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Editors' showcase: Obstetrics and gynecology

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Editors' showcase: Obstetrics and gynecology

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Editorial: Editors' showcase: obstetrics and gynecology

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Editorial on the Research Topic
Editorial: Editors' showcase: obstetrics and gynecology

We launched the *Editors' showcase: obstetrics and gynecology* to attract groundbreaking research articles and reviews for our section, Frontiers in Medicine/Obstetrics and Gynecology. Our invited authors and authors did not disappoint. We received many articles for the Editors' Showcase, 17 of which were ultimately published. They ranged over the whole breadth of OB/Gyn specialties, including Reproductive Medicine, Epidemiology, Endocrinology, Ultrasound and Prenatal Genetic Testing, Traditional Chinese Medicine, and Basic Research. All together the 17 publications attracted some 16,000 total views, over 15,000 article views, over 5,000 downloads and counting.

Six articles looked at topics in Reproductive Medicine, four examining various ovarian pathologies. Mu et al. reviewed Resistant Ovary Syndrome (ROS), a rare gynecological endocrine disorder impacting women's reproductive health. Patients with ROS display typical female sex characteristics and karyotype and a standard ovarian reserve, yet suffer from elevated gonadotropin and low estrogen levels, leading to primary or secondary amenorrhea. Despite numerous case reports over five decades, ROS's pathogenesis remains unclear, with no effective treatment strategies established. Their review collated all available ROS reports, summarizing its pathogenesis and treatment options, aiming to guide clinical management and set a foundation for future research.

Also focusing on the ovary, Bai and Wang discussed Premature Ovarian Failure (POF), a growing concern affecting women under 40, characterized by secondary amenorrhea and hormonal imbalances. Though hormone replacement therapy is advised, a comprehensive approach is vital for patient wellbeing. The core cause is linked to the depletion of the ovarian reserve due to issues with primordial follicle activation, emphasizing the need for pathway-based interventions.

Biernacka-Bartnik et al. investigated the threshold value for HOMA-IR to identify insulin resistance using the SHBG level in Caucasian women with polycystic ovary syndrome (PCOS). Using data from 854 women with PCOS, the study found that those with low SHBG levels ($<$26.1 nmol/L) also had higher HOMA-IR values. The determined cut-off value for HOMA-IR indicating insulin resistance was $\geq$2.1, aligning with European standards for insulin resistance.

Su et al. studied the effects of hematopoietic cell transplantation (HCT) on reproductive functions in female survivors. Involving 55 females under 40, the study found 72.7% showed signs of premature ovarian insufficiency (POI) post-HCT. The likelihood of POI was influenced by age during transplantation, conditioning regimen, and blood disease type, with those aged 21–40 at HCT time being most affected.

Two articles addressed topics in optimizing IVF treatment. Wan et al. explored the optimal timing for frozen embryo transfer (FET) post-oocyte retrieval by comparing immediate FET (within 60 days of retrieval) to delayed FET (>60 days post-retrieval) through a retrospective analysis. Analyzing 5,549 patients, the study found no significant differences in various pregnancy outcomes, including live birth rates, between the two groups. Both immediate and delayed FET yielded comparable pregnancy results.

Jiang et al. devised an open-label, non-inferiority RCT to evaluate the necessity of luteal phase support (LPS) during natural cycle frozen-thawed embryo transfer (NC-FET), given the existing uncertainties about its importance in ART success. This study involving 1,010 ovulatory women will compare outcomes with and without LPS.

Five articles in the RT investigated a range of topics in Obstetrics. Wang et al. performed a meta-analysis of 41 articles from 1980 to 2021 to investigate the impact of pregnancy interval on maternal outcomes. Their review revealed that short interpregnancy intervals (IPI < 6 months) are associated with higher risks of preterm birth, low birth weight, and other adverse outcomes, but not gestational hypertension or diabetes. The study calls for further research to inform pregnancy guidelines for childbearing-aged women.

Zhou et al. explored the impact of insulin on severe hypertriglyceridaemia (HTG) during the third pregnancy trimester. Comparing insulin-treated women with a control group on a low-fat diet, the study revealed the insulin group had significantly reduced prenatal lipid levels, decreased complications like HTG-AP, and improved pregnancy outcomes, including lower neonatal weight and reduced intensive care unit (ICU) admissions.

Herzberg et al. explored the gynecological and fertility complications after conservative treatment for placenta accreta spectrum (PAS). In a study involving 134 women with conservatively managed PAS and 134 matched controls, women with PAS had a higher need for postpartum operative procedures. However, there was no significant difference in fertility outcomes between the two groups post-treatment.

Zhang et al. conducted a meta-analysis to assess the impact of small-angle episiotomy on postoperative recovery in primiparous women. Analyzing 25 RCTs with 6,366 cases, the study found that small-angle episiotomy significantly decreased incisional tearing, reduced suturing time, and minimized incisional bleeding. However, there was no notable difference in severe laceration rates. The technique can be beneficially applied in clinical settings, considering maternal and fetal conditions.

Liu et al.'s study investigated the influence of excessive gestational weight gain (GWG) before and after 28 weeks on the delivery mode among women attempting trial of labor after cesarean (TOLAC), considering pre-pregnancy BMI. Analyzing data from a Chinese hospital between 2016 and 2022, the study found that 71.1% of 512 women achieved vaginal birth. While no link was identified between excessive GWG before 28 weeks and vaginal birth after cesarean (VBAC) rates, excessive GWG after 28 weeks significantly decreased VBAC rates across all BMI categories, regardless of weight gain before 28 weeks.

Topics in Gynecology included a wide range of different topics within the specialty, including Chinese medicines, endometriosis, pelvic organ prolapse and cystocele. Zhong et al. conducted a Bayesian network meta-analysis comparing Chinese patent medicines combined with hormone replacement therapy (HRT) for treating premature ovarian failure (POF). Analyzing 64 randomized controlled trials with 5,675 participants, they found HRT combined with Chinese patent medicines, especially Zuogui pills, to be more effective than HRT alone. However, due to the low quality of included studies, further high-quality, multi-center trials are needed.

Wang et al. investigated the fallopian tube's potential role in the development of ovarian endometriosis by studying the expression of the folate receptor-alpha gene (FOLR1) and its protein (FRA). Analyzing 144 tissue samples, they found that FOLR1 was highly expressed in fallopian tube and ovarian endometriosis tissues, with significantly lower levels in endometrial samples. The results suggest that the fallopian tube may play a key role in ovarian endometriosis, and FRA expression could offer potential therapeutic targets.

Wu et al. reviewed the molecular mechanisms underlying pelvic organ prolapse (POP), a common gynecological issue in middle-aged and elderly women. Despite the high incidence and significant clinical impact, current treatments remain suboptimal. The study delves into the relationships between POP and factors like MMPs/TIMPs, cyclins, microRNAs, and oxidative stress. The goal is to identify precise biomarkers or molecular targets for better POP prevention, diagnosis, and treatment. Further research is essential for improved understanding and interventions.

Yin et al. examined factors affecting cystocele and its Green classification in 357 primiparous women using three-dimensional ultrasound. Results showed 242 had cystocele. Factors like body mass index (BMI) at delivery and prolonged second stage of labor were linked to cystocele and bladder abnormalities. The study suggests weight control during and post-pregnancy and minimizing the second stage of labor to reduce cystocele risk.

Two papers focused on the importance of genetic diagnosis, both prenatally and in a pregnant patient. Kang et al. presented two cases highlighting inconsistencies between non-invasive prenatal testing (NIPT) and invasive testing for trisomy 21. Despite NIPT's advancements, invasive tests remain essential because placental DNA doesn't fully represent fetal genetic information. They emphasized that positive NIPT results need confirmation through invasive testing, and even negative NIPT results necessitate continuous monitoring due to potential placental mosaicism.

Huang et al. presented the first case of a pregnant woman with TSC2/PKD1 contiguous gene deletion syndrome, a rare condition combining tuberous sclerosis and polycystic kidney disease symptoms. The patient exhibited multiple clinical signs, including renal cysts and angiomyolipoma. There was an observed increase in the size of renal abnormalities during pregnancy. Enhanced monitoring and prenatal genetic testing can ensure optimal outcomes for both mother and fetus.

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Gynecological complications in long-term survivors after allogeneic hematopoietic cell transplantation—a single-center real-life cross-sectional study

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Background and objectives: Hematopoietic stem cell transplantation (HCT) is a treatment for hematopoietic diseases. However, most cured female patients may suffer from premature ovarian insufficiency (POI) after HCT, which is mainly caused by the pre-HCT conditioning regimen. Hence, this study aims to explore the impact of HCT treatment on reproductive and ovarian functions in female survivors.

Methods: A total of 55 female participants under the age of 40, who underwent HCT and met the inclusion criteria were enrolled. Data related to blood disease, menstruation, and fertility in the 3 years following HCT were collected.

Results: The involved patients received transplantation at different age stages, ranging from 8 to 37. All patients, except those with aplastic anemia (AA; 5/55), received a myeloablative conditioning regimen, usually modified total body irradiation/cyclophosphamide (TBI/Cy; 25/55) or modified Busulfan/cyclophosphamide (Bu/Cy; 23/55). Among women (42/55) who menstruated before HCT, 16.67% (7/42) had a spontaneous menstrual relapse and 83.3% (35/42) had amenorrhea after HCT. 72.7% (40/55) could be regarded as having POI. This proportion included 100% (25/25) of women aged 21–40 at the time of HCT, 62.5% (15/24) of those aged 11–20, and 0% (0/6) of those ≤10 years old. Patients with AML were more likely to have POI (95.7%). Patients aged ≤10 years (0%) or 11–20 years (16.7%) at the time of HCT were less likely to have moderate to severe menopause than those 21–40 years old (44%).

Conclusion: The prevalence of POI following HCT was high and POI was associated with age, conditioning regimen, and type of blood disease.

KEYWORDS

hematopoietic stem cell transplantation, premature ovarian insufficiency, conditioning regimen, myeloablative conditioning regimen, obstetrics and gynecology

1. Introduction

Hematopoietic stem cell transplantation (HCT) is an established treatment for many congenital or acquired disorders of the hematopoietic system and some other life-threatening diseases (1–5). More than 50,000 patients worldwide receive the treatment annually, including children, adolescents, and women of childbearing age. In China, the annual number of transplants reached more than 10,000 for the first time in 2019. The number of patients with pediatric (≤18 years of age) was 12.79%. The number of haploidentical donor (HID) HCT first exceeded 5,000 per year, which is much higher than that in the US or Europe (1,769 and 3,538 [10] in 2019) (6, 7). The group from Peking University established and enriched the Beijing Protocol which makes up 94% of HID HCTs in China. Further studies have shown that the "Beijing Protocol' in HID can provide comparable outcomes to matched sibling donors (MSD) or unrelated donor (URD) HCT in both benign diseases and hematologic malignancies (8). Over the last decades, survival rates of childhood, adolescent, and young adulthood (CAYA) cancer have remarkably increased thanks to substantial improvements in the comprehension of cancer molecular biology, refinement of diagnostic techniques, and novel treatment strategies (9–11). Hypogonadism secondary to antineoplastic treatment is called hypergonadotropic hypogonadism (characterized by elevated levels of luteinizing hormones and FSH owing to the lack of negative feedback from the gonads) (12). According to multiple published analyses, the incidence of ovarian failure ranges from 44 to 100% among transplant recipients during childhood, with clinical and demographical heterogeneity of different study cohorts accounting for most of this variability (13–15).

Cancers occurring in childhood and adolescence differ markedly from cancers in adults in their incidence and tumor characteristics. Worldwide, the average annual incidence in children aged less than 15 years is 140 new cases per million children, although there are three-fold variations between world regions and ethnic groups. The most common cancers in children are leukemia and lymphoma, while the major cancers among adults, such as carcinoma of the lung, breast, or colon, are rare in children. Cancer treatments are improving, but they are also often reproductive toxicity, leading an increasing number of young cancer survivors to seek personalized fertility preservation strategies (16, 17). The loss of fertility can negatively impact the quality of life (QOL) of young cancer survivors (18, 19), and women diagnosed with cancer show that the ability to have children in the future is very important (20). In fact, among young women diagnosed with cancer, the potential loss of fertility can sometimes be more stressful than the cancer diagnosis itself (21). The American Society of Clinical Oncology recommends that, as part of pre-cancer treatment education and informed consent, healthcare providers address infertility risks in patients treated during their reproductive years and be prepared to discuss fertility preservation options and/or refer all patients to fertility specialists (22). These referrals are essential because studies have shown that receiving counseling for precancer treatment regarding fertility preservation significantly improves QOL scores after cancer treatment in women of childbearing age (23). In addition, counseling with a fertility specialist and subsequent attempts to preserve fertility were associated with increased quality of life compared to women who only received counseling from an oncologist (23).

The myeloablative regimen conditioning (MAC) mBuCy regimen in MSD-HCT and the mBuCy+ATG regimen in haplo-HCT are the most popular in China and achieve remarkable results. Reduced-intensity conditioning (RIC) or intensified conditioning regimen is also used for subgroups of patients. High-dose radiotherapy and chemotherapy, especially the TBI and alkylating agents involved in the MAC cause damage to oocytes, granulosa cells, and ovarian stroma resulting in higher rates of POI. In detail, when the conditioning regimen administered in adult women includes total body irradiation (TBI), gonadal failure is extremely frequent and affects almost 100% of the patients for exposures above 10 Gy (24–26). The incidence of POI in hematological patients receiving conventional chemotherapy before HCT is 65–86% (27), rising to close to 100% following MAC, giving a probability of future pregnancy of less than 1% in the latter group (28). It has been established that factors affecting reproduction and ovarian function after HCT include the type of conditioning regimen, age, and pubertal status at the time of HCT, type of HCT, and types of hematologic disorders. In addition, osteoporosis, cardiovascular, neurological, and genitourinary tract diseases are common long-term risks that contribute to mortality among patients with POI (29).

In 2019, the total number of HCTs in China reached more than 10,000 for the first time benefiting from the Beijing Protocol. Although some studies have been conducted around the world, there is little information regarding the impact of HCT on reproduction and ovarian function in Chinese women. Protective measures and hormone replacement therapy post-HCT have not been fully investigated. To the best of our knowledge, this paper would be the first retrospective and prospective study on reproduction and ovarian function in Chinese women following allogeneic HCT. An exploration of the factors contributing to ovarian damage is expected to give insights into future protective practices.

2. Methods

2.1. Study design and procedure

The present study consisted of data analysis of a cross-sectional study of baseline data collected by questionnaires. First of all, we obtained the information of all female patients

who visited the Hebei Yanda Ludaopei Hospital between 1 January and 31 December 2017 through the case database, and then obtained the patient's informed consent through outpatient or telephone or WeChat. Briefly, patients after HCT with the hematopoietic disease were recruited from outpatient hematology clinics. A data collection form was utilized to collect study-related information which included age at HCT, height, weight, disease type, lines of chemotherapy, conditioning regimen, menarche status, menopausal symptoms, and so on. they were followed up for 5 years. During the non-epidemic period, patients were usually followed up in the clinic every 6 months. During the outbreak, follow-up assessments were performed *via* telephone. All participants provided written informed consent before. This study was approved by the Medical Ethics Committee of Peking University People's Hospital and the Medical Ethics Committee of Hebei Yanda Ludaopei Hospital (NO. 2020PHB017-01).

2.2. Patients

Patients, less than age 40 at the time of HCT for blood disease were included in the study. Exclusion criteria included the following: (1) presence of POI, premature ovarian failure, or sexual development abnormalities before treatment (0 patient); (2) history of ovarian surgery (0 patient); (3) receipt of second transplantation (2 patients).

2.3. Data variables

An independently designed questionnaire was adapted to gather the following information: general information (age, height, weight), information on blood disorders [type of hematologic disease, lines of chemotherapy before transplantation, donor type, conditioning regimen, acute graft-vs.-host disease (aGVHD) grade], and information related to gynecology (menstrual status before and after HCT, parity status, awareness of the protection of reproductive function, and hormone replacement therapy) were collected. The modified Kupperman menopausal index (KMI) was used to evaluate the severity of the menopausal symptoms. Data were collected by telephone or network questionnaire and analyzed by gynecologists or hematologists about 3 years after transplantation.

2.3.1. Definitions of variables

Premature ovarian insufficiency was defined as a clinical condition in postmenarchal women $<$ 40 years of age and characterized by the absence of menstrual cycles (amenorrhea) for $\geq$4 months and 2 elevated serum follicle-stimulating hormones (FSH) levels in the menopausal range, or delayed or arrested pubertal progression in girls $\geq$ 13 years.

Menopausal symptoms were determined by the modified Kupperman Index and classified as none (total score $<$ 6), mild (6 $\leq$ total score $\leq$ 15), or moderate (16 $\leq$ total score $\leq$ 30), and severe (total score $>$ 30). Please refer to the literature (30).

2.4. Statistics

All statistical analyses were performed using a two-tailed test with a value of $p < 0.05$ being considered statistically significant. Data are presented as mean $\pm$ SD. The Chi-square test and Fisher's exact test were used for significance analyses of categorical variables and Fisher's exact test was used to compare results with significant differences. IBM SPSS software v20.0 was used for all statistical analyses.

3. Results

3.1. Baseline characteristics of the patients

Of the total of 74 patients under the age of 40 who underwent allo-HCT at Hebei Yanda Ludaopei Hospital, 2 women did not meet the inclusion criteria: 2 cases received secondary transplants. Among the remaining 72 cases, 10 women refused to participate in the study, and 7 cases lost follow-up. Eventually, 55 cases completed the survey. The flowchart is shown in Figure 1.

3.1.1. Baseline characteristics

Among the 55 cases, the average age of transplantation was 20.45 years (ranging from 8 to 37), so various participants of childhood, adolescence, and childbearing ages were involved.

Malignant hematological diseases were chiefly represented by acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) (80%). Patients with AA accounted for only 9.1% and the remaining 10.9% of patients suffered from hypocellular leukemia, lymphoma, chronic active EBV infection, and lymphoid blast phase of chronic myeloid leukemia. All patients, except those with aplastic anemia (AA), were treated with a myeloablative conditioning regimen (MAC) (90.9%), of whom 45.5% received the modified total body irradiation (TBI)/ cyclophosphamide (Cy) regimen and 41.8% the modified busulfan (Bu)/Cy regimen. cGVHD was present in 50.9% of patients. Baseline characteristics of the study cohort are given in Table 1.

TABLE 1 Baseline characteristics of the patients ($N = 55$).

Characteristics ($n = 55$)	Number (%)
Age of HCT (year)	
≤10	6 (10.9)
11–15	16 (29.1)
16–20	7 (12.7)
21–40	26 (47.3)
Disease type	
ALL	21 (38.2)
AML	23 (41.8)
AA	5 (9.1)
Other	6 (10.9)
Lines of chemotherapy (not including conditioning regimen)	
0	6 (10.9)
≤5	24 (43.6)
5–10	15 (27.3)
>10	10 (18.2)
Type of allogeneic HCT	
Haploidentical	38 (69.1)
Unrelated	10 (18.2)
Matched sibling	7 (12.7)
HLA match	
5/10	23 (41.8)
6/10-9/10	19 (34.5)
10/10	13 (23.6)
Conditioning regimen	
MAC	51 (92.7)
TBI/Cy	25 (45.5)
Bu/Cy	23 (41.8)
Flu+Ara-C/Cy	3 (4.3)
RIC	4 (7.3)
aGVHD grade	
0	31 (56.4)
I	11 (20.0)
II	8 (14.5)
III	3 (5.5)
IV	2 (3.6)
cGVHD	
No	27 (49.1)
Yes	28 (50.9)
Relapse	
No	52 (94.5)
Yes	3 (5.5)

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; AA, aplastic anemia; HLA, human leukocyte antigen; MAC, myeloablative conditioning regimen; TBI, total body irradiation; Cy, cyclophosphamide; Bu, busulfan; Flu, fludarabine; Ara-C, cytosine arabinoside; RIC, reduced-intensity conditioning; aGVHD, acute graft-vs.-host disease.

