrium (n = 50) and ULs explants (n = 50) before and after UA treatment (**B**). The qPCR gene expression profile of signal transducers (SMAD2, and SMAD3) in normal myometrium (M) (n = 100) and ULs tissues of nontreated (NT-L) (n = 150) and UA-treated (UA-L) (n = 100) women (**C**), as well as in healthy myometrium (n = 50) and ULs explants (n = 50) before and after UA treatment (**D**). Release of TGF-β1 (**E**) and TGF-β3 (**F**) to the medium by normal myometrium (n = 50) and ULs explants (n = 50) with or without UA treatment. Effects of UA and P4 with or without the SMAD3 inhibitor (**G**) or with or without the TGF-β receptor I and II inhibitor (**H**) on ULs primary cell (n = 30) viability after 24 hours of treatment were measured using the MTT assay. The cell viability of the treated groups is presented as the percentage of the control, considered to be 100%. Immunohistochemical staining of SMAD3 and phosphorylated SMAD3 (pSMAD3) in ULs from nontreated (NT-L) (n = 150) and SMAD3 and pSMAD3 in ULs from UA-treated (UA-L) (n = 100) women (**I**). Scale bar, 200 μm. Immunocytochemical localization of SMAD3 without or with UA treatment in ULs primary cells (n = 30) (**J**). Scale bar, 20 μm.
Each bar represents the ratio of the investigated gene to the housekeeping gene (*PPIA* ± SEM). *Statistically significant differences between nontreated and treated groups (*, $P<.05$; **, $P<.01$; ***, $P<.001$; ****, $P<.0001$) (one-way ANOVA with the post hoc Bonferroni's test).
C/NT-L = control/nontreated ULs; iSMAD3 = SMAD3 inhibitor; iTGF-βRI/II = TGF-β receptor I and II inhibitors; NT-L = nontreated ULs; M, healthy myometrium; MTT assay = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay; P4 = progesterone treated; UA-L = ulipristal acetate treated ULs; ULs = uterine leiomyomas.

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Ulipristal acetate affects nuclear translocation of SMAD3 in ULs

Transforming growth factor β may promote tumor growth through SMAD-dependent and/or independent pathways (9). To determine whether P4 and UA activate TGF-β1-SMAD signaling, we investigated their effects on the ratio of phosphorylated SMAD3 (pSMAD3) and nonphosphorylated SMAD3 in ULs. The nontreated (NT-L) group showed abundant expression of SMAD3 and pSMAD3, whereas the UA-L group samples demonstrated weaker cytoplasmic SMAD3 and nuclear pSMAD3 staining (Fig. 2I). Automatic quantification and OD evaluation revealed significantly decreased SMAD3 and pSMAD3 expression in the UA-L group compared with the NT-L group (Supplemental Fig. 4A to C, available online). In accordance, immunocytochemical localization of pSMAD3 could be found only in the NT-L cell nuclei, and no pSMAD3 translocation was found in the UA treatment samples (Fig. 2J).

ULIPRISTAL ACETATE AND P4 REGULATE VEGF AND INTERLEUKIN-6 EXPRESSION IN ULs

To screen for other potentially relevant factors affecting UL biology, we checked the expression of VEGF, interleukin 6 (IL-6), and collagen 1A1 (COL1A1) (5, 18–23). Abundant VEGF staining was observed in the NT-L group, whereas VEGF expression was very weak in the UA-L group (Fig. 3A). Automatic quantification and OD evaluation revealed a significantly decreased VEGF expression in the UA-L group compared with the NT-L group (Supplemental Fig. 5A, available online). Human recombinant VEGF (0–50 ng/mL) stimulation showed no effect on UL cell viability (Supplemental Fig. 5B, available online). Vascular endothelial growth factor A levels did not differ from healthy myometrium in nontreated UL (NT-L) tissue, was unaffected by P4, but was suppressed by UA (UA-L) (Fig. 3B). Vascular endothelial growth factor B level was up-regulated in NT-L tissue vs. myometrium, and P4 did not affect UL, whereas UA suppressed its levels (Supplemental Fig. 6A, available online). *VEGFC* and *VEGFR2* were down-regulated in NT-L tissue vs. myometrium, and P4 up-regulated *VEGFC* but had no effect on VEGFR2, whereas UA suppressed both genes (Supplemental Fig. 6B and C, available online). UA inhibited the VEGF release from UL explants, whereas iSMAD3 abolished this UA-induced effect (Fig. 3D).

Nontreated UL tissue showed abundant IL-6 staining, whereas this expression was weak in UA-L tissue (Fig. 3A). Automatic quantification and OD evaluation revealed significant IL-6 expression in UA-L tissue compared with NT-L tissue (Supplemental Fig. 7A, available online). Treatment with human recombinant IL-6 (0–30 ng/mL) significantly up-regulated UL cell viability (Supplemental Fig. 7B, available online). In UL explants, IL-6 and IL-6R were significantly up-regulated after P4 treatment and significantly down-regulated after UA treatment (Fig. 3C and, Supplemental Fig. 7A and C, available online). Ulipristal acetate treatment of UL explants significantly decreased their VEGF and IL-6 production, whereas the addition of iSMAD3 partially abolished this UA effect (Fig. 3D to E and Supplemental Fig. 8B, available online). Furthermore, iSMAD3 also reduced the P4-stimulated IL-6 release (Fig. 3E).

The overexpression of COL1A1 and excessive production of procollagen I α-1 (proCOLIA1) have been associated with fibrosis and were potentially regulated by P4 (19). In UL explants, P4 treatment increased *COL1A1* expression as well as the release of proCOLIA1, whereas UA treatment decreased collagen type I expression and proCOLIA1 release (Fig. 3F and g). Furthermore, iSMAD3 significantly suppressed P4-stimulated *COL1A1* expression but showed no additive or synergistic effects with UA (Fig. 3F).

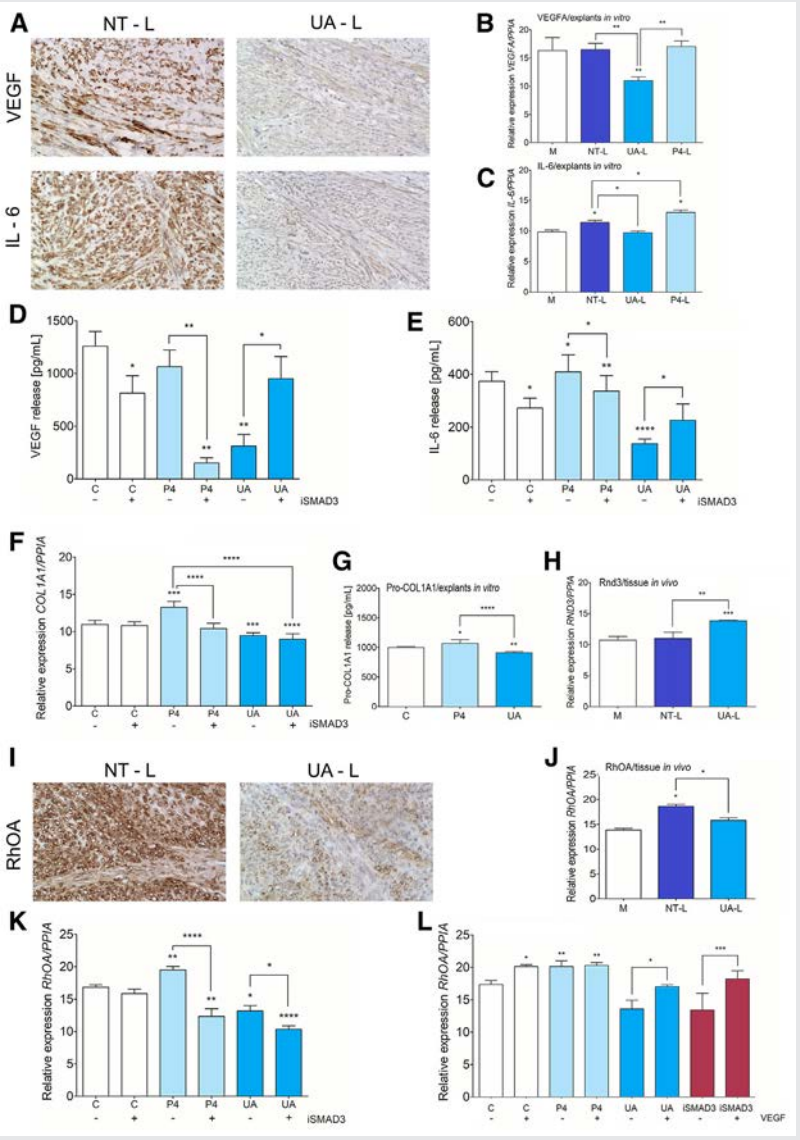
Progesterone and UA regulate the RhoA pathway in ULs

Ras homolog family member A, a key regulator of ECM accumulation, is activated by TGF-β and VEGF in several fibrotic or neoplastic diseases (20–22). We found abundant RhoA staining in the NT-L tissue, whereas only weak expression and levels were found after UA treatment (Fig. 3I). Automatic quantification and OD evaluation revealed significantly decreased RhoA expression in UA-L tissue compared with NT-L tissue (Supplemental Fig. 4C, available online). In addition, UA significantly down-regulated RhoA expression levels in UL tissues and explants (Fig. 3J and K). Progesterone up-regulated RhoA expression in UL explants, and this effect could be abolished by iSMAD3 (Fig. 3K). The addition of iSMAD3 to UA showed an additive effect on the down-regulation of RhoA expression in UL explants (Fig. 3K). Co-treatment of VEGF with P4 did not enhance the stimulating effects of P4 (Fig. 3I). VEGF abolished the UA and iSMAD3-dependent down-regulation of RhoA (Fig. 3I). Furthermore, UA treatment up-regulated the RhoA antagonist Rnd3 expression levels in UL tissues (Fig. 3H).

DISCUSSION

The functional implications of P4 actions through activation of the nuclear and membrane PRs are still rather poorly understood in the biology of ULs, although up-regulated PGR expression levels in them have been demonstrated (24). Among the two distinct isoforms of the human progesterone receptor (PGRA and PGRB), the transcriptional effects of PGRA and PGRB on progestin-responsive promoters differ (25). In general, PGRB functions as a transcriptional activator of progesterone-responsive genes, whereas PGRA is an inhibitor of steroid hormone receptors, as well as for the PGRB isoform (26). The current study showed that the expression of *PGRB* was down-regulated after UA, and the expression of total *PGRA/B* was up-regulated, indirectly suggesting that UA increases the expression of the *PGRA* isoform in ULs. Our results, therefore, suggested that UA may inhibit the transcriptional action of PGRB through an antagonistic effect. Conversely, UA might activate the inhibitory effect of PGRA as its agonist, causing double inhibition of the activity of PGR in ULs. Moreover, UA showed no effect on PGR and PGRMC2 but down-regulated the mPRα, mPRβ, mPRγ, and PGRMC1 membrane receptors at gene and/or protein levels in ULs. These novel findings suggested that not only PGRs but also the membrane P4 receptors may play a role in UL

FIGURE 3



The effect of ulipristal acetate (UA) and progesterone (P4) treatments on vascular endothelial growth factor (VEGF), interleukin 6 (IL-6), collagen type I α-1 chain (COL1A1), and Ras homolog family member A (RhoA) expression and on the VEGF, IL-6, and proCOL1A1 release by uterine leiomyoma (UL) cells and explants. Immunohistochemical (IHC) staining of VEGF and IL-6 (**A**) in ULs from nontreated (NT-L) (n = 150) and UA-treated (UA-L) (n = 100) women. The quantitative polymerase chain reaction (qPCR) analysis of VEGFA (**B**) and IL-6 (**C**) expression in NT-L, UA-L, and P4-treated (P4-L) ULs explants (n = 50). Release of VEGF (**D**) and IL-6 (**E**) levels in the control, P4, and UA with or without iSMAD3 in ULs cells (n = 30). The qPCR gene expression analysis of COL1A1 in ULs primary cells (n = 30) after treatment with UA, P4, and iSMAD3 (**F**). Release of proCOL1A1 (**G**) levels in the NT-L, P4-, and UA-treated ULs explants (n = 50). The qPCR gene expression analysis of Rnd3 in normal myometrium (M) (n = 100), ULs tissue from nontreated (NT-L) (n = 150), and UA-treated (UA-L) (n = 100) (**H**) women. Immunohistochemical staining of RhoA (**I**) in ULs from nontreated (NT-L) (n = 150) and UA-treated (UA-L) (n = 100) women. The qPCR gene expression analysis of RhoA in UL tissue from nontreated (NT-L) (n = 150) and UA-treated (UA-L) (n = 100) (**J**) women. The qPCR gene expression analysis of RhoA in UL explants (n = 50) after treatment with UA, P4, iSMAD3 (**K**), and VEGF (**I**).
Each bar represents the ratio of the investigated gene to the housekeeping gene (*PPIA* ± SEM). * Statistically significant differences between nontreated control and treated groups (*, *P*<.05; **, *P*<.01; ***, *P*<.001; ****, *P*<.0001) (one-way ANOVA with the post hoc Bonferroni's test). C/NT-L, control/nontreated ULs; iSMAD3, an inhibitor of SMAD3; M, healthy myometrium; P4, progesterone treated ULs; RhoA, Ras homolog family member A; UA-L, ulipristal acetate treated ULs. Scale bar, 200 μm.

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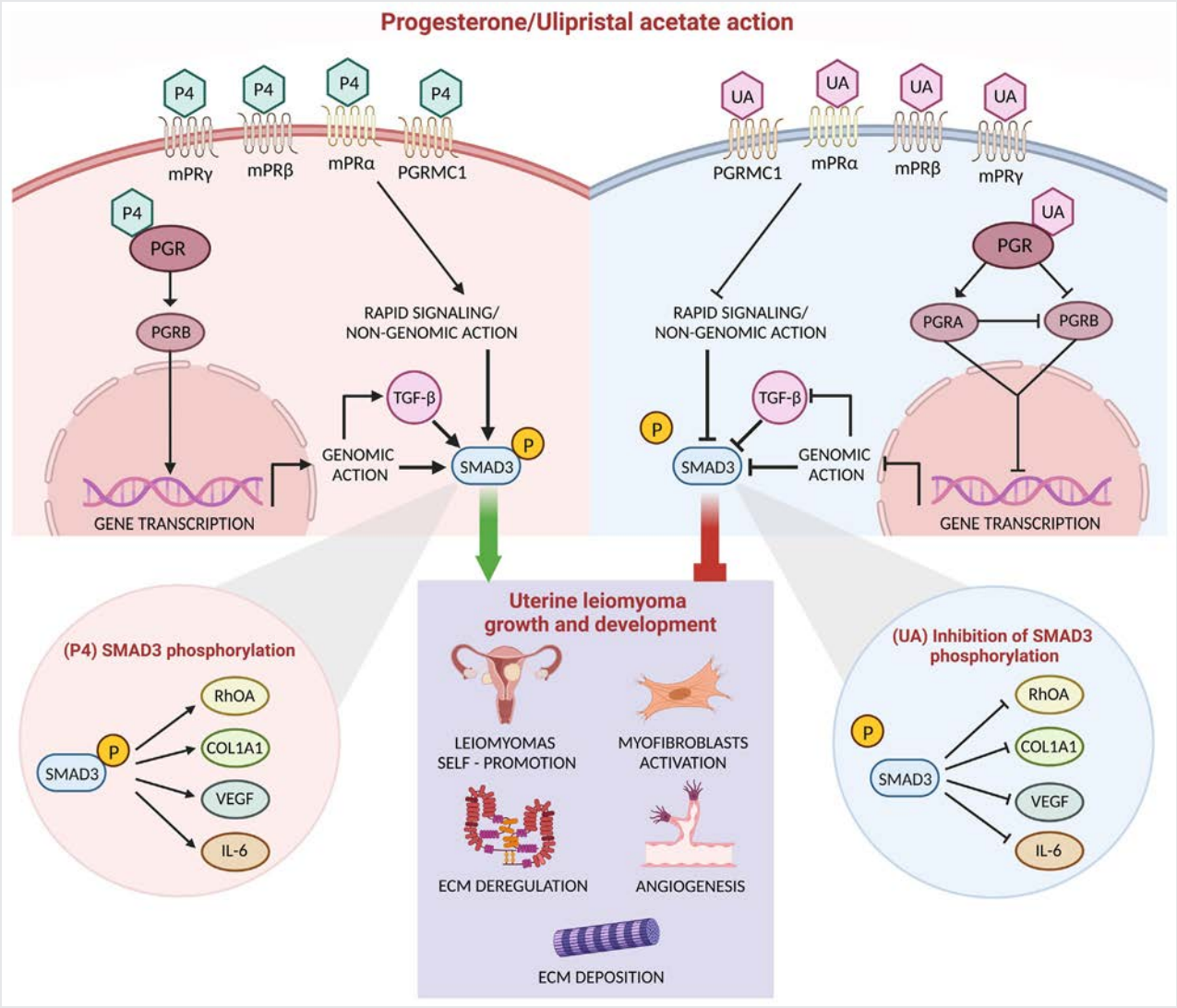
pathobiology, which might be of future importance in UL treatment strategies along with UA.

Excessive accumulation of ECM components plays an important role in the formation of ULs (5). P4 has been shown to activate the signaling pathways involved in the control of ECM deposition and cell proliferation (5). The present study emphasized a major role for P4 in ULs in the regulation of the TGF-β signaling pathway. Besides SMADs, TGF-β can

FIGURE 4



Overview of progesterone (P4) and ulipristal acetate (UA) action via genomic and nongenomic signaling in uterine leiomyomas (UL) cells. Progesterone induces the classical signaling by binding to PGR located within the cytoplasm. This conformation activates genomic action by triggering the transcription of target genes in UL cells. One of the effects of genomic P4 action is SMAD3 phosphorylation, which controls tumor growth and development. Nontreated ULs have high gene and protein expression of mPRs such as mPRα, mPRβ and PGRMC1. Progesterone initiates a rapid nonclassical signaling of mPRs by activating downstream targets, leading to the tumor's growth and development. One of these targets is SMAD3, whose phosphorylation is stimulated by P4. On the contrary, UA demonstrated specific antagonistic effects on PRs. Ulipristal acetate down-regulates the expression of mPRα, mPRβ, mPRγ, and PGRMC1 membrane receptors, but shows no effect on PGR and PGRMC2 at gene and/or protein levels in ULs. Through PR blockage, UA inhibited UL proliferation, growth, or ECM accumulation via the TGF-β and SMAD3 signaling pathway and downstream specific targets. Figure 4 was created with BioRender.com.
P4 = progesterone; PR = progesterone receptor; mPR = membrane progesterone receptor.

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activate other signaling molecules, modulating cellular downstream responses (27, 28). Transforming growth factor β receptors may regulate the Rho GTPases like Ras, RhoA, Cdc42, and Rac1 nonSMAD factors through phosphorylation or direct interaction (29). However, these kinases can also activate the canonical SMAD pathway (27). Ras homolog family member A plays an important role in different processes, including cell growth, proliferation, and modulation of the actin cytoskeleton and ECM (30, 31). We demonstrated the overexpression of RhoA in ULs. Progesterone and UA regulated differentially RhoA expression in an SMAD3-dependent manner, P4 with up-regulation and UA with down-regulation, which suggested RhoA action in ULs may also be regulated by canonical SMAD signaling. It has been demonstrated that SMAD3 regulates RhoA activation and cytoskeletal reorganization by controlling neuroepithelial

cell transforming 1 in TGF-β1-induced cells (32). Interestingly, in the present study, UA up-regulated the RhoA antagonist Rnd3 in ULs, which was a novel finding with future target application potential.

Overexpression of collagen subtypes, an abnormal collagen structure (5), and TGF-β3 are the main factors for the induction of collagen expression in ULs (23). Additionally, the SMAD3 mediation is known to play an important role in TGF-β-induced transcription of fibrosis-related genes, including collagens (33, 34). Hereby, we found that P4 increased *COL1A1* expression and proCOL1A1 production by UL explants; both were suppressed by UA. It has been shown that SMAD3 down-regulation may decrease the gene expression of procollagen types I and III in fibroblasts and reduce the ECM deposition and fibrosis processes in keloid disease fibroblasts (35). Our findings provide additional evidence that UA treatment might affect several central fibrotic factors, such as collagens, potentially influencing the abnormal ECM deposition in ULs.

Besides TGF-β, other cytokines, like interleukins, may regulate the pathobiology of ULs (18). It has been shown that IL-6 may regulate ECM remodeling and induce collagen production (36). Our study revealed significantly up-regulated IL-6 and IL-6R expression after P4 treatment but no effects on IL-6 release. Interleukin-6 down-regulation by UA highlighted the involvement of this interleukin in UL biology. In addition, *SMAD3* silencing has been shown to attenuate IL-6-induced collagen synthesis in dermal fibroblasts (36). Thus, the inhibition of IL-6 release after SMAD3 blockage may suggest that the canonical TGF-β signaling pathway regulates its activity in ULs.

Because of excessive production and accumulation of ECM, ULs have been considered to possess poor vascularization (37). We hereby show that UA treatment down-regulated the high VEGF expression and their release in ULs. Activation of the canonical TGF-β pathway has been shown to play a key role in the regulation of VEGF release (12, 13). We did not observe any P4-induced up-regulation of VEGF production in ULs. However, when we blocked SMAD3, it significantly inhibited the P4-support of VEGF release, suggesting that P4 may sustain VEGF activation via the TGF-β-SMAD3 pathway. In addition, VEGF has been shown to stimulate angiogenesis in a Rho GTPase-dependent manner in endothelial cells. The VEGFA and VEGF2 axes stimulate the activation of RhoA, Cdc42, and Rac1, leading to vascular development and the formation of cytoplasmic migratory structures (20, 21, 38). Our present analysis revealed that VEGF up-regulated RhoA expression in UL explants, and UA inhibited this effect. The mechanistic sequence of events might involve the release of VEGF by the ULs to stimulate intratumor angiogenesis, which was enhanced by P4 through SMAD3 signaling. The RhoA pathway was likely involved not only in the ECM reorganization but also in its regulation of vascular processes inside the ULs. Extracellular matrix deposition and structure are crucial for fibrotic tumor formation (39).

A better mechanistic understanding of the synthesis and accumulation of ECM is critical for the development of further novel therapeutic strategies for ULs. However, for clinical translation, several key points still need to be addressed (like combining UA with direct and indirect ECM targeting with SMAD3, TGF-βRI and II, RhoA, VEGF, or COL1A1). This proof of the principal and concept experiments could be a limitation of this study. The most important next step would be to investigate the combination therapy of UA with RhoA and ROCK inhibitors, as well as with VEGF or IL-6 inhibitors, to determine their effectiveness in the regulation of ECM synthesis and accumulation in ULs in vitro, followed by in vivo studies. Subsequently, the tolerance and safety of these therapies should be assessed. We assert that identifying and targeting fibrogenic signaling hubs like RhoA could serve as a strategy for the design, characterization, and translation of new antineoplastics against ULs and other neoplasms with excessive fibrosis.

CONCLUSIONS

In summary, P4 activated the TGF-β and SMAD3 signaling pathways and stimulated proliferation, growth, and ECM remodeling in ULs (Fig. 4). On the contrary, SPRM-presentative UA showed novel opposite effects on UL proliferation and growth or ECM accumulations through the TGF-β and SMAD3 signaling pathways, namely negatively acting through SMAD3, TGF-β RI and II, RhoA, VEGF, or decreasing the fibrosis-related gene *COL1A1* and p*roCOLIA1* production. Our present findings therefore highlight a novel treatment option for ULs through combining selected TGF-β and SMAD3 signaling pathway members with SPRMs.

CRediT Authorship Contribution Statement

Conceptualization: G.M., D.P.T., P.B., O.L., M.Sz., M.S., A.P.P., M.K., T.B., M.Z.K., J.T.; Investigation: G.M., D.P.T., P.B., O.L., M.Sz., M.S., A.P.P., M.K., T.B., M.Z.K., A.P., J.T.; Funding acquisition: N.A.R., G.M.; Supervision: G.M., D.P.T., X.L., I.H., S.W., N.A.R.; Writing – original draft: G.M., D.P.T., S.W., I.H., N.A.R.; Writing – review and editing: G.M., D.P.T., S.W., I.H., and N.A.R.

Declaration of Interests

G.M. has nothing to disclose. D.P.T. has nothing to disclose. P.B. has nothing to disclose. O.L. has nothing to disclose. M.Sz. has nothing to disclose. M.S. has nothing to disclose. A.P.P. has nothing to disclose. M.K. has nothing to disclose. T.B. has nothing to disclose. M.Z.K. has nothing to disclose. A.P. has nothing to disclose. J.T. has nothing to disclose. X.L. has nothing to disclose. I.H. has nothing to disclose. S.W. has nothing to disclose. N.A.R. has nothing to disclose.

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Evidencia funcional de dos mecanismos de acción distintos de progesterona y un modulador selectivo del receptor de progesterona en los leiomiomas uterinos

Objetivo: Estudiar los mecanismos específicos a través de los cuales la progesterona y los moduladores selectivos del receptor de progesterona impactan el crecimiento, síntesis y acumulación de la matriz extracelular en los leiomiomas uterinos.

Diseño: Estudio de laboratorio.

Lugar: Instituciones de Investigación Académica.

Paciente(s): Este estudio involucró a mujeres en edad reproductiva con diagnóstico de infertilidad asociada a leiomiomas uterinos que se sometieron a miomectomía ya sea después de un tratamiento con el modulador selectivo del receptor de progesterona acetato de ulipristal (UA) o sin ningún tratamiento previo farmacológico. Las muestras control incluyeron tejido de miometrio sano (n = 100). Las muestras se obtuvieron del Departamento de Reproducción y Endocrinología Ginecológica y del Biobanco de la Universidad Médica de Bialystok, Polonia.

Intervención(es): Se recolectaron muestras de tejido miometrial inmediatamente después de cirugía para su análisis y evaluación transcripcional de pacientes con leiomiomas uterinos tratadas durante dos meses con UA diario (5mg/d) (n = 100) y de pacientes que no recibieron tratamiento (n = 150) o miometrio sano normal (n = 100).

Principal(es) Medida(s) de Resultado(s): Se evaluó la activación inducida por progesterona de las vías de señalización relacionadas con la síntesis, el depósito y el crecimiento de la matriz extracelular de los leiomiomas uterinos, así como el perfil de expresión de los receptores de progesterona en los leiomiomas uterinos.

Resultado(s): Los resultados indicaron que la progesterona activó las vías de señalización del factor de crecimiento transformador-β y SMAD3 y promovió la proliferación, el crecimiento y la remodelación de la matriz extracelular en los leiomiomas uterinos mediante la regulación a la alta de SMAD3, el receptor del factor de crecimiento transformador-β (TGF-β) tipo I y II, el homólogo A de Ras, el factor de crecimiento vascular endotelial, o aumentando la producción del gen de colágeno relacionado con fibrosis, tipo I, -1, y procolágeno, tipo I, -1. Por el contrario, el UA tuvo efectos inhibidores sobre estos procesos. El estudio también mostró que los receptores de progesterona tanto nucleares como de membrana desempeñan funciones distintas en la biopatología del leiomioma uterino.

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Predicting risk of endometrial failure: a biomarker signature that identifies a novel disruption independent of endometrial timing in patients undergoing hormonal replacement cycles

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Objective: To propose a new gene expression signature that identifies endometrial disruptions independent of endometrial luteal phase timing and predicts if patients are at risk of endometrial failure.
Design: Multicentric, prospective study.
Setting: Reproductive medicine research department in a public hospital affiliated with private fertility clinics and a reproductive genetics laboratory.
Patients: Caucasian women (n = 281; 39.4 ± 4.8 years old with a body mass index of 22.9 ± 3.5 kg/m^2) undergoing hormone replacement therapy between July 2018 and July 2021. Endometrial samples from 217 patients met RNA quality criteria for signature discovery and analysis.
Intervention(s): Endometrial biopsies collected in the mid-secretory phase.
Main Outcome Measure(s): Endometrial luteal phase timing-corrected expression of 404 genes and reproductive outcomes of the first single embryo transfer (SET) after biopsy collection to identify prognostic biomarkers of endometrial failure.
Results: Removal of endometrial timing variation from gene expression data allowed patients to be stratified into poor (n = 137) or good (n = 49) endometrial prognosis groups on the basis of their clinical and transcriptomic profiles. Significant differences were found between endometrial prognosis groups in terms of reproductive rates: pregnancy (44.6% vs. 79.6%), live birth (25.6% vs. 77.6%), clinical miscarriage (22.2% vs. 2.6%), and biochemical miscarriage (20.4% vs. 0%). The relative risk of endometrial failure for patients predicted as a poor endometrial prognosis was 3.3 times higher than those with a good prognosis. The differences in gene expression between both profiles were proposed as a biomarker, coined the endometrial failure risk (EFR) signature. Poor prognosis profiles were characterized by 59 upregulated and 63 downregulated genes mainly involved in regulation (17.0%), metabolism (8.4%), immune response, and inflammation (7.8%). This EFR signature had a median accuracy of 0.92 (min = 0.88, max = 0.94), median sensitivity of 0.96 (min = 0.91, max = 0.98), and median specificity of 0.84 (min = 0.77, max = 0.88), positioning itself as a promising biomarker for endometrial evaluation.

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Conclusion(s): The EFR signature revealed a novel endometrial disruption, independent of endometrial luteal phase timing, present in 73.7% of patients. This EFR signature stratified patients into 2 significantly distinct and clinically relevant prognosis profiles providing opportunities for personalized therapy. Nevertheless, further validations are needed before implementing this gene signature as an artificial intelligence (AI)-based tool to reduce the risk of patients experiencing endometrial failure. (Fertil Steril® 2024;122:352–64. ©2024 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: artificial intelligence, precision medicine, patient stratification, endometrial-factor infertility, gene expression signature

A key factor for reproductive success in assisted reproduction treatments is the status of the maternal endometrium during embryo implantation and fetal development. The endometrial cycle is reflected by the cyclic structural and functional changes of the endometrium across the menstrual cycle, particularly during the mid-secretory phase, to prepare for the "window of implantation" (WOI) (1–5). After successful embryo implantation, the decidua (specialized layer of the endometrium) actively encapsulates the trophectoderm to support placentation and provide adequate vascularization for optimal fetal growth (6). Thus, approaches that predict and prevent endometrial-factor infertility could substantially improve assisted reproduction treatment outcomes by supporting the establishment and maintenance of pregnancy (7, 8).

There is a lack of consensus on the minimum number of implantation failures with good-quality embryos derived from ovum donation or with confirmed euploid karyotypes required to diagnose recurrent implantation failure (RIF) (9, 10). The disparities in clinical classification and heterogeneous etiology of RIF confound its diagnosis and leave the exact prevalence unclear (9–14). As clinical symptoms cannot be prevented and do not provide sufficient criteria to stratify patients, patients might be misclassified and receive inefficient treatment. Indeed, most studies using clinical criteria for RIF identified no or few differentially expressed genes between samples of affected patients (15, 16), reflecting the molecular heterogeneity of RIF.

To overcome these healthcare challenges, high-throughput molecular technologies are combined with artificial intelligence (AI) algorithms to provide a deeper understanding of infertility-related pathogenesis (7, 17–21). These molecular insights are then applied in genomics-driven precision medicine approaches to characterize complex diseases, and ultimately, improve patient diagnosis and prognosis (22). Employing genomics in reproductive medicine has the potential to go beyond treating the clinical manifestations of disease progression (23), by leveraging the patients' heterogeneous molecular profiles for precise stratification and revealing new targets for preventive measures (24–26).

In this regard, current diagnostic tools for endometrial-factor infertility use transcriptomics to date the endometrial biopsy in the mid-secretory phase, exceeding the accuracy and objectivity of traditional endometrial progression staging by Noyes' histological criteria (1, 7, 17, 19, 20). However, the clinical utility of synchronizing the endometrium by adjusting the timing of progesterone (P4) administration on the basis of the patient's transcriptomic endometrial dating profile remains controversial (27–34). Independent randomized clinical trials found no improvement in reproductive outcomes (34), indicating the inefficacy of this clinical intervention and highlighting the need for alternative treatments and/or a refined target patient population that benefits from this strategy.

Combining these precision medicine approaches with transcriptomics and AI algorithms; our group recently proposed a novel molecular taxonomy of RIF and a new clinical algorithm for endometrial-factor infertility that considered 2 molecular causes of implantation failure: a WOI displacement that indicates a dysregulated endometrial timing; and a WOI disruption that indicates an endometrial alteration independent of endometrial timing (18). Transcriptomic analysis revealed these 2 WOI phenotypes were presented exclusively or simultaneously and patients with RIF had significantly more WOI disruptions but a similar frequency of WOI displacements, compared with controls (18). These findings suggested that WOI disruptions had stronger associations with implantation failure than WOI displacements and shifted the paradigm for the study and treatment of endometrial-factor infertility (18). In addition, this work by Sebastian-Leon et al. (15, 16) highlighted the importance of removing the transcriptomic variation due to cyclical endometrial tissue changes (ie, the effect of endometrial timing) that, if not corrected, could mask the significant disruptions in endometrial gene expression. However, given this hypothesis-driven research was conducted retrospectively and in silico with publicly available data, the reproductive outcomes of the cycle after the biopsy collection were not available (15). This clinical follow-up is required to discover biomarkers for WOI disruption in patients with a higher risk of endometrial failure.

On the basis of these genomics-driven precision medicine strategies, this proof-of-concept study aimed to discover a biomarker panel that could identify endometrial disruption, independent of endometrial timing, to predict, and ultimately prevent, poor reproductive outcomes in in vitro fertilization (IVF) patients.

MATERIAL AND METHODS

Ethics statement

This study was approved by the Ethics Committee of the Instituto Valenciano de Infertilidad, Valencia, Spain (1810-FIVI-066-PD/1810-FIVI-048-PD). Written informed consent was obtained from all recruited patients (n = 281).

Participants, collection of endometrial biopsies, and reproductive outcomes

This prospective, multicentric study was conducted between July 2018 and July 2021 at 5 private fertility clinics in Spain. Participants (n = 281) were scheduled for endometrial evaluation due to medical indications or ≥2 implantation failures of idiopathic origin. All patients met the following inclusion criteria: 18–50 years old, with a body mass index (BMI) of 19–30 kg/m^2, undergoing hormone replacement therapy for single embryo transfer (SET), presenting an endometrial thickness >6.5 mm with trilaminar structure in proliferative phase before P4 administration, and serum levels of estradiol >100 pg/mL and P4 <1 ng/mL on the 10th day of estrogen treatment (17). Endometrial biopsies were collected after an average of 114.5 ± 7.2 h of P4 (duration ranged from 82 to 172 h), and processed as we previously described (17).

Baseline patient characteristics including age, BMI, the number of IVF attempts, the quality of the embryo transferred (defined in the Supplemental Methods, available online), and reproductive outcomes (ie, pregnancy, live birth, and biochemical and clinical miscarriage) for each attempt, were obtained from internal medical records. Information about IVF attempts in other fertility clinics was included if recorded (only in 47.7% of patients).

Study design

First, a custom RNA-seq gene panel (the endometrial failure risk [EFR] panel) comprising 404 biomarkers of endometrial luteal phase timing and/or potential disruption was designed and employed to determine the risk of endometrial failure among 217 IVF patients (Supplemental Fig. 1, step 1 available online). The endometrial timing-corrected expression of the EFR panel was used to study the differential expression associated with endometrial disruption together with clinical characteristics of IVF patients to stratify the population as having poor or good endometrial prognoses, according to a semi-supervised learning process (Supplemental Fig. 1, step 2). The distinct transcriptomic patterns of each prognosis group were associated with the differences in reproductive outcomes of SET after biopsy collection, and the differentially expressed gene signature was proposed as a biomarker of endometrial disruption (Supplemental Fig. 1, step 3). Finally, the gene expression signature was used to supervise machine learning algorithms to evaluate the biomarker capacity of this signature to distinguish endometrial competence. The predictive performance of the models was assessed through the accuracy, sensibility, and specificity over 100 iterations of fivefold stratified cross-validation (Supplemental Fig. 1, step 4).

Constructing the EFR gene panel

Supplemental Figure 2 illustrates the workflow for the EFR panel gene selection. A whole-transcriptome endometrial data set (GSE58144, n = 115) retrieved from Gene Expression Omnibus (35) was processed to correct the endometrial progression effect, using linear models in limma (version 3.46.0) (36), and unmask genes related to endometrial disruption (16). These genes were ranked according to an informativity score (CorrelationAttributeEval) in Weka (version 3.8.2, December 22, 2017) (37) to evaluate their worth by measuring their correlation with endometrial pathology. Gene sets with increasing gene signature size were developed by adding the most informative genes in a stepwise manner, and their predictive performance was analyzed using Support Vector Machine (38) and Random Forest (39) algorithms. After calculating predictive performance with a stratified fivefold cross-validation (default parameters; 80:20; 100 iterations) using the Weka R package (40), the optimal signature size was determined for each algorithm, as the one with the fewest genes and highest accuracy. Finally, genes we previously prioritized for transcriptomic endometrial dating (17) were also included in the EFR panel to be able to remove the endometrial luteal phase timing variation.

Endometrial biopsy processing, sequencing, and transcriptomic preprocessing

Total RNA was extracted, and only samples that met our quality criteria were sequenced using the TruSeq Targeted RNA Illumina protocol and our custom EFR panel, in the Illumina NextSeq 550 platform with a paired-end design of 150 cycles and 4 M reads/sample, as previously described (17). Raw transcriptomic data was processed and normalized using limma, to filter low-quality raw counts (Q < 30) and low-read samples (<2 M) (17). Possible batch effects were detected using principal components analysis and corrected with linear models in limma. Finally, endometrial luteal phase timing effects were detected using our transcriptomic endometrial dating (TED) model (17) (see Supplemental Methods) and removed using limma.

EFR biomarker signature identification

A preliminary classification of patients was based on the patients' history of reproductive outcomes and transcriptomic patterns corrected for endometrial luteal phase timing. This approach eliminated the gene expression variation related to the cyclic tissue changes that mask endometrial disruption (16, 18) and was used before applying semi-supervised machine learning on the basis of self-labeled techniques (see Supplemental Methods) (41).

Once, the genomic-driven disease taxonomy was established, the differentially expressed genes obtained between the final good and poor prognosis groups were proposed as the EFR biomarker signature. The limma R package was used to identify the statistically significant differentially expressed genes (false discovery rate [FDR]-adjusted P value <.05).

Functional characterization of the EFR signature

The biomarker signature genes were annotated for functional interpretation databases by consulting Kyoto Encyclopaedia of Genes and Genomes human pathways (42) (release 99.0, August 1, 2021) and Gene Ontology (version July 2, 2021; employing only experimental annotations) (43). Functional terms were manually summarized into categories according

to their description in the database and presented as a bar plot using ggplot2 (44).

EFR signature capability as a biomarker of endometrial disruption

To estimate the prediction capability of the EFR signature to distinguish poor and good endometrial prognosis profiles, we implemented predictive models on the basis of the Support Vector Machine algorithm. Using a stratified fivefold cross-validation process (repeated 100 times), the accuracy, sensitivity, and specificity were estimated. The range of values obtained across the 100 iterations was presented as a boxplot using ggplot2.

Statistical analysis

Rates for reproductive outcomes (ie, pregnancy rate [PR], cumulative pregnancy rate [CPR], live birth rate [LBR], biochemical miscarriage rate [BMR], and clinical miscarriage rate [CMR]) were calculated as defined in the Supplemental Methods. Descriptive statistics were used for transcriptomic and clinical characteristics of patients to compare prognosis or WOI displacement groups. Continuous variables were presented as an overall mean ± standard deviation whereas discrete variables were presented as counts and percentages. Groups were compared using the Wilcoxon rank-sum test for continuous variables and Fisher's exact test for discrete variables. All statistical analyses were conducted in R. All graphical results were generated with ggplot2.

Gene expression validation by quantitative real-time polymerase chain reaction

Quantitative real-time polymerase chain reaction (RT-qPCR) was performed to validate the expression of the top 4 differentially expressed genes between patients with poor (n = 10) and good (n = 10) endometrial prognoses. RNA (200–220 ng) was reverse transcribed into cDNA using the PrimeScript Reagent Kit (Perfect Real Time, Takara, Japan) on a Thermocycler T3000 (Biometra, Ireland). Quantitative real-time polymerase chain reaction was performed on a StepOnePlus Real-Time PCR System (Applied Biosystems, USA) using Power-Up SYBR Green (Thermo Fisher Scientific, USA) and standard conditions. Supplemental Table 1 (available online) lists the specific primers (Invitrogen, Thermo Fisher Scientific) used. Universal human RNA (Agilent, Spain) and water were respectively included as positive and negative controls. Each sample was analyzed in duplicate and relative gene expression (normalized to beta actin) was calculated using the ΔΔCt method (45, 46).

RESULTS

Preliminary sequencing with the 404-gene EFR panel

The EFR panel evaluated 404 genes: 139 that predict endometrial disruption and 310 that serve as biomarkers of endometrial dating or timing. Notably, 45 genes overlapped in these 2 endometrial factors.

Of the 281 endometrial samples collected, we excluded 56 samples for having low-quality or insufficient RNA; 6 samples containing a low number of sequencing reads (Supplemental Fig. 3A); and 2 samples that had low gene expression after normalization (Supplemental Fig. 3B). Thus, 217 patients were considered in the downstream analysis. After correcting batch effects from sequencing runs (Supplemental Fig. 3C) and removing the transcriptomic variation due to endometrial cyclic changes (endometrial luteal phase timing; Supplemental Fig. 3D), there were no other confounding technical or clinical factors (Supplemental Fig. 4).

A genomic-driven endometrial taxonomy for patient stratification on the basis of a novel endometrial disruption independent of endometrial timing

Forty-four patients were initially categorized as acute RIF on the basis of the inability to carry a pregnancy to term after 3–9 SETs. Alternatively, 40 patients were categorized as acute Fertile because they reached full-term pregnancy at the first SET after biopsy. Notably, after this clinical acute criteria, 133 (61.3%) patients remained unclassified for having insufficient attempts (Supplemental Fig. 5A), and no transcriptomic differences were found between the acute RIF and acute Fertile groups (the lowest FDR was 0.3027). Incorporation of transcriptomic data with this initial acute clinical classification allowed us to stratify patients with poor (n = 137, 63.1%) or good (n = 49, 22.6%) endometrial prognoses; leaving only 31 samples (14.3%) unclassified because of the lack of recognition by the algorithms (Supplemental Fig. 5B).