3.2. The menstruation and menopausal symptoms of patients before and after HCT

As shown in Table 2, before transplantation, 13/55 (23.6%) had no menarche, 33/55 (60%) had relatively regular menstrual cycles (menstrual cycle ≤60 days), and 9/55 (16.4%) had irregular menstrual cycles (menstrual cycle>60 days). Only 5/55 (9.1%) patients said they were aware of pre-transplantation reproductive and ovarian protection treatments, such as egg-freezing, ovarian cryopreservation, and GnRH-a injection. No patient received fertility protection. Among women without pre-transplantation menarche, 8/13 (61.5%) later experienced spontaneous menarche; 7/42 (16.67%) had a spontaneous menstrual relapse and 35/42 (83.3%) had amenorrhea after transplantation. Only 1/55 (1.8%) had severe menopausal symptoms.

3.3. Correlations between the incidence of POI/menopausal symptoms and different clinical factors

As shown in Table 3, age at transplantation ($p < 0.001$), conditioning regimen with TBI/Cy ($p < 0.001$), conditioning regimen with Bu/Cy regimen ($p = 0.001$) and AML ($p < 0.01$) are factors affecting POI. Pairwise comparisons revealed that the probability of POI in patients aged ≤10 years at transplantation (0%) was significantly lower than that in patients aged 11–20 (62.5%; $p < 0.001$) or 21–40 (100%; $p < 0.001$). For the 6 patients aged ≤10 years at transplantation, the median age was 9 years (range, 8–10 years). They all had spontaneous menarche or spontaneous menstrual relapse. The probability of POI in patients aged 11–15 years was 56.3%, and the probability of POI in patients aged 16–20 years was 71.4%. For the 5 patients who had no menarche, the median age was 12.2 years (range, 11–16 years) and all of them had serum FSH at menopausal level (>40 IU/l). The probability of POI in patients aged 11–20 years was significantly lower than in patients aged 21–40 ($p < 0.01$). The probability of POI in patients receiving the TBI/Cy regimen (50%) was significantly lower than in those receiving chemotherapy alone (96.3%; $p < 0.001$). The probability of POI in patients receiving the Bu/Cy conditioning regimen (96%) was significantly higher than in those receiving other regimens (53.3%; $p < 0.001$). In addition, patients with AML were more likely to have POI (95.7%; $p < 0.01$). Kupperman scores were significantly correlated with age at transplantation. Although no significant difference was shown by pairwise comparison, the occurrence of moderate and severe menopausal-related symptoms (Kupperman score > 15) was lower in patients aged ≤10 (0%) or 11-20 years (16.7%) than in those of 21-40 years. Among older patients, 44% had Kupperman scores of >15. In

TABLE 2 The menstruation and menopausal symptoms of patients before and after HCT.

Symptoms ($n = 55$)	Age	Number (%)
Menstruation before transplantation		
No menarche	12 ± 5.7	13 (23.6)
Regular	22.5 ± 20.5	33 (60.0)
Irregular	24 ± 18.4	9 (16.4)
Sexually active before transplantation		
Yes	31 ± 8.4	21 (38.2)
No	12.5 ± 6.3	34 (61.8)
Given birth before transplantation		
Yes	34 ± 4.2	18 (32.73)
No	16.5 ± 0.7	37 (67.27)
Aware of the protection of reproductive function before transplantation		
Yes	22.5 ± 20.5	5 (9.1)
No	21.0 ± 12.7	50 (90.9)
Reproductive function protection before transplantation		0 (0)
Menstruation after transplantation		
No menarche		5 (9.1)
Spontaneous menarche		8 (14.5)
Spontaneous menstrual relapse		7 (12.7)
Amenorrhea		35 (63.6)
Sexually active after transplantation		
Yes	29.0 ± 2.1	12 (21.8)
No	19.0 ± 2.8	36 (78.2)
Pregnancy after transplantation		0 (0)
Whether to diagnosed with POI		
Yes	18.5 ± 3.5	40 (72.7)
No	12.5 ± 6.4	15 (27.3)
Kupperman index after transplantation		10 ± 8.41
<6 (Asymptomatic)	12.0 ± 5.7	24 (43.6)
6–15 (Mild)	11.5 ± 0.7	16 (29.1)
16–30 (Moderate)	12.5 ± 2.1	14 (25.5)
>30 (Severe)	32.0 ± 0.0	1 (1.8)
Hormone replacement therapy		
Yes	9.5 ± 2.1	22 (40.0)
No	22.0 ± 14.1	33 (60.0)

addition, HLA matching, aGVHD grade, and cyclosporine use were also analyzed but none showed a correlation with POI diagnosis or Kupperman score. One-way correlation analysis of the decision to accept HRT and the Kupperman score revealed no significant difference.

3.4. Factors affecting POI in children and adolescent females

Since the post-HCT incidence of POI in women aged 21–40 years reached 100%, a stratified analysis of factors affecting POI in children and adolescents of ≤ 20 years was conducted. POI incidence (6.7%) in patients receiving the TBI/Cy regimen was significantly lower ($p < 0.001$). In addition, the POI incidence of (92.3%) in those receiving the Bu/Cy conditioning regimen was significantly higher ($p < 0.001$). Patients with AML were more likely to have POI (90.9%; $p < 0.001$). Patients with transplantation performed after menarche are more likely to appear with POI (83.3%; $p = 0.003$).

4. Discussion

Hematopoietic stem cell transplantation involves the elimination of abnormal hematopoietic cells through conditioning regimens, such as radiotherapy and chemotherapy, followed by transplantation of donor or autologous hematopoietic stem cells to replenish the hematopoietic and immune systems. Worldwide, the number of HCTs has shown sustained growth for decades. With the annual global frequency of HCT increasing and prolonged post-HCT survival times, protection of reproductive and ovarian function becomes increasingly important. The last report from the Chinese Blood and Marrow Transplantation Registry Group (CBMTRG) described a continued growth of transplant activity in China (8).In 2019, the total number of HCTs in China reached more than 10,000 for the first time, to date, the reproductive and ovarian function of post-HCT Chinese has received little attention. The current study aimed to address this deficit.

Most patients enrolled in the current study suffered from ALL or AML (80%) and their ages at the time of transplantation ranged from 8-37 years. Except for those patients who had anemic hematological diseases, all were treated with myeloablative pre-HCT conditioning regimes, such as modified TBI/Cy and Bu/Cy programs. Such programs are compatible with various individualized medications, including Me-CCNU, idarubicin (IDA), antithymocyte globulin (ATG), and fludarabine (FLU). The present study is restricted to a consideration of TBI and alkylating agents which have the greatest impact on the ovary. The current study is a comprehensive description of pregnancy, menstruation, and menopause-related symptoms of post-HCT Chinese patients 3 years after transplantation. The number of women who were sexually active following HCT (21.8%) was fewer than before transplantation (38.2%) and the pregnancy rate was 0. Patients generally are advised to avoid pregnancy from the time of pretransplant evaluation through at least 2 years (malignant blood disease) or 3-5 years (benign blood disease) post-HCT and often longer because of the risk of relapse and continued use of potentially teratogenic, transplant-related medications, although this recommendation is tailored for individual patients (31). Sanders et al. (13) have reported a 4.5% pregnancy rate in post-pubertal women following transplantation. Vatanen et al. (32) evaluated the ovarian function among 92 adult or pubertal female survivors during 1978–2000, at a mean age of 9±4.3 years (range 1–19). Ten

TABLE 3 Correlations between the incidence of POI/menopausal symptoms and different clinical factors.

	POI			Kupperman score >15		
	Number	Proportion (%)	*P*	Number	Proportion (%)	*P*
Age at transplantation						
≤10	0	0	<0.001	0	0	0.028
11-20	15	62.5		4	16.7	
21-40	25	100		11	44	
Lines of chemotherapy						
0	5	83.3	NS	0	0	NS
≤5	19	79.2		8	33.3	
6-10	12	80		5	33.3	
>10	4	40		2	20	
Type of disease						
AML	22	95.7	<0.01	8	34.8	NS
ALL	11	52.4		7	33.3	
AA	4	80		0	0	
Other	3	50		0	0	
Type of transplantation						
Matched Sibling	6	85.7	NS	2	28.6	NS
Haploidentical	26	68.4		11	28.9	
Unrelated	8	80		2	20	
TBI/Cy						
No	26	96.3	<0.001	8	29.6	NS
Yes	14	50		7	25	
Bu/Cy						
No	16	53.3	0.001	8	26.7	NS
Yes	24	96		7	28	
cGVHD						
No	17	63	NS	7	25.9	NS
Yes	23	82.1		8	28.6	
Menarche at treatment						
pre menarche	13	38.5	0.003	1	7.7	NS
post menarche	42	83.3		14	33.3	

women out of the 92 survivors had a total of 14 pregnancies and gave birth to 12 children. The current study reveals a very low awareness rate (9.1%) regarding protective treatment for reproductive function among women receiving HCT and none had undergone such treatment. In 2017, local doctors were not aware that transplants could cause POI, were unaware of alternative fertility protection methods, and did not inform them of the risks in advance. At that time, this situation was also widespread in other hospitals in China, but it has improved significantly now, and we have published relevant consensuses (33). Such findings indicate the crucial nature of appropriate cooperation between departments of hematology and obstetrics and gynecology for the future. Hematologists should fully inform the patient before transplantation and make a referral to the obstetrician and reproductive specialist for consultation and treatment. Post-HCT ovarian function was evaluated, revealing that 72.7% (40) of participants in the current study could be reliably diagnosed with POI. Of 13 recipients of treatment during childhood who had no menarche before transplantation, 8 experienced spontaneous menarche, as did 7 women who did have menarche before transplantation. All women aged >20 years at the time of transplantation had amenorrhea. These observations suggest that a minority of women retain ovarian function after HCT. Whereas some young women experience post-HCT menstrual cramps, hormone tests show values for FSH > 40U/L, indicating that POI has developed. In women > 20 years old, the probability of POI is 100%. The current study included one subject who was 20 at the time of transplantation and had a voluntary menstrual blast for 4 years post-transplantation, resulting in FSH = 6U/L and recovery of ovarian function. However, further observations are required to demonstrate full

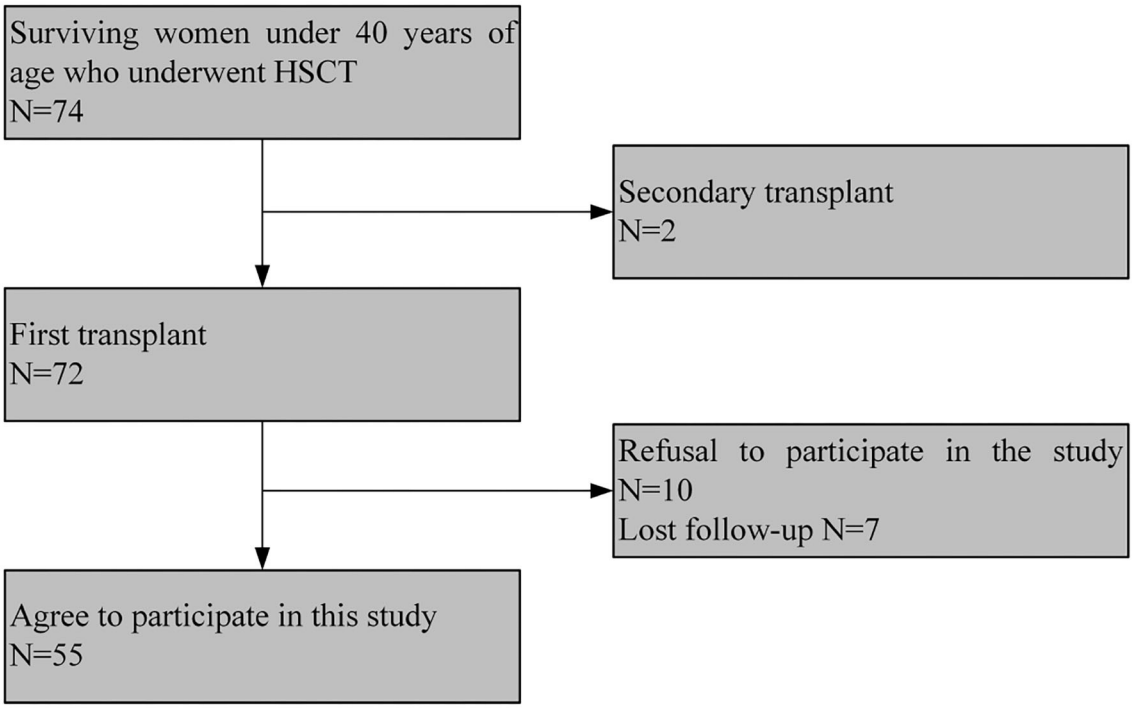

FIGURE 1
Consort diagram of patients.

recovery of ovarian function. Menopausal-related symptoms experienced post-transplantation were relatively mild, perhaps due to the younger ages of enrolled patients. Only 40% of patients received post-operative HRT. We believe that HRT should always be given in the post-transplantation period, even to women with spontaneous menarche or relapse, to achieve the therapeutic effect of inducing puberty and primary prevention (34). Consistent with the results of previous studies, age is the most important factor influencing the incidence of POI: the younger the age at transplantation, the lower the risk of POI. POI incidence among those of 21–40 years was 100%; 62.5% for those aged 11–20 and 0% for those aged ≤10 years (32). Pascale et al. (24) suggested that the younger group showed clinical evidence of ovarian function after BMT significantly more often than the older group (71 vs. 22%; $P < 0.01$). Logistic regression analysis confirmed an independent protective effect of young age at the time of BMT ($P = 0.004$). These findings are likely to be related to the gradual atresia of ovarian follicles after birth. In addition, this study finds that women receiving TBI/Cy are less susceptible to developing POI than those receiving chemotherapy alone. This result is inconsistent with previous research works carried out by Jadoul et al. (24) and Vatanen et al. (27), and their detailed results can be found in the corresponding literature cited here. Differences in radiotherapy and chemotherapy regimens in different centers may account for these discrepancies. TBI administered by the center scrutinized in the current study adopted accelerated hyperfractionated radiation therapy. Following 3 days of irradiation, there was no special protection for the pelvic cavity. Such an approach may be less damaging to the ovaries. By contrast, the home Bu/Cy regimen involves a relatively large dose of intravenous medication for 4 days with no liver first-pass effect. The probability of POI in patients with AML (95.7%) was higher than that in patients with ALL (52.4%) or patients with AA (80%), perhaps due to variations in radiotherapy and chemotherapy dosage. Stratified analysis was conducted to clarify factors related to POI onset among women of < 20 years at transplantation. The TBI/Cy regimen was associated with low POI incidence and the Bu/Cy regimen with high incidence. To the best of our knowledge, the current retrospective prospective study is the first to report a complete analysis of post-HCT reproductive and ovarian function in Chinese women. A novel finding is that the use of linear accelerators and hypersegmentation schemes during the TBI/Cy conditioning regimen program was less damaging to the ovaries. We acknowledge some shortcomings in our research. The sample size was small, confounding factors could not be satisfactorily controlled and multivariate analysis could not be performed. Moreover, the follow-up time was short. However, we believe that there is an urgent need to improve the protection of ovarian function before transplantation and to provide HRT treatment after transplantation to bring about increased survival rates and quality of life for patients with HCT.

5. Conclusion

Premature ovarian insufficiency incidence in women after HCT is 72.7%, including rates of 100% for transplant recipients aged 21–40, 62.5% for those aged 11–20, and 0% for those ≤10 years old. Protective factors for the development of post-HCT POI include young age at transplantation and a modified TBI/Cy conditioning regimen. Risk factor includes the Bu/Cy conditioning regimen program and AML. Symptoms related to menopause were related to the age at transplantation with younger women having lower Kupperman scores. Children, adolescents, and young women with POI should be managed by a multidisciplinary team including gynecologists, pediatricians, endocrinologists, dietitians, and psychologists.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study was approved by the Medical Ethics Committee of Peking University People's Hospital (Project no.2018PHB085-01). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

HS and XZ wrote the article. HS wrote ethical materials and a cooperation agreement. YZ and XY conceived and designed the experiments. HS, XZ, and YZ collected the data and analyzed experimental data. YL, DL, and JZ revised the manuscript critically during the revision stage. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Resistant ovary syndrome: Pathogenesis and management strategies

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Resistant ovary syndrome (ROS) is a rare and difficult gynecological endocrine disorder that poses a serious risk to women's reproductive health. The clinical features are normal sex characteristics, regular female karyotype, and usual ovarian reserve, but elevated endogenous gonadotropin levels and low estrogen levels with primary or secondary amenorrhea. Although there have been many case reports of the disease over the past 50 years, the pathogenesis of the disease is still poorly understood, and there are still no effective clinical management strategies. In this review, we have collected all the current reports on ROS and summarized the pathogenesis and treatment strategies for this disease, intending to provide some clinical references for the management and treatment of this group of patients and provide the foothold for future studies.

KEYWORDS

resistant ovary syndrome, etiology, pathogenesis, infertility, management strategies

Introduction

Resistant ovary syndrome (ROS), also known as ovarian insensitivity syndrome or Savage syndrome, is a rare gynecological condition with heterogeneous etiology that was first identified and named by de Moraes-Ruehsen and Jones (1).

Patients present clinically with primary or secondary amenorrhea before the age of 30 years of age, with a low response to exogenous gonadotropins and biochemical tests suggesting elevated endogenous gonadotropin levels and low estrogen levels. Moreover, ROS patients present with normal karyotype and normal ovarian reserve, i.e., normal Anti-Mullerian Hormone (AMH) and inhibin B (INB) levels, and with normal numbers of small follicles on vaginal ultrasound and laparoscopic ovarian histology (2–4).

According to the World Health Organization (WHO) classification (5), ROS belongs to type III amenorrhea characterized by hypergonadotropic hypogonadism (6, 7). It is often difficult to distinguish clinically from primary ovarian insufficiency (POI) or premature ovarian failure (POF) and is considered a subtype of POI or POF.

Etiology and pathogenesis

Current research on ROS is still in its preliminary stage. Given its low prevalence, it has predominantly been reported as scattered cases, and no large sample-size studies have been conducted. Studies on the mechanism have mostly focused on gonadotropin pre-receptor and partial receptor levels. Deficiency of follicle-stimulating growth factors, mutations in the follicle-stimulating hormone (FSH) receptor or beta subunit, abnormal gonadotropin signaling, and autoimmune problems are potential causes of this disorder. It has been established that the mechanism of ROS involves the failure of gonadotropins to act effectively on the follicles. Accordingly, the follicles fail to develop normally and stagnate in a resting state.

Inactive mutations of follicle-stimulating hormone

Current evidence suggests that primordial follicles develop to the primary stage mediated by the PI3K/AKT/mTOR signaling pathway (8) (initial recruitment), while most primordial follicles remain inhibited until they receive activation signals. Once growth begins, the primordial follicles develop into sinus follicles in response to local cellular regulators in the ovary, such as keratinocyte growth factor (KGF) (9), platelet-derived growth factor (PDGF) (10), basic fibroblast growth factor (bFGF) (11), and so on. Although most early sinus follicles undergo atresia, at least one sinus follicle will progress to preovulation (circulating recruitment) under pituitary FSH and luteinizing hormone (LH) stimulation (12). During the later stages of follicular development, FSH provides the primary stimulus (13, 14; **Figure 1**). Intriguingly, inactivating FSH mutations result in many sinus follicles developing without the support of endogenous FSH and failing to develop into dominant follicles, remaining in the primary stage. Clinically, several small follicles without dominant follicles can be observed under vaginal B-ultrasound.