EFR biomarker signature identification and functional characterization

A total of 122 genes were identified as significantly different (30.2% of all panel genes, FDR < 0.05) between patients with good and poor endometrial prognoses, including 63 downregulated and 59 upregulated in patients with poor endometrial prognosis (Table 1 and Fig. 1A), and proposed as the EFR biomarker signature. Transcriptomically, endometrial biopsies clustered by prognosis (Fig. 1B), and the signature genes had functions that were mainly related to regulation (17%); metabolism (8.4%); the immune system and inflammation (7.8%); protein processes (7.3%), signaling (6.9%), transport (6.1%), and the cell cycle, proliferation, and differentiation (5.3%) (Fig. 1C and Supplemental Table 2). The poor prognosis profile was distinguished by a downregulation of 27/39 (69.2%) functional groups. We highlight the functions with higher proportions of downregulated genes as steroidogenesis and steroid signaling (77.8%), hormone-related (80.0%), the nervous system (81.3%), gametogenesis and reproduction (85.7%), and oxidative stress and response to O_2 (88.9%), as well as cell action potential and polarization (100%) (Fig. 1D). Few functional groups, including cell migration, extracellular matrix, and glucose homeostasis contained a larger proportion of upregulated genes (60.9%, 62.5%, and 62.5% upregulated genes, respectively; Fig. 1D).

TABLE 1

Endometrial Failure Risk (EFR) signature's genes.

Gene ID	Gene name	FC	FDR
PAEP	Progestagen Associated Endometrial Protein	3.1873	9.12E-07
GPR110	Adhesion G Protein-Coupled Receptor F1	2.3304	3.68E-05
CHST1	Carbohydrate Sulfotransferase 1	2.0292	0.0004
POSTN	Periostin	1.8936	0.0009
BCL2L10	BCL2 Like 10	1.8549	0.0001
TSPAN8	Tetraspanin 8	1.8098	0.0179
CYP3A5	Cytochrome P450 Family 3 Subfamily A Member 5	1.7966	0.0092
SFRP4	Secreted Frizzled Related Protein 4	1.7778	1.23E-06
BAI2	Adhesion G Protein-Coupled Receptor B2	1.7138	0.0018
ITGA8	Integrin Subunit Alpha 8	1.7033	0.0005
PMEPA1	Prostate Transmembrane Protein, Androgen Induced 1	1.7026	3.35E-07
CLU	Clusterin	1.6771	3.68E-05
MUC16	Mucin 16, Cell Surface Associated	1.6288	0.0001
MYH7B	Myosin Heavy Chain 7B	1.6034	0.0116
SCGB2A2	Secretoglobin Family 2A Member 2	1.5859	0.0326
STC1	Stanniocalcin 1	1.5800	0.0033
FOXP1	Forkhead Box P1	1.5332	3.94E-13
C10orf10	DEPP1 Autophagy Regulator	1.5093	0.0004
LRRC17	Leucine Rich Repeat Containing 17	1.5020	0.0001
SERTAD4	SERTA Domain Containing 4	1.4805	7.63E-07
COL16A1	Collagen Type XVI Alpha 1 Chain	1.4786	0.0024
C1orf133	SERTAD4 Antisense RNA 1	1.4381	0.0065
MAPK8IP3	Mitogen-Activated Protein Kinase 8 Interacting Protein 3	1.4275	0.0003
ABCC3	ATP Binding Cassette Subfamily C Member 3	1.4181	0.0124
MFAP5	Microfibril Associated Protein 5	1.4030	0.0033
MFAP2	Microfibril Associated Protein 2	1.3990	0.0005
GPX3	Glutathione Peroxidase 3	1.3960	0.0198
EDN3	Endothelin 3	1.3627	0.0053
SORCS1	Sortilin Related VPS10 Domain Containing Receptor 1	1.3563	0.0322
DUSP2	Dual Specificity Phosphatase 2	1.3545	0.0264
SLPI	Secretory Leukocyte Peptidase Inhibitor	1.3463	0.0197
BICD1	BICD Cargo Adaptor 1	1.3427	1.99E-05
CRABP2	Cellular Retinoic Acid Binding Protein 2	1.3395	0.0040
ARID5B	AT-Rich Interaction Domain 5B	1.3247	1.31E-05
EVC	EvC Ciliary Complex Subunit 1	1.3157	0.0011
C2CD4B	C2 Calcium Dependent Domain Containing 4B	1.3091	0.0109
ANO1	Anoctamin 1	1.3014	0.0053
CLDN4	Claudin 4	1.2867	0.0216
IGF2	Insulin Like Growth Factor 2	1.2854	0.0134
SYNE2	Spectrin Repeat Containing Nuclear Envelope Protein 2	1.2756	5.71E-06
VAV3	Vav Guanine Nucleotide Exchange Factor 3	1.2644	0.0286
ITGA9	Integrin Subunit Alpha 9	1.2562	0.0084
OBFC2A	Nucleic Acid Binding Protein 1	1.2266	0.0032
WEE1	WEE1 G2 Checkpoint Kinase	1.2190	6.54E-06
RAB40B	RAB40B, Member RAS Oncogene Family	1.2100	0.0042
BCL6	BCL6 Transcription Repressor	1.1891	0.0084
ID4	Inhibitor Of DNA Binding 4, HLH Protein	1.1878	0.0156
CD55	CD55 Molecule (Cromer Blood Group)	1.1806	0.0493
LETM2	Leucine Zipper And EF-Hand Containing Transmembrane Protein 2	1.1754	0.0179
CYBRD1	Cytochrome B Reductase 1	1.1647	0.0116
LIMK2	LIM Domain Kinase 2	1.1614	0.0022
C14orf45	Basal Body Orientation Factor 1	1.1526	0.0053
C16orf70	Phagosome Assembly Factor 1	1.1514	0.0022
FOSL2	FOS Like 2, AP-1 Transcription Factor Subunit	1.1412	0.0193
SCAMP1	Secretory Carrier Membrane Protein 1	1.1296	0.0264
CTDSP2	CTD Small Phosphatase 2	1.1099	0.0166
RNF19A	Ring Finger Protein 19A, RBR E3 Ubiquitin Protein Ligase	1.1058	0.0250
HMGN3	High Mobility Group Nucleosomal Binding Domain 3	1.0988	0.0286
ACTN1	Actinin Alpha 1	1.0983	0.0322
ENY2	ENY2 Transcription And Export Complex 2 Subunit	−1.0932	0.0466
PSMB10	Proteasome 20S Subunit Beta 10	−1.1231	0.0053
ARHGDIA	Rho GDP Dissociation Inhibitor Alpha	−1.1245	0.0433
PRPF4	Pre-MRNA Processing Factor 4	−1.1397	0.0348
COPG1	COPI Coat Complex Subunit Gamma 1	−1.1433	0.0006
RACGAP1	Rac GTPase Activating Protein 1	−1.1496	0.0042
PNP	Purine Nucleoside Phosphorylase	−1.1519	0.0143
STEAP4	STEAP4 Metalloreductase	−1.1550	0.0270

Diaz-Gimeno. Endometrial Failure Risk signature. Fertil Steril 2024.

TABLE 1

Continued.

Gene ID	Gene name	FC	FDR
BDH1	3-Hydroxybutyrate Dehydrogenase 1	−1.1744	0.0030
ECI2	Enoyl-CoA Delta Isomerase 2	−1.1862	0.0178
ANK3	Ankyrin 3	−1.2084	0.0433
IL15	Interleukin 15	−1.2233	0.0198
GSN	Gelsolin	−1.2241	0.0076
GLIPR1	GLI Pathogenesis Related 1	−1.2251	0.0166
CBR3	Carbonyl Reductase 3	−1.2252	0.0124
ATP6V0E2	ATPase H+ Transporting V0 Subunit E2	−1.2304	0.0082
DYNLT3	Dynein Light Chain Tctex-Type 3	−1.2323	0.0199
S100A4	S100 Calcium Binding Protein A4	−1.2478	0.0041
BUB1B	BUB1 Mitotic Checkpoint Serine/Threonine Kinase B	−1.2482	0.0451
IDH1	Isocitrate Dehydrogenase (NADP(+)) 1	−1.2488	0.0124
PLA2G16	Phospholipase A And Acyltransferase 3	−1.2498	0.0124
EPHB3	EPH Receptor B3	−1.2530	0.0396
OAZ3	Ornithine Decarboxylase Antizyme 3	−1.2581	0.0013
PYCR1	Pyrroline-5-Carboxylate Reductase 1	−1.2597	0.0007
FAM155B	NALCN Channel Auxiliary Factor 2	−1.2618	0.0116
ECHS1	Enoyl-CoA Hydratase, Short Chain 1	−1.2621	3.68E-05
CSRP2	Cysteine And Glycine Rich Protein 2	−1.2647	0.0023
TLR4	Toll Like Receptor 4	−1.2716	0.0065
CTSW	Cathepsin W	−1.2764	0.0322
CEP55	Centrosomal Protein 55	−1.3021	0.0264
BARD1	BRCA1 Associated RING Domain 1	−1.3064	0.0014
PLA2G4A	Phospholipase A2 Group IVA	−1.3101	0.0008
ATP1B1	ATPase Na+/K+ Transporting Subunit Beta 1	−1.3202	0.0113
FOXO1	Forkhead Box O1	−1.3330	0.0018
BIRC3	Baculoviral IAP Repeat Containing 3	−1.3496	0.0007
CRISP3	Cysteine Rich Secretory Protein 3	−1.3498	0.0397
LMCD1	LIM And Cysteine Rich Domains 1	−1.3627	0.0012
PSMB8	Proteasome 20S Subunit Beta 8	−1.3783	3.68E-05
CD81	CD81 Molecule	−1.3888	0.0104
ALDH1A3	Aldehyde Dehydrogenase 1 Family Member A3	−1.3891	0.0007
SQLE	Squalene Epoxidase	−1.3905	0.0001
KMO	Kynurenine 3-Monooxygenase	−1.4107	0.0434
RPRM	Reprimo, TP53 Dependent G2 Arrest Mediator Homolog	−1.4121	0.0061
SPP1	Secreted Phosphoprotein 1	−1.4130	0.0242
ANGPTL1	Angiopoietin Like 1	−1.4381	0.0017
HPRT1	Hypoxanthine Phosphoribosyltransferase 1	−1.4394	1.43E-05
ATP6V1A	ATPase H+ Transporting V1 Subunit A	−1.4484	0.0040
CDK1	Cyclin Dependent Kinase 1	−1.4651	0.0084
ENPEP	Glutamyl Aminopeptidase	−1.4704	0.0441
TH	Tyrosine Hydroxylase	−1.5149	0.0113
G0S2	G0/G1 Switch 2	−1.5183	0.0226
PROS1	Protein S	−1.5297	0.0025
STAR	Steroidogenic Acute Regulatory Protein	−1.5342	0.0005
DKK1	Dickkopf WNT Signaling Pathway Inhibitor 1	−1.5425	0.0005
BANK1	B Cell Scaffold Protein With Ankyrin Repeats 1	−1.5553	0.0005
KCNJ2	Potassium Inwardly Rectifying Channel Subfamily J Member 2	−1.6602	0.0119
SLC7A2	Solute Carrier Family 7 Member 2	−1.6919	0.0038
PROK1	Prokineticin 1	−1.7218	0.0106
OLFM4	Olfactomedin 4	−1.7759	0.0458
IFNG	Interferon Gamma	−1.9666	0.0033
CXCL13	C-X-C Motif Chemokine Ligand 13	−2.0690	0.0001
SLC16A6	Solute Carrier Family 16 Member 6	−2.2113	9.12E-07
FGB	Fibrinogen Beta Chain	−2.3652	0.0108

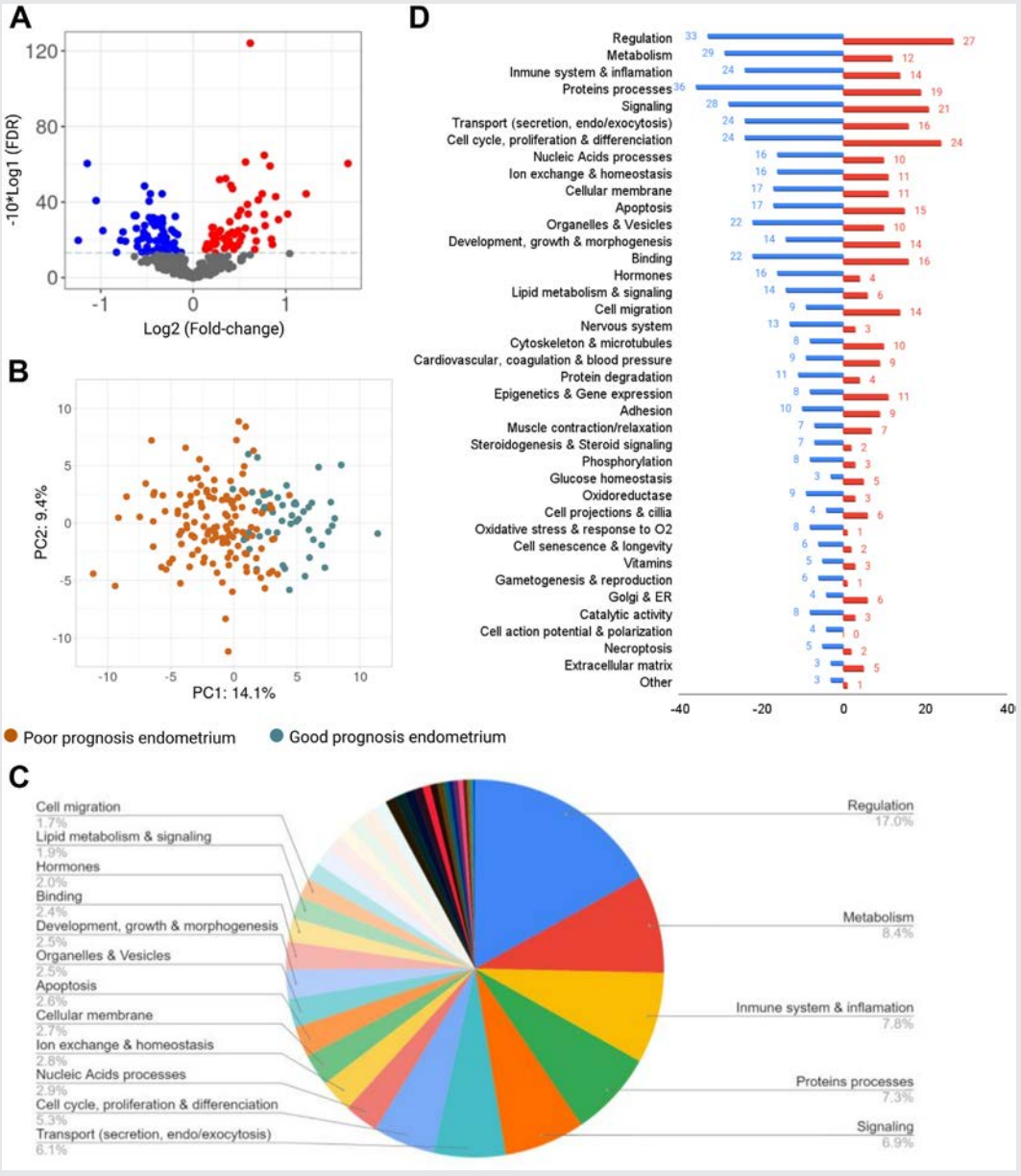
Complete list of the 122 genes included in the endometrial failure risk (EFR) signature. Gene name and ID, fold change (FC) related to poor prognosis, and adjusted *P* value by false discovery rate (FDR) are indicated.

Diaz-Gimeno. Endometrial Failure Risk signature. Fertil Steril 2024.

Differences in clinical reproductive outcomes authenticated the EFR signature's clinical relevance

There were no significant differences in the age or BMI of patients predicted to have good/poor endometrial prognosis (all $P>.05$). Women predicted to have poor endometrial prognosis previously underwent significantly more SETs than those with good endometrial prognosis (2.8 vs. 1.6; $P<.0001$), however, there were no significant differences in embryo quality ($P=.4679$) between groups (Supplemental Table 3). The lack

FIGURE 1



Transcriptomic and functional characterization of the endometrial failure risk (EFR) signature. (**A**) Volcano plot showing the 122 EFR signature genes. Red and blue dots were used to differentiate EFR genes that were significantly upregulated or downregulated in poor prognosis samples, respectively, whereas gray dots represented genes (n = 282) whose expression was not significantly different (FDR > 0.05) between poor and good prognoses groups and were not included in EFR signature. (**B**) Principal component analysis highlighting the transcriptomic behavior of endometrial samples from patients predicted to have poor (orange) or good (green) endometrial prognosis on the basis of genes included in EFR signature. Notably, the transcriptomic variance explained by the first and second principal components (PC1 and PC2, respectively) reached 23.1%. (**C**) Pie chart depicting the overall proportion of EFR signature genes included in each functional category. (**D**) The EFR signature genes were classified into 39 functional groups. Red and blue bars were used to differentiate genes that were significantly upregulated or downregulated in poor prognosis samples, respectively. FDR = false discovery rate. Figure created with BioRender.com.

Diaz-Gimeno. Endometrial Failure Risk signature. Fertil Steril 2024.

of differences in the proportion of out-of-phase endometria (57% in poor vs. 47% in good, $P=.25$) between good and poor prognosis groups (Supplemental Table 3) distinguished this signature from established endometrial luteal phase timing phenotypes (displaced or on-time). Therefore, the significant differences ($P<.0001$) between the reproductive outcomes of women predicted to have poor and good endometrial prognoses validated the clinical relevance of our transcriptomic signature. Specifically, the poor endometrial prognosis was associated with a 34.5% decline in PR

(*P*=3.8E-05), a 52% reduction in LBR (*P*=5E-10), a 19.6% rise in CMR (*P*=.0066), and 20.4% increase in BMR (*P*=.0023) (Fig. 2A). Furthermore, patients with a poor endometrial prognosis only achieved a CPR of 42.3% after the ninth SET, whereas patients with a good prognosis had an 83.7% CPR at the fifth attempt (Fig. 2B).

The relative risk of implantation failure or miscarriage in women predicted to have poor endometrial prognosis was respectively 2.7 and 11.5 compared with women with good endometrial prognosis. However, when failures in implantation or ongoing pregnancy were considered together, the relative risk of endometrial failure (not achieving a live birth in the next SET) for patients with poor endometrial prognosis was 3.3.

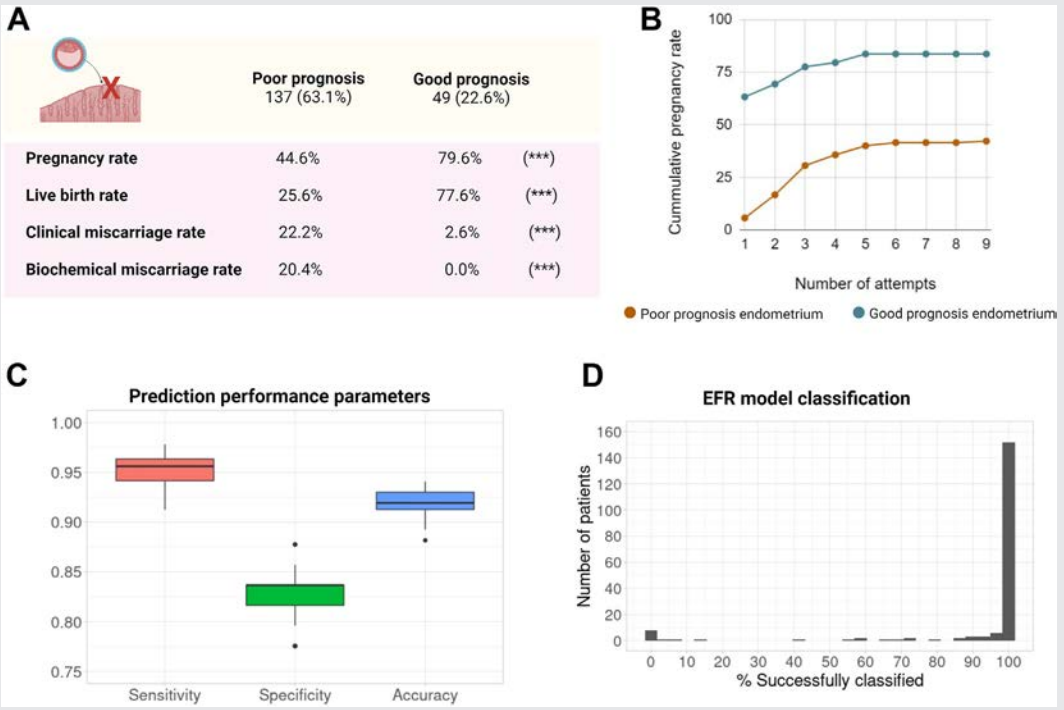
EFR signature biomarker's predictive capability

The 186 samples labeled with poor (n = 137) or good (n = 49) endometrial prognoses were used to evaluate the signature's predictive performance. The 122 differentially expressed genes between poor/good endometrial prognoses proposed as the EFR biomarker signature were employed to train the model. Cross-validation yielded a median accuracy of 0.92 (min = 0.88, max = 0.94), sensitivity of 0.96 (min = 0.91, max = 0.98), and specificity of 0.84 (min = 0.77, max = 0.88) (Fig. 2C). Notably, 163 of 186 (87.6%) samples were classified correctly in almost 90% of all cross-validation models, whereas 151 of 186 (81.2%) samples were well classified correctly in 100% of the iterations. Twelve and eleven samples were misclassified consistently, in <50% or 50%–90% of models, respectively (Fig. 2D).