Abnormalities in the regulation of follicle-stimulating factors

Besides FSH, C-type natriuretic peptide (CNP) is a follicle-stimulating factor that has been identified in recent years (12, 15). CNP belongs to the natriuretic peptide family and is characterized by a highly conserved 17-membered ring structure formed by intramolecular disulfide bonds (16). It was found that the precursor protein natriuretic peptide precursor C (NPPC) of CNP and its cognate receptor natriuretic peptide receptor-B (NPRB) were expressed in sinus follicles and preovulatory follicles (17). CNP is produced by the sinus follicle and binds to its receptor to stimulate follicle development by activating the guanylate cyclase coupled receptor to produce cyclic guanosine monophosphate (cGMP) (18, 19). In addition, in the preovulatory follicle, CNP stimulates cGMP production by activating NPRB, which is expressed by perivitelline and oocyte-associated cumulus cells (CCs) and diffuses into oocytes *via* gap junctions (20). In oocytes, cGMP inhibits phosphodiesterase 3A, thereby preventing cAMP hydrolysis (20). Overwhelming evidence suggests that adenosine 3′, 5′-cyclic phosphate (cAMP) is a second messenger in various central cellular functions. In the ovaries, cAMP regulates ovulation, enhances primordial follicle growth, and provides a key signal for gonadotropin action on the gonads. High levels of cAMP can promote growth in primordial follicles (21) and inhibit oocyte maturation (20). Sato et al. (18) reported that daily injections of CNP to infant mice could promote follicle growth, and ovulation was successfully induced by gonadotropins. This was also demonstrated by *in vitro* studies (22). This finding suggests that CNP is essential for follicular growth and development. Regional follicular growth and development in ROS patients may be related to the abnormal regulation of this factor (**Figure 2**).

Mutations in the follicle-stimulating hormone receptor gene

The interaction of FSH with its receptor is critical for follicle development and maturation. Interestingly, any variation in the FSHR genotype may alter the receptor's ability to bind FSH and induce downstream signaling pathways. Inactivating mutations in the FSHR gene have been associated with loss of ovarian function in women, and these mutations lead to impaired receptor function (23). Abel et al. (24) found that follicles developed to the antral follicle stage in mice with disrupted FSHR gene, suggesting that the FSHR defect does not affect the development of the antral follicle, accounting for a large number of primordial follicles in the ovary of ROS patients with no sinus follicle development.

It is well-established that mutations in the FSHR are rare, and only 25 loss-of-function mutations in FSHR have been found in women with ovarian dysgenesis, primary amenorrhea, and secondary amenorrhea (**Table 1**). Of these, the C 566 T mutation is only seen in Finns, suggesting a possible interethnic difference (25). Mutations in the FSHR gene are reportedly rare in UK women with ROS (26). However, most studies had small sample sizes and did not provide robust evidence that FSHR gene polymorphisms have pathophysiological significance in ovarian function.

Abnormal regulation of granulocyte proliferation factors

At least three oocyte-derived factors have been shown to promote the growth of granulosa cells, including R-spondin2, growth differentiation factor 9 (GDF9), and

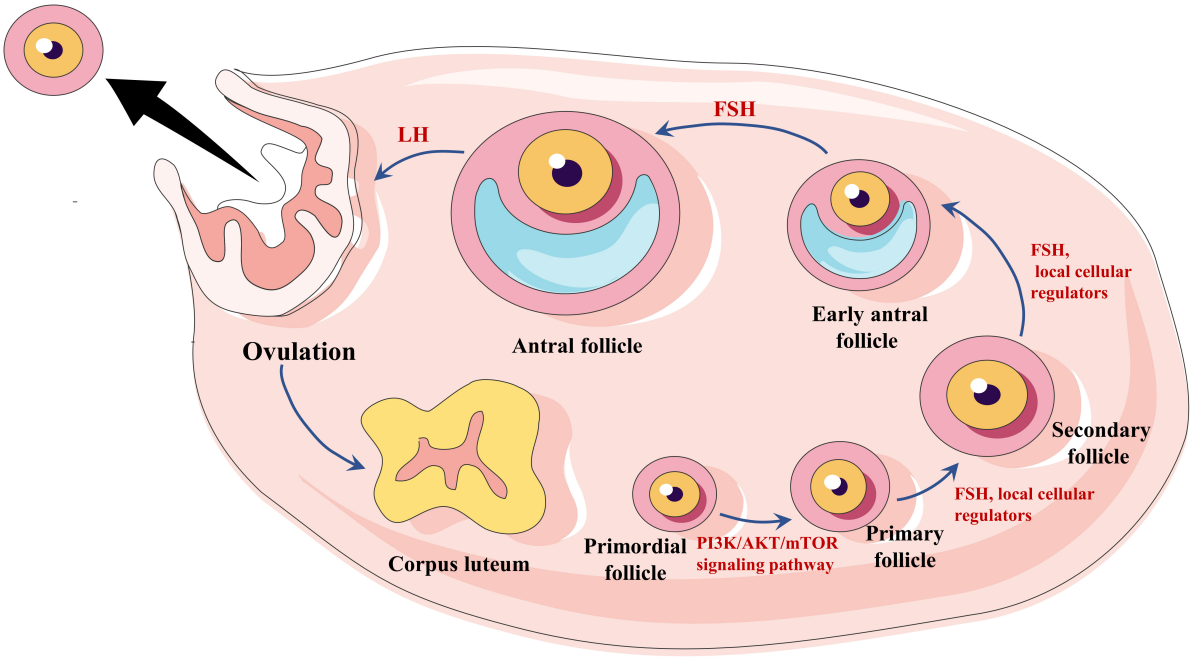

FIGURE 1
Hormonal regulation of follicle growth and expulsion. Primordial follicles are regulated by the PI3K/AKT/mTOR signaling pathway to a primary stage of development, followed by the development of sinus follicles by the action of follicle-stimulating hormone (FSH) and local cellular regulators. Eventually, the oocyte matures under the influence of LH, and ovulation occurs.

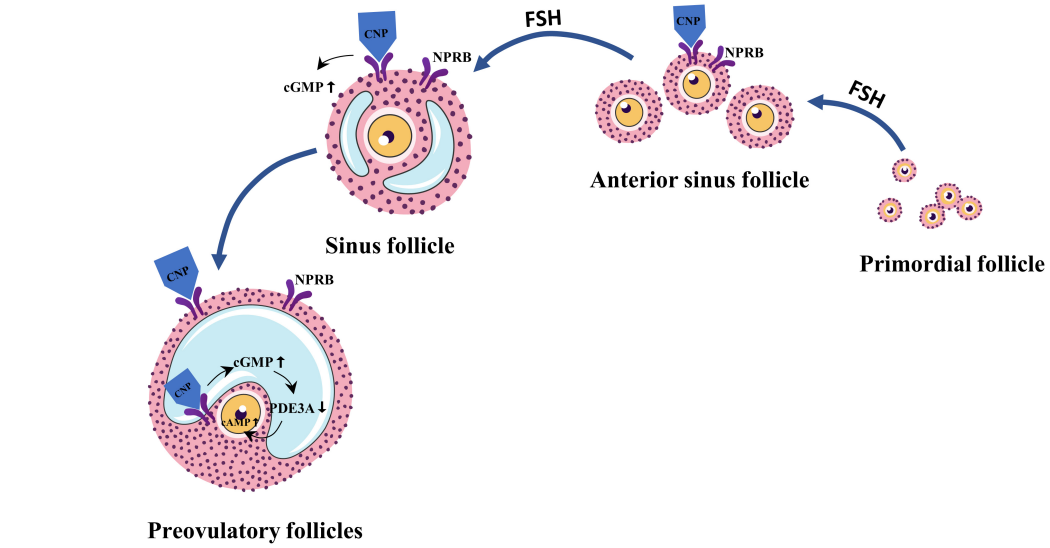

FIGURE 2
C-type natriuretic peptide (CNP) is an important follicle growth regulator secreted by granulosa cells in secondary and sinus follicles in response to follicle-stimulating hormone (FSH) stimulation. Cyclic guanosine monophosphate (cGMP) and cyclic phosphate (cAMP) levels are regulated by CNP in combination with natriuretic peptide receptor-B (NPRB), thereby regulating follicle development and maturation.

bone morphogenetic protein 15 (BMP15) (12). Studies in mice have revealed that the transcripts of R-spondin2 are only present in primary oocytes and oocytes of larger follicles but not in the initiating follicles (44). The decrease of R-spondin2 level may lead to failure of follicle development in the late reproductive phase (12), which may contribute to the fact that in ROS patients, during repeated ovulation stimulation, even when follicles develop, they reach atresia occurs before dominance. If

TABLE 1 Inactivating mutations of follicle-stimulating hormone receptor (FSHR) previously reported in women with ovarian dysgenesis, primary amenorrhea, and secondary amenorrhea.

No.	Nucleotide change (exon number)	Amino acid change	Phenotype of subjects	References
1	c.566C>T (exon 7)	p.Ala189Val	Primary amenorrhea with increased serum FSH levels	(27)
2	c.662T>G (exon 8)	p.Val221Gly	Primary amenorrhea	(28)
3	c.671A>T (exon 7) c.1801C>G (exon 10)	p.Asp224Val p.Leu601Val	Primary amenorrhea with increased serum FSH levels	(23)
4	c.1043C>G (exon 10)	p.Pro348Arg	Primary amenorrhea with increased serum FSH levels	(29)
5	c.1255G>A (exon 10)	p.Ala419V al	Primary amenorrhea with increased serum FSH levels	(30)
6	c.1555C>A (exon 10)	p.Pro519Thr	Primary ovarian failure with increased FSH levels	(31)
7	c.1760C>A (exon 10)	p.Pro587His	Primary amenorrhea	(32)
8	c.1723C>T (exon 10)	p.Ala575Val	Primary amenorrhea with hypergonadotropic hypogonadism	(25)
9	c.175C>T (exon 2)	p.Arg59Term	Primary ovarian insufficiency with increased serum FSH levels	(33)
10	c.1222G>T (exon 10)	p.Asp408Tyr	Primary amenorrhea with increased serum FSH levels	(34)
11	c.1253T>G (exon 10)	p.Ile418Ser	Primary ovarian failure and hypergonadotropic hypogonadism	(35)
12	c.1298C>A (exon 10)	p.Ala433Asp	Hypergonadotropic hypogonadism	(36)
13	c.419delA c.1510C>T c.44G>A (exons 1 and 2)	p.Lys140Argfs*16 p.Pro504Ser p.Gly15Asp	Primary ovarian insufficiency with resistant ovary syndrome	(37)
14	I423T (exon 10)	–	Primary amenorrhea with primary ovarian failure	(38)
15	c.479C>T (exon 6) c.1717C>T (exon 10)	p.Ile160Thr p.Arg573Cys	Secondary amenorrhea with increased serum FSH levels	(39, 40)
16	c.182T>A (exon 2) c.2062C>A (exon 10)	p.Ile61Asn p.Pro688Thr	Secondary amenorrhea with resistant ovary syndrome	(41)
17	c.793A>G (exon 9) c.1789C>A (exon 10)	p.M265V p.L597I	Secondary amenorrhea with premature ovarian insufficiency	(42)
18	c.646G>A (exon 8) c.1313C>T (exon 10)	p.Gly216Arg p.Thr438Ile	Secondary amenorrhea with premature ovarian insufficiency	(43)

similar R-spondin2 effects are identified in humans, R-spondin agonists could provide a new therapeutic approach for infertile women (12).

In addition to R-spondin2, GDF9 and BMP15 are local factors produced by oocytes that stimulate follicle development. They are members of the TGF-β superfamily of cystine junctional proteins (45) and bind to receptor serine kinases (RSK) to stimulate downstream signaling (46). Both factors bind to type II RSK BMP receptor II (47) and recruit type I RSK for GDF9, and BMP15 to regulate downstream SMAD proteins in granulosa cells. Current evidence suggests that GDF9 treatment enhances the growth and differentiation of cultured prezygotic follicles (48). *In vivo*, treatment with GDF9 promotes the development of primordial follicles to primary and antral follicles (49). GDF9 also has antiapoptotic effects during early sinus follicle development (50). In the ovaries of ROS patients, follicles are often present in the primordial state, which may be associated with a lack of or abnormal regulation of the GDF9 factor.

Immunity-related issues

Many studies have shown that the pathogenesis of ROS is related to immune factors. Most studies found that patients with ROS may have autoantibodies against FSHR that block the ovarian response to gonadotropin stimulation (51–55). In this respect, Rogenhofer et al. (6) identified antibodies against human menopausal gonadotropin (HMG) that were not recombinant-FSH (re-FSH) in a patient with ROS. It has also been found that IgG can block DNA synthesis of granulosa cells stimulated by FSH in ROS patients (56). In addition, Chiauzzi

et al. (52) found immunoglobulin (Ig-FSHR) in the blood of patients with ROS that inhibited the binding of FSH to its receptor by detecting the level of circulating immunoglobulin in patients with ROS. Escobar et al. (57) reported a case of myasthenia gravis with ROS whereby a substance behaving like gamma globulins could inhibit binding to FSH-specific receptors in an *in vitro* system.

In contrast, Starup and Pedersen (58) found no circulating gonadotropin antibodies in a 21 years-old woman with ROS. Consistently, Board et al. (59) found no autoimmune antibodies in ROS patients. Moreover, recent case reports of ROS in which patients were examined for immune disorders showed no signs of immune disorders (anti-nuclear antibodies, antiphospholipid antibodies, lupus antibodies, antibodies to semicarbazide, adrenocortical autoantibodies, steroid cell autoantibodies, serum 21-hydroxylase, 17-hydroxylase, and P450 side-chain cleavage enzyme autoantibodies were all negative) (60–62).

Management strategy

The clinical treatment varies for women of different ages, with various complaints and other clinical manifestations. For girls during puberty, the main aim is to promote the development of secondary sexual characteristics, maintain normal menstrual flow and protect the function of the ovaries, mainly using hormone supplementation; for women of childbearing age without fertility requirements, the basic principle of treatment is to provide physiological supplementation and prevent diseases in other systems caused by hormone deficiency. In contrast, assisted reproductive techniques such as ovulation promotion and *in vitro* culture of immature oocytes can be performed for women of childbearing age with fertility requirements. All specific treatment options suggested are based on case reports (**Figure 3**).

Management strategy of resistant ovary syndrome patients without fertility requirements

Hormone replacement therapy

For adolescents or women of childbearing age who do not require fertility, treatment of ROS usually begins with hormone replacement therapy (HRT) to maintain normal menstruation. Estrogen (e.g., estradiol valerate 2–4 mg/d) and progestin (e.g., norgestrel 500 mg/d) are administered for 22–28 days (63, 64). Withdrawal bleeding occurs on days 3–7 after discontinuation, and the next cycle is administered on the 5th day of menstruation, usually for three consecutive cycles. In addition, short-acting oral contraceptive pills (OCP) can be used to establish an artificial menstrual cycle for patients.

Treatment of resistant ovary syndrome patients with fertility requirements

Hormone replacement therapy

For ROS patients with fertility requirements, ovulation induction therapy or other assisted fertility methods are usually performed based on HRT (usually $\geq$3 months). However, cases of ROS having spontaneous pregnancies and successful live births during or after treatment with HRT have been extensively reported in the literature (63–68).

Mueller et al. (63) reported on a 26 years-old woman with primary infertility with decreased serum FSH and LH levels and increased levels of estradiol, progesterone, and inhibin A during the third month of HRT, leading to spontaneous follicular growth, maturation, and ovulation. One cycle after discontinuation of the drug, there was still spontaneous ovulation of follicles, and the patient successfully conceived. Lim et al. (69) reported a 24 years-old ROS woman with secondary amenorrhea and primary infertility. After 2 months of cyclic estrogen-progestin replacement therapy, insufficient inhibition of FSH and LH was observed. Subsequently, she was prescribed 100 pg of Ethinyl Estradiol daily for 23 consecutive months and successfully conceived.

It is possible that exogenous estrogen suppressed excessive gonadotropins in the body, resulting in increased sensitivity of FSH receptors on follicular membrane cells and increased sensitivity to gonadotropins, allowing follicles to become sensitive to ovulatory drugs or spontaneously ovulate and conceive (70). Although individual cases cannot indicate whether the treatment was effective or occurred by chance, it has been established that approximately 13% of ROS patients can become pregnant spontaneously after low-dose HRT. Accordingly, pregnancy is still possible after HRT treatment in ROS patients (71).

Combined letrozole and human menopausal gonadotropin for ovulation

Letrozole is a third-generation aromatase inhibitor, and its peripheral action is key to the successful induction of ovulation in patients with ROS. In the periphery, letrozole blocks the conversion of androgens to estrogens at the ovarian level by inhibiting aromatase activity, leading to a transient accumulation of androgens in the ovary, which in turn stimulates the expression of insulin-like growth factor I and other autocrine and paracrine factors and increases ovarian responsiveness to gonadotropins (72). The combination of HMG and letrozole can maximize its effect and reduce the dosage of HMG. Mu et al. (73) reported the successful induction of dominant follicle development and ovulation in a patient with ROS after ovulation promotion with letrozole combined with HMG, resulting in the live birth of a healthy male infant.

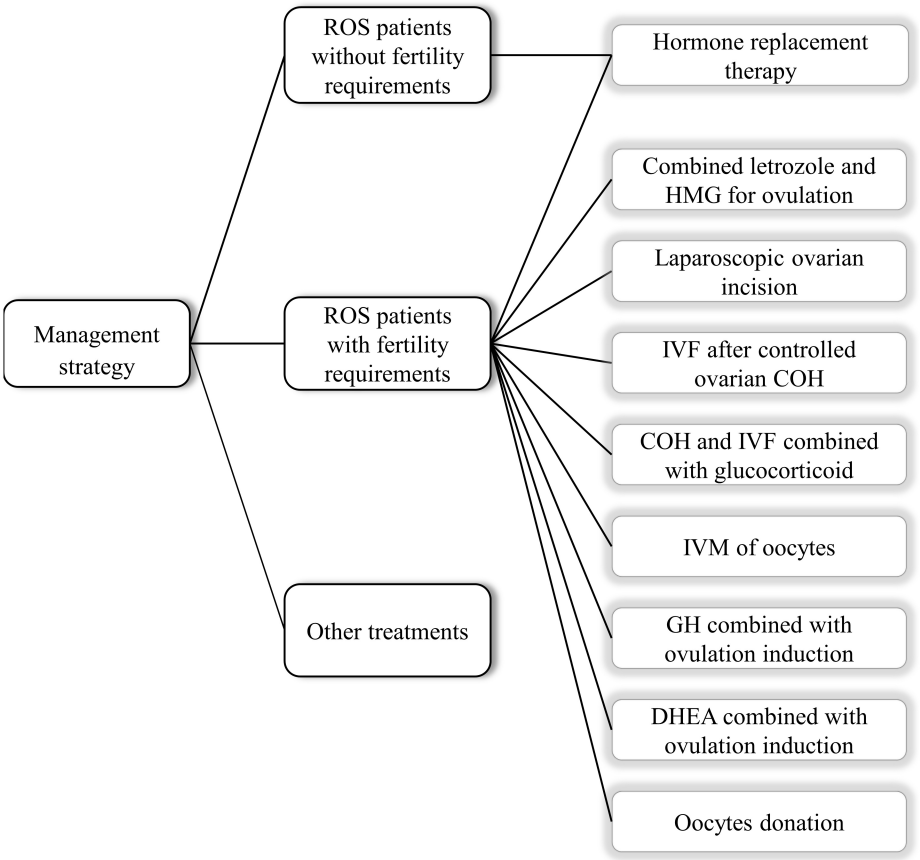

FIGURE 3
The specific clinical treatments of resistant ovary syndrome (ROS) patients.