Clinical algorithm results after evaluating both WOI factors

Next, we grouped samples according to both potential causes of implantation failure, endometrial luteal phase timing (displaced or on-time) (Fig. 3A), and endometrial disruption (good or poor prognosis) (Fig. 2A) and compared the patients' reproductive outcomes. There were no significant differences between the patients with displaced and on-time WOI in terms of BMI (*P*=.8740), the average number of SETs (*P*=.0879), and embryo quality (*P*=1). However, patients with a displaced WOI were significantly older (40.4 vs. 38.3 years old; *P*=.0044) (Supplemental Table 4). Once patients were stratified by prognosis (endometrial disruption), the proportion of

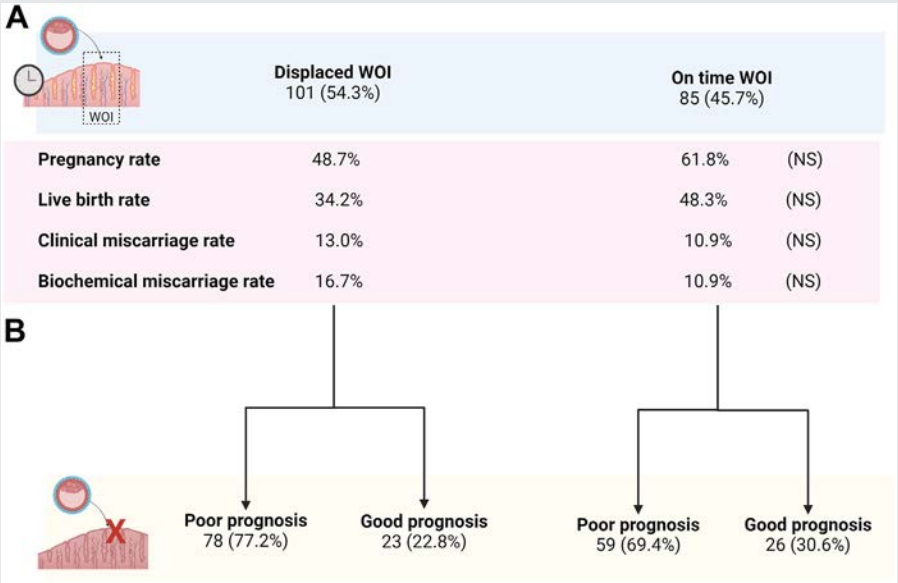
FIGURE 2



Clinical meaning and prediction capability of the EFR signature. (**A**) Clinical outcomes of the single embryo transfer after biopsy collection. Rates for pregnancy, live birth, and clinical and biochemical miscarriage were calculated as described in the Supplemental Methods. Statistical differences were evaluated using Fisher's exact test. (**B**) Comparison of cumulative pregnancy rates. Notably, many patients predicted to have a good endometrial prognosis achieved pregnancy with a single transfer (63.3%) and reached the maximum number of pregnancies (83.7%) at the fifth transfer, whereas patients predicted to have poor endometrial prognosis were only able to achieve a cumulative pregnancy rate of 42.3% at the ninth transfer. (**C**) Boxplots for the EFR signature prediction parameters (sensitivity, specificity, and accuracy) were calculated over 100 iterations of fivefold stratified cross-validation. (**D**) Histogram showing the proportion of patients that were successfully classified during the aforementioned cross-validation procedure. Figure created with BioRender.com.

Diaz-Gimeno. Endometrial Failure Risk signature. Fertil Steril 2024.

FIGURE 3



Evaluation of reproductive outcomes after single embryo transfer (SET) on the basis of the 2 WOI factors. Patients were initially classified according to transcriptomic endometrial dating by TED (28) (**A**), as per current clinical practice. This approach distinguishes between patients whose window of implantation (WOI) was considered displaced or on time. These groups were then stratified according to their endometrial disruption (**B**), as determined by our semi-supervised stratification. In all cases, the reproductive outcomes of the first SET after biopsy collection were compared using Fisher's exact test. NS = no significant differences; TED = transcriptomic endometrial dating. *P<.05; **P<.01; ***P<.001. Figure created with BioRender.com.

Diaz-Gimeno. Endometrial Failure Risk signature. Fertil Steril 2024.

patients with poor prognosis was similar (P=.2458) between displaced and on-time groups (Fig. 3B).

Experimental validation of genes related to the EFR signature

The expression of the top 4 differentially expressed genes in the poor prognosis group was validated by RT-qPCR. Progestagen-associated endometrial protein and G-coupled protein receptor 110, which were found upregulated by RNA-seq, were significantly overexpressed in the poor prognosis group, compared with the good prognosis group (fold change [FC] = 6.157 [P=.0288] and FC = 11.803 [P=.0350], respectively; Supplemental Figs. 6A and B). Meanwhile, the expression of fibrinogen beta chain and solute carrier 16 member 6, which were found downregulated by RNA-seq, did not reach a statistically significant difference (FC = 1.582 [P=.2475] and FC = 2.311 [P=.4376], respectively; Supplemental Figs. 6C and D).

DISCUSSION

Advances in the diagnosis and treatment of RIF have been limited by its elusive etiology, the discrepancies in clinical classification (mainly due to the lack of consensus over the minimum number of pregnancy attempts) (9, 10), and the heterogeneous molecular profiles of affected patients (15). On the basis of current clinical practice, patients with RIF may be misclassified and/or receive inefficient treatment (9, 10). Thus, the aim of this study was to propose a novel gene expression signature that could identify endometrial disruptions independent of endometrial luteal phase timing. Using a semi-supervised learning AI strategy together with the endometrial gene expression obtained from our custom EFR panel, we stratified patients according to poor or good endometrial prognoses and demonstrated how this signature reliably predicted gene expression patterns that corresponded with significant differences in PR, LBR, CMR, and BMR. When considering both WOI factors (disruption and timing) in our study cohort, poor endometrial prognoses (disruption) affected 73.7% of patients, whereas WOI displacements only affected 54.3%; 41.9% of patients presented a displaced WOI and were predicted to have a poor endometrial prognosis, whereas only 12.4% of patients presented with a displaced WOI and were predicted to have a good endometrial prognosis. These results offer a possible explanation as to why other studies reported there was no clinical benefit of personalizing embryo transfers on the basis of transcriptomic dating of the endometrium (34). According to our clinical algorithm, only patients with a displaced WOI and a good endometrial prognosis (12.4% of our cohort) would be better off with this approach. Patients with poor endometrial prognosis (73.7% of our cohort) would have suboptimal reproductive outcomes even though their WOI was on-time. This highlights an unmet healthcare need for IVF patients with endometrial disruptions (18) and underscores the importance of additional prospective studies to further characterize patients on the basis of these 2 molecular causes for trying tailored treatments.

Contemplating endometrial luteal phase timing together with a prevalent disruption or poor endometrial prognosis shifts the paradigm in studies about the endometrium. Our innovative methodology based on transcriptomics and AI models led us to develop new endometrial biomarker taxonomies related to endometrial luteal phase timing and/or endometrial disruption (16, 18). There were 30 genes overlapping between the previously published TED signature (17) and the EFR signature presented herein, emphasizing their correlation despite the lower FC of biomarkers for endometrial disruption versus endometrial timing (16).

AI algorithms offer powerful approaches for patient stratification, by combining high-throughput molecular data and clinical variables (47). Clinical translation of these approaches will require careful supervision of how the machine learning is initially trained (48). However, a semi-supervised learning methodology implementing accurate clinical diagnostic criteria and patient-specific genomic data to establish relevant molecular taxonomies will be indispensable for advancing precision medicine (23), especially within the context of endometrial-factor infertility. Predictive biomarkers can stratify patients with endometrial failure even if the number of their attempts was insufficient to meet clinical RIF classification criteria (41). To our knowledge, this is the first study to apply semi-supervised learning to the endometrium, conferring an advantage over previous models that were only trained with clinical classifications and did not leverage the underlying molecular heterogeneity of the condition (15). The lack of gene expression differences between RIF and control patients with our initial clinical classification reinforced findings from Macklon's group and the molecular heterogeneity among patients with RIF (15, 16).

The EFR signature comprised 122 genes, including 44 genes newly associated with an endometrial gene expression signature and a single common gene with Koot's pioneer endometrial timing-independent signature for endometrial pathology (15). Here, we only considered biopsies collected in the endometrial mid-secretory phase, when patients had received P4 for 82–172 h. Although this signature is independent of endometrial luteal phase timing, further studies are required to confirm if the signature is detectable in other phases of the endometrial cycle. Progestagen-associated endometrial protein is well characterized in endometrial tissue and was the most upregulated gene (FC = 3.18) in the poor endometrial prognosis profile. However, the levels we observed were 10-fold lower compared with previous studies where changes in endometrial timing were considered (19, 49). Alternatively, fibrinogen beta chain, related to the plasma membrane and vesicle transport by secretory granules, was the most downregulated gene. This gene accumulated polymorphisms that hinder the production of fibrinogen and its associated anti-inflammatory activity (50) and was recently related to the dysregulated pathways in RIF (51). FOXP1, the most predictive gene, is a transcription factor mediated by estrogen that has established functions in regulation, leukocyte differentiation, and response to lipids and chemokines. This gene is associated with breast (45) endometrial cancer (52), and endometriosis-related fibrosis (53), but was never included in an endometrial gene signature until now. With *FOXP1* and the FOX transcription factor family being associated with the regulation of the menstrual cycle and implantation (54–57), this evidence reinforces our prior study (58) and affirms that alterations in endometrial regulation are an underlying cause of endometrial failure.

Our clinical algorithm considers the 2 WOI factors, endometrial timing and disruption, to stratify IVF patients on the basis of 4 possible phenotypes: displaced/on-time and poor/good prognosis. This model is promising for preventive and precision medicine in endometrial-factor infertility, as it combines the robust prediction parameters of the TED model (17) and the EFR biomarker signature's predictive capability reported herein. In contrast to other transcriptomic-based endometrial dating tools (17, 19, 59–61), our bioinformatic methodology removes the molecular variation associated with the cyclic menstrual changes, providing deeper insights for identifying a new kind of disruption independent of endometrial luteal phase timing by the EFR signature. Furthermore, the EFR biomarker signature was developed on the basis of reproductive outcomes and effectively classifies patients predicted to have positive or negative reproductive outcomes in the first SET after biopsy collection. All these facts, point out to a more potential clinical utility than existing tools that only predict the WOI without showing significant differences in reproductive outcomes as have been reported (27–34) and we have shown in our findings. Although our strategy appears promising, the classification presented herein needs further refinement before clinical implementation. Larger sample sizes and validation in independent samples are required to ensure model generalization (62). Healthcare professionals will need to be adequately trained to ensure technical reproducibility. Longitudinal assessments including several menstrual cycles in the same patient are recommended to confirm inter-cycle reproducibility and to gain a comprehensive understanding of the reliability of the EFR panel. Additional studies should assess the relationship between a poor endometrial prognosis and the presence of benign disorders that potentially compromise endometrial function and/or embryo implantation (63).

Inspired by the MammaPrint risk signature for breast cancer (64, 65), the EFR signature could become a next-generation tool used to classify patients on the basis of endometrial prognosis, to predict reproductive outcomes before undergoing IVF, potentially experiencing RIF and/or losing valuable embryos. Although treatments for patients with poor endometrial prognosis remain to be determined, clinically identifying these patients is the first step toward improving their care.

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CRediT Authorship Contribution Statement

Original idea and study design was conceived by P.D.-G. and A.P. Patient population was defined by A.P., M.C.V., I.S.-R., and P.D.-G. Ethical committee was passed by P.D-G., with the help of M.C.V. Sample collection and clinical data management were performed by A.P., N.P., M.C.V., I.S.-R., with the help of J.M.S.-R., D.M.-G., A.M.-M., and P.S.-L., supervised by P.D.-G. RNA-seq custom panel was designed by P.D.-G. and implemented by P.S.-L. and J.M.S.-R. Selection and bioinformatic preprocessing of transcriptomic data sets from GEO were performed by A.D.-P. and P.S.-L. and supervised by P.D.-G. Sample preprocessing and RNA extraction were done by J.M.S.-R. and supervised by P.D.-G. Targeted RNA-seq experimental protocol and sequencing was refined and implemented by K.S., coordinated by P.D.-G., and supervised by D.W. Validation design and real-time PCRs were done by D.M.-G and A.M.-M. and supervised by P.D.-G. Prediction model design and implementation were done by P.S.-L. and supervised by P.D.-G. Transcriptomic analysis and statistics were analyzed by P.S.-L., with the help of A.D.-P. and supervised by P.D.-G. Clinical data analysis was done by P.S.-L. and supervised by P.D.-G. Data interpretation and conclusions were provided by P.D.-G., with the help of P.S.-L. and A.D.-P. and supervised by A.P. Tables and figures were designed by P.D.-G., with the help of P.S.-L. and implemented by P.S.-L. Manuscript writing was done by P.D.-G. and P.S.-L., with the help of D.M.-G. supervised by A.P., and revised by all authors.

Declaration of Interests

P.D.-G., P.S.-L., and A.P. report planned European patent IVI RMA GLOBAL. K.S. has nothing to disclose. D.M.-G. has nothing to disclose. J.M.S.-R. has nothing to disclose. M.D.C.V. has nothing to disclose. A.D.-P. has nothing to disclose. I.S.-R. has nothing to disclose. A.M.-M. has nothing to disclose. N.P. has nothing to disclose. D.W. has nothing to disclose.

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Predecir el riesgo de fallo endometrial: una firma biomarcada que identifica una nueva disrupción independiente de la sincronización endometrial en pacientes sometidas a ciclos de reemplazo hormonal

Objetivo: Proponer una nueva firma de expresión de genes que identifica disrupciones independientes de la sincronización de la fase lútea y predice si las pacientes están en riesgo de fallo endometrial.

Diseño: Estudio prospectivo multicéntrico.

Pacientes: Mujeres caucásicas ((n= 281; 39.4± 4.8 años con índice de masa corporal 22.9 ±3.5 Kg/m2) sometidas a terapia de reemplazo hormonal entre Julio 2018 y Julio 2021. Muestras endometriales de 217 pacientes cumplieron los criterios de calidad del RNA para el descubrimiento y análisis de la firma.

Intervención (es): Biopsias endometriales recogidas en la fase secretora media.

Variable principal (es): Expresión corregida para el tiempo de la fase lútea endometrial de 404 genes y resultados reproductivos de la primera transferencia de embrión único (SET) tras recogida de biopsia para identificar biomarcadores pronósticos de fallo endometrial.

Resultados: La eliminación de la variación del tiempo endometrial de los datos de expresión génica permitió estratificar a las pacientes en grupos de mal (n=137) o buen (n=49) pronóstico endometrial en base a sus perfiles clínico y transcriptómico. Se encontraron diferencias significativas entre los grupos de pronóstico endometrial en términos de tasas reproductivas: gestación (44.6% vs. 79.6%), recién nacido (25.6% vs. 77.6%), aborto clínico (22.2% vs. 2.6%), y aborto bioquímico (20.4% vs. 0%). El riesgo relativo de fallo endometrial en pacientes predichas como mal pronóstico endometrial fue 3.3 veces mayor que en aquellas con buen pronóstico. Las diferencias en la expresión génica entre ambos perfiles fueron propuestas como biomarcador, acuñando la firma de riesgo de fallo endometrial (EFR). Los perfiles de bajo pronóstico se caracterizaron principalmente por 59 genes sobreexpresados y 63 infraexpresados relacionados con la regulación (17.0%), metabolismo (8.4%), respuesta inmune e inflamación (7.8%).
Esta firma EFR tenía una mediana de precisión de 0.92 (min=0.88, max=0.94), mediana de sensibilidad de 0.96 (min = 0.91, max = 0.98), y una mediana de especificidad de 0.84 (min = 0.77, max = 0.88), posicionándose como un biomarcador prometedor para la evaluación endometrial.

Conclusión: La firma EFR reveló una disrupción endometrial nueva, independiente del tiempo de la fase lútea, presente en el 73.7% de las pacientes. Esta firma de EFR estratificó a los pacientes en dos perfiles de pronóstico significativamente distintos y clínicamente relevantes, brindando oportunidades para una terapia personalizada. Sin embargo, es necesaria mas validación antes de implementar esta firma genética como una herramienta basada en inteligencia artificial (AI) para reducir el riesgo que las pacientes experimenten fallo endometrial.

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Analysis of factors affecting the prognosis of patients with intrauterine adhesions after transcervical resection of adhesions

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Objectives: To study the factors affecting the prognosis of patients with intrauterine adhesions (IUAs) after transcervical resection of adhesions (TCRA), analyze the reproductive outcome, and guide prognostic improvements.
Design: Prospective study.
Patients: Our study included 292 patients diagnosed with IUAs who underwent follow-up office hysteroscopy at Shenyang Women's and Children's Hospital between June 2018 and June 2022.
Interventions: Patients were divided into case (52 patients whose hysteroscopy results indicated the presence of IUAs) and nocase (240 patients whose uterine cavity had returned to normal shape without obvious adhesion) groups on the basis of the results of a 2-month follow-up hysteroscopy following TCRA. Clinical data were collected and compared with various influencing factors, and the combined effect of these factors was assessed using multifactorial logistic regression analysis. A nomogram prediction model was constructed and internally validated on the basis of multifactorial analysis.
Main Outcome Measures: Intrauterine re-adhesion was observed at a 2-month follow-up after TCRA.
Results: Postoperative re-adhesion occurred in 52 of 292 patients with IUAs. Multifactorial binary logistic regression analysis showed that IUA barrier gel reapplication 5 days after TCRA was a protective factor. In contrast, the preoperative American Fertility Society scores demonstrated that severe IUAs and chronic endometritis were risk factors. The results of the multifactorial analysis were used to build a nomogram model, and the area under the curve value of the nomogram model for predicting postoperative recurrence was 0.914 (95% confidence interval: 0.864–0.956). The bootstrap method was subsequently used to resample 1,000 times for internal validation. The results showed that the internal validation C-index was 0.9135, and the calibration and ideal curves were well-matched.
Conclusion: The prognosis of patients with IUAs after TCRA is related to the severity of preoperative IUAs, presence of chronic endometritis, and IUA barrier gel reapplication 5 days after TCRA. Therefore, clinicians should monitor patients using targeted data to reduce recurrence risk after TCRA and improve the prognosis of patients with IUAs. (Fertil Steril® 2024;122:365–72. ©2024 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Intrauterine adhesion, re-adhesion, transcervical resection of adhesions, influence factors, alignment diagram prediction model

Intrauterine adhesions (IUAs) obstruct the uterine cavity because of endometrial damage, endometrial fibrosis, and scarring. They often lead to severe clinical complications such as menstrual disorders (amenorrhea), infertility, and recurrent miscarriage, affecting reproductive capacity (1). Intrauterine adhesions comprise 36.6% of infertility cases (24.6% of secondary infertility and 12.0% of primary infertility cases) and are a prevalent cause of secondary infertility; this trend is increasing annually (2). Transcervical resection of adhesions (TCRA) is the standard treatment strategy for IUA in patients with fertility needs (3). However, the high recurrence rate after TCRA remains a major challenge in IUA treatment (re-adhesion rate up to 62.5%) (4). Therefore, adjuvant therapeutic measures are available to improve IUA prognosis after TCRA, such as simultaneous intrauterine device (IUD) placement, intrauterine balloon, or

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gel application during TCRA to serve as a barrier to the anterior and posterior uterine walls and prevent re-adhesion (5–7). Nonetheless, IUA recurrence is high, with an estimated 14%–48% of patients experiencing re-adhesion formation after adjuvant therapies (8–12). This study aimed to explore the factors influencing IUA prognosis after TCRA, analyze reproductive outcomes and provide guidelines for improving outcomes.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of Shenyang Women's and Children's Hospital (approval number: 202325). There are no conflicts of interest to declare.

Study population

Patients with IUAs who underwent hysteroscopy due to reproductive needs in the outpatient department of Shenyang Women's and Children's Hospital from June 2018 to June 2022 were selected, and subsequent treatments and follow-up were performed. The inclusion criteria were as follows: moderate and severe IUAs confirmed by office hysteroscopy in our hospital, age $\leq$45 years, fertility requirements, complete outpatient review data, and complete clinical and imaging data. The exclusion criteria were as follows: previous TCRA with recurrent IUA, infertility due to uterine factors other than IUA (including congenital uterine anomalies and severe uterine adenomyosis), contraindications to the use of estrogen and progesterone (coronary heart disease, venous thromboembolism, stroke, transient ischemic attack, active liver disease, breast cancer, high-risk endometrial cancer, or unexplained vaginal bleeding), allergy to intrauterine adhesion barrier gel, and presence of severe intercurrent illness (e.g., coagulative disorders, systemic disease, severe cardiopathy). Ultimately, 292 patients were included. On the basis of hysteroscopy results from approximately 2 months postoperatively, the patients were divided into case group (whose hysteroscopy results suggested that uterine adhesions remained) and nocase group (whose uterine cavity returned to its normal shape without obvious adhesions).