Laparoscopic ovarian incision

In recent years, laparoscopy has been widely used in treating gynecological diseases due to its low invasiveness, good surgical field, rapid healing, low risk of infection, low impact on the abdominal organs and high safety profile. LOI is a novel and simple laparoscopic procedure that promotes follicular growth in patients with follicular maturation disorders by mechanical damage to the ovarian cortex (74) to provide the intrinsic stimulation needed for dormant follicles.

Tanaka et al. (75) performed LOI in 11 patients with ROS, followed by controlled ovarian hyperstimulation (COH) for at least 1 year. Four ROS patients became pregnant and delivered three healthy babies with one ongoing pregnancy.

Controlled ovarian hyperstimulation and *in vitro* fertilization

Unlike conventional COH, the COH scheme of ROS patients may be slightly different due to the lack of antibodies or receptors in the ovary in ROS patients and there is no clear clinical consensus on this.

Rogenhofer et al. (6) reported a 26 years-old woman with secondary amenorrhea with fertility appeal that was given HRT. On day 20 of the third cycle, Gonadotropin-releasing hormone agonist (GnRH-a) was used for downregulation, and 75IU rec-FSH-β and 225 IU HMG were injected subcutaneously on day 10 of downregulation. Ultrasound examination showed 12 dominant follicles 14 days after treatment. Intracytoplasmic sperm injection (ICSI) was performed after egg removal, and a boy was born naturally after transplantation. The patient received a steady and sustained suppression of gonadotropins, thus increasing ovarian sensitivity, which may be attributed to pituitary downregulation (76).

Controlled ovarian hyperstimulation and *in vitro* fertilization combined with glucocorticoids

Glucocorticoids (GC) are extremely important regulatory molecules in the body and play an important role in regulating the development, growth, metabolism and immune function of the body. In clinical practice, they are

widely used as anti-inflammatory and immunosuppressive agents (77). Dexamethasone is a long-acting GC widely used in allergic and autoimmune inflammatory diseases. Li et al. (78) reported a case of ROS with serological evidence of antibodies against FSHR, who eventually achieved a live birth after ovarian stimulation combined with dexamethasone treatment. Hydrocortisone is a short-acting GC with anti-inflammatory and anti-allergic effects and is widely used in immune disorders. Riestenberg et al. (79) documented a case of Ig-FSHR-related POI initially resistant to maximal dose gonadotropin stimulation that eventually underwent successful COH and oocyte cryopreservation using short-term high-dose prednisone for transient immunosuppression. The above two cases indicate that GC suppression of abnormal autoimmune antibodies may be used for ROS treatment.

In vitro maturation of oocytes

In vitro maturation is a method of obtaining immature cumulus-oocyte complexes from antral follicles with or without ovulation medication, culturing them *in vitro* under suitable conditions to mature to the MII stage, followed by IVF/ICSI to achieve pregnancy (80). Indications for IVM include cases of ROS with impaired oocyte maturation. Since 2013, several cases of ROS in women successfully conceiving following IVM have been reported clinically (62, 81–87). Galvao et al. (83) reported that nine patients with ROS underwent 24 IVM cycles and found that the live birth rate was 16.7% per started cycle and 33.3% per patient. Therefore, IVM offers a meaningful approach to fertility claims in ROS patients, but it is more costly.

Growth hormone combined with ovulation induction

It is widely acknowledged that the reproductive and growth axes often interact, and GH indirectly affects ovarian development and its sensitivity to gonadotropins during puberty through gonadotropins and insulin-like growth factor 1 (IGF-I) (88). Growth hormone deficiency or insufficiency leads to delayed puberty and affects normal ovarian function. Studies in recent years have shown that growth hormone can improve ovarian responsiveness, promote endometrial growth, improve ovulation treatment in patients with low ovarian response, and increase pregnancy rates with cyclic ovulation. However, the use of growth hormone in the promotion of ovulation in patients with ROS warrants further investigation (89–92). Mueller et al. (63) reported a case of ROS in which follicle growth was not successfully induced with growth hormone. There are no reports in the literature on the effectiveness of growth hormones in promoting ovulation in patients with ROS.

Dehydroepiandrosterone combined with ovulation induction

Dehydroepiandrosterone (DHEA) is a steroid abundant in human blood circulation (93). It enters the circulation mainly in the form of DHEA sulfate (DHEA-S), which has weak androgenic effects and is converted to testosterone and estradiol in peripheral tissues (94). Interestingly, Zangmo et al. (95) found an increase in the number of oocytes, fertilization rates, and whole embryos in IVF cycles in patients with poor ovarian response to DHEA. Similarly, some studies confirmed that DHEA could optimize the fertility of POI patients and lead to successful pregnancy (96, 97). However, Wong et al. (98) found no benefit of DHEA supplementation on functional improvement in POI patients through a prospective observational study.

Oocyte donation

With the development of assisted reproductive technology, the technical challenges of oocyte donation have largely been resolved, but the ethical issues it raises are also a hot topic of the current debate on the technology (99). It is widely thought that donor oocytes can be used for *in vitro* fertilization transplantation only in ROS patients who have not responded well to long-term ovulation-promoting drugs.

Other treatments

In addition to the above treatments, appropriate calcium and vitamin D supplementation can prevent osteoporosis due to estrogen deficiency in ROS patients (100); appropriate psychological support is also helpful in restoring follicular development and ovulation in ROS patients (101). In addition, Chinese medicine has been reported to assist in treating ROS patients *via* kidney tonification. However, specific treatment effects need to be studied in large sample size studies.

Conclusion

Research is still ongoing to understand the complex pathogenesis of ROS, given its intricacy. The exact mechanisms remain largely unclear, and currently available approaches are often ineffective. The heterogeneity in the etiology of ROS account for the wide range of individual treatment options, and treatment modalities such as psychological support, artificial cycles, and ovulation promotion do not address the root of the patient's problem. The poor effectiveness of treatment is often accompanied by the psychological and financial strain of long-term medication on patients. Accordingly, an in-depth understanding of the pathogenesis of the disease is an important prerequisite for studying ROS management strategies. With the

development of assisted reproductive technology, new assisted reproductive methods such as IVM, GH, and DHEA addition may be able to solve the fertility problems of this patient population, but more comprehensive and effective management strategies need to be further investigated.

Author contributions

LL conceived and directed the manuscript writing. ZM reviewed the literature and wrote the manuscript. SS reviewed the literature. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Long-term gynecological complications after conservative treatment of placenta accreta spectrum

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Objective: To examine the association between conservative treatment for PAS (placenta accreta spectrum) and subsequent gynecological and fertility complications.

Methods: All women who underwent conservative treatment for PAS between January 1990 and December 2000 were included in this retrospective cohort study conducted in a tertiary teaching hospital. Gynecological and fertility complications experienced after the index delivery were collected from the medical records and telephone questionnaires. This data was compared to an age and parity-matched control group of women without PAS.

Results: The study group included 134 women with PAS managed conservatively and 134 controls with normal deliveries matched by parity and age. Women in the PAS group required significantly more postpartum operative procedures such as hysteroscopy or D&C (OR = 6.6; 95%CI: 3.36–13.28; $P = <0.001$). Following the index delivery, there were 345 pregnancies among 107 women who attempted conception following conservative treatment for PAS vs. 339 pregnancies among 105 women who attempted conception in the control group. Among women who attempted conception following conservative treatment for PAS 99 (92.5%) delivered live newborns (a total of 280 deliveries) vs. 94 (89.5%) in the control group, (a total of 270 live newborns, $p = 0.21$). The need for fertility treatments was not different between the two groups (OR = 1.22; 95%CI: 0.51–2.93; $P = 0.66$).

Conclusion: After conservative treatment for PAS, significantly more women required complementary procedures due to retained placenta and/or heavy vaginal bleeding. There was no evidence of fertility impairment in women post-conservative treatment for PAS.

KEYWORDS

conservative treatment, gynecological complications, fertility, invasive placentation, placenta accreta spectrum

Synopsis

Following conservative treatment for invasive placentation women required more additional procedures such as hysteroscopy or dilatation and curettage due to retained placental tissue and heavy vaginal bleeding. There was no evidence of fertility impairment in this population.

Introduction

Placenta accreta spectrum (PAS) is a major obstetric complication with rising incidence (1–9) caused by abnormal uterine placental attachment due to the absence of the decidua basalis (10). PAS is a general term encompassing abnormal placentation with varying degrees of involvement (total, partial or focal) and levels of invasion (placenta accreta, increta, and percreta) (10). Recent guidelines (11) suggest a planned cesarean–hysterectomy for cases with high suspicion of PAS, thus, avoiding the risk **of** complication (e.g., hemorrhage) (12).

We previously reported obstetrical outcomes following conservative treatment for PAS using an extirpative technique (13). Similar to other studies we found a higher risk of postpartum hemorrhage and recurrent PAS in subsequent pregnancies. However, when PAS is diagnosed yet uterine conservation was achieved, women may attempt future pregnancies (14). There is a paucity of data regarding other parameters such as quality of life, impact on fertility, and gynecological outcomes after this conservative management. Prior publications of case reports and case series focused on pregnancy outcomes but did not examine other gynecologic problems and subsequent fertility outcomes (15–18).

In this retrospective observational study, we aimed to compare those who underwent conservative treatment for PAS versus a matched cohort of women who did not have PAS for gynecological complications and subsequent fertility.

Methods

This retrospective cohort study was performed in the Feto-Maternal unit of Hadassah-Hebrew University Medical Center in Jerusalem, Israel, a tertiary teaching hospital. The Institutional Review Board of Hadassah Medical Organization approved the study (decision number 0263-10-HMO), and verbal consent was obtained from all women during a telephone questionnaire.

Our database is described previously (13, 19) and summarized here briefly. The study group cohort comprised all women who underwent conservative treatment for PAS between January 1, 1990, and December 31, 2000, who could be contacted and agreed to participate. Women were allocated to the study group when met clinical or histopathological inclusion criteria for PAS.

The common practice in our unit, when there is a substantial risk for PAS in the ultrasound examination, we usually recommend doing an elective cesarean section with a multidisciplinary team to get prepared for massive bleeding and a hysterectomy when needed. However, in other cases when there is an assessment of PAS during the placenta extraction in normal vaginal delivery or cesarean section, we may consider conservative treatment that usually includes manual lysis of the placenta or D&C.

After using the common procedures of manual revision of uterine cavity or D&C we consider another complementary procedure in cases with clinical suspicion due to symptoms or signs of retained placenta parts in the ultra-sound exam and not as a determined procedure.

Conservative management of PAS was defined as the removal of the placenta with uterine preservation.

Demographic characteristics and matching criteria are presented in Appendix 1.

The control group cohort was selected from the labor and delivery unit electronic medical record following this process: each delivery with PAS from the study group was matched to a normal delivery composing the control group, and according to the consecutive chronological order. The study and control group were matched by maternal age, mode of delivery, and previous live births.

Once the study (prior PAS) and control (normal pregnancy and delivery) groups were identified, the hospital medical records and the Ministry of Health Central Bureau of Statistics data were reviewed to obtain information about obstetrics, fertility, and gynecological parameters until 2010. In addition, we conducted a complementary telephone questionnaire to obtain information regarding complications that appeared after the index delivery (e.g., postpartum operative intervention, the need for fertility treatments, etc.) (Appendix 2).

Outcomes

The primary outcome measure was unusual postpartum vaginal bleeding that required operative interventions. The secondary outcomes were menstrual cycle irregularity, gynecologic clinic visit frequency, and the need for fertility treatments.

Statistical analysis was performed using SPSS (SPSS Inc., Chicago, IL, USA). Continuous parametric variables are presented using mean SD, and the difference between the two groups was assessed using the student *t*-test. Categorical variables are presented as count (percentage), and the differences between the study and control group for each of the categorical variables were analyzed using $\chi 2$ or Fischer exact test. Odds ratios (ORs) were calculated using a multivariable logistic regression model and are presented with 95% confidence

TABLE 1 Questionnaire responses following the index delivery for women following conservative treatment for placenta accreta spectrum vs. age-parity matched controls.

Characteristics	Study group ($n = 134$)	Control group ($n = 134$)	OR (95% CI)	*P* value
Strong pain after delivery	18 (14.40%)	9 (6.98%)	2.24 (0.97–5.20)	0.06
Unusual bleeding after delivery	20(16.26%)	8 (6.15%)	2.96 (1.25–7.00)	0.01
Dilatation and Curettage (D&C) or hysteroscopy	53(40.77%)	12 (9.38%)	6.6 (3.36–13.28)	<0.0001
Increase in the number of gynecologic clinic visits	7 (5.22%)	5 (3.79%)	1.4 (0.43–4.52)	0.57
Change in the frequency of the menstrual cycle	14 (10.61%)	9 (6.92%)	1.59 (0.66–3.83)	0.29

Values are given as a number (percentage) unless stated otherwise.

TABLE 2 Pregnancy outcomes following the index delivery for women following conservative treatment for placenta accreta spectrum vs. age-parity matched controls.

Characteristics	Pregnancies in the study group ($n = 345$)	Pregnancies in the control group ($n = 339$)	OR (95% CI)	*P*-value
Term-delivery	267 (77.39%)	252 (74.33%)	1.18 (0.83–1.68)	0.35
Pre-term delivery	13 (3.77%)	19 (5.60%)	0.66 (0.32–1.36)	0.26
Miscarriage	57 (16.52%)	62 (18.29%)	0.88 (0.59–1.31)	0.54
Ectopic pregnancy	3 (0.87%)	3 (0.88%)	0.98 (0.20–4.90)	0.98
Termination of pregnancy	5 (1.45%)	3 (0.85%)	1.65 (0.39–6.94)	0.50

Values are given as a number (percentage) unless stated otherwise.

intervals. A $p < 0.05$ was considered statistically significant. Missing variables were not imputed.

Results

Over the ten-year study period, there were 34,450 deliveries at Hadassah-Hebrew University Medical Center, and 260 deliveries had prior PAS and met the study group inclusion criteria. Ninety-nine (38.1%) women were lost to follow-up, 5 (1.9%) women lacked a matched control, and 22 (8.5%) women declined participation. Thus, the final study group included 134 women following conservative treatment for PAS and 134 control women. The groups were similar for demographic and obstetrical parameters.

More women following conservative treatment for PAS reported an unusual vaginal bleeding during the postpartum period (OR = 2.96 95% CI 1.25–7.00; P = 0.01). Additionally, these women required significantly more postpartum operative procedures such as hysteroscopy or "dilation and curettage" secondary to abnormal uterine bleeding (40.8 vs. 9.4%; OR = 6.6 [95%CI = 3.36–13.28], $P = <0.0001$). There were no differences between women following conservative treatment for PAS vs. the control group for menstrual cycle frequency and gynecologic clinic visits in the subsequent years (Table 1).

Following the index delivery until 2010, there were 345 pregnancies among 107 women who attempted conception following conservative treatment for PAS vs. 339 pregnancies among 105 women who attempted conception in the control group. Many women had more than one pregnancy. Among women who attempted conception following conservative treatment for PAS 99 (92.5%) delivered live newborns (a total of 280 deliveries) vs. 94 (89.5%) in the control group, (a total of 270 live newborns, $p = 0.21$). There were no stillbirths in either group. The proportion of term deliveries, preterm deliveries, miscarriages, and ectopic pregnancies was similar in both groups (Table 2). The number of women who complained about strong pain after the index delivery was not significantly different between the groups (OR = 2.24; 95% CI 0.97–5.20; $p = 0.06$).

Following the index delivery, the subsequent need for fertility treatments was similar in both groups (OR = 1.22 95% CI 0.51–2.93; $p = 0.66$) (Table 3).

Discussion

The main finding of the study is that ∼40% of women who were treated conservatively for PAS required complementary procedures (i.e., hysteroscopy or dilatation and curettage), due to residual placenta and vaginal bleeding in the puerperium period. This finding emphasizes the importance of close monitoring and prompt intervention in the post-partum period

TABLE 3 Fertility treatments following the index delivery for women following conservative treatment for placenta accreta spectrum vs. age-parity matched controls.

	Study group ($n = 134$)	Control group ($n = 134$)	OR (95% CI)	*P*-value
Ovulation induction	7 (5.30%)	7 (5.30%)	NS	NS
IVF	5 (3.79%)	3 (2.27%)	1.22 (0.51–2.93)	0.66

Values are given as a number (percentage) unless stated otherwise. NS = Non-Significant.

in women who undergo conservative treatment for abnormal placentation.

Due to the high risk for intervention in this population, monitoring should include careful assessment of abnormal uterine bleeding and a sonographic evaluation upon necessity. Numerous studies have demonstrated the utility of postpartum ultra-sound in evaluating residual trophoblastic tissue (20–22). It should be noted that although ultrasound has multiple advantages in the evaluation of residual trophoblastic tissue, it has high false-positive results (23). By using a combination of clinical judgment and sonographic imaging, an accurate diagnosis can be made (24).

When residual trophoblastic tissue is suspected, the gold standard for evaluation is diagnostic hysteroscopy followed by removal of the residua by operative hysteroscopy (25, 26).

The fertility potential after conservative treatment for PAS is unknown. One theory considers these women to be at increased risk for unsuccessful embryo implantation due to abnormal placentation in a prior pregnancy. However previous studies on conservative treatment in cases of PAS found no adverse effect on fertility. Sentilhes et al. reported a retrospective study on women with a history of conservative management for PAS in France. Among 91 women, 9% had severe intrauterine synechiae and were amenorrheic; 30% desired more children; 24 women conceived 34 pregnancies, and 21 deliveries were resulting in healthy babies. The authors concluded that successful conservative treatment for abnormal placentation does not appear to hinder subsequent fertility (15). In our study, the fertility potential of women who underwent conservative treatment in cases of PAS was not affected in comparison to the control group.

This study addresses important issues regarding fertility and gynecological outcomes following conservative treatment for PAS. Additionally, our study provides important information for counseling women regarding their fertility potential and gynecological follow-up care.