Data collection

The following clinical data were collected: age, body mass index, age at menarche, menstrual cycle (regular or irregular), number of pregnancies, number of deliveries, history of missed abortion dilation and curettage (D&C), abortion D&C, and diagnostic curettage, severity at first presentation of IUA, presence of other common gynecological conditions (including the combination of endometriosis, fibroids, and ovarian cysts), presence of chronic endometritis (CE), preoperative endometrial thickness, preoperative endometrial volume, preoperative endometrial receptivity, preoperative uterine arteries, preoperative estrogen treatment, intrauterine adhesion barrier gel reapplication 5 days after TCRA, preoperative and postoperative American Fertility Society (AFS) scores, and preoperative and postoperative menstrual volume visual analogue scale (VAS) scores; and reproductive outcomes.

Hysteroscopy procedure

The patients underwent office hysteroscopy 3–7 days after menstruation at our hospital. The timing of office hysteroscopy for women with secondary amenorrhea was not limited. All examinations were conducted by the same team of experienced outpatient gynecologists using the same equipment. Specialist physicians graded IUA severity using the 1988 AFS (13) scoring criteria, which included adhesion extent, nature, and menstrual status and classified IUA into mild, moderate, and severe. Patients with moderate and severe IUAs confirmed by office hysteroscopy were admitted to the hospital for TCRA.

Surgical methods and postoperative treatment procedures

Women with IUA underwent TCRA 3–7 days after menstruation; secondary amenorrhea did not affect the time of the procedure. Preoperatively, endometrial thickness, endometrial volume, and uterine artery blood flow were determined by transvaginal ultrasound. A hysteroscopic surgery system (Karl Storz, Tuttlingen, Germany) was used to perform TCRA. The same team of gynecologists performed each procedure while the patients were under combination spinal anesthesia or general anesthesia. Under ultrasound guidance, the adherent tissue was carefully separated intraoperatively to restore the shape of the uterine cavity; the operative video and/or image data were retained during the operation. Endometrial samples were collected intraoperatively for CD138 immunostaining for CE diagnosis. CD138-positive cells $\geq$5 per 10 high-power fields are diagnostic of CE. Oral doxycycline was administered as regular postoperative treatment in cases in which the patient had CE. Intraoperatively, 3 mL of intrauterine adhesion barrier gel (Bairuiji Biomedical Co., Ltd., National Machinery Registration, No. 20153141542) was injected for the first time; an intrauterine balloon tube with 3–5 mL volume (depending on the size of the uterine cavity and degree of adhesion) was placed in the uterine cavity and removed 5 days later. In some patients, 3 mL of intrauterine adhesion barrier gel was reinjected when the balloon tube was removed. The first intrauterine balloon dilatation with a 16-Fr Foley catheter (Wellead, Guangzhou, China) was performed 10 days after TCRA, and office hysteroscopy was performed 20 days after TCRA (hysteroscopy was delayed until the end of menstruation in cases where patients were menstruating); patients were treated for 2–3 cycles in the described order. When membranous adhesions in any part of the uterine cavity or muscular adhesions that were $<$1/3 of the uterine cavity were detected during office hysteroscopy, the adhesions were immediately separated using a scope or micro-scissors. All patients were administered sequential estrogen-progesterone treatment immediately after TCRA to promote endometrial repair.

Criteria for judging intrauterine re-adhesion

Currently, there is no uniform standard for the duration of postoperative follow-up. The Chinese Expert Consensus on Clinical Diagnosis and Treatment of Uterine Adhesions (14) recommends that postoperative follow-up be conducted once a month for 3 months and subsequently once every 6 months

until 1 year for TCRA. The American Association of Gynecological Laparoscopists (AAGL) (1) recommends re-evaluation of uterine morphology 2–3 months postoperatively. Meanwhile, we have shown previously (15) that early second-look hysteroscopy combined with intrauterine balloon dilatation after hysteroscopic TCRA might improve the prognosis and postoperative pregnancy rate in women with IUA. By comparing intraoperative imaging data of TCRA and postoperative follow-up hysteroscopy, the following were used as the criteria for intrauterine re-adhesion: both uterine horns were visible at TCRA, but 1 or both horns were not visible at follow-up, and membranous, fibrous, or muscular adhesions were found to have reappeared in any part of the uterine cavity at follow-up.

Statistical methods

SPSS version 26.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Normally distributed data were expressed as mean±SD, and non-normally distributed data as M (P25, P75). The t-test was used to compare groups of normally distributed data; a nonparametric test (rank-sum test for paired samples and 2 independent samples) was used to compare non-normally distributed data. Categorical data were tested using the Chi-square ($\chi 2$) (for paired data) or Fisher's test. Risk factors were analyzed using multifactorial logistic regression analysis with backward regression (likelihood ratio). R software (R Foundation for Statistical Computing, Vienna, Austria) was used to construct the column-line graph model. The predictive value of the model was analyzed using the receiver operating characteristic (ROC) curve; internal validation was performed using the bootstrap method with computer simulation of adequate sampling. Statistical significance was set at $P<.05$.

RESULTS

Occurrence of postoperative re-adhesion

Approximately 2 months after hysteroscopic adhesion separation, 52/292 (17.81%) patients with IUA had postoperative re-adhesion formation.

Recovery of patients with IUA after TCRA

Comparison of preoperative and postoperative AFS scores. Among the 292 patients, the pre- and postoperative AFS scores 2 months postoperatively were 8 (7, 8) and 2 (2, 2), respectively, with significant difference ($P<.05$) (Table 1).

Comparison of mean pre- and postoperative menstrual volume VAS scores. Among the 292 patients, the mean pre- and postoperative menstrual volume VAS scores were 4 (3, 5) and 5 (4, 6), respectively, with significant difference ($P<.05$) (Table 2).

Reproductive outcomes

By April 2023, 220 patients had been followed up for 11 to 44 months. Notably, 114 (51.8%) patients became pregnant, with 85 (74.6%) live births; 15 (13.2%) reported missed abortion, spontaneous abortion, inevitable abortion, or unplanned pregnancy with a request for abortion; 2 (1.7%) reported induced abortions; and 12 (10.5%) were in gestational states. Of the patients who became pregnant, 45 (39.5%) had spontaneous pregnancies, and 69 (60.5%) had assisted reproductive technology pregnancies (17 ovulation induction and 52 in vitro fertilization–embryo transfer pregnancies). Of the 85 live births, 16 (14.0%) were preterm, and 69 (60.5%) were full-term. Of the patients who had live births, 14 (16.5%) had vaginal deliveries, and 71 (83.5%) had cesarean births. Of the 85 deliveries, 41 (48.2%) patients experienced obstetric complications, including 25 (29.4%) placenta accreta, 2 (2.4%) placenta increta, 1 (1.2%) low-lying placenta, 10 (11.8%) postpartum hemorrhage, 3 (3.5%) placental abruptions, and no uterine ruptures.

TABLE 1

Comparison of preoperative and postoperative AFS scores.

	Value	*P* value
AFS scores		
Preoperative	8 (7, 8)	0
Postoperative	2 (2, 2)	

AFS, American Fertility Society.

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Analysis of factors influencing postoperative re-adhesion in patients with IUA

A comparison of gravidity, number of prior uterine surgeries, preoperative endometrial thickness, preoperative endometrial volume, preoperative AFS classification, IUA and CE co-occurrence, intrauterine adhesion barrier gel reapplication 5 days after TCRA, and preoperative estrogen treatment in patients with and without postoperative re-adhesion formation showed significant differences ($P<.05$) (Table 3).

Logistic regression analysis of factors influencing postoperative re-adhesion in patients with IUA

Using variables that were found to differ in univariate analyses as independent variables, multifactorial logistic regression analyses using backward regression showed that preoperative AFS classification, patients with IUA and CE, and intrauterine adhesion barrier gel reapplication 5 days after TCRA were all factors influencing the occurrence of postoperative re-adhesion in patients with IUA. The risk of re-adhesion formation in patients with severe AFS was 11.8

TABLE 2

Comparison of preoperative and postoperative menstrual volume VAS scores.

	Value	*P* value
Menstrual volume VAS scores		
Preoperative	4 (3, 5)	0
Postoperative	5 (4, 6)	

VAS, visual analogue score.

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TABLE 3

Analysis of factors affecting postoperative re-adhesion in patients with IUA.

		Case group (52)	Nocase group (240)	*P* value
Age (y)		32.23 ± 5.25	32.47 ± 4.94	.754
Height (cm)		162.00 (158.0, 166.8)	161.00 (159.0, 165.0)	.385
Weight (kg)		61.500 (55.0, 66.8)	60.000 (53.0, 69.8)	.707
BMI (kg/m^2)		23.315 (20.2, 25.5)	23.000 (20.2, 26.0)	.892
Age at menarche (y)		14.000 (13.0, 14.0)	14.000 (13.0, 14.0)	.632
Menstrual cycle	Irregular	24 (46.15)	78 (32.50)	.061
	Regular	28 (53.85)	162 (67.50)	
Number of pregnancies		2.000 (1.0, 3.8)	2.000 (1.0, 2.8)	.002[b]
Number of deliveries		0.000 (0.0, 0.0)	0.000 (0.0, 0.0)	.348
Infertility	Yes	24 (46.15)	118 (49.17)	.694
Number of prior uterine surgery		2.000 (1.0, 3.0)	1.000 (1.0, 2.0)	.009[b]
Combined endometrial polyps	Yes	1 (1.92)	21 (8.75)	.161
Combined uterine Fibroids	Yes	10 (19.23)	24 (10.00)	.06
Combined ovarian cysts	Yes	1 (1.92)	12 (5.00)	.546
History of hysteroscopic endometrial Polypectomy	Yes	2 (3.85)	13 (5.42)	.906
History of hysteroscopic Submucosal myomectomy	Yes	1 (1.92)	2 (0.83)	.446
History of missed abortion D & C	Yes	15 (28.85)	91 (37.92)	.218
History of abortion D & C	Yes	33 (63.46)	118 (49.17)	.061
History of spontaneous abortion D & C	Yes	1 (1.92)	11 (4.58)	.624
Diagnostic curettage	Yes	1 (1.92)	8 (3.33)	.928
History of induced labor D & C	Yes	3 (5.77)	7 (2.92)	.545
History of residue placenta D & C	Yes	1 (1.92)	0 (0.00)	.178
History of hydatidiform mole D & C	Yes	2 (3.85)	3 (1.25)	.218
Preoperative endometrial receptivity typing	A-type	6 (11.54)	53 (22.08)	.099
	B-type	26 (50.00)	124 (51.67)	
	C-type	20 (38.46)	63 (26.25)	
Preoperative endometrial thickness (cm)		0.400 (0.3, 0.5)	0.500 (0.4, 0.6)	.017[a]
Preoperative endometrial volume (mL)		1.295 (0.8, 2.0)	1.600 (1.0, 2.1)	.047[a]
Preoperative uterine artery resistance index RI (right side)		0.830 (0.8, 0.9)	0.840 (0.8, 0.9)	.138
Preoperative uterine artery resistance index RI (left side)		0.840 (0.8, 0.9)	0.840 (0.8, 0.9)	.99
Preoperative uterine artery pulsatility index PI (right side)		2.23 ± 0.52	2.33 ± 0.58	.244
Preoperative uterine artery pulsatility index PI (left side)		2.38 ± 0.51	2.37 ± 0.57	.91
AMH (ng/mL)		2.710 (1.9, 5.6)	3.210 (1.9, 5.8)	.729
Preoperative AFS classification	Moderate	16 (30.77)	211 (87.92)	0[b]
	Severe	36 (69.23)	29 (12.08)	
Presence of CE	Yes	41 (78.85)	31 (12.92)	0[b]
Reapplication of intrauterine adhesion barrier gel 5 days after TCRA	Yes	2 (3.85)	37 (15.42)	.026[a]
Preoperative estrogen treatment	Yes	15 (28.85)	34 (14.17)	.010[a]

AFS, American Fertility Society, AMH, anti-müllerian hormone; BMI, body mass index; CE, chronic endometritis; D & C, dilation and curettage, IUA, intrauterine adhesions; PI, pulsatility index; RI, resistance index; TCRA, transcervical resection of adhesions.
[a] $P < .05$.
[b] $P < .01$.

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times higher than in those with moderate IUA. The risk of re-adhesion formation in IUA patients with CE was 26.5 times higher than in those without CE. The risk of re-adhesion formation with intrauterine adhesion barrier gel reapplication 5 days after TCRA was 0.2 times higher than in those without gel reapplication (Table 4).

Establishment and validation of an alignment diagram prediction model for postoperative re-adhesion in patients with IUA

The independent variables identified in the multifactorial analysis were incorporated into the prediction model constructed using R software. The model included 3 variables: preoperative AFS classification, IUA and CE co-occurrence, and intrauterine adhesion barrier gel reapplication 5 days after TCRA, the values of which were entered to obtain the corresponding scores in the first row. The corresponding probability of re-adhesion after TCRA was obtained by calculating the total score. The C-indices of the 3 variables that individually predicted postoperative re-adhesion in patients with IUA and CE, intrauterine adhesion barrier gel reapplication 5 days after TCRA, and preoperative AFS classification were 0.830, 0.558, and 0.78, respectively. This result showed that the generated nomogram model significantly improved prediction accuracy (Supplemental Fig. 1A, available online).

According to the ROC analysis results, the area under the curve of the alignment diagram for predicting re-adhesion in a patient after TCRA was 0.914 (95% confidence interval [CI]: 0.864–0.956), indicating better predictive ability of the model (Supplemental Fig. 1B). The bootstrap method was subsequently used to resample the scoring system 1,000 times for internal validation. The results showed that the C-index of the internal validation was 0.9135, and the calibration curve fitted well with the ideal curve, indicating that the scoring system was stable (Supplemental Fig. 1C).

DISCUSSION

Since Asherman's first systematic report on IUA in 1948, there has been a gradually increasing interest in the importance of IUA. The detection rate of IUA has increased with the introduction of noninvasive examination methods such as uterine ultrasonography and the widespread use of hysteroscopy (16). Schenker et al. (17) demonstrated that abortion curettage, missed abortion D&C, diagnostic curettage, uterine artery embolism, endometrial tuberculosis infection, and IUD placement were closely associated with IUA development in 1,856 IUA cases, among which abortion curettage was the most prevalent cause of IUA. The traditional method of separating IUAs using instruments, such as dilation rods, probes, and biopsy forceps, increases the risk of uterine perforation, myometrial wall damage, and uterine cavity "false channel formation" due to the blind surgical procedure. Hysteroscopy allows the visualization of the entire uterine cavity and clarifies the location, extent, and nature of adhesions, uterine horns, and tubal ostiums, avoiding blind procedures and improving the treatment effect and surgical safety (4). Therefore, TCRA is the first-line treatment for IUA; however, the incidence of re-adhesion formation after TCRA remains high (3.1%–23.5%), especially severe adhesions (20%–62.5%) (4). Meanwhile, the results of this study showed that approximately 2 months after TCRA, 52 (17.81%) of 292 patients with IUA had postoperative re-adhesion, indicating that the incidence of postoperative re-adhesion requires reduction. Moreover, there is no expert consensus on a systematic treatment plan to prevent intrauterine re-adhesion. Therefore, the factors causing postoperative re-adhesion in patients with IUA should be investigated, and a systematic treatment plan should be developed. Two hundred ninety-two patients with moderate to severe IUA were enrolled between June 2018 and June 2022 and underwent TCRA with concomitant endometrial biopsy, first-time injection of intrauterine adhesion barrier gel, and indwelling intrauterine balloon tube during the procedure. Postoperatively, the patients were administered secondary intrauterine adhesion barrier gel, intrauterine balloon dilatation, early second-look hysteroscopy, and sequential estrogen-progesterone treatment. This systematic and comprehensive management improved treatment and reproductive outcomes.

The formation time of postoperative re-adhesion is 5 to 7 days (18). The AAGL (1) recommends IUA barrier gel for IUA treatment. Therefore, we initially employed the barrier gel intraoperatively to prevent adhesions. However, current studies rarely mention the timing of IUA barrier gel application. Since IUA barrier gel starts to degrade after 7 days and is completely degraded and absorbed after 14 days, we reapplied this gel treatment while removing the intraoperative indwelling intrauterine balloon tube 5 days postoperatively, aiming to reduce the risk of re-adhesion formation. Our results also confirmed that the risk of re-adhesion formation with IUA barrier gel reapplication 5 days after TCRA was 0.2 times higher compared with not reapplication. The reapplication of IUA barrier gel 5 days after surgery was negatively correlated with intrauterine re-adhesion formation. However, in the current study, we reapplied IUA barrier gel at only one time point. We consider this a limitation, and further in-depth research on reapplication time points is warranted in the future.

The safety and efficacy of intrauterine balloon dilatation are crucial in preventing recurrent IUAs after TCRA to reduce the area, probability, and duration of traumatic contact, controlling factors that promote adhesion occurrence. Meanwhile, given the time it takes for postoperative re-adhesion formation, our patients underwent intrauterine balloon dilatation on postoperative day 10 to bluntly separate fresh loose adhesions in the cavity and lower segments of the uterus and prevent the formation of dense and muscular adhesions. The intervention area expansion achieved at this stage extends the time for endometrial repair, improving postoperative adhesion control, uterine cavity recovery, endometrial repair, and menstrual cycle (8).

Although intrauterine balloon dilatation can prevent the wound from adhering to each other through the barrier effect, the uterine cavity cannot be visualized. In addition, owing to the shape of the Foley catheter, adhesion recurrence at the uterine horns could not be prevented. Therefore, we conducted early office hysteroscopy 20 days postoperatively. Patients were treated for 2–3 cycles in the described order. Patients were administered sequential estrogen-progesterone treatment immediately after TCRA to promote endometrial repair.

The AFS classification score demonstrates IUA prognosis and has been shown to directly influence conception and lead to different pregnancy outcomes (19, 20). The study found a significant decrease in postoperative AFS scores and improvement in menstrual flow in 188 of 292 patients, consistent with previous reports (14, 19, 20). The study also reported a significant increase in postoperative menstrual

TABLE 4

Logistic regression analysis of factors influencing postoperative re-adhesion in patients with IUA.

	P	OR	(95% CI)
Preoperative AFS classification	0	11.8	4.68–29.73
Presence of CE	0	26.5	10.51–66.73
Reapplication of autocrosslinked hyaluronic acid gel 5 days after TCRA	.035	0.2	0.04–0.89

AFS, American Fertility Society; IUA, intrauterine adhesions; TCRA, transcervical resection of adhesions; CE, chronic endometritis; OR, odds ratio, CI, confidence interval.

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volume VAS scores, indicating that the proposed systemic treatment regimen enhances IUA prognosis.

We successfully followed up on 220 patients with IUA and assessed their reproductive outcomes; the postoperative clinical pregnancy rate was 51.8%. The live birth rate was 74.6%, with a full-term live birth rate of 60.5% and a preterm birth rate of 14.0%. However, clinicians should pay more attention to the fact that damage to the basal layer of the endometrium, inadequate endometrial blood supply, and deformation or volume reduction of the uterine cavity caused by IUAs present a risk for postpregnancy comorbidities in patients with IUA, such as placental implantation abnormalities, postpartum hemorrhage, and other serious obstetric complications. The results of this study showed that the incidence rates of placenta adherence and postpartum hemorrhage were 29.4% and 11.8%, respectively. These values were higher than the 10.1% and 11.4% incidence rates reported previously (21). The high preoperative AFS scores 8 (7,8) of the participants in this study suggest that adhesions were more severe, which may be the reason for a higher incidence of obstetric complications.

The multifactorial logistic regression analysis results of the present study showed that a major risk factor influencing re-adhesion after TCRA was IUA severity at first detection. Valle and Sciarra (22) reported that the recurrence rate after TCRA was 62.5%, whereas the present study demonstrated that the risk of re-adhesion formation was 11.8 times higher in patients with severe IUA than in those with moderate IUA. Patients with severe IUA initially have significantly scarred endometrium. The TCRA removes existing scar tissue; however, it does not affect the fibrosis-promoting mechanism that sets off after endometrial damage. Therefore, patients with severe IUA at initial examination are at a higher risk of postoperative re-adhesion (23, 24).

Furthermore, this study revealed that the combination of CE in patients with IUA was a risk factor for re-adhesion (odds ratio 26.481, $P<.05$). There was a positive correlation between CE and IUA in patients with IUA. Liu et al. (25) found that the expression of uterine transforming growth factor $\beta 1$ (which promotes fibrosis and inhibits extracellular matrix degradation) was elevated in the endometrium of patients with IUA and CE, whereas the expression of matrix metalloproteinase 9, which stabilizes fibrosis, was reduced. The risk of re-adhesion may be higher in patients with IUA. Routine endometrial biopsy during TCRA is recommended to diagnose CE as early as possible to standardize the treatment of patients with IUA and CE.

Despite extensive research, the nomogram model is rarely utilized in clinical practice. This model visualizes the results of multifactorial logistic regression and displays the relative significance of each factor in the form of scores that are convenient to calculate and easy to understand. We constructed an alignment diagram prediction model for postoperative re-adhesion in patients with IUA on the basis of multifactorial analysis and validated the results of the nomogram model with ROC analysis and bootstrap method. This approach confirmed the high predictive accuracy and discriminability of the column chart model. Presenting the nomogram to the patients, helps them to visualize the risk factors for re-adhesion and their degree of significance, thus improving their understanding of the disease and the importance of treatment adherence.

CONCLUSION

The IUA prognosis after TCRA is related to severe preoperative IUA, presence of CE, and IUA barrier gel reapplication 5 days after TCRA. Patients with moderate to severe IUA should undergo precise surgery and long-term management, including IUA barrier gel reapplication 5 days after TCRA, intrauterine balloon dilatation, early second-look hysteroscopy, and sequential estrogen-progesterone treatment. In addition, this study notes that in nonexperimental studies, the decision to use IUA barrier gel may be influenced by a variety of factors. Although the model in this study considered several key factors, there may still be unmeasured confounding factors (such as the hospital's geographical location and the patients' demographic characteristics). Therefore, although the predictive model in this study performed well in assessing the risk of patient re-adhesion, it is not directly useful for causal reasoning. The inference of causality requires caution, and the influence of confounding factors should be fully controlled. Moreover, this study has certain limitations, being a single center study. Multicenter prospective studies conducted in future would allow in-depth analysis of the multiple factors that affect the prognosis of IUA patients after TCRA.

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CRediT Authorship Contribution Statement

Jiantong Zhang: Writing – original draft, Writing – review & editing, Investigation, Sata curation. **Cong Shi:** Conceptualization, Methodology. **Jianhua Sun:** Supervision, Conceptualization. **Jumin Niu:** Conceptualization, Methodology, Writing – review & editing, Supervision.

Declaration of Interests

J.Z. has nothing to disclose. C.S. has nothing to disclose. J.S. has nothing to disclose. J.N. has nothing to disclose.

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Análisis de los factores que afectan el pronóstico de los pacientes con adherencias intrauterinas después de la resección transcervical de adherencias

Objetivo: Estudiar los factores que afectan el pronóstico de pacientes con adherencias intrauterinas (AIU) después de la resección transcervical de adherencias (RTCA), analizar el resultado reproductivo y orientar las mejoras pronósticas.

Diseño: Estudio prospectivo.

Pacientes: Nuestro estudio incluyó a 292 pacientes diagnosticadas con AIU que se sometieron a una histeroscopia de consultorio de seguimiento en el Shenyang Women's y Children's Hospital entre junio de 2018 y junio de 2022.

Intervenciones: Los pacientes se dividieron en casos (52 pacientes cuyos resultados de histeroscopia indicaron la presencia de AIU) y no-casos (240 pacientes cuya cavidad uterina había vuelto a su forma normal sin adherencias evidentes) según los resultados de 2 meses de seguimiento con histeroscopias después de la RTCA. Se recopilaron datos clínicos y se compararon con varios factores influyentes, y el efecto combinado de estos factores se evaluó mediante un análisis de regresión logística multifactorial. Se construyó un modelo de predicción nomográfico y se validó internamente sobre la base del análisis multifactorial.

Principales medidas de resultado: Observar re-adherencia intrauterina a los 2 meses de seguimiento después de RTCA.

Resultados: La readhesión posoperatoria se produjo en 52 de 292 pacientes con AIU. El análisis de regresión logística binaria multifactorial mostró que la reaplicación del gel de barrera AIU 5 días después de la TCRA fue un factor protector. Por el contrario, las puntuaciones preoperatorias de la Sociedad Estadounidense de Fertilidad demostró que las AIU graves y la endometritis crónica eran factores de riesgo. Los resultados del análisis multifactorial se utilizaron para construir un modelo de nomograma, y el valor del área bajo la curva del modelo de nomograma para predecir la recurrencia posoperatoria fue 0,914 (95%intervalo de confianza: 0,864–0,956). Posteriormente se utilizó el método bootstrap para volver a muestrear 1000 veces para la validación interna. Los resultados mostraron que el índice C de validación interna era 0,9135 y que las curvas de calibración e ideales coincidían bien.

Conclusión: El pronóstico de los pacientes con AIU después de RTCA está relacionado con la gravedad de las AIU preoperatorias, la presencia de endometritis crónica y la reaplicación del gel de barrera AIU 5 días después de RTCA. Por lo tanto, los médicos deben monitorear a los pacientes utilizando datos específicos para reducir riesgo de recurrencia después de TCRA y mejorar el pronóstico de los pacientes con AIU.

The effect of improved metabolic syndrome parameters on live birth

Multiple underlying mechanisms often link obesity and metabolic syndrome conditions, which are associated with poorer reproductive outcomes (1–3). Women with obesity and unexplained infertility are often counseled on preconception weight loss as an initial step to achieving a successful pregnancy with intrauterine insemination (IUI). Despite this practice, there is no Level 1 evidence supporting increased live birth with improved weight or metabolic parameters in this cohort.

Using data from the "Improving Reproductive Fitness Through Pretreatment With Lifestyle Modification in Obese Women With Unexplained Infertility" (FIT-PLESE) Trial (4), the authors hypothesized that improved metabolic syndrome parameters improve live birth in women with unexplained infertility and that any loss of weight among obese women with metabolic syndrome improves live birth, compared with no weight loss.

MATERIALS AND METHODS

Study design

This prospective cohort analysis used data from 379 regularly cycling women (ages 18–40 years) with obesity (BMI $\geq$ 30kg/m^2) and unexplained infertility enrolled in the FIT-PLESE trial (4). In addition to comparing the number of metabolic syndrome diagnostic parameters met, we utilized the metabolic syndrome *z* scoring system to compare changes in metabolic syndrome status among participants.

Metabolic Syndrome Diagnostic Parameters: Investigators used widely accepted metabolic syndrome diagnostic criteria (Table 1).

Metabolic Syndrome Severity (*z* score): This score augments the "present/not present" dichotomous nature of metabolic syndrome criteria and is frequently used to assess risks associated with diabetes and cardiovascular disease (5).

$$\text{z score} = \left[2 \times \left(\frac{\text{waist (cm)}}{\text{height(cm)}}\right)\right] + \frac{\text{glucose}\left(\frac{\text{mg}}{\text{dL}}\right)}{100} + \frac{\text{triglycerides}\left(\frac{\text{mg}}{\text{dL}}\right)}{150} + \frac{\text{SBP (mmHg)}}{130} - \frac{\text{HDL}\left(\frac{\text{mg}}{\text{dL}}\right)}{50}$$

Statistical analysis was performed using logistic regression modeling and odds ratios in addition to chi-square for univariate associations. $P<.05$ was regarded as statistically significant. Interaction terms were not included in logistic models for simplicity of analysis and interpretation.

RESULTS

Metabolic parameters and live birth

A total of 191 participants were diagnosed with metabolic syndrome, and 168 were not (Table 1). Ten of the 30 women (33.3%) with metabolic syndrome who reduced their metabolic syndrome criteria had a live birth following IUI. Thirty-two of the 161 women (19.9%) whose metabolic syndrome parameters did not reduce also achieved a live birth ($P=.102$).

z score and live birth

Metabolic syndrome *z* scores followed a normal distribution with a mean of 3.7 (SD=0.5). Live birth during the fertility treatment phase of the study was 17.2% (33/192) for those with metabolic syndrome who improved their *z* score and 20.8% (20/96) for those whose score was unchanged or worsened ($P=.055$).

Weight loss and live birth

Among women with metabolic syndrome at the preconception phase, live birth was achieved in 20.6% of those who lost weight (29/141) compared with 26.7% (12/45) of those who did not lose weight ($P=.390$).

CONCLUSION

This secondary analysis of the FIT-PLESE trial does not support the hypothesis that fertility or live birth outcomes are better in women who improved their metabolic syndrome parameters through lifestyle intervention prior to IUI. This lack of improvement was consistent across a comprehensive interrogation of both the *number* of abnormal metabolic parameters as well as the metabolic syndrome *z* score before and after intervention, regardless of the amount of weight lost.

Potential study limitations include age and small sample size due to the nature of a secondary analysis, which may have led to residual confounding (Supplemental materials, available online). Education is a limitation as most women (80%) in the FIT-PLESE trial reported at least some college education and received IUI treatment(s). Higher levels of formal education may confound results and limit their generalizability. Obese women with lower educational/socioeconomic backgrounds may still benefit from improvements in metabolic parameters, given their overall higher risk for poorer reproductive

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TABLE 1

Baseline characteristics (at time of FIT-PLESE trial initiation).

	Metabolic Syndrome No (N = 168)	Metabolic Syndrome Yes (N = 191)	*P* value
Age (years)	32.0 (29.0, 35.0)	33.0 (30.0, 36.0)	.133
Ethnicity			.775
Non-Hispanic/ Latino	159/168 (94.6)	178/191 (93.2)	
Hispanic	4/168 (2.4)	7/191 (3.7)	
Unknown	5/168 (3.0)	6/191 (3.1)	
Smoking history			.441
Current	12/168 (7.1)	20/191 (10.5)	
Former	47/168 (28.0)	46/191 (24.1)	
Never	109/168 (64.9)	125/191 (65.5)	
Alcohol history			.570
Current	152/168 (90.5)	166/191 (86.9)	
Former	11/168 (6.6)	17/191 (8.9)	
Never	5/168 (3.0)	8/191 (4.2)	
How long has the patient been attempting conception (months)?	30.0 (18.0, 48.0) n = 167	25.0 (18.0, 48.0) n = 188	.700
BMI	36.6 (33.3, 41.5)	39.1 (34.8, 45.1)	<.001
Metabolic Syndrome parameters			
Waist (cm)	111.5 (101.5, 119.0)	117.0 (106.5, 130.0)	<.001
Fasting glucose (mg/dL)	89.1 (83.0, 94.0)	98.2 (89.3, 108.4)	<.001
Triglycerides (mg/dL)	94.5 (75.0, 119.0)	154.0 (107.0, 197.0)	<.001
Systolic Blood Pressure (mmHg)	118.5 (112.0, 126.5)	128.0 (119.0, 135.0)	<.001
HDL (mg/dL)	42.0 (36.0, 49.0)	37.0 (32.0, 43.0)	<.001

Metabolic syndrome diagnostic criteria (at least three of the following parameters): Fasting glucose (>100 mg/dL); HDL (<50 mg/dL); Triglycerides (>150 mg/dL); Waist circumference (≥35 inches or 88.9 cm), and Blood pressure (Systolic >130 mmHg, Diastolic >85 mmHg).

Spitzer. Metabolic syndrome and live birth. Fertil Steril 2024.

outcomes. Conclusions should be limited to those undergoing IUI.

Although we were not able to detect an association between metabolic syndrome improvements and live birth in this cohort, the results of this study should not encourage women with obesity not to lose weight or make efforts to improve other metabolic parameters. Instead, the findings underscore the multifaceted nature of obesity, metabolic health, and reproductive outcomes and should help guide evidence-based discussions between providers and patients prior to treatment.

CRediT Authorship Contribution Statement

Trimble Spitzer: Writing – review & editing, Writing – original draft. **Michael P. Diamond:** Writing – review & editing, Conceptualization. **Robert Wild:** Writing – review & editing, Conceptualization. **Richard Legro:** Writing – review & editing, Supervision. **Heping Zhang:** Writing – review & editing, Formal analysis. **Fangbai Sun:** Writing – review & editing, Formal analysis. **Shen Ling:** Writing – review & editing, Formal analysis. **Nanette Santoro:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of Interests

This work was prepared in part by a military employee of the US Government as part of official duties of T.S. and, therefore, is in the public domain and does not possess copyright protection. The opinions and assertions expressed herein are those of the author(s) and do not reflect the official policy or position of the Uniformed Services University of the Health Sciences or the Department of Defense. M.P.D. reports serving as a member of the Board of Directors and a stockholder of Advanced Reproductive Care; and serving as a Consultant for Seikagaku, Actamax, Temple Therapeutics, and ARC Medical Devices. R.W. reports Abiacare PCOS, Amgen Repatha in Pg, and Partners Mass General Menopause Reviews outside of the submitted work. R.L. reports consulting fees from InSupp, Ferring, Bayer, Abbvie and Fractyl and funding from UL1 TR002014 National Center for Advancing Translational Sciences, 5 R01AT009484-02 Inositol Supplementation to Treat Polycystic Ovary Syndrome: A Double-Blind Dose Ranging RCT (INSUPP-P) NIH/NCCIH, 5 R01 HD091350-04 The COMET-PCOS Trial: Comparing the effects of Oral Contraceptive Pills versus Metformin in the medical management of overweight/obese women with Polycystic Ovary SyndromeNIH/University of Pennsylvania, Guerbet USA Therapeutic Effect of Sonographic Hysterosalpingography: Oil vs. Water Based Media: The SHOW Pilot Trial, 5 R01 HD083323-04 Functional Analysis of PCOS Candidate Genes NIH/NICHD, 1 R01HD100630-01 AMH Signaling Pathway Variation in PCOS NIH, Hass Avocado Board A Multi-Site Randomized Clinical Trial to Compare Healthy Eating Recommendations for Six Months on Changes in Visceral Adiposity in Overweight/Obese Americans (Penn State Avocado Study), 5 U10HD055925-10REV Data Coordinator Center for the RMN; consulting fees from Organon, Covis Pharma GmbH (2022), Novo Nordisk (12/2021), and Insudd (2020); honorarium from Honorary Professor, National Research Center for Assisted Reproductive Technology and Reproductive

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Influences of race, ethnicity, and other social factors on coronavirus disease 2019 vaccination uptake among patients undergoing in vitro fertilization

OBJECTIVE

Pregnancy is a major risk factor for severe coronavirus disease 2019 (COVID-19), yet uptake of the COVID-19 vaccination among pregnant persons and those trying to conceive is lower than in the general population, particularly among Black women (1). Vaccine hesitancy in the US has been associated with age, sex, race and ethnicity, education, socioeconomic status, and concerns about the harms of vaccination on health and fertility (2, 3). Persons who refused or delayed vaccination were likely to be younger, identify as Black or Hispanic, and have lower income and education.

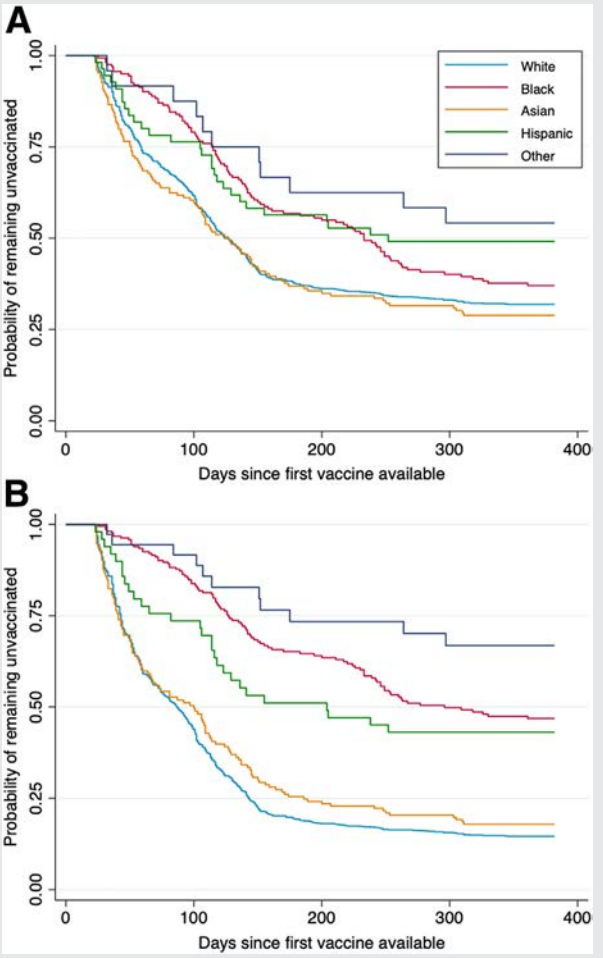
Despite substantial evidence of vaccine safety in pregnancy and before conception, infertile patients have expressed fears about potential effects on fertility outcomes (4, 5). No prior studies have examined vaccination rates or factors influencing vaccination among patients seeking fertility treatment. Patients undergoing in vitro fertilization (IVF) treatment are predominantly White and tend to have higher education and socioeconomic resources—factors associated with vaccination—yet they may also be uniquely sensitive to concerns about fertility. This study investigates COVID-19 vaccination among patients undergoing IVF treatment during the pandemic and focuses on the influences of race, ethnicity, and other sociodemographic factors on vaccination uptake.

STUDY DESIGN

After approval by the Institutional Review Board, patients aged ≥18 years who underwent IVF treatment cycles at the University of Pennsylvania from January 1, 2020, to December 31, 2021, were included. Primary exposure was self-identified race and ethnicity: White and non-Hispanic; Black or African American and non-Hispanic; Asian and non-Hispanic; Hispanic or Latino; and other and non-Hispanic. The outcome was time to full vaccination, defined as days from December 14, 2020 (date of first vaccine availability) to completion of two-dose Pfizer or Moderna vaccine regimens or one dose of the Johnson & Johnson Janssen vaccine.

Survival analysis was conducted with Cox proportional hazards regression and accelerated failure time models, adjusting for age, intention for pregnancy, and social vulnerability indices. Pregnancy intention was defined as the intent to undergo embryo transfer within the year after oocyte retrieval. Social vulnerability was defined by socioeconomic, household, housing, and transportation indices from the Centers for Disease Control for US Census tracts in 2020 (Supplemental Methods, available online).

FIGURE 1



Survival curves for time to vaccination by race and ethnicity among patients undergoing in vitro fertilization treatment. (**A**) Unadjusted and (**B**) adjusted for age, socioeconomic status, household social vulnerability, and intention for pregnancy.

Humphries. COVID vaccine by race/ethnicity in IVF. Fertil Steril 2024.

RESULTS

Of 1,250 patients undergoing IVF treatment, 848 (67.8%) had received at least one vaccine dose, and 816 (65.3%) were vaccinated fully as of December 31, 2021. These vaccination rates were similar to those for the general US population at that time (73% and 62%, respectively) (6). In our cohort, 834 (66.7%) patients identified as White, 168 (13.4%) Black, 153 (12.2%) Asian, 55 (4.4%) Hispanic, and 24 (1.9%) other race. Compared with White patients, Black and Hispanic patients had higher social vulnerability (Supplemental Table 1, available online).

TABLE 1

Hazard ratios for time to vaccination across race and ethnicity groups using Cox proportional hazards regression.

	Unadjusted HR (95% CI)	Adjusted HR[a] (95% CI)
White	1.00	1.00
Black	0.73 (0.59–0.91)	0.79 (0.63–0.99)
Hispanic	0.62 (0.42–0.91)	0.65 (0.44–0.95)
Asian	1.08 (0.88–1.33)	1.07 (0.87–1.32)
Other	0.49 (0.27–0.90)	0.50 (0.27–0.90)

Note: HRs of <1 indicate a lower hazard (risk) of vaccination.
CI = confidence interval; HR = hazard ratio.
[a] Adjusted for age, socioeconomic status, household social vulnerability, and pregnancy intention.

Humphries. COVID vaccine by race/ethnicity in IVF. Fertil Steril 2024.

Coronavirus disease 2019 vaccination rates differed significantly across race and ethnicity groups (Fig. 1). After adjusting for age, socioeconomic status, household vulnerability, and pregnancy intention, probability of vaccination remained lower for Black, Hispanic, and other race individuals compared with White patients (Table 1). No differences were observed for Asian patients. We identified a bimodal curve in the hazard distribution for Black patients (Supplemental Fig. 1, available online), with a delayed peak 250 days after vaccine availability, which coincided with policies at Philadelphia employers requiring vaccination by August 2021. Because of this time interaction, we applied a generalized gamma-accelerated failure time model and found consistent results. Adjusted time ratios demonstrated a longer time to vaccination for Black and other race patients. Independent of race, vaccination rates were similar across the spectrum of socioeconomic vulnerability (high: 67.4%, moderate: 64.6%, and low: 67.4%) and housing and transportation strata. Our findings were consistent across indications for IVF treatment, including among patients actively seeking pregnancy and those pursuing fertility preservation.

CONCLUSION

Black, Hispanic, and other race patients undergoing IVF treatment from 2020 to 2021 had lower COVID-19 vaccination rates compared with White patients. Unlike prior studies, socioeconomic status and access to care were not independently associated with vaccination. We hypothesize that other factors, such as community norms, medical mistrust, and bias in medicine, may underlie this disparity. Strengths of the study were a relatively diverse dataset that was representative of surrounding urban and suburban communities, a large sample size, and low missingness of exposure and outcome (<3%); limitations include a lack of information about reasons for vaccination delay or refusal, limited generalizability beyond a university-based clinic with a large catchment area, and potential misclassification or oversimplification of race and ethnicity categories. Studies should continue to analyze pandemic-era events to identify vaccination strategies that promote health equity.

TABLE 2

Time ratios for time to vaccination across race and ethnicity groups using accelerated failure time models.

	Unadjusted TR (95% CI)	Adjusted TR[a] (95% CI)
White	1.00	1.00
Black	1.50 (1.29–1.75)	1.47 (1.25–1.74)
Hispanic	1.23 (0.89–1.43)	1.09 (0.86–1.40)
Asian	0.92 (0.79–1.06)	0.92 (0.79–1.08)
Other	1.45 (1.02–2.07)	1.48 (1.03–2.12)

Note: TRs of >1 indicate longer time to vaccination (deceleration in the time-to-event).
CI = confidence interval; TR = time ratio.
[a] Adjusted for age, socioeconomic status, household social vulnerability, and pregnancy intention.

Humphries. COVID vaccine by race/ethnicity in IVF. Fertil Steril 2024.

CRediT Authorship Contribution Statement

Leigh A. Humphries: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jeremy Applebaum:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Florencia G. Polite:** Writing – review & editing, Methodology. **Elizabeth Kravitz:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Clarisa R. Gracia:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Dara S. Berger:** Writing – review & editing, Visualization, Supervision, Investigation, Formal analysis, Conceptualization.

Declaration of Interests

L.A.H. has nothing to disclose. J.A. has nothing to disclose. F.G.P. has nothing to disclose. E.K. has nothing to disclose. C.R.G. has nothing to disclose. D.S.B. has nothing to disclose.