In conclusion, approximately 40% of women undergoing conservative treatment for PAS require complementary procedures for uterine emptying, but future fertility potential is not affected.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and the Institutional Review Board of Hadassah Medical Organization approved the study (Decision Number 0263-10-HMO), and verbal consent was obtained from all women during a telephone questionnaire. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

SH, YE, and DK are the primary authors of this article. SH, YE, RH, HH, and DK developed the original design, conducted the study, and contributed to the writing of the final version of the article. All authors fulfilled the definition of authorship, contributed to the article, and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.992215/full#supplementary-material

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Efficacy of insulin in treating severe hypertriglyceridaemia in the third trimester of pregnancy

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This study aims to investigate the efficacy of insulin in treating severe hypertriglyceridaemia (HTG) during the third trimester of pregnancy. Women with severe HTG (TG $\geq$ 11.30 mmol/L) in the third trimester of pregnancy who received clinical examination and delivered in Hubei Maternal and Child Health Hospital from 01 January 2017 to 30 September 2021 were recruited. Patients with TG $\geq$ 11.30 mmol/L at 30–32 weeks of gestation were treated with a low-fat diet and insulin as the insulin treatment group. For the control group, patients with TGs of 5.65–11.30 mmol/L at 30–32 weeks of gestation who developed severe HTG (TG $\geq$ 11.30 mmol/L) before delivery were treated with a low-fat diet only. General maternal information, delivery, perinatal treatment and laboratory examination information were collected from electronic medical records and compared. We found that in the insulin treatment group, there were higher values of progestational body mass index (BMI) ($Z = -2.281$, $P = 0.023$), higher incidence of diabetes ($\chi^2 = 20.618$, $P < 0.001$) and higher incidence of fatty liver ($\chi^2 = 4.333$, $P = 0.037$) than in the control group but also a higher pregnancy weight gain compliance rate ($\chi^2 = 4.061$, $P = 0.044$). Laboratory examination before delivery revealed that compared with the control group, insulin treatment significantly decreased prenatal TG ($Z = -10.392$, $P < 0.001$), cholesterol ($Z = -8.494$, $P < 0.001$), low-density lipoprotein ($Z = -3.918$, $P<0.001$), apolipoprotein A1 ($t = 2.410$, $P = 0.019$), cystatin ($Z = -4.195$, $P < 0.001$), incidence of hypocalcaemia ($P = 0.036$), and absolute number of lymphocytes ($Z = -3.426$, $P = 0.001$). Delivery outcomes were also improved in the insulin treatment group compared with the control group, including lower neonatal weight ($Z = -2.200$, $P = 0.028$), incidence of macrosomia ($\chi^2 = 4.092$, $P = 0.043$), gestational age ($Z = -3.427$, $P = 0.001$), and rate of intensive care unit (ICU) conversion ($P = 0.014$). In conclusion, insulin therapy for HTG in the third trimester of pregnancy could increase the pregnancy weight gain compliance rate, decrease blood lipid levels and the incidence of severe complications such as HTG acute pancreatitis (HTG-AP), and improve pregnancy outcomes.

KEYWORDS

insulin, third trimester, severe, curative effect, hypertriglyceridaemia

Introduction

Typically, triglycerides (TGs) are mildly elevated during pregnancy and show a gradual decrease to prepregnancy levels after delivery at 6 weeks postpartum. However, hypertriglyceridaemia (HTG) (≥ 5.65 mmol/L) in pregnant women has shown an increasing trend in recent years in China and indicates pregnancy risk. Particularly in the third trimester of pregnancy (>28 weeks), severe HTG (TG ≥ 11.30 mmol/L) can lead to HTG acute pancreatitis (HTG-AP) and other severe complications, such as premature delivery, abortion and even maternal and/or infant death (1). Dietary adjustment is still the basis of lipid management for the treatment of severe HTG in the third trimester of pregnancy (2). The combination of omega-3 fatty acids in the diet could reduce the synthesis of liver TG, increase the activity of lipoprotein lipase (LPL) and safely reduce the TG level of pregnant women by 25–30%. Fenofibrate could be safely used for HTG in the second trimester of pregnancy with slow effects (3). Heparin can reduce serum TG levels, but long-term use of heparin results in reduced chylomicron decomposition, increased TG levels, and rebound HTG as well as potential risk of bleeding. Nicotinic acid was only used in early pregnancy with potential teratogenic effects (4). Blood purification treatment (including haemofiltration, haemoperfusion and plasmapheresis) was safely used to reduce TG levels during pregnancy by replacing deficient LPL or APO when HTG-AP occurred (5). Gene therapy for hyperlipidaemia was reported to reduce the risk of pancreatitis by 70%, but no clinical trials were conducted on pregnant women (6). Insulin was shown to reduce TG by enhancing LPL activity and degradation of chylomicrons. Intensive therapy with insulin during pregnancy was a safe, effective and inexpensive approach for TG reduction, expected to result in reductions of 50–75% within 2–3 days with obvious effects (7). However, there are few clinical studies on the treatment of severe hypertriglyceridaemia with insulin in the third trimester of pregnancy.

Low-fat diet treatment is required for hypertriglyceridaemia (HTG) (TG> 5.65 mmol/L) in pregnant women at 30–32 weeks of gestation in our hospital (The Maternal and Child Health Hospital of Hubei Province). With low-fat diet treatment, only approximately 5% of less severe HTG patients (TG of 5.65–11.30 mmol/L) would become severe HTG (TG ≥ 11.30 mmol/L) before delivery. However, for those with severe HTG (TG ≥ 11.30 mmol/L) at 30–32 weeks of gestation, there is a high risk of developing HTG-AP given low-fat diet treatment alone. Therefore, this study evaluated the efficacy of insulin treatment in patients with severe HTG at 30–32 weeks of gestation compared with a non-insulin-treated patients who had less severe HTG at 30–32 weeks of gestation but developed severe HTG before delivery. We found that patients in the insulin treatment group achieved even better clinical outcomes than those in the control group, although the latter had less severe HTG than the treatment group. Therefore, we concluded that insulin was effective in treating severe hypertriglyceridaemia in the third trimester of pregnancy.

Materials and methods

Objects and groups

This study was a retrospective study with parturients who were examined and delivered in the Maternal and Child Health Hospital of Hubei Province from 01 January 2017 to 30 September 2021. All the parturients provided written informed consent for the collection and publication of their medical information during their first visit to the hospital. This study was approved by the Ethics Committee of the Maternal and Child Health Hospital of Hubei Province [Approval Number: [2021] IEC (LW037)].

The objects and groups of this study are shown in the flow diagram (Supplementary Figure 1). Case inclusion criteria: (1) 18–45 years old; (2) examination and delivery in the Maternal and Child Health Hospital of Hubei Province; (3) delivery of a single live birth; (4) HTG in the third trimester (TG> 5.65 mmol/L) (8). Case exclusion criteria: (1) incomplete medical records; (2) non-compliance with medical advice for treatment.

There were a total of 118,622 live parturient women in the Maternal and Child Health Hospital of Hubei Province from 01 January 2017 to 30 September 2021. According to the inclusion and exclusion criteria, there were 48 severe HTG (TG ≥ 11.30 mmol/L) patients who received both a low-fat diet and insulin treatment (insulin treatment group, $n = 48$) and 7,056 with less severe HTG (TG of 5.65–11.30 mmol/L) who only received low-fat diet treatment at 30–32 weeks of gestation. Among the less severe HTG patients, 365 patients developed severe HTG (control group, n=365) before delivery, while the remaining 6,691 patients had non-progressive HTG (i.e., TG remained at 5.65–11.30 mmol/L). Four hundred non-progressive HTG patients were randomly selected for statistical analysis using 6,691 patient IDs as input and the R function "sample" for calculation.

Therapy

1) Low-fat diet: The main guideline for patients was as follows: Total fat should be limited to 20% of daily calories (9), and foods with a high glycaemic index should be avoided, supplemented with omega-3 fatty acids. The specific type of omega-3 supplements were fish oil soft capsules, and each capsule contained 1000 mg fish oil with 183 mg eicosapentaenoic acid (EPA) and 120 mg docosahexaenoic acid (DHA). The pregnant women took one capsule along with a meal twice a day.
2) Intensive insulin treatment: Insulin was administered when TG ≥ 11.30 mmol/L, with an initial dose of 0.1–0.3 U/kg body

weight/hour and continuous intravenous pumping. Blood glucose was monitored once every 1 to 4 h to adjust the pumping dose of insulin. When blood glucose was 8.30–11.10 mmol/L, it was necessary to guard against the occurrence of hypoglycaemia, and 5% glucose (glucose:insulin of (4–6 g):1 U) was to be given at the same time to prevent the occurrence of hypoglycaemia. Insulin pumping was suspended when TG $\leq$ 5.65 mmol/L. In the event of persistent hypoglycaemia occurs, insulin was to be discontinued (9).

3) Blood purification treatment was guided as follows: If pregnant women have HTG-AP and TG $\geq$ 11.3 mmol/L at 24–48 h after admission or the decrease in TG does not reach 50% after conservative treatment, blood purification treatment should be adopted, mainly including haemofiltration, haemoperfusion and plasmapheresis (5). For pregnant women without HTG-AP, if the decrease in blood lipids is not significant or the disease progresses and is complicated with hyperlipidaemia pancreatitis, pregnancy is terminated in time and combined with drug therapy and/or blood purification treatment (4).

Data collection

All information and data were obtained through the hospital information system.

1) General data of puerperas: hospitalization number, age, body mass index (BMI) before pregnancy, weight gain during pregnancy and determination of whether weight gain during pregnancy met the standard were included. A BMI $<$ 18.5 kg/m^2 was defined as low weight, and the recommended weight gain during pregnancy ranged from 12.5 to 18.0 kg. A BMI of 18.5–24.9 kg/m^2 was defined as normal body weight, and the recommended weight gain range during pregnancy was 11.5–16.0 kg. A BMI of 25.0–29.9 kg/m^2 was defined as overweight, and the recommended weight gain range during pregnancy was 7.0–11.5 kg. A BMI $\geq$ 30.0 kg/m^2 was defined as obesity, and the recommended weight gain during pregnancy ranges from 5.0 to 9.0 kg (10). The number of deliveries and complications, such as gestational hypertension, gestational diabetes mellitus, biliary stones and fatty liver, were all recorded.
2) Delivery: These data included methods of delivery (including vaginal delivery, cesarean section and forceps-assisted delivery), gender of the newborn, weight of the newborn, whether the newborn was macrosomic, gestational age of delivery, whether the newborn was premature, whether the newborn was asphyxia, whether the newborn was complicated with HTG-AP, whether the newborn was transferred to the neonatal intensive care unit (NICU) and whether the pregnant woman was transferred to the intensive care unit (ICU).
3) Laboratory examination before delivery: These data included TG, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), apolipoprotein (APO) A1, APO B, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (γ-GGTP), total bilirubin (TBIL), direct bilirubin (DBIL), albumin, urea nitrogen (BUN), creatinine, urinary inhibition, potassium, sodium, calcium, hypocalcaemia, white blood cells, platelets, absolute neutrophil count (ANC), absolute lymphocyte count (ALC), ANC/ALC (N/L) ratio, hypersensitive C-reactive protein (hs-CRP) and plasma D-dimer.
4) Blood purification treatment: These data included haemoperfusion, plasma exchange and continuous renal replacement therapy (CRRT).

All data were merged into a complete database by hospitalization numbers.

Quality control

One obstetrician and one ICU doctor were selected for standardized guidance and training. Information on obstetrician patients (including pregnant women transferred to the ICU) with TGs $\geq$ 11.30 mmol/L was screened through the clinical laboratory system. The electronic medical record system was matched according to name and hospitalization number, and basic information about pregnant women and related treatment information were checked in electronic medical records. Those eligible for inclusion had to be registered with complete medical records.

Statistical analysis

SPSS 25.0 software was used for statistical analysis. Measurement data conforming to a normal distribution were described by ($x \pm$ s), and comparison between groups was performed by t-test (or corrected t-test). Measurement data that did not conform to a normal distribution were described by M (P_{25}, P_{75}), and the rank-sum test was used for comparisons between groups. Statistical data are expressed as the number of cases and percentage. The χ^2 test (or Fisher's exact probability method) was used for intergroup comparisons, and the trend test was used for intergroup comparisons of grade variables. $P < 0.05$ was considered statistically significant.

Results

Comparison of the general data of puerperas

To evaluate the effects of insulin treatment, we compared the general maternal data. We observed that the insulin treatment

TABLE 1 Comparison of general data of puerperas.

Item	Control group ($n = 365$)	Insulin treatment group ($n = 48$)	χ^2/Z– value	P–value
Age (P_{25}, P_{75})	31 (28, 34)	31 (29, 34)	−0.050	0.960
Progestational BMI (P_{25}, P_{75})	21.9 (20.7, 23.6)	22.3 (21.6, 24.6)	−2.281	0.023
Pregnant women with weight up to standard rate, %	74.2	87.5	4.061	0.044
Rate of primiparity, %	58.9	54.2	0.392	0.531
Rate of hypertension during pregnancy, %	22.5	20.8	0.065	0.798
Gestational diabetes			20.618[a]	<0.001[a]
Rate of pregnant women without diabetes, %	65.8	39.6		
Rate of pregnant women with diet–controlled diabetes, %	30.7	41.7		
Rate of pregnant women with insulin–treated diabetes, %	3.6	18.8		
Rate of pregnant women with biliary calculi, %	0.3	2.1	0.350	0.554
Rate of pregnant women with fatty liver, %	0.3	4.2	4.333	0.037

[a]Trend test the unmarked: rank-sum test or chi-square test. The measurement data conforming to the normal distribution were described by x ± s, the measurement data not conforming to the normal distribution were described by M (P_{25}, P_{75}), and the counting data were presented by percentage.

group had patients with higher BMIs before pregnancy ($Z = -2.281$, $P = 0.023$) and a higher proportion of pregnant women with diabetes ($\chi^2 = 20.618$, $P < 0.001$) and fatty liver ($\chi^2 = 4.333$, $P = 0.037$) than the control group. There were no significant differences in age, primiparity, hypertension during pregnancy and presence of biliary calculi between the treatment and control groups. However, the insulin treatment group achieved a greater gestational weight gain compliance rate ($\chi^2 = 4.061$, $P = 0.044$) than the control group (Table 1). These results suggested that insulin treatment could improve the gestational weight gain compliance rate.

Comparison of laboratory examination before delivery

Laboratory examination and the percentage of blood purification treatments before delivery were compared between the insulin treatment and control groups. Patients in the insulin treatment group had lower prenatal TG ($Z = -10.392$, $P < 0.001$), cholesterol ($Z = -8.494$, $P < 0.001$), LDL ($Z = -3.918$, $P < 0.001$), APO A1 ($t = 2.410$, $P = 0.019$), cystatin ($Z = -4.195$, $P < 0.001$), incidence of hypocalcaemia ($P = 0.036$), and ALC ($Z = -3.426$, $P = 0.001$) and higher DBIL ($Z = -2.338$, $P = 0.019$), N/L ratio ($Z = -2.751$, $P = 0.006$), and Ca in blood ($Z = -2.944$, $P = 0.003$) (Table 2). There were no significant differences between the treatment and control groups for other laboratory examinations, including HDL, APO B, TBIL, albumin, blood BUN, creatinine, K, Na, white blood cells, platelets, ANC, hs-CRP, D–dimer contents and ALT, AST and γ-GGT activities in blood. The percentages of all blood purification treatments (blood perfusion, plasma exchange and CRRT) were also not significantly different between the two groups. These results indicated that insulin was effective in the treatment of severe hypertriglyceridaemia in the third trimester of pregnancy.

Comparison of delivery outcomes

Delivery outcomes were compared between the insulin treatment group and the control group. We found earlier gestational age ($Z = -3.427$, $P = 0.001$), lower neonatal weight ($Z = -2.200$, $P = 0.028$), lower incidence of macrocephaly ($\chi^2 = 4.092$, $P = 0.043$) and lower incidence of maternal referral to ICU treatment ($P = 0.014$) in the insulin treatment group than in the control group (Table 3). There were no significant differences in delivery mode, gender of newborn, neonatal asphyxia rate, incidence of premature delivery, HTG-AP or transfer to NICU treatment between the two groups. These results revealed that insulin treatment improved the delivery outcomes of severe hypertriglyceridaemia in the third trimester of pregnancy.

Discussion

The low incidence of severe HTG restricts the study of the risk factors for this disease. Therefore, there is still a lack of clear guidance for the prevention of severe HTG. HTG is mostly caused by gene mutation or familial HTG and non-hereditary and non-familial factors, such as pregnancy, obesity, alcohol dependence, uncontrolled diabetes, hypothyroidism, chronic renal failure and drug usage (namely, estrogen drugs, glucocorticoids, immunosuppressants and antipsychotics) (11). In this study, we found that a higher BMI before pregnancy and a higher proportion of pregnant women with diabetes and fatty liver were associated with earlier occurrence of severe HTG in

TABLE 2 Comparison of laboratory examination and blood purification treatment before delivery.

Item	Control group ($n = 365$)	Insulin treatment group ($n = 48$)	χ^2/Z–value	P–value
TG (P_{25}, P_{75})	13.47(12.06, 15.85)	8.67 (8.37, 10.52)	−10.392	< 0.001
Cholesterol (P_{25}, P_{75})	8.25 (7.29, 9.63)	5.72 (4.69, 6.61)	−8.494	< 0.001
LDL (P_{25}, P_{75})	3.26 (2.48, 3.81)	2.23 (1.22, 3.23)	−3.918	< 0.001
HDL (P_{25}, P_{75})	1.37 (1.16, 1.56)	1.40 (1.06, 1.81)	−1.048	0.295
APO A1	2.32 ± 0.38	2.13 ± 0.53	2.410[c]	0.019[c]
Apo B	1.22 ± 0.28	1.20 ± 0.44	0.363[c]	0.718[c]
ALT (P_{25}, P_{75})	9.0 (7.0, 12.0)	10.0 (7.0, 14.7)	−1.067	0.286
AST (P_{25}, P_{75})	17.0 (14.4, 20.0)	17.6 (15.0, 22.0)	−0.963	0.335
γ-GGT (P_{25}, P_{75})	12.0 (8.4, 18.0)	15.7 (8.0, 20.5)	−1.112	0.266
TBIL (P_{25}, P_{75})	7.0 (5.4, 8.7)	7.2 (5.3, 10.5)	−0.995	0.320
DBIL (P_{25}, P_{75})	1.1 (0.7, 1.9)	1.4 (0.8, 2.6)	−2.338	0.019
Albumin (P_{25}, P_{75})	35.3 (33.5, 37.1)	34.9 (33.8, 36.0)	−1.137	0.255
BUN (P_{25}, P_{75})	3.69 (3.09, 4.40)	3.76 (2.93, 4.86)	−0.700	0.484
Creatinine (P_{25}, P_{75})	44.7 (38.3, 50.0)	45.5 (38.9, 56.8)	−1.036	0.300
Cystatin (P_{25}, P_{75})	1.25 (1.08, 1.51)	1.10 (0.91, 1.24)	−4.195	< 0.001
K (P_{25}, P_{75})	3.97 (3.79, 4.16)	4.05 (3.85, 4.23)	−1.435	0.151
Na (P_{25}, P_{75})	135.0 (133.4, 136.0)	134.9 (132.8, 135.9)	−1.450	0.147
Ca (P_{25}, P_{75})	2.27 (2.20, 2.34)	2.30 (2.24, 2.39)	−2.944	0.003
Rate of Hypocalcaemia	8.2	0.0	—	0.036[b]
Leukocytes (P_{25}, P_{75})	8.59 (7.31, 10.15)	8.68 (7.48, 10.42)	−0.496	0.620
Platelets (P_{25}, P_{75})	196 (158, 232)	186 (153, 216)	−1.097	0.273
ANC (P_{25}, P_{75})	6.40 (5.29, 7.75)	6.64 (5.23, 7.94)	−0.594	0.552
ALC (P_{25}, P_{75})	1.51 (1.27, 1.91)	1.26 (1.03, 1.72)	−3.426	0.001
N/L (P_{25}, P_{75})	4.07 (3.16, 5.28)	5.17 (3.24, 9.33)	−2.751	0.006
hs–CRP (P_{25}, P_{75})	2.76 (1.49, 4.68)	2.92 (1.37, 4.66)	−0.250	0.802
Plasma D–dimer (P_{25}, P_{75})	1.80 (1.35, 2.59)	1.83 (1.31, 2.32)	−0.418	0.676
Rate of pregnant women receiving blood perfusion, %	0.5	2. 1	0.075[a]	0.784[a]
Rate of pregnant women receiving plasma exchange, %	0.8	0.0	—	1.00[b]
Rate of pregnant women receiving haemofiltration, %	0.5	0.000	—	1.00[b]

TG, triglyceride; LDL, low–density lipoprotein; HDL, high–density lipoprotein; APO, apolipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GGT, gamma glutamyl transferase; TBIL, total bilirubin; DBIL, direct bilirubin; BUN, urea nitrogen; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; N/L, ANC/ALC; hs–CRP, hypersensitive C–reactive protein.