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Effects of a eucaloric high-fat diet on anterior pituitary hormones and adipocytokines in women with normal weight

OBJECTIVE

Obesity in women is associated with metabolic disorders and may be a contributor to decreased fertility and adverse pregnancy outcomes (1). Previous studies report that obesity in women is correlated with diminished luteinizing hormone (LH) pulse amplitude and LH levels, decreased corpus luteum stimulation, lower levels of luteal progesterone, and heightened estradiol-induced negative feedback (2). We have termed the interplay of obesity, hyperlipidemia, hyperinsulinemia, and relative hypogonadotropic hypogonadism as "reprometabolic syndrome" (1) and shown that this phenotype can be induced in normal-weight women by a eucaloric high-fat diet (HFD) (3). Understanding the mechanisms underlying obesity-related pituitary-gonadal dysfunction is crucial to developing effective clinical interventions. This study investigates the effects of a 1-month eucaloric HFD on nonreproductive anterior pituitary trophic hormones and adipocytokines in healthy, normal-weight women.

STUDY DESIGN

This research presents a secondary analysis of a parent study (3). The protocol was approved by the University of Colorado Multiple Institutional Review Board and registered at ClinicalTrials.gov (NCT02653092). All study participants provided informed consent for the procedures as described.

Nineteen normal-weight (body mass index 18.0–24.9 kg/m^2) healthy, eumenorrheic women were enrolled. Participants underwent frequent blood sampling during the early follicular phase of the menstrual cycle before and after the 1-month eucaloric HFD intervention (48% calories from fat). Serum levels of thyroid-stimulating hormone (TSH), free thyroxine T4 (fT4), total triiodothyronine (tT3), cortisol, growth hormone (GH), prolactin (PRL), insulin-like growth factor 1 (IGF-1), adiponectin, and leptin were measured using immunoassay. Detailed methods are described in Supplementary Materials (available online).

RESULTS

The clinical characteristics and baseline demographics of the 18 participants were consistent with a healthy, young population (Supplemental Table 1, available online). One participant was not included in the final analysis because of nonadherence to the prescribed diet. All blood draws during the prediet and postdiet cycles were completed within the 2–7-day early follicular phase window per protocol.

There was a small but significant decrease in tT3 levels (P=.01) and cortisol levels (P=.02) after the HFD (Table 1 and Fig. 1C, D). A small increase in GH levels approaching statistical significance (P=.08) was observed (Table 1 and Fig. 1F). There were no statistically significant differences in the levels of TSH, fT4, PRL, IGF-1, adiponectin, or leptin in response to the HFD (Table 1, Fig. 1A, B, E, and G, and Supplemental Fig. 1, available online).

There were no changes in body weight before and after the diet. Participants' adherence to the prescribed diet appeared uniformly excellent, as lipidomic and metabolomic analysis demonstrated a significant increase in red blood cell fatty acids during the HFD compared with baseline (Supplemental Fig. 2). No adverse events were recorded among participants.

CONCLUSION

We previously observed that infusion of lipid and insulin in normal-weight women inducing hyperinsulinemia and hyperlipidemia was associated with decreased basal and gonadotropin-releasing-hormone stimulated levels of LH and follicle-stimulating hormone (4), with no apparent impact on other anterior pituitary trophic hormones (5). Additionally, exposure to a 1-month HFD was linked to a significant reduction of mean LH levels and suppressed LH and follicle-stimulating hormone responses to gonadotropin-releasing hormone (3).

Herein, we observed a small but significant decrease in tT3 levels in response to an HFD, although levels of TSH

TABLE 1

Hormone and adipocytokine levels pre- and post-HFD (n = 18).

Analyte	Estimate of mean difference (95% CI)	*P* value
Adiponectin (ng/mL)	−0.04 (−0.12, 0.04)	.27
Cortisol (μg/dL)	**−2.31 (−4.15, −0.47)**	**.02**
fT4 (ng/dL)	0.05 (−0.05, 0.15)	.31
GH (ng/mL)	0.43 (−0.05, 0.92)	.08
IGF-1 (ng/mL)	104.25 (−313.43, 521.93)	.61
Leptin (ng/mL)	−0.76 (−1.66, 0.15)	.1
PRL (ng/mL)	−0.37 (−1.35, 0.61)	.44
TSH (μIU/mL)	−0.01 (−0.17, 0.14)	.84
tT3 (ng/mL)	**−0.1 (−0.17, −0.03)**	**.01**

Note: Bold text indicates statistically significant differences $P < .05$. CI = confidence interval; fT4= free T4; GH = growth hormone; HFD = high-fat diet; IGF-1 = insulin-like growth factor 1, PRL = prolactin; TSH = thyroid-stimulating hormone; Tt3 = total T3.

Nguyen. High-fat diet and pituitary hormones. Fertil Steril 2024.

A.P.B. and N.S. should be considered similar in author order.

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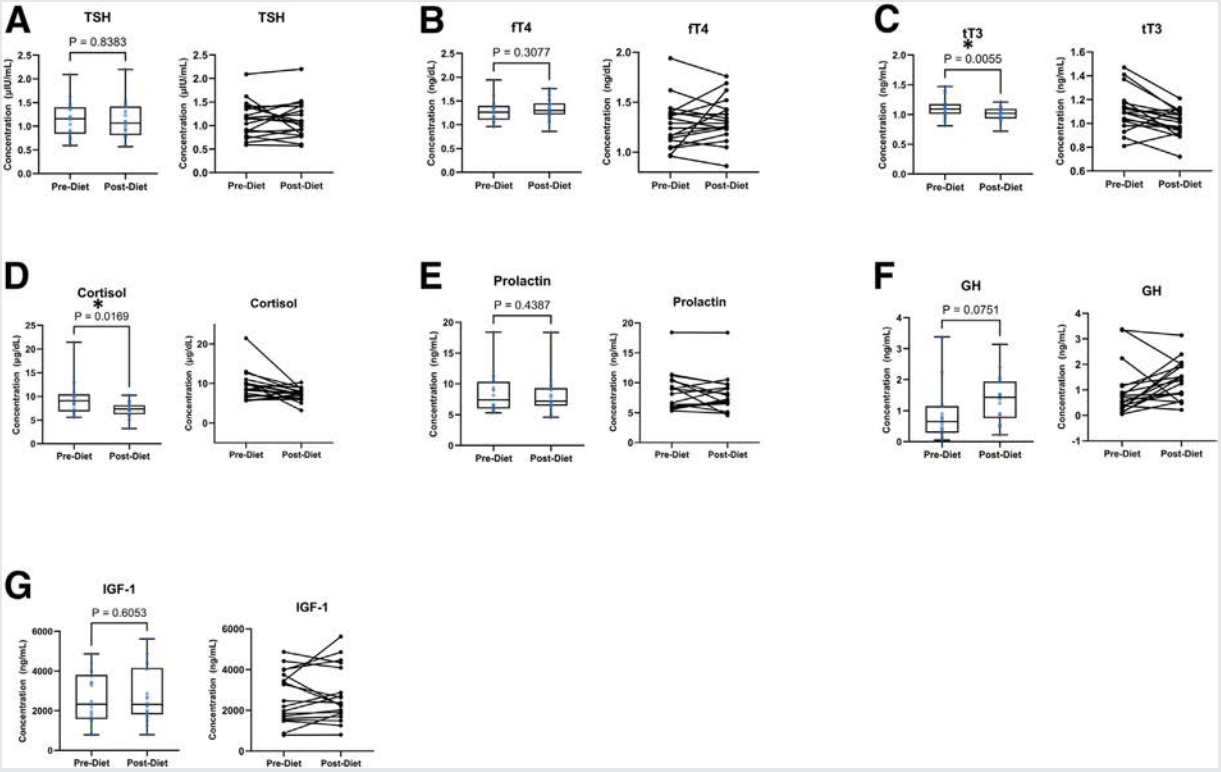
Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

FIGURE 1



Effect of HFD on TSH, fT4, tT3, cortisol, PRL, GH, and IGF-1. Pooled steady-state levels of (**A**) serum TSH (**B**) serum fT4, (**C**) serum tT3, (**D**) cortisol, (**E**) prolactin, (**F**) GH, and (**G**) IGF-1 were measured as described in methods. Box and whisker plots represent the distribution of values, medians, and maximum and minimum values. Spaghetti plots demonstrate paired data points for each participant before and after the HFD. Serum tT3 (*P*=.01) and cortisol (*P*=.02) decreased after a 1-month exposure to the HFD and there were no changes in TSH, fT4, PRL, GH, or IGF-1 (n = 18). One-sample *t*-tests were used to compare hormone levels before and after the HFD intervention by subtracting a participant's prediet measure from her postdiet measure, then testing mean differences against zero. *P* values are reported for each comparison with 0.05 set as the level of statistical significance (alpha) for all analyses. CI = confidence interval; fT4= free T4; GH = growth hormone; HFD = high-fat diet; IGF-1 = insulin-like growth factor 1, PRL = prolactin; TSH = thyroid-stimulating hormone; Tt3 = total T3.

Nguyen. High-fat diet and pituitary hormones. Fertil Steril 2024.

and fT4 remained unchanged. Notably, thyroid hormone levels stayed well within normal limits, suggesting that the diet-induced changes in tT3 levels may not be of clinical significance. We acknowledge time-varying confounding as a potential source of bias in this paired analysis.

Additionally, we observed a post-HFD decrease in cortisol levels, suggesting a potential impact on adrenal function. However, no significant changes in other nongonadotropin pituitary hormones or adipocytokines, including the levels of PRL, GH, IGF-1, adiponectin, and leptin were observed, in response to the HFD (Table 1).

Our results suggest that a 1-month eucaloric HFD, shown to induce reprometabolic syndrome and suppress gonadotropins (3), had no significant effect on other anterior pituitary hormones or adipocytokines. Thus, the observed impact of the HFD on anterior pituitary gonadotropins (3) appears cell-type specific and not a consequence of global suppression or nonspecific, obesity-related lipotoxicity-induced pituitary dysfunction.

CRediT Authorship Contribution Statement

Thy Nguyen: Writing – original draft, Investigation. **Katherine Kuhn:** Writing – review & editing, Project administration, Investigation, Formal analysis. **Angela Fought:** Formal analysis, Data curation. **Matthew Bolt:** Formal analysis. **Andrew P. Bradford:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Nanette Santoro:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of Interests

T.N. has nothing to disclose. K.K. has nothing to disclose. A.F. has nothing to disclose. M.B. has nothing to disclose. A.P.B. is a consultant for Amazon. N.S. reports consulting fees from Ansh Labs; is a Scientific Advisory Board member for Menogenix, Inc., Astellas Pharma, Que Oncology, and Amazon; is a scientific consultant for FertilityIQ and Ansh Labs; and is a Medical Advisory Board member for Project Ember/Amazon.

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Check for updates

New neovagina-creating technique on the basis of a fasciocutaneous flap for Müllerian agenesis

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Objective: To present a new surgical technique on the basis of an internal thigh fasciocutaneous flap for generating a compliant and sensitive neovagina with preservation of the external genitalia.
Design: Video demonstration of the surgical steps.
Patient(s): An 18-year-old woman with Müllerian agenesis confirmed at ultrasound and magnetic resonance imaging. The residual vagina was 3 cm long and 1.5 cm wide. After counseling by a gynecologist and plastic surgeon, in which all available techniques with pros and cons were exposed, the patient opted for the new technique. The long time required by conservative approaches and the desire to preserve the external genitalia with the chance to have a sensitive vagina guided the choice.
Intervention(s): The cul-de-sac of the vaginal stump was incised transversally. A 4-cm–wide and 9-cm–long canal bounded anteriorly by the bladder, posteriorly by the rectum, and superiorly by the peritoneum of Douglas was developed by blunt dissection. Fasciocutaneous flaps of 12 per 5 cm on the anteromedial aspect of the thighs were developed, identifying the vascular—from the pudendal artery—and nervous pedicles. A tunnel between the flap pedicles and neovagina introitus was created between fascia and subcutaneous tissue, detaching the vulvar structures from the ischiopubic ramus. Flaps were tunneled up to the neovagina introitus and sutured together by interrupted suture to form a tube with outside skin. The flaps were transposed into the canal everting the tube to obtain the skin lining the internal neovagina. The inferior margins of the flaps were sutured to the vaginal stump mucosa. No internal stitches were placed. Antibiotic prophylaxis was used during surgery. The entire procedure lasted 6 hours. During the postoperative period, no special positioning or ambulation restrictions were used.
Main Outcome Measure(s): Compliance and sensitivity of the neovagina, esthetic result, and perioperative and long-term complications.
Result(s): The postoperative course was uneventful, with early mobilization. The length of hospital stay was 16 days to allow proper vaginal dilator use; initial daily followed by intermittent use was planned. At a 2-year follow-up, the neovagina was sensitive and patent, allowing sexual intercourse. No complications were reported, and the patient was satisfied with the functional and esthetic result.
Conclusion(s): The new surgical technique was feasible and effective, preserving the external genitalia and avoiding graft healing and bowel secretion drawbacks without an intra-abdominal surgical step and related risks. However, more cases—2 cases performed to date with similar results—and long-term follow-up are needed to confirm the efficacy. In this regard, the regular use of vaginal dilators and forecast adherence between flaps and the connective tissue of the bladder and rectum are expected to prevent neovagina prolapse without any anchoring to the pelvic structures.
(Fertil Steril® 2024;122:382–4. ©2024 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Mayer-Rokitansky-Küster-Hauser syndrome, vaginoplasty, multidisciplinary surgery

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CRediT Authorship Contribution Statement

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Declaration of Interests

S.U. has nothing to disclose. L.G. has nothing to disclose. E.V. has nothing to disclose. C.D. has nothing to disclose. R.D.P. has nothing to disclose. S.G. has nothing to disclose. A.E. has nothing to disclose.

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Nueva técnica de creación de una neovagina basada en un colgajo fascio-cutáneo para la agenesia Mulleriana

Antecedentes: La agenesia mülleriana, conocida como síndrome de Mayer-Rokitansky-Küster-Hauser, se caracteriza por la ausencia de útero, cérvix y dos tercios superiores de la vagina proximal. Para facilitar las relaciones sexuales, un enfoque conservador basado en dilatadores y la vaginoplastia de Vecchietti genera una tracción progresiva del muñón vaginal hasta lograr un tamaño de vagina adecuado. Otras técnicas crean la neovagina utilizando injertos muco-cutáneos, peritoneales o ileo-sigmoideos o un injerto cutáneo de tejido genital para rellenar el nuevo espacio formado entre la vejiga y el recto. La desventaja de la primera técnica es el prolongado tiempo requerido, mientras que los inconvenientes de las segundas son la estenosis, dehiscencia, pobres resultados estéticos o ausencia de sensibilidad vaginal.

Objetivo: Presentar una nueva técnica quirúrgica basada en un colgajo fascio-cutáneo del muslo interno para generar una neovagina con capacidad adecuada y con sensibilidad, junto con la preservación de los genitales externos.

Diseño: Video-demostración de los pasos quirúrgicos.

Paciente(s): Una mujer de 18 años con agenesia Mulleriana confirmada por ecografía y resonancia nuclear magnética. La vagina residual tenía 3 cm de longitud y 1,5 cm de ancho. Después del asesoramiento por parte del ginecólogo y cirugía plástica, en el que se expusieron todas las técnicas disponibles con sus pros y contras, la paciente optó por la nueva técnica. El largo tiempo requerido para el enfoque conservador y el deseo de preservar los genitales externos junto con la opción de tener una vagina con sensibilidad guiaron la elección.

Intervención(es): Se realizó una incisión transversal en el muñón del fondo de saco vaginal. Un canal de 4 cm de ancho y 9 cm de longitud limitado anteriormente por la vejiga, posteriormente por el recto y en la parte superior por el peritoneo de Douglas fue desarrollado mediante disección roma. Se desarrollaron injertos fascio-cutáneos de la cara antero-medial del muslo, identificando los pedículos vasculares -de la arteria pudenda- y nerviosos. Se creó un túnel entre los pedículos del injerto y el introito de la neovagina, entre la fascia y el tejido subcutáneo, separando las estructuras vulvares de la rama isquio-púbica. Los colgajos fueron tunelizados hasta el introito de la neovagina y suturados juntos por puntos de sutura interrumpidos para formar un tubo con la piel exterior. Los injertos fueron traspuestos en el canal evertiendo el tubo para obtener el forro de piel de la neovagina interna. Los bordes inferiores de los colgajos se suturaron a la mucosa del muñón vaginal. No se dejaron puntos internos. Durante la cirugía se utilizó profilaxis antibiótica. El procedimiento completo duró 6 horas. Durante el postoperatorio no se indicó ninguna postura especial ni hubo restricciones a la deambulación.

Medida del resultado(s) principal(es): amplitud y sensibilidad de la neovagina, resultado estético, complicaciones perioperatorias y a largo plazo.

Resultado(s): El curso postoperatorio transcurrió sin incidencias, con movilización precoz. La duración de la estancia hospitalaria fue de 16 días para permitir el uso apropiado del dilatador vaginal, el cual se planificó a diario al inicio seguido por el uso intermitente. A los 2 años de seguimiento la neovagina tenía sensibilidad y era permeable permitiendo las relaciones sexuales. No se informaron complicaciones y la paciente estaba satisfecha con los resultados estéticos y funcionales.

Conclusión(es): La nueva técnica quirúrgica fue factible y efectiva, preservando los genitales externos y evitando los inconvenientes de la cicatrización del injerto y de la secreción intestinal, sin paso quirúrgico intraabdominal y los riesgos asociados. Sin embargo, se necesitan más casos y seguimiento a largo plazo -hasta ahora 2 casos realizados con resultados similares- para confirmar la eficacia. En este aspecto, el uso regular de dilatadores vaginales y la previsible adherencia entre los colgajos y el tejido conectivo de la vejiga y el recto se espera que prevengan el prolapso de la neovagina sin ningún anclaje a las estructuras pélvicas.

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Ovarian tissue biopsy for cryopreservation by vaginal natural orifice transluminal endoscopic surgery: a new approach for a minimal invasive ovarian biopsy

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Objective: Vaginal natural orifice transluminal endoscopic surgery (vNOTES) is an emerging surgical procedure that combines the advantages of the vaginal approach with laparoscopic vision and instrumentation. Shorter hospitalization and lesser postoperative pain associated with vNOTES may be explained by the advantages of this innovative surgical approach (e.g., absence of abdominal incisions, shorter operative time, and lower insufflation pressure). Ovarian tissue cryopreservation allows to preserve reproductive and endocrine functions in young women with oncological disease at risk of premature ovarian insufficiency (POI) caused by gonadotoxic treatments. Ovarian tissue biopsy for cryopreservation consists of a large biopsy of 1 or both ovaries that is usually performed by laparoscopy. Then, the removed ovarian tissue is cryopreserved for the future transplant after cancer remission. The volume of ovarian biopsy ranges from 50% of the ovary for women at moderate risk of POI to 70%–100% of it for those at high risk. The inclusion criteria for ovarian tissue cryopreservation are women aged <35 years who cannot delay start of oncological treatments for follicle cryopreservation, with a moderate or high risk of POI and good chance of 5-year survival. Ovarian tissue cryopreservation cannot be performed if tumor treatments include uterine irradiation or for tumors at risk of ovarian metastases (as in the case of ovarian cancer, leukemia, neuroblastoma, or Burkitt lymphoma). Despite widespread adoption of vNOTES in gynecology, ovarian biopsy for cryopreservation has never been performed using this route.

Design: Step-by-step explanation of the procedure with descriptive text and narrated video footage.

Setting: Tertiary-level referral academic center.

Patient(s): A 27-year-old patient recently diagnosed with low-grade follicular non-Hodgkin lymphoma was referred to our center for ovarian tissue cryopreservation before chemotherapy. The patient included in this study gave informed consent for publication of the video and posting of the video online including social media, the journal website, scientific literature websites (e.g., PubMed, ScienceDirect, and Scopus), and other applicable sites. Because of the nature of the study, institutional review board approval was not required.

Intervention(s): Access to the peritoneal cavity was created by a 3-cm posterior colpotomy. The peritoneum was then opened using cold scissors and temporarily fixed to the posterior vaginal wall. The GelPOINT Mini Advanced Access Platform (Applied Medical, Rancho Santa Margarita, CA), with 1 10-mm and 2 5-mm trocars, was used as the vNOTES port. The inner Alexis ring of the GelPOINT was inserted through the colpotomy into the pouch of Douglas. A hysterometer was placed into the uterine cavity to keep the uterus anteverted during the surgery. A pneumoperitoneum was created to a pressure of 8 mm Hg, and the operating table was tilted to a 20° Trendelenburg position. A 10-mm rigid 30° camera was inserted in the inferior and larger trocar, and both ovaries were visualized. Seventy percent of the left ovary was removed with cold scissors to minimize trauma on the surgical specimen. After removal of the GelPOINT cap, ovarian biopsy was immediately picked up by the biologist of our fertility center. The ovary was coagulated with a bipolar instrument. The hysterometer was then replaced by a uterine manipulator to perform tubal patency

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test, and blue dye passage through both salpinges was observed. Finally, the Alexis retractor and stich on the posterior peritoneum were removed, and the vagina was sutured using interrupted stiches. The total operative time was 25 minutes.
Main Outcomes Measure(s): Ovarian tissue biopsy for cryopreservation by vNOTES.
Result(s): No intraoperative and postoperative complications were reported, and the patient was discharged after 24 hours from surgery.
Conclusion(s): Vaginal natural orifice transluminal endoscopic surgery may be a feasible alternative approach to laparoscopy for ovarian tissue cryopreservation: it allows an easy access to the ovaries and removal of different tissue volumes. Patients undergoing ovarian cryopreservation may benefit from the vNOTES approach because a rapid postoperative recovery is crucial to start chemotherapy in a short time. As for other vNOTES procedures, accurate selection of patients seems to be crucial for a successful ovarian tissue cryopreservation. We believe that the inclusion and exclusion criteria reported for other gynecologic procedures performed through vNOTES may also be valid for ovarian tissue cryopreservation by vNOTES. Women at high risk of pelvic adhesions (e.g., coexistent endometriosis, previous pelvic surgery, or inflammatory pelvic disease), those with an increased body mass index or enlarged uterus, and those with cervical, vaginal, or uterine cancer cannot be considered for this approach because all these factors are associated with failure of vNOTES. On the other hand, women with no history of surgery, endometriosis, and large myomas may benefit from the vNOTES approach, and these women represent most of patients who undergo ovarian tissue cryopreservation. Further and larger studies are needed to assess the efficacy and safety of this new approach.
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El resumen está disponible en Español al final del artículo.