[a]Corrected chi–square test, [b]Fisher's exact test, [c]Corrected t–test, the unmarked: rank-sum test. The measurement data conforming to the normal distribution were described by x ± s, the measurement data not conforming to the normal distribution were described by M (P_{25}, P_{75}), and the counting data were presented by percentage.

late pregnancy. In addition, less severe HTG patients (5.65–11.30 mmol/L) at 30–32 weeks of gestation who developed severe HTG before delivery, even with low-fat diet treatment, had a higher progestational BMI, lower gestational weight gain compliance rate, higher TG and cholesterol before delivery, higher rates of macrosomia, HTG-AP and pregnant women shifted to the NICU during delivery than non-progressive HTG patients (Supplementary Table S1). Thus, overweight or obesity before pregnancy could be a risk factor for severe HTG, and weight control during pregnancy could improve pregnancy outcomes. Pregnant women with elevated TGs in early pregnancy had an increased risk of gestational diabetes and macrosomia (12). Therefore, for intervention with a low-fat diet and exercise, the optimal time to screen for dyslipidaemia is prepregnancy or early pregnancy (13). For late pregnancy, proper weight gain during pregnancy, management of pregnancy complications (diabetes, hypothyroidism, etc.) and intensive insulin treatment should also be emphasized.

Approximately 15–20% of severe HTG patients develop HTG-AP (7), and up to 50% of acute pancreatitis in pregnancy is associated with HTG (14). HTG is the second major cause of acute pancreatitis in China, and the trend is that it occurs in younger pregnant women with more severe disease (15). Acute pancreatitis in pregnancy (APIP) has led to maternal and fetal mortality rates of 37 and 60%, respectively, in the past (4). With the improvement of timely identification and treatment, the mortality rate has decreased to 1 and 20%, respectively, which is better but still threatens the health of

TABLE 3 Comparison of delivery between insulin treatment group and control group.

Item	Control group ($n = 365$)	Insulin treatment group ($n = 48$)	χ^2/Z–value	P–value
Delivery way			0.818	0.624
Rate of eutocia, %	32.6	27.1		
Rate of cesarean delivery, %	65.8	72.9		
Rate of forceps delivery, %	1.6	0.0		
Rate of delivery boy, %	52.1	60.4	1.191	0.275
Neonatal weight (P_{25}, P_{75})	3400 (3093, 3700)	3200 (2805, 3605)	−2.200	0.028
Rate of macrosomia, %	16.4	4.2	4.092[a]	0.043[a]
Rate of neonatal asphyxia, %	1.4	4.2	0.667[a]	0.414[a]
Delivery gestational age (P_{25}, P_{75})	39.0 (38.1, 39.4)	38.1 (37.2, 39.1)	−3.427	0.001
Percentage			0.733	1.000
Rate of mature birth, %	89.9	91.7		
Rate of premature birth, %	9.9	8.3		
Rate of stillbirth, %	0.3	0.0		
Rate of HTG–AP, %	2.7	0.0	—	0.614[b]
Rate of pregnant women shifted to NICU, %	7.7	8.3	0.000	1.000
Rate of pregnant women shifted to ICU, %	10.4	0.000	—	0.014[b]

HTG–AP, hypertriglyceridaemia acute pancreatitis; NICU: neonatal intensive care unit; ICU, intensive care unit.
[a]Corrected chi–square test, [b]Fisher's exact test, the unmarked: rank-sum test or chi-square test. The measurement data conforming to the normal distribution were described by x ± s, the measurement data not conforming to the normal distribution were described by M (P_{25}, P_{75}), and the counting data were presented by percentage.

mothers and children (2). Consistent with a previous study (4), the incidence of HTG-AP in this study was 0.8/10000 (10/118622), all of which occurred in the control group in contrast with none in the insulin treatment group, suggesting an effect of insulin in the third trimester on preventing the occurrence of HTG-AP. Therefore, in midwifery institutions, it is recommended that blood lipid levels be monitored every 1–2 weeks for pregnant women with TG > 5.65 mmol/L in the third trimester of pregnancy and intensive insulin therapy be initiated in a timely manner when TG > 11.30 mmol/L to reduce the occurrence of HTG-AP and avoid adverse pregnancy outcomes.

There were some limitations in this study. The timing of severe HTG occurrence in the treatment and control groups was not the same; this difference might introduce bias but not alter the conclusion. This treatment group in this study included patients with severe HTG (TG ≥ 11.30 mmol/L) that occurred at 30–32 weeks of gestation who were treated with a low-fat diet and insulin. The best control group would have been severe HTG patients at the same stage who were treated with a low-fat diet. However, the incidence of severe HTG in pregnancy from our hospital was 0.348% (413/11862). Among them, only 48/413 severe HTG cases occurred at 30–32 weeks of gestation. The remaining severe HTG (365/413) patients had TGs of 5.65–11.30 mmol/L at 30–32 weeks of gestation, received low-fat diet treatment, but still developed severe HTG before delivery (at 37–41 weeks of pregnancy). Their conditions were not as severe as in the treatment group due to delayed disease occurrence as well as lower TG and BMI before pregnancy and the proportion of pregnant women with diabetes and fatty liver at 30–32 weeks of gestation. However, patients in the insulin treatment group achieved even better clinical outcomes, including higher gestational weight gain compliance rates, lower TG levels before delivery and better delivery outcomes than those in the control group. Therefore, we concluded that insulin was effective in treating severe HTG in the third trimester of pregnancy. In the future, a study of large samples from multiple centers can be designed to analyse the risk factors for severe HTG more comprehensively and dynamically and to determine the effects of insulin on HTG at the same level of severity. Moreover, the standardized management model for severe HTG during pregnancy should be adopted to reduce bias in the study as well as to prevent HTG-AP occurrence.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Maternal and Child Health Hospital of Hubei Province. The patients/participants

provided their written informed consent to participate in this study.

Author contributions

DZ: research design, implementation, data collection, analysis, and paper writing. GS and JH: participated in research design and guidance. QG: participated in research design, implementation, supervision of data collection and analysis, paper revision, and financial support. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.977620/full#supplementary-material

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Impact of progesterone-free luteal phase support following natural cycle frozen embryo transfer: Study protocol for a multicenter, non-inferiority, randomized controlled trial

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Introduction: Nowadays, frozen-thawed embryo transfer (FET) has become one of the standard treatments for infertility in the field of assisted reproductive technology (ART). Natural cycle FET (NC-FET) has many advantages, such as simplicity and economics, no effect on patients' menstrual cycles, estrogen and progesterone levels, as well as no interference in endometrial growth and transformation, which is aligned with the natural physiological state of embryo implantation. Nonetheless, there is a controversy regarding the need for luteal phase support (LPS) during NC-FET cycles. The purpose of this study is to assess whether LPS was not inferior to non-LPS in terms of OPR in NC-FET cycles.

Methods and analysis: This study including 1,010 ovulatory women undergoing *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles with an elective freeze-all strategy followed by NC-FET will be performed at four university-affiliated reproductive centers. Participants will be randomly assigned in a 1:1 ratio to receive LPS treatment or not. This study is designed as an open-label, non-inferiority, randomized controlled trial (RCT), and the primary statistical strategies were intention-to-treat (ITT) and per-protocol (PP) analysis.

Discussion: There may not have been any significant difference in the chance of a live birth after FET if no progesterone was supplemental during the luteal phase. However, due to the limited number of previous studies, which are mainly retrospective, evidence is still limited. Thus, by conducting this multicenter RCT, we intend to evaluate whether LPS is necessary in NC-FET.

Ethics and dissemination: A Reproductive Ethics Committee of the Affiliated Hospital of Shandong University of Traditional Chinese Medicine (SDUTCM) has approved this study. This study will handle the data as required by general data protection regulations. Participants will sign a written informed consent

regarding participation in the study and storage of blood samples in a biobank for future research. This study will be monitored by study personnel trained in Good Clinical Practice who are not involved in the study. The results of this study will be disseminated through publication in international peer-reviewed scientific journals.

Clinical trial registration: [https://www.chictr.org.cn/], identifier [ChiCTR2200057498].

KEYWORDS

luteal phase support (LPS), natural cycle, frozen embryo transfer (FET), spontaneous ovulation, assisted reproductive technology (ART)

Highlights

- The first clinical trial to demonstrate that NC-FET without LPS can be non-inferior to NC-FET with LPS, minimizing the pain and need for medication.
- The purpose of this study is to provide more evidence-based support through a multicenter, non-inferiority, randomized controlled trial.
- Physicians and participants could not be blinded to treatment allocation.

Introduction

In recent years, with the significant improvement of ovarian stimulation regimens and embryo cryopreservation and thawing techniques, the frozen-thawed embryo transfer (FET) procedure has gained more popularity in assisted reproductive technology (ART) than fresh embryo transfer (1). Additionally, it has been shown that FET is effective in reducing the incidence of ovarian hyperstimulation syndrome (OHSS), preserving remaining embryos, and increasing the cumulative live birth rate (LBR) (2, 3). In general, NC-FET is indicated for patients who experience regular ovulation (4, 5). The NC-FET is cost-effective, does not affect the menstrual cycle, does not interfere with estrogen and progesterone (P_4) levels, or endometrial growth and transformation, and conforms to the natural physiological state of embryo implantation, despite the disadvantages of repeated monitoring and high cancelation rates (6). Furthermore, a recent large retrospective study involving 14,421 cycles demonstrated a lower early pregnancy loss rate with NC-FET, while the LBR was higher.

In NC-FET cycles, the necessity of luteal phase support (LPS) is still controversial. An investigation of 84 UK IVF clinics revealed that, in natural FET cycles, 31% administer LPS always, while 44% administer it sometimes (7). In another online survey of 179 IVF clinics around the world (representing an estimated 39 thousand FET cycles annually), it was found that more than half (57%) of clinics are using LPS in natural FET cycles, and 49% of clinics are using P_4 exclusively (8). Despite common use, LPS is not universally used in natural FET cycles, as both surveys underscore. In 2013, a RCT with small sample size (n = 102) conducted by Eftekhar et al. comparing NC-FET with no LPS, LPS did not result in higher clinical pregnancy and implantation rates or lower miscarriage rates (9). Coincidentally, Waldman et al. reported similar results in their retrospective study (10). Nevertheless, several recent studies showed that patients undergoing LPS with vaginal P_4 in NC-FET had higher LBR and lower miscarriage rates (11–13). In addition to LPS with P_4, patients who received LPS by intramuscular human chorionic gonadotropin (hCG) had a higher ongoing pregnancy rate (OPR) in NC-FET, whereas the RCT results of Lee et al. indicated that this LPS protocol did not exert a similar effect on the OPR (14, 15). Recent meta-analyses indicated a positive impact of P_4 supplementation after FET in natural cycles (16–18), but further large RCTs are required to confirm these findings.

Therefore, it is still unclear whether or not we should perform LPS in NC-FET. Additionally, previous studies still have limitations such as single center, small sample size, large population heterogeneity, different types, routes and times of P_4 administration, and inconsistent study endpoint settings. Thus, to determine whether LPS affects reproductive outcomes in NC-FETs, we conducted this well-designed RCT.

Methods and analysis

Study design

The study is designed as an open-label, non-inferiority RCT, including reproductive centers of four tertiary care hospitals in mainland China. The flow chart and study process schedule are shown in **Figure 1** and **Table 1**.

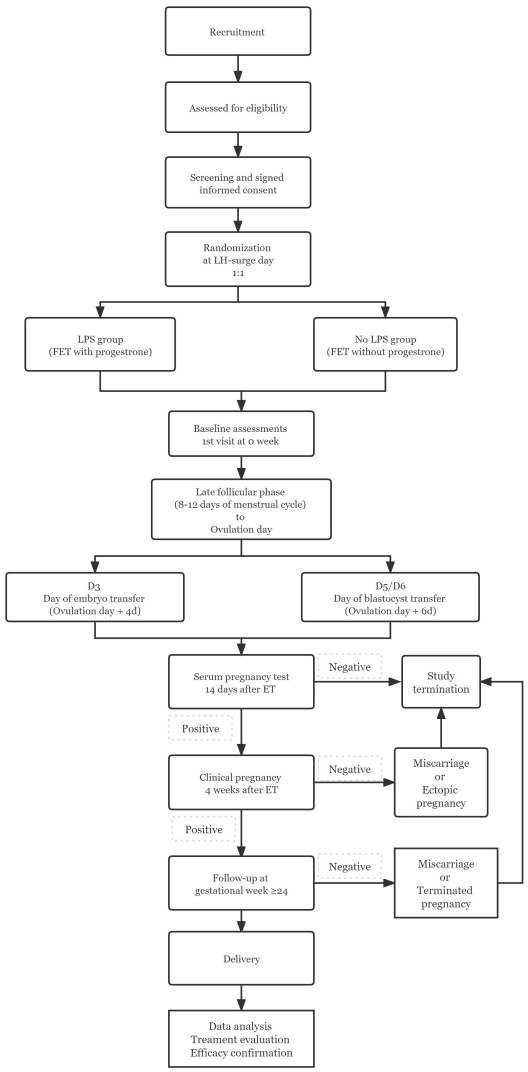

FIGURE 1
Study flow chart.

Objectives

At present, there is no clear evidence to suggest that LPS is required for NC-FET cycles following spontaneous ovulation. Therefore, there is still a fierce debate in clinical practice about whether patients treated with NC-FET should receive LPS. Our hypothesis is that no LPS is not inferior to OPRs in patients treated with LPS.

Eligibility criteria

Inclusion criteria

Having regular ovulatory cycle; at least one embryo or blastocyst available for transfer.

Exclusion criteria

Age $\geq$ 45 years old, body mass index (BMI) $\geq$ 30 kg/m^2; oocyte donation cycle; chromosomal abnormalities in both or one of the couples.; egg donation cycle; history of pelvic chemoradiotherapy; polycystic ovarian syndrome (PCOS); ovarian endometrioma, uterine fibroids, endometrial polyps or intrauterine adhesions requiring surgical treatment; history of repeated implantation failure (Infertile patients $<$ 40 years old experience $\geq$ 3 oocyte retrieval cycles, and fresh or FETs cumulatively transfer at least four high-quality embryos without clinical pregnancy) and recurrent pregnancy loss (two or more pregnancy losses). Patients may withdraw from the study at any time without reason.

Study population and recruitment

The study population include patients undergoing elective freeze-all strategy following IVF/ICSI cycles. Morphological evaluation of cleavage-stage embryos and blastocysts will be performed according to the Racowsky et al. (**19**) and the Gardner Scoring System (**20**), respectively. At least one grade I or II embryo with D3 blastomeres $>$ 6 or high-quality blastocysts with a score $\geq$ 3BB will be cryopreserved. The investigators will contact all patients interested and eligible for the study by telephone. Patients interested in participating in the study will visit the corresponding reproductive center on days 2–4 of the menstrual cycle, after having fully understood the content of the study. Here, they further understand the details of the study and sign an informed consent. Each individual may be included only once and only in the first FET cycle after the oocyte retrieval. All medical personnel in the study will receive all necessary information and training to uniformly handle patients at each reproductive center. All sites staff have sufficient experience in conducting clinical trials.

Randomization

Randomization will be performed by an independent statistician using a computer-generated randomization schedule with block randomization (block size of four) stratified by female age ($<$ or $\geq$ 35 years) at LH-surge day in NC-FET cycle. Patients will be randomized in a 1:1 ratio to one of the following groups: (i) LPS group: Patients will receive vaginal combined intramuscular P_4 treatment starting on the day of ovulation. (ii) Non-LPS group: Neither LPS nor any other therapies are administered to patients.

Interventions

Frozen-thawed embryo transfer will be performed in NC with spontaneous ovulation. Transvaginal ultrasonography

TABLE 1 Overview of study visits.

	Baseline * (2–4 days at menstruation)	Late follicular phase (8–12 days of menses) –ovulation day	D3 of embryo transfer (ovulation day + 4 days)	Blastocyst transfer (ovulation day + 6 days)	Day of pregnancy test (ET day + 14 days)	Pregnancy follow-up	Follow-up 1 year
Information and counseling	*						
Signing of informed consent	*						
Treatment-related data collection	*				*	*	*
Randomisation	*						
Transvaginal ultrasound scan	*	*				*	
Blood sample	*	*	*	*	*		
Quality of life questionnaire						*	

*All participants.

(TVUS) is used to monitor follicular development and endometrial growth. In the late follicular phase, that is, on days 8–12 of the cycle (depending on the length of the patient's menstrual cycle), when the dominant follicle develops to a mean diameter of more than 14 mm, serum LH level is monitored every other day until the LH surge appeared (21). When the endometrial thickness reaches more than 8 mm and the endometrium is classified as type A or B, embryo transfer is performed 4 days (D3 embryos) or 6 days (D5/D6 blastocysts) after the appearance of the LH surge. If luteinization of unruptured follicles occurs, embryo transfer is canceled. Plasma hCG levels were measured 14 days after embryo transfer.

Patients in the LPS group are given P_4 sustained-release gel Crinone® 8% (Crinone, Merck Serono, Switzerland) vaginally, 90 mg/time, once a day and P_4 injection (Zhejiang Xianju Pharmaceutical Co., Ltd., Zhejiang, China) intramuscularly, 20 mg/vial, twice a day until 14 days after FET (21). For pregnant women, LPS treatment should be continued until 10 weeks of gestation.

Data collection

Relevant treatment data of patients are collected at the corresponding time points: (1) basic data (2–4 days of menstruation); (2) dominant follicle development to an average diameter of more than 14 mm to the day of ovulation under TVUS monitoring; (3) D3 embryo transfer day (ovulation day + 4 days); (4) blastocyst transfer day (ovulation day + 6 days); (5) pregnancy test day (embryo transfer day + 14 days). In the case of pregnancy and delivery, data will be collected from the patient's medical records as well as the birth records of the newborn for registration of obstetric and neonatal outcomes up to 1 year after delivery. Any protocol deviations or unanticipated effects on the conduct of the trial will be registered. All study personnel will be trained in data collection and entry, handling of data discrepancies, and procedures performed during study visits. Data collection forms are available by contacting the study steering committee.

Blood sample collection

As part of the basic data stage, follicle-stimulating hormone (FSH), LH, estradiol (E_2), and P_4 should be measured, followed by LH, E_2, and P_4 until an LH surge occurs. E_2 and P_4 should be measured on the day of embryo transfer, and E_2, P_4, and β-hCG should be measured on the day of pregnancy test.

Research biobank and biobank for future research projects

In addition to samples for serial analysis, a total of 12 ml of blood samples (serum, and plasma) will be drawn at each sampling time and stored in a -20°/-80° freezer at Rigs Hospitalet. Samples will be identified by anonymous subject ID numbers to maintain subject confidentiality. In this study, the sample can be used as a backup of continuous analytical samples or stored in a biological sample bank for possible future research projects. Patients will be asked to sign a separate informed consent form to store the blood samples in a biobank for future research. Additional approval from the Ethics Committee will be required for future projects. If samples are not used, they will be destroyed according to the biomaterial destruction rules after the end of the study or no later than 5 years after the last patient is enrolled.

Transvaginal ultrasound

During FET cycles, Transvaginal ultrasonography is required as per clinical routine. Transvaginal ultrasonography is used to determine endometrial thickness and antral follicle number on days 2–5 of menstruation in a treatment cycle. Endometrial thickness and the size of the dominant follicle are estimated in the late follicular phase, i.e., on days 8–12 of the cycle depending on the length of the patient's menstrual cycle. TVUS was repeated until the dominant follicle reached more than 17–18 mm and an LH surge appeared. If conception occurs, an early pregnancy scan will be performed at pregnancy 7–8 to assess fetal viability and crown-rump length.

Data management

According to the informed consent form signed by all study participants, the study staff and relevant regulatory authorities can directly access the relevant data of patients to study and follow up the relevant conditions of patients and facilitate the quality control. All data for the study will be uploaded into electronic case report forms in the study's electronic data capture system for ease of data integration and management. The study electronic data capture system had a complete audit trail based on anonymous subject identification numbers used in the study. The system will interval program numeric data to detect possible input errors in the study. This platform is protected by a password-protected access system. The system will automatically backup data daily and store it on the server. Documents containing patient identifying information will be stored separately in a document with limited access. Source documents will be reviewed by Good Clinical Practice-trained study personnel (not participating in the study) to ensure completeness and accuracy of the data. The reviewer will assess the overall quality of the data and confirm that the site meets the protocol requirements. Data will be processed in accordance with the Data Protection Act and approved by the Data Review Center. The principal study site will develop a unified data processing agreement form with other cooperative centers and strictly implement it.

Data sharing plan

Study data will be shared according to International Committee of Medical Journal Editors guidelines. Data sharing may occur with parties who provide the purpose associated with the detailed use of data. The other party's study must obtain the corresponding study approval. The study data will not be shared with the same team as the purpose of this study. Data sharing will take place 3 months after the publication of papers involving the study's primary and secondary outcomes. Any new research project must be conducted under the premise of approval. The party requesting data sharing will be charged accordingly.

Outcome measures

Primary outcome

The primary outcome is OPR. Ongoing pregnancy is defined as intrauterine pregnancy confirmed by TVUS examination at more than 12 weeks of gestation accompanied by normal fetal heart beats.

Secondary outcomes

(1) Positive pregnancy: positive pregnancy refers to serum β-hCG $\geq$ 10 mIU/ml 14 days after embryo transfer; (2) Embryo implantation rate: the number of gestational sacs determined by TVUS examination divided by the total number of embryos transferred; (3) Pregnancy loss: pregnancy loss refers to the loss of an intrauterine pregnancy that is less than 28 weeks gestational age; (4) Ectopic pregnancy: ectopic pregnancy refers to the implantation and development of embryos in sites other than the uterine coelom; (5) Multiple pregnancy: multiple pregnancy refers to the simultaneous presence of two or more fetuses in the uterine cavity; (6) Live birth: live birth refers to newborns with gestational age at delivery $\geq$ 24 weeks and heartbeat and respiration; (7) Pregnancy-related complications: including preeclampsia, gestational hypertension, cesarean section, and postpartum hemorrhage (>1,000 ml); (8) Obstetric complications: including gestational diabetes (GD), placental pathology (accreta, previa), specify spontaneous preterm birth or induced preterm birth; macrosomia; small for gestational age, large for gestational age; low birth weight: [absolute weight, relative weight compared to mean at specific gestational local reference curve (p or Z-value)], and perinatal death.

Non-inferiority design and power calculation

The present study will adopt the non-inferiority design. Due to previous studies showing that NC-FET treated with LPS does not achieve the same outcome as NC-FET treated without LPS, and that treatment without LPS has the advantages of being less costly and less painful. Specifically, we assumed a 10% margin of non-inferiority and a 50% OPR in two arms based on our center's experience with FET. The number of cases in each group was calculated at 429, according to the design of 1:1 parallel non-inferiority, the unilateral test with alpha = 0.05, and the power of 90%. Assuming a drop rate of 15%, there are 505 cases per group and 1,010 cases for the two groups.

Drop-outs and cancel cycles

Dropout refers to the study participants' voluntary decision to withdraw from the study due to personal subjective factors. The canceled cycles were those who were forced to cancel the cycles due to endometrial lesions found in TVUS, failure of follicular development or failure of embryo thawing and resuscitation up to 21 days in the cycle. The researchers will make a detailed summary of the number and causes of dropout and cancelation in both groups and completion characteristics within and between groups. Based on our experience, the dropout rate will be up to 15%. If the actual dropout rate is higher than expected, we will discuss the potential bias, analyze the differences between the results and draw conclusions accordingly.

Statistical analysis and interpretation of data

The intention-to-treat (ITT) analysis includes both dropout and cancelation cycles. The per-protocol (PP) analysis includes all patients who strictly followed the study protocol. In the as-treated analysis, patients who subsequently received LPS but not NC-FET are excluded, however, those who subsequently received LPS from NC-FET until 10 weeks of gestation are included. We measured OPRs and identified differences between groups based on relative risk (RR) and 95 confidence interval (CI). PP analyses will be also performed for all reproductive outcomes.

Continuous data were compared using Student's *t*-test and results are presented as mean (standard deviation, SD) or median (inter-quartile range, IQR). Categorical data with expected frequencies less than five is assessed using χ^2 analysis and Fisher's exact test. *P*-values less than 0.05 is considered statistically significant. Data analysis will be performed using SPSS 26.0 and R 4.1.3. Multivariate logistic regression analysis will be performed to identify variables independently associated with OPR.

Patient and public involvement statement

Patients and the public are not involved in developing research questions or study design. The results of the study will be disseminated to the participants and their families by telephone and the patient's attending physician.

Ethics and dissemination

This study has received ethical approval from the Reproductive Ethics Committee of the Affiliated Hospital of SDUTCM (SDUTCM-RM-2022076) and all participating hospitals. The researchers had obtained written informed consent from each patient participating in the study before the start of the study. Any amendments to the protocol that could affect the design, conduct, and safety of the study will be implemented after formal amendment by a committee. Data will be reviewed and approved by an external Data and Safety Monitoring Board. Details of data management will be given elsewhere in this paper.

It is sufficiently assured that the trial personnel's safety is assured. Whether they are administered LPS after NC-FET make the difference between the two regimens. We don't expect there to be a difference in the OPR between those who receive LPS and those who do not receive LPS. In most cases, the study will not cause discomfort or harm to the patient. When blood is drawn and P_4 is injected intramuscularly, the patient may feel pain and discomfort and may experience minor bruising. Neither will participants incur additional financial costs nor will they receive financial compensation for participating in the clinical study.

The study will be presented at national and international scientific meetings by presenting the results in scientific journals and the Chinese Clinical Trial Registry (ChiCTR) and published in high impact peer-reviewed international scientific journals for reproductive medicine. The results of the common interest will be reported in the public media.

Discussion

At present, there is no clear evidence that LPS is required for NC-FET cycles following spontaneous ovulation. Therefore, Weissman conducted a web survey of FET in 2020 involving 179 IVF centers in 56 countries involving 39152 FET cycles (8). In this survey, it was found that 44% of participants did not

administer LPS to patients during NC-FET cycles. At present, relevant studies have the limitations of multi-center, insufficient sample size, different types and routes of P_4 administration, and different administration time, so larger RCTs are needed for further study. We designed this multi-center large RCT by a group of experienced professionals for this purpose. In our hypothesis, patients without LPS do not experience a lower OPR than those who receive LPS treatment. Provided that the hypothesis is validated, we can minimize the pain and economic burden caused by medication. The results of this study may be implemented clinically immediately after publication. Consequently, we hope that this study will lead to the development of new standards in NC-FETs on both a national and international scale.

Trial status

The trial was registered on 14 March 2022 (ChiTR). The actual study start date was 1 July 2022 and the expected study end date was 31 December 2023. The enrollment start date was 15 July 2022; the anticipated enrollment end date was 31 May 2023.

Author contributions

J-YS and Z-GS participated in the conception, design, writing, and editing of the study protocol. W-JJ wrote the first draft. All authors were involved in the critical revision of this manuscript and approved the final version of the manuscript prior to submission.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Chinese patent medicines combined with hormone replacement therapy for premature ovarian failure: A Bayesian network meta-analysis

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Objective: The objective of this study was to compare the efficacy differences between Chinese patent medicines combined with hormone replacement therapy (HRT) in the treatment of premature ovarian failure (POF) by the Bayesian network meta-analysis (NMA) method.

Methods: Randomized controlled trials (RCTs) reporting Chinese patent medicine combined with HRT for POF included Medline (*via* PubMed), Embase, Cochrane Library, China National Knowledge Infrastructure Database (CNKI), Wanfang Database (Wanfang), VIP Database (VIP), and China Biology Medicine Database (CBM) from the inception of the databases to July 2022. Two researchers independently screened the articles, extracted data, and evaluated the quality. The literature that met the inclusion criteria was screened out, the quality and risk of bias of the included studies were assessed according to the Cochrane 5.1 manual and RevMan 5.4, and NMA was performed using Stata 15.0 and R software.

Results: Sixty-four RCTs involving 5,675 individuals containing 12 oral Chinese patent medicines combined with HRT were enrolled into the current NMA. The results showed that when compared with patients using only HRT, the total clinical response rate is greater in patients using HRT combined with one of these 12 oral Chinese patent medicines. Among them, Zuogui pills + HRT [odds ratio (OR) = 3.92; 95% credible interval (CrI) = 0.86, 23.84; SUCRA = 73.76%] is most likely to be the best intervention, and the suboptimal intervention is Guishen pills + HRT (OR = 3.22, 95% CrI = 1.16, 9.44, SUCRA = 70.60%).

Conclusion: Chinese patent medicines combined with HRT were more effective than HRT alone in the treatment of POF. Zuogui pills are good at decreasing follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and more effective in the improvement of total clinical response rate; Xuefu Zhuyu capsule is also good at decreasing FSH. Ziheche capsule is an expert in improving estradiol level; Kuntai capsule shows the lowest incidence of

adverse reactions. However, the quality of the literature included in this study is relatively low, so it may affect the results of the study. Therefore, higher quality and multi-center trial would be necessary for supporting these results.

Systematic review registration: [www.crd.york.ac.uk/prospero], identifier [CRD42022350587].

KEYWORDS

Chinese patent medicine, hormone replacement therapy (HRT), premature ovarian failure (POF), network meta-analysis (NMA), validity

Introduction

Premature ovarian failure (POF) refers to the cessation of ovarian function before the age of 40 and is one of the prominent problems and diseases of female reproductive health. In recent years, the incidence of POF has gradually increased and shows a younger trend. The incidence rate before the age of 40 is about 1%, and the incidence of POF is 1 in 100 women before 40 years of age and 1 in 1,000 women before 30 years of age (1, 2). The main pathogenesis of POF is related to iatrogenic factors, immune factors, and genetic factors. The clinical features of POF are hypoestrogenism or estrogen deficiency, elevated gonadotropin levels, and lack of mature follicles. Estrogen deficiency can cause menopausal symptoms, such as sweating, hot flashes, vaginal and urinary symptoms, and vaginal dryness. However, decreased fertility and even infertility are the top POF-related concerns for women of every reproductive age. In addition, the negative effects of POF include an increased risk of cardiovascular disease, osteoporosis, and sexual dysfunction (3, 4).

In, ESHRE published a guideline for premature ovarian insufficiency, and this review presented the hormone replacement therapy (HRT) options for women with ovarian failure until natural menopause (5). Studies showed that HRT can compensate for estrogen deficiency, resulting in relief of menopausal symptoms (6). Also, it reduces the risk of cardiovascular disease (7, 8) and the impact on bone health in the long run (9–11). Since HRT has been used for a long time and cannot restore ovarian function, some researchers began to find out whether only using traditional Chinese medicine or combined with HRT can enhance the efficacy and gradually restore ovarian function without increasing adverse reactions. A meta-analysis study showed that Kuntai capsule alone had no significant difference compared with HRT in improving clinical efficacy, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E_2) (12). With the publication of studies on Chinese patent medicine combined with HRT in the treatment of POF, a published systematic review has confirmed the advantages of Chinese patent medicine combined with HRT in the treatment of this disease (13). At present, there are a variety of proprietary Chinese medicines for the treatment of POF, but there are few related clinical studies, and there is a lack of objective evidence-based medical evidence to confirm their safety and effectiveness. At the same time, there is currently a lack of comparison between the efficacy of different Chinese patent medicines. So, it is difficult to evaluate the efficacy and safety of various Chinese patent medicines combined with HRT in the treatment of POF. Therefore, the aim of our study was to rank the effects of various interventions using directly or indirectly available evidence through a Bayesian network meta-analysis (NMA) to gain insight into the strengths and weaknesses of these interventions to provide evidence for clinical treatment.

Methods

Registration

This systematic review and NMA are reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA-NMA) statement. The study is registered with the International Prospective Register of Systematic Reviews (registration number CRD42022350587). Ethics approval or patient consent was not required, as all analyses were based on previously published studies.

Search strategies

Randomized controlled trials (RCTs) reporting Chinese patent medicine combined with HRT for POF included Medline (*via* PubMed), Embase, Cochrane Library, China National Knowledge Infrastructure Database (CNKI), Wanfang Database (Wanfang), VIP Database (VIP), and China Biology Medicine Database (CBM) from the inception of the databases to July 2022. The following Medical Subject Headings [MeSH] and keywords incorporating Boolean operators were applied: "premature ovarian insufficiency," "primary ovarian insufficiency," "POF," "Chinese herbal," "Traditional Chinese Medicine," "Chinese and Western Medicine," "capsule," "grain," "Oral liquid," "pill," "Dan," "Gao." We also implemented a recursive manual search to search full-text studies from obtained tracking bibliographies or similar systematic reviews to check for potentially qualified studies that we missed first.

Inclusion criteria

The **inclusion criteria** were constructed around the PICOS standard:

1)participants: The included subjects are all patients with POF [at least 4 months of oligomenorrhea or amenorrhea; two random measurements (> 4 weeks) of FSH > 40 IU/L] (14);

2)interventions: Chinese patent medicine combined with HRT;

3)comparison: HRT alone;

4)outcomes: main outcomes: total clinical response rate [(recovery number of cases + efficiency number of cases)/total number of cases]; secondary outcomes: serum FSH, LH, estradiol (E_2) levels, and adverse reactions;

5)study design: randomized clinical trial (RCT).

Exclusion criteria

The exclusion criteria are as follows:

1)observation group or control group combined with other treatment methods;

2)literature that cannot extract complete outcome indicators or the full text cannot be obtained;

3)self-control studies, non-randomized controlled trial, experimental studies, experience summary, reviews, and case reports;

4)excluded studies in which the full text could not be obtained, the same data were repeatedly published, the use of HRT courses were unreasonable, and there were no diagnostic criteria for the disease.

Literature screening and data extraction

Selecting the studies will be accomplished by importing them into Endnote 20 (version 20.1.0, Clarivate Analytics) to manage and remove duplicate entries. Having independently screened the literature to determine whether it meets inclusion criteria, two researchers then read the abstracts and full texts to determine whether they meet the criteria. Data extraction content includes publication, patient information, intervention and control measures, treatment course, and outcome indicators.

Quality evaluation

The quality of assessment was according to Cochrane Handbook for Systematic Reviews of Interventions (15), including the following seven domains. Each of these options was evaluated as high, low, or unclear. For selection bias, it was defined as low risk of bias if the study described the method of sequence generation and allocation concealment, otherwise it was considered high risk of bias. For performance bias, the study was considered low risk of bias if it describes the method used to blind subjects, otherwise it was considered high risk of bias. For measurement bias, studies were considered low risk of bias if they described all methods of blinding outcome assessors, otherwise high risk of bias. For follow-up bias, studies were considered low risk of bias if the study described completeness of outcome data for each primary outcome (including loss to follow-up, data excluded from analysis), and high risk of bias otherwise. For reporting bias, studies were considered low risk of bias if they described how systematic reviewers examined selective outcome reporting that may have occurred, and high risk of bias otherwise. For other biases, studies were considered to be at high risk of bias if the study design was imprecise, or reporting was significantly inconsistent with previous studies. Seven items were rated as "unclear risk" when the study did not mention relevant items. Any disagreement will be resolved through discussion with the superior researcher.

Certainty of the evidence

The grading of recommendations assessment, development, and evaluation (GRADE) approach (16–18) for NMA was used to rate the certainty of the evidence of NMA estimates. Comparisons were initially rated as high-quality evidence and were downgraded accordingly, based on study limitations, imprecision, inconsistency, indirectness, and publication bias. We downgraded the study quality by one level in the study limitation, including concerns about selection bias, performance bias, detection bias, attrition bias, reporting bias, or other bias; as for imprecision item, if the sample size is insufficient or an imprecise estimate of the wide confidence interval is produced in this comparison, we will downgrade. For inconsistencies, we downgraded it by one level if study heterogeneity was found in the comparison, particularly local inconsistencies between direct and indirect evidence. If heterogeneity is observed according to four areas, namely demographic disparities, interventions, outcome measurement, and indirect comparisons, indirect projects will be downgraded. For publication bias item, we judge by asymmetric funnel diagrams. After the above assessment, the quality of evidence will be classified into one of four levels, including high, moderate, low, and very low quality. Two investigators rated the certainty of consulting with a third party.

Statistical analyses

The direct pairwise meta-analysis and bias evaluated were performed with RevMan 5.4. Variables with continuous and

categorical effects were measured using the odds ratio (OR) and mean difference (MD). OR and MD were calculated using 95% credible interval [CrI]. In terms of heterogeneity, I^2 represents the statistical value of 25, 50, and 75% of mild, moderate, and high heterogeneity, respectively, and was used to measure the presence or absence of substantial heterogeneity (19).

Network transitivity was considered a crucially important assumption in NMA, and its evaluation will further directly influence our analysis (20). Therefore, to ensure that multiple treatment comparisons were sufficiently similar, we estimated transmissibility by comparing clinical and methodological characteristics (e.g., patients and experimental design) across all included studies (15). The STATA/SE version 15.0 (StataCorp, College Station, TX) was used to drawn network plots and comparison-adjusted funnel plot. We employed R Version 5.0 (Mac OS X 12_5_1) with the GeMTC package to conduct NMA. The parameters in GeMTC were set as follows: initial value, 2.5; number of simulation iterations, 50,000; number of annealing times, 20,000; thinning factor, 1; and number of chains, 4. The convergence of iterations can be monitored in terms of potential scale reduction factors (PSRFs). To rank the effects of the intervention, we used the cumulative ranking probability curve (SUCRA, surface under the cumulative ranking area) and found that higher SUCRA values indicate greater efficacy (21). We did not implement the hypothesis of consistency because of non-close loops. Finally, identifying evidence of small-sample effects in networks by plotting comparative corrected funnel plots.