Key Words: Vaginal surgery, vaginal natural orifice transluminal endoscopic surgery (vNOTES), cryopreservation, ovarian tissue, fertility preservation

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CRediT Authorship Contribution Statement

Renato Seracchioli: Conceptualization, Formal analysis, Methodology, Supervision. **Manuela Maletta:** Writing – review & editing, Conceptualization, Writing – original draft. **Enrico Pazzaglia:** Writing – original draft, Software. **Antonio Raffone:** Conceptualization. **Rossella Vicenti:** Conceptualization, Supervision. **Stefano Scarperi:** Supervision, Visualization. **Valentino Bergamini:** Formal analysis, Supervision. **Diego Raimondo:** Conceptualization, Supervision, Validation.

Declaration of Interests

R.S. has nothing to disclose. M.M. has nothing to disclose. E.P. has nothing to disclose. A.R. has nothing to disclose. R.V. has nothing to disclose. S.S. has nothing to disclose. V.B. has nothing to disclose. D.R. has nothing to disclose.

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Biopsia de tejido ovárico para criopreservación mediante cirugía endoscópica transluminal por orificio natural vaginal: Un nuevo abordaje para una biopsia ovárica mínimamente invasiva.

Objetivo: La cirugía endoscópica transluminal por orificio natural vaginal (vNOTES) es un procedimiento quirúrgico emergente que combina las ventajas del abordaje vaginal con la visión y la instrumentación laparoscópica. La hospitalización más corta y el menor dolor postoperatorio asociados con vNOTES pueden explicarse por las ventajas de este abordaje quirúrgico innovador (como la ausencia de incisiones abdominales, menor tiempo operatorio y menor presión de insuflación). La criopreservación de tejido ovárico permite preservar las funciones reproductiva y endocrina en mujeres jóvenes con enfermedades oncológicas en riesgo de insuficiencia ovárica prematura (IOP) causada por tratamientos gonadotóxicos. La biopsia de tejido ovárico para criopreservación consiste en una biopsia extensa de uno o ambos ovarios, que generalmente se realiza por laparoscopia. Posteriormente, el tejido ovárico extraído se criopreserva para un futuro trasplante después de la remisión del cáncer. El volumen de la biopsia ovárica varía desde el 50% del ovario en mujeres con riesgo moderado de IOP hasta el 70%–100% en aquellas con alto riesgo. Los criterios de inclusión para la criopreservación de tejido ovárico son mujeres menores de 35 años que no pueden retrasar el inicio de los tratamientos oncológicos, para la criopreservación de folículos, con riesgo moderado o alto de IOP y buena probabilidad de supervivencia a 5 años. La criopreservación de tejido ovárico no puede realizarse si los tratamientos contra el tumor incluyen irradiación uterina o para tumores con riesgo de metástasis ováricas (como en el caso de cáncer ovárico, leucemia, neuroblastoma o linfoma de Burkitt). A pesar de la amplia adopción de vNOTES en ginecología, la biopsia ovárica para criopreservación nunca se ha realizado utilizando esta vía.

Diseño: Explicación paso a paso del procedimiento con texto descriptivo y video narrado.

Entorno: Centro académico de referencia de nivel terciario.

Paciente(s): Una paciente de 27 años recientemente diagnosticada con linfoma no Hodgkin folicular de bajo grado, fue referida a nuestro centro para criopreservación de tejido ovárico antes de la quimioterapia. La paciente incluída en este estudio dió su consentimiento informado para la publicación del video y su difusión en línea, incluyendo redes sociales, sitios web de revistas científicas (por ejemplo, PubMed, ScienceDirect y Scopus), y otros sitios aplicables. Debido a la naturaleza del estudio, no se requirió la aprobación del comité de ética institucional.

Intervención(es): Se creó acceso a la cavidad peritoneal mediante una colpotomía posterior de 3 cm. Luego, se abrió el peritoneo con tijeras frías y se fijó temporalmente a la pared vaginal posterior. Se utilizó la plataforma de acceso avanzado GelPOINT Mini (Applied Medical, Rancho Santa Margarita, CA), con 1 trocar de 10 mm y 2 de 5 mm, como puerto vNOTES. El anillo interno Alexis del GelPOINT se introdujo a través de la colpotomía en el fondo de saco de Douglas. Se colocó un histerómetro en la cavidad uterina para mantener el útero en anteversión durante la cirugía. Se creó un neumoperitoneo a una presión de 8 mm Hg y la mesa operatoria se inclinó a una posición Trendelenburg de 20 grados. Se insertó una cámara rígida de 30 grados y 10 mm en el trocar inferior y más grande, visualizando ambos ovarios. Se extrajo el 70% del ovario izquierdo con tijeras frías para minimizar el trauma en la muestra quirúrgica. Después de retirar la tapa del GelPOINT, la biopsia ovárica fue recogida inmediatamente por el biólogo de nuestro centro de fertilidad. El ovario se coaguló con un instrumento bipolar. Luego, el histerómetro fue reemplazado por un manipulador uterino para realizar una prueba de permeabilidad tubárica, observando el paso del tinte azul a través de ambas trompas de Falopio. Finalmente, se retiraron el retráctor Alexis y las suturas en el peritoneo posterior, y se suturó la vagina con puntos interrumpidos. El tiempo operatorio total fue de 25 minutos.

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Hysteroscopic subchorionic injection of methotrexate followed by laparoscopic excision of the gestational sac for the management of cesarean scar ectopic pregnancy: an innovative dual approach of a challenging pathology

Maria del M. Tejerizo Fe, M.D.,[a] Paola E. Benitez, M.D.,[a] Alejandro M. Gonzalez, M.D., Ph.D.,[a] Kyara Marquez, M.D.,[b] Joelle Mouhanna, M.D.,[b] and Jose Carugno, M.D.[b]

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Objective: To describe an effective two-step surgical approach for the management of cesarean scar ectopic pregnancies (CSEPs). CSEPs occur at an estimated frequency of 1 in 1,800 pregnancies, constituting approximately 6% of ectopic pregnancies in women with a history of prior cesarean delivery [1, 2]. Despite numerous recommended therapeutic approaches, the most effective treatment strategy remains uncertain [3].
Design: We present an innovative double-step technique for the management of a patient with a CSEP involving hysteroscopic subchorionic injection of methotrexate (MTX), followed by laparoscopic resection of the residual gestational sac and simultaneous repair of the uterine defect.
Setting: Academic tertiary hospital.
Patient: A 34-year-old G2P1001 with a history of prior cesarean section presented at 10 weeks of gestation. Ultrasound revealed a gestational sac within the niche of the previous cesarean scar, confirming the diagnosis of a CSEP. The patient included in this video gave consent for publication of the video and posting of the video online, including on social media, the journal website, scientific literature websites (such as PubMed, ScienceDirect, and Scopus, among others), and other applicable sites.
Intervention: The initial treatment involved hysteroscopic administration of MTX within the placental intervillous spaces, ensuring precise medication delivery. The administered dose of MTX was 1 mg/kg. Following the normalization of beta-human chorionic gonadotrophin (β-hCG) levels, laparoscopic resection of the remaining gestational sac and reconstruction of the uterine wall defect were performed.
Main Outcome Measures: We have implemented a management strategy focusing on ectopic pregnancy removal and addressing defect revision. The hysteroscopic approach allows for a clear assessment of the ectopic pregnancy and facilitates precise MTX administration, enhancing its effectiveness by increasing drug concentration within the placental intervillous space. Delaying surgical repair until after the β-hCG levels have decreased reduces the risk of excessive bleeding during the procedure, as lower β-hCG levels are associated with reduced vascularity at the ectopic site. Subsequent laparoscopic resection allows for complete removal of the remaining products of conception and repair of the defect, preserving the uterus and restoring normal anatomy. Compared to

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other surgical approaches, our two-step approach enables a more precise evaluation of placental implantation, making it a highly effective surgical method.
Results: We successfully managed a CSEP using a double-step technique. This involved hysteroscopic injection of subchorionic MTX, followed by laparoscopic resection of the residual gestational sac. Concurrently, we repaired the uterine defect. Both procedures were performed in an outpatient setting without complications detected during or after treatment. At the follow-up visit, the patient reported good health, and subsequent ultrasound confirmed an empty isthmocele.
Conclusion: This sequential hysteroscopic and laparoscopic approach represents a definitive and effective minimally invasive surgical option for the treatment of CSEP.
(Fertil Steril® 2024;122:388–90. ©2024 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Cesarean scar ectopic pregnancy, minimally invasive surgery, fertility-sparing, hysteroscopy

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CRediT Authorship Contribution Statement

Maria del Milagro Tejerizo Fe: Visualization. **Paola E. Benitez:** Visualization. **Alejandro M. Gonzalez:** Conceptualization. Methodology. Writing - review & editing. **Kyara Marquez:** Writing - original draft. Writing - review & editing. Visualization. **Joelle Mouhanna:** Writing - original draft. **Jose Carugno:** Conceptualization. Methodology. Writing - review & editing.

Declaration of Interests

M.M.T.F. has nothing to disclose. P.E.B. has nothing to disclose. A.M.G. has nothing to disclose. K.M. has nothing to disclose. J.M. has nothing to disclose. J.C. has nothing to disclose.

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Inyección subcorionica histeroscópica de metotrexato seguida de escisión laparoscópica del saco gestacional para el tratamiento del embarazo ectópico en cicatriz de cesárea: un enfoque dual innovador de una patología desafiante

Objetivo: Describir un abordaje quirúrgico eficaz en dos pasos para el tratamiento de embarazos ectópicos en cicatriz de cesárea (EECC). Los EECC ocurren con una frecuencia estimada de 1 de cada 1,800 embarazos, lo que constituye aproximadamente el 6% de los embarazos ectópicos en mujeres con antecedentes de parto previo por cesárea [1, 2]. A pesar de los numerosos enfoques terapéuticos recomendados, la estrategia de tratamiento más eficaz sigue siendo incierta [3].

Diseño: Presentamos una técnica innovadora de doble paso para el tratamiento de una paciente con un EECC que incluye la inyección subcoriónica histeroscópica de metotrexato (MTX), seguida de resección laparoscópica del saco gestacional residual y reparación simultánea del defecto uterino.

Lugar: Hospital universitario de tercer nivel.

Paciente: G2P1001 de 34 años con antecedentes de cesárea previa se presentó a las 10 semanas de gestación. La ecografía reveló un saco gestacional dentro del nicho de la cicatriz de la cesárea anterior, lo que confirma el diagnóstico de EECC. La paciente incluida en este video dio su consentimiento para la publicación del video y su difusión en línea, incluidas las redes sociales, el sitio web de la revista, sitios web de literatura científica (como PubMed, ScienceDirect y Scopus, entre otros) y otros sitios aplicables.

Intervención: El tratamiento inicial implicó la administración histeroscópica de MTX dentro de los espacios intervellosos placentarios, asegurando una administración precisa de la medicación. La dosis administrada de MTX fue de 1 mg/kg. Tras la normalización de los niveles de gonadotropina coriónica humana beta (b-hCG), se realizó la resección laparoscópica del saco gestacional restante y la reconstrucción del defecto de la pared uterina.

Principales medidas de resultado: Hemos implementado una estrategia de manejo centra en la extirpación del embarazo ectópico y abordaje de la revisión del defecto. El abordaje histeroscópico permite una evaluación clara del embarazo ectópico y facilita la administración precisa de MTX, mejorando su efectividad al aumentar la concentración del fármaco dentro del espacio intervelloso placentario. Retrasar la reparación quirúrgica hasta que los niveles de b-hCG hayan disminuido reduce el riesgo de sangrado excesivo durante el procedimiento, ya que los niveles más bajos de b-hCG se asocian con una vascularización reducida en el sitio ectópico. La resección laparoscópica posterior permite la eliminación completa de los productos restantes de la concepción y la reparación del defecto, preservando el útero y restaurando la anatomía normal. Comparado con otros enfoques quirúrgicos, nuestro enfoque de dos pasos permite una evaluación más precisa de la implantación placentaria, lo que lo convierte en un método quirúrgico altamente eficaz.

Resultados: Gestionamos con éxito una EECC utilizando una técnica de doble paso. Esto implicó una inyección histeroscópica de MTX subcoriónico, seguida de resección laparoscópica del saco gestacional residual. Al mismo tiempo, reparamos el defecto uterino. Ambos procedimientos se realizaron de forma ambulatoria sin que se detectaran complicaciones durante o después del tratamiento. En la visita de seguimiento, el paciente refirió buen estado de salud y la ecografía posterior confirmó un istmocele vacío.
Conclusión: Este abordaje histeroscópico y laparoscópico secuencial representa una opción quirúrgica mínimamente invasiva definitiva y efectiva para el tratamiento de la EECC.

Response letter to "Some common, fatal flaws in systematic reviews of observational studies"

We express our gratitude for the insightful response to our previously published manuscript "Endometrial Receptivity Array before frozen embryo transfer cycles: A systematic review and meta-analysis," provided by Drs. Jack Wilkinson and Katie Stocking (1, 2). This article has raised the following 2 major concerns:

- The manuscript underscores an examination of the methodological underpinnings characterizing systematic reviews (SRs) with a specific focus on non-randomized studies of interventions (NRSIs). It highlights flaws in critical appraisal methods, particularly the use of the Newcastle-Ottawa Scale (NOS), which may overlook critical confounding factors. The later review, using the Risk Of Bias in Non-randomized Studies-of Interventions (ROBINS-I) tool, is deemed superior in assessing bias.
- The manuscript also discusses the misuse of meta-analysis in NRSIs, emphasizing the necessity of adjusting for confounding variables. Despite similar conclusions between the reviewed SRs, the investigators stress the importance of methodological rigor in SRs to prevent misleading conclusions. They caution readers to critically assess SRs and advocate for maintaining high methodological standards in literature reviews (1). Although we agree with the concern about the limitations of the use of the NOS, especially with regards to confounding control and the difficulty in replicating assessments for final scores in the SRs of NRSIs, it is important to note that these concerns have already been addressed in the main text by the investigators. Moreover, the scoring of the NOS for each of the included studies has been conducted by 2 independent and proficient researchers to ensure the repeatability of the scoring and, thus, the consistency and reliability of the scoring process (2). Despite the challenges associated with its application, the NOS offers a pragmatic solution for researchers seeking to evaluate the methodological quality of NRSIs, particularly when robust or more accurate alternatives such as the ROBINS-I tool are not feasible. Thus, while acknowledging its limitations, the NOS continues to be a relevant tool and widely used because of its established history and familiarity among researchers (3, 4).

We, in fact, found that sensitivity and publication bias analyses consistently showed similar findings regarding the impact of including or excluding the randomized controlled trial (RCT) (5) in the SR alongside the NRSIs (2). This could suggest that the inclusion of the multicenter RCT with the NRSIs is methodologically appropriate because it appears to be devoid of significant publication bias (6). The methodological quality of the included RCT has also been assessed using the ROBINS-I checklist (2).

In conclusion, we extend our appreciation to Drs. Jack Wilkinson and Katie Stocking for their insightful contributions. Their manuscript underscores the crucial need for methodological checking in the SRs of NRSIs. Although we acknowledge the concerns regarding the limitations of the NOS, particularly in addressing confounding factors, we argue for its continued relevance and utility in the absence of more robust alternatives. Our analysis suggests that sensitivity, subgroup, and publication bias analyses support the methodological appropriateness of the included RCTs alongside NRSIs in SRs, given consistent findings. As such, on the basis of current RCTs, there are not sufficient data to support the routine use of endometrial receptivity array in the patient population with recurrent implantation failure.

CRediT Authorship Contribution Statement

Sara E. Arian: Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Conceptualization. **Shayan Mostafaei:** Methodology, Validation, Formal analysis, Investigation, Resources, Data curation.

Declaration of Interests

S.E.A. has nothing to disclose. S.M. has nothing to disclose.

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Reply of the Authors: Response to Arian and Mostafaei

A systematic review of endometrial receptivity array was used to illustrate some common errors in reviews of nonrandomized studies of interventions (NRSIs) (1). The investigators' response does not allay the concerns and introduces some new ones (2). Using the ERA review as an example, we described how the use of Newcastle-Ottawa Scale (NOS) resulted in inappropriate assessments of study quality. For example, studies were assessed as being good quality, despite making no attempt to control for confounding whatsoever. The investigators respond that there was good agreement between team members when using the NOS. This point is not in doubt but highlights the problem we describe—NOS consistently leads researchers to inappropriate assessments of study quality. The investigators indicate that limitations were addressed; however, the review describes critically flawed studies as being "low risk of bias." In the discussion, the investigators present that it "may not be completely true" to assert that control for 2 confounders is sufficient to deal with confounding, which is something of an understatement. We referred to ROBINS-I as superior; however. the investigators describe it as being "not feasible," without explanation. Indeed, another ERA review by Glujovsky et al. (3) did use ROBINS-I, casting some doubt on the claim that this is not possible. The investigators found all NRSIs to be a moderate or critical risk of bias.

We also described the error of failing to control for confounding in meta-analysis, even when the original studies did so. The investigators do not address this point. They defend the decision to combine randomized controlled trials and NRSIs in meta-analysis as appropriate because of an absence of "significant publication bias." We had not mentioned pooling of randomized controlled trials and NRSIs but would emphasize that this should be avoided and that a test of publication bias is irrelevant to the decision of whether or not to pool studies of different designs. Publication bias refers to a phenomenon where studies are more or less likely to be published according to their findings, such that meta-analyses are based on a distorted subset of all researches conducted. The fact that the investigators present this in support of the decision to combine studies adds to concerns about methodological standards. We emphasize that this review is typical of those regularly published in journals. Reviews featuring serious methodological misunderstandings contribute to treatment decisions on a daily basis. Although we appreciate the study of Arian et al., it is time to raise the acceptable standard of research.

CRediT Authorship Contribution Statement

Jack Wilkinson: Writing – original draft, Methodology, Conceptualization. **Katie Stocking:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization.

Declaration of Interests

J.W. is a statistical/methodological editor for Cochrane Gynaecology and Fertility, *Fertility and Sterility*, *BJOG: An International Journal of Obstetrics & Gynaecology*, *Reproduction and Fertility*, and *Journal of Hypertension*. K.S. is a statistical editor for Cochrane Gynaecology and Fertility and methodological editor for *Fertility and Sterility*.

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3. Glujovsky D, Lattes K, Miguens M, Pesce R, Ciapponi A. Personalized embryo transfer guided by endometrial receptivity analysis: a systematic review with meta-analysis. Hum Reprod 2023;38:1305–17.

Check for updates

Erratum for Practice Committee of the American Society for Reproductive Medicine. Tobacco or marijuana use and infertility: a committee opinion. Fertil Steril 2024;121:589–603.

A typographical error occurred in the last sentence in the left column on page 596 and should read "In an ongoing prospective cohort study enrolling men presenting to an infertility clinic, although no appreciable associations were found with LH levels, FSH levels were modestly depressed among marijuana smokers…"

The citation on page 597, first column, line 16, should be "Wise LA, Wesselink AK, Hatch EE, Rothman KJ, Mikkelsen EM, Sørensen HT, et al. Marijuana use and fecundability in a North American preconception cohort study. J Epidemiol Community Health 2018;72:208–215."

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Check for updates

Amin AF, Abd el-Aal DE, Darwish AM, Meki AR. Evaluation of the impact of laparoscopic ovarian drilling on Doppler indices of ovarian stromal blood flow, serum vascular endothelial growth factor, and insulin-like growth factor-1 in women with polycystic ovary syndrome. Fertil Steril. 2003 Apr;79(4):938-41. doi:10.1016/s0015-0282(0204849-5). PMID: 12749434

The ASRM Research Integrity Committee and the Editor in Chief examined this article after concerns were raised by a reader. While the author responded to the inquiry, the underlying data were not available due to the long interval since publication (21 years). Based on the data presented in the paper, the Committee identified errors of which future readers of the article should be aware. Specifically, the footnote in Table 3 states that all the p-values for the comparisons of Normal vs Pre-LOD groups are <0.001. However, this is incorrect; all p-values for these comparisons are >0.05. The correct p-values for the 4 p-values are: 0.5258, 0.384, 0.589, 0.447. The results comparing pre-LOD to post-LOD cannot be verified without the data.

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