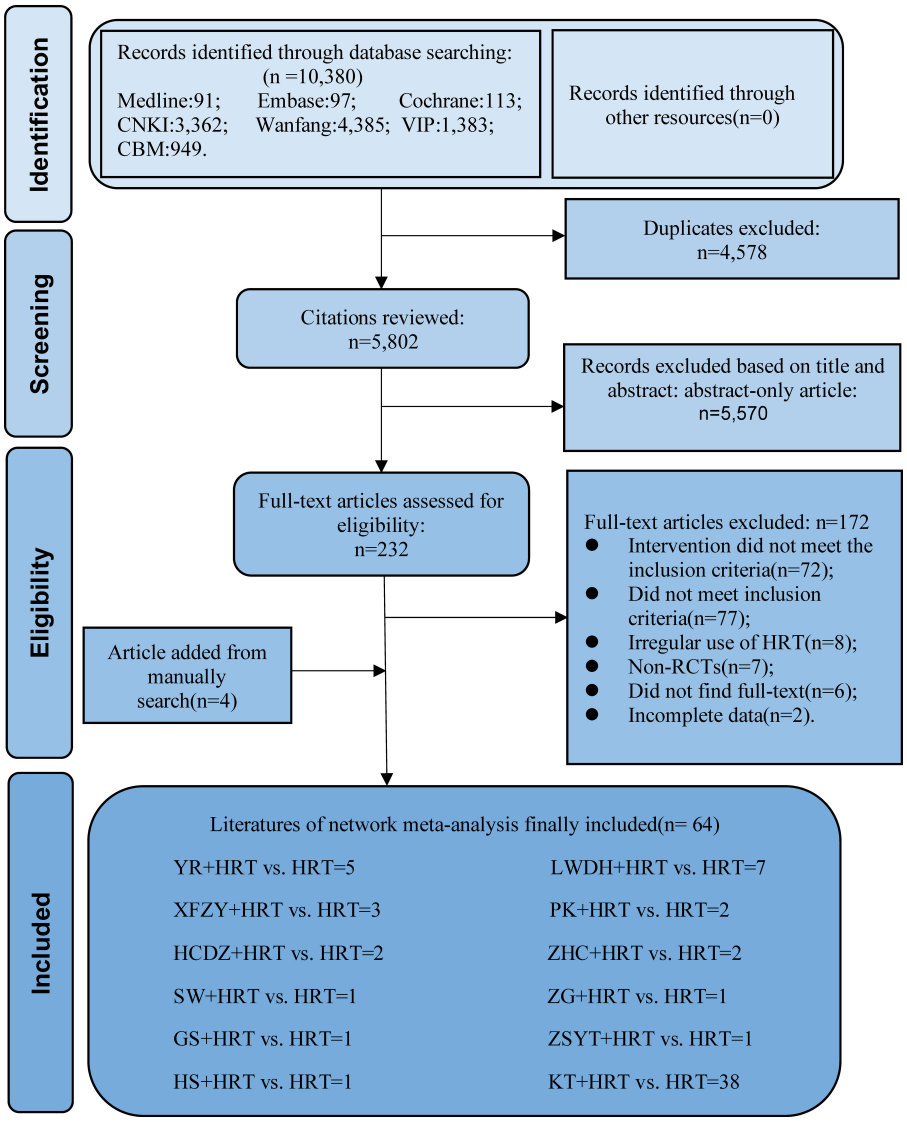

FIGURE 1
Literature review flowchart.

Results

Literature retrieval process and results

A total of 10,380 literature from seven databases were included in this study. First, we have removed duplicate 4,578 studies and, by reading the title and abstract, removed incompatible 5,570 studies. Second, by reading the full text, 127 incompatible studies were removed. The reasons for exclusion are as follows: (1) intervention did not meet the inclusion criteria (n = 72); (2) did not meet inclusion criteria (n = 77); (3) irregular use of HRT (n = 8); (4) non-RCTs (n = 7); (5) did not find full text (n = 6); (6) incomplete data (n = 2). Finally, 64 articles (22–85) were included for research. The selection process is illustrated in Figure 1.

Quality of the included studies

All 64 RCTs were mentioned randomization, of which 30 were random number table method, one was simple random method, and one was the envelope lottery method, rated as low risk. The rest were only described as "random" and rated as unclear risk; in terms of allocation concealment, blinding of participants or doctors, and blinding of outcome evaluator, all articles were not described and rated as unclear risk; all articles with complete data and no selective reporting were rated as unclear risk, unable to judge other sources of bias, rated as unclear risk. The assessment results of the risk of bias test are shown in Supplementary Figure 1. The GRADE level of evidence for the primary outcome was rated very low to low quality, shown in Supplementary Table 1.

Network meta-analysis

Figure 2 shows all outcome measures, with all included Chinese patent medicines combined with HRT being compared at least once, while there is a lack of closed loop between various types of Chinese patent medicines combined with HRT.

Primary outcome

Total clinical response rate

A total of 52 studies involving 4,702 patents reported the total clinical response rate. Among them, GS + HRT, HCDZ + HRT, SW + HRT, KT + HRT, XFZY + HRT, YR + HRT, and LWDH + HRT compared with HRT have statistical significance (see Table 1). ZG + HRT [odds ratio (OR) = 3.92; 95% credible interval (CrI) 0.86, 23.84; SUCRA = 73.76%] had the highest rate of impact on POF patients in terms of the other 11 Chinese patent medicines combined with HRT. Next, the second was GS + HRT (OR = 3.22, 95% CrI = 1.16, 9.44, SUCRA = 70.60%) and HCDZ + HRT (OR = 2.95, 95% CrI = 1.35, 6.51, SUCRA = 68.41%) was third. The comparison-correction funnel plot showed that not all studies were symmetrically distributed around the X = 0 line, and two

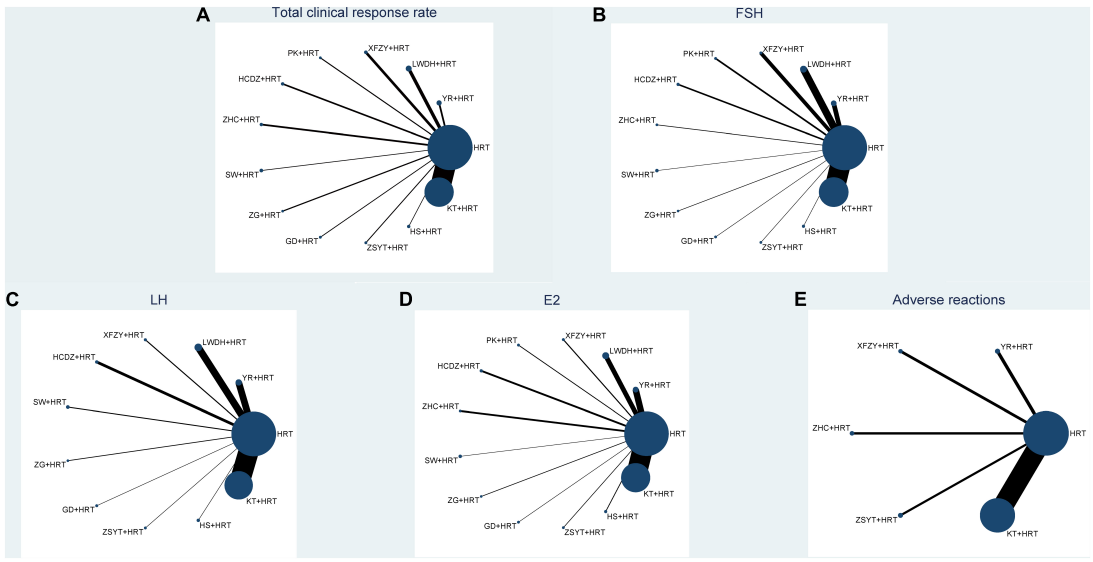

FIGURE 2
Network plot of all the trials based on the outcomes. P.S: **(A)** total clinical response rate; **(B)** FSH; **(C)** LH; **(D)** E2; **(E)** adverse reactions; HRT, hormone replacement therapy; YR, Fuke Yangrong Capsule; LWDH, Liuwei Dihuang Pills; XFZY, Xuefu Zhuyu Capsule; PK, Peikun pills; HCDZ, Heche Dazao pills; ZHC, Ziheche Capsule; SW, Siwu Mixture; ZG, Zuogui pills; GS, Guishen pills; ZSYT, Zishen Yutai pills; HS, Huanshao Capsule; KT, Kuntai Capsule.

TABLE 1 Relative effect sizes of efficacy at post-treatment according to network meta-analysis.

A

73.76%												
ZG + HRT	**70.60%**											
1.23 (0.19, 9.41)	**GS + HRT**	**68.41%**										
1.33 (0.24, 9.68)	1.09 (0.30, 4.05)	**HCDZ + HRT**	**66.38%**									
1.38 (0.22, 10.29)	1.13 (0.27, 4.40)	1.01 (0.31, 3.24)	**SW + HRT**	**60.04%**								
1.46 (0.17, 13.81)	1.17 (0.19, 6.71)	1.08 (0.20, 5.15)	1.06 (0.18, 5.24)	**PK + HRT**	**54.08%**							
1.71 (0.37, 10.50)	1.42 (0.50, 4.17)	1.30 (0.57, 2.89)	1.26 (0.53, 3.31)	1.18 (0.31, 5.53)	**KT + HRT**	**52.15%**						
1.73 (0.29, 13.81)	1.44 (0.37, 5.74)	1.29 (0.40, 4.34)	1.26 (0.37, 4.61)	1.21 (0.24, 6.95)	1.02 (0.41, 2.50)	**ZHC + HRT**	**50.76%**					
1.78 (0.32, 12.21)	1.43 (0.41, 5.47)	1.35 (0.46, 3.65)	1.32 (0.42, 4.15)	1.22 (0.27, 6.46)	1.03 (0.49, 2.13)	1.02 (0.32, 3.15)	**XFZY + HRT**	**43.64%**				
1.95 (0.40, 12.81)	1.61 (0.51, 5.30)	1.45 (0.59, 3.81)	1.42 (0.52, 4.32)	1.35 (0.33, 6.87)	1.14 (0.66, 2.00)	1.12 (0.40, 3.23)	1.10 (0.48, 2.69)	**YR + HRT**	**41.77%**			
2.14 (0.28, 17.66)	1.72 (0.33, 8.26)	1.56 (0.37, 6.11)	1.51 (0.34, 6.90)	1.42 (0.23, 9.49)	1.22 (0.35, 3.88)	1.20 (0.27, 5.10)	1.15 (0.29, 4.54)	1.06 (0.27, 3.90)	**ZSYT + HRT**	**38.82%**		
2.13 (0.37, 15.95)	1.78 (0.47, 6.86)	1.58 (0.50, 5.30)	1.58 (0.47, 5.68)	1.48 (0.30, 8.58)	1.25 (0.52, 2.99)	1.24 (0.36, 4.29)	1.20 (0.41, 3.66)	1.1 (0.40, 2.98)	1.04 (0.25, 4.71)	**HS + HRT**	**26.00%**	
2.47 (0.51, 16.25)	2.07 (0.67, 6.51)	1.87 (0.77, 4.65)	1.83 (0.69, 5.33)	1.73 (0.43, 8.40)	1.45 (0.90, 2.37)	1.43 (0.54, 3.93)	1.42 (0.62, 3.36)	1.28 (0.63, 2.51)	1.2 (0.35, 4.53)	1.17 (0.45, 3.02)	**LWDH + HRT**	**3.54%**
3.92 (0.86, 23.84)	3.22 (1.16, 9.44)	2.95 (1.35, 6.51)	2.88 (1.21, 7.49)	2.69 (0.73, 12.36)	2.28 (1.91, 2.75)	2.26 (0.94, 5.55)	2.22 (1.11, 4.57)	2.01 (1.18, 3.38)	1.87 (0.60, 6.50)	1.83 (0.78, 4.34)	0.64 (0.41, 0.99)	**HRT**

B

72.90%												
XFZY + HRT	**70.46%**											
−0.48 (−14.26, 13.27)	**ZG + HRT**	**63.32%**										
−1.72 (−15.27, 11.85)	−1.20 (−14.66, 12.26)	**GS + HRT**	**61.78%**									
−2.52 (−12.49, 7.36)	−2.02 (−11.78, 7.73)	−0.82 (−10.43, 8.71)	**KT + HRT**	**57.57%**								
−2.61 (−16.19, 10.92)	−2.09 (−15.62, 11.45)	−0.90 (−14.28, 12.49)	−0.06 (−9.78, 9.57)	**ZSYT + HRT**	**50.11%**							
−3.74 (−15.85, 8.45)	−3.21 (−15.22, 8.86)	−2.00 (−13.93, 9.88)	−1.20 (−8.61, 6.27)	−1.14 (−13.03, 10.80)	**HCDZ + HRT**	**49.99%**						
−3.70 (−14.54, 7.22)	−3.21 (−13.86, 7.59)	−2.00 (−12.42, 8.69)	−1.20 (−6.16, 3.99)	−1.13 (−11.62, 9.63)	0 (−8.58, 8.75)	**YR + HRT**	**49.75%**					
−3.73 (−14.36, 6.82)	−3.24 (−13.71, 7.29)	−2.03 (−12.34, 8.30)	−1.20 (−5.66, 3.27)	−1.14 (−11.4, 9.22)	0 (−8.41, 8.34)	0 (−6.46, 6.23)	**LWDH + HRT**	**48.66%**				
−3.93 (−17.55, 9.67)	−3.44 (−16.9, 9.99)	−2.23 (−15.45, 11.10)	−1.39 (−10.97, 8.10)	−1.34 (−14.76, 11.95)	−0.23 (−12.11, 11.69)	−0.21 (−10.93, 10.20)	−0.21 (−10.59, 10.04)	**HS + HRT**	**46.55%**			
−4.25 (−18.08, 9.57)	−3.73 (−17.47, 9.95)	−2.57 (−16.06, 10.97)	1.74 (−8.21, 11.62)	−1.66 (−15.31, 11.98)	−0.55 (−12.71, 11.63)	−0.57 (−11.49, 10.32)	−0.54 (−11.06, 10.06)	−0.33 (−13.96, 13.34)	**ZHC + HRT**	**36.95%**		
−5.48 (−17.69, 6.62)	−5.00 (−17.06, 7.07)	−3.76 (−15.64, 8.15)	−1.74 (−11.62, 8.21)	−2.93 (−14.82, 9.06)	−1.78 (−12.17, 8.55)	−1.78 (−10.66, 6.86)	−1.77 (−10.27, 6.55)	−1.56 (−13.51, 10.42)	−0.55 (−12.71, 11.63)	**PK + HRT**	**33.88%**	
−6.24 (−19.64, 7.36)	−5.67 (−19.13, 7.74)	−4.52 (−17.86, 8.94)	−3.68 (−13.25, 5.85)	−3.63 (−16.93, 9.79)	−2.46 (−14.36, 9.34)	−2.48 (−13.06, 7.95)	−2.48 (−12.74, 7.85)	−2.29 (−15.56, 11.13)	−1.91 (−15.47, 11.51)	−0.71 (−12.60, 11.3)	**SW + HRT**	**8.02%**
−9.30 (−19.12, 0.38)	−8.79 (−18.41, 0.83)	−7.61 (−17.00, 1.81)	−6.78 (−8.47, -5.09)	−6.72 (−16.16, 2.84)	−5.57 (−12.80, 1.64)	−5.59 (−10.48, −0.92)	−5.57 (−9.69, −1.48)	−5.37 (−14.76, 4.06)	−5.03 (−14.82, 4.66)	−3.81 (−11.09, 3.52)	−3.11 (−12.48, 6.30)	**HRT**

(Continued)

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TABLE 1 (Continued)

C

72.18%										
GS + HRT	**68.06%**									
−0.39 (−8.49, 7.72)	**ZG + HRT**	**68.02%**								
−0.32 (−8.63, 7.98)	−0.04 (−8.61, 8.54)	**SW + + HRT**	**62.94%**							
−0.93 (−8.78, 6.94)	−0.56 (−8.62, 7.46)	−0.59 (−8.95, 7.74)	**HS + HRT**	**61.01%**						
−1.12 (−8.92, 6.72)	−0.74 (−8.84, 7.33)	−0.80 (−9.12, 7.57)	−0.18 (−8.06, 7.67)	**ZSYT + HRT**	**60.06%**					
−1.35 (−7.40, 4.70)	0.98 (−5.46, 7.42)	−1.03 (−7.70, 5.75)	−0.42 (−6.43, 5.68)	−0.23 (−6.31, 5.86)	**LWDH + HRT**	**55.43%**				
−1.66 (−7.31, 3.92)	−1.29 (−7.33, 4.72)	−1.34 (−7.62, 5.00)	−0.73 (−6.40, 4.91)	−0.55 (−6.26, 5.10)	−0.31 (−3.05, 2.37)	**KT + HRT**	**44.94%**			
−2.50 (−8.81, 3.94)	−2.13 (−8.77, 4.60)	−2.19 (−9.09, 4.82)	−1.58 (−7.94, 4.89)	−1.38 (−7.76, 5.10)	−1.15 (−5.17, 2.93)	−0.83 (−4.19, 2.62)	**YR + HRT**	**42.09%**		
−2.89 (−10.26, 4.46)	−2.49 (−10.25, 5.12)	−2.56 (−10.46, 5.34)	−1.95 (−9.39, 5.43)	−1.76 (−9.22, 5.55)	−1.53 (−7.06, 3.91)	−1.22 (−6.27, 3.79)	−0.38 (−6.27, 5.35)	**HCDZ + HRT**	**13.37%**	
−5.53 (−11.05, −0.03)	−5.15 (−11.06, 0.77)	−5.21 (−11.39, 1.07)	−4.59 (−10.14, 0.96)	−4.41 (−10.01, 1.12)	−4.17 (−6.65, −1.70)	−3.87 (−4.95, −2.72)	−3.03 (−6.27, 0.17)	−2.65 (−7.51, 2.28)	**HRT**	**1.86%**
−8.61 (−15.42, −1.72)	−8.22 (−15.37, −1.1)	−8.28 (−15.66, −0.83)	−7.67 (−14.50, −0.77)	−7.46 (−14.33, −0.60)	−7.24 (−11.99, −2.47)	−6.93 (−11.09, −2.7)	−6.09 (−11.31, −0.96)	−5.72 (−12.01, 0.66)	−3.07 (−7.09, 0.98)	**XFZY + HRT**

D

95.95%											
ZHC + HRT	**86.84%**										
10.70 (−11.15, 32.66)	**YR + HRT**	**81.97%**									
13.34 (−12.02, 38.50)	2.62 (−14.00, 18.97)	**ZG + HRT**	**78.59%**								
15.40 (−9.74, 40.47)	4.74 (−11.85, 20.98)	2.14 (−18.61, 22.86)	**ZSYT + HRT**	**65.94%**							
22.45 (−2.83, 48.00)	11.78 (−5.26, 28.67)	9.17 (−11.82, 30.18)	7.06 (−13.83, 27.97)	**HS + HRT**	**49.05%**						
31.15 (10.39, 51.88)	20.46 (12.23, 28.40)	17.82 (2.90, 32.69)	15.66 (0.99, 30.46)	8.65 (−6.66, 23.94)	**KT + HRT**	**43.21%**					
31.96 (8.47, 55.23)	21.32 (7.44, 34.71)	18.69 (0.10, 37.02)	16.54 (−1.93, 34.73)	9.51 (−9.56, 28.38)	0.86 (−10.73, 12.35)	**HCDZ + HRT**	**32.49%**				
35.04 (13.79, 56.28)	24.38 (14.73, 33.83)	21.76 (6.08, 37.48)	19.60 (4.12, 35.33)	12.61 (−3.62, 28.75)	3.92 (−2.36, 10.30)	3.09 (−9.46, 15.78)	**LWDH + HRT**	**31.19%**			
35.63 (10.58, 60.53)	24.93 (8.93, 40.89)	22.27 (1.91, 42.72)	20.20 (−0.08, 40.36)	13.14 (−7.31, 33.71)	4.44 (−9.82, 18.92)	3.61 (−14.19, 21.83)	0.55 (−14.59, 15.79)	**GS + HRT**	**28.95%**		
36.27 (11.49, 61.05)	25.61 (9.48, 41.56)	23.01 (2.87, 43.34)	20.81 (0.63, 41.14)	13.90 (−7.04, 34.37)	5.19 (−9.16, 19.52)	4.32 (−13.58, 22.48)	1.29 (−13.96, 16.39)	0.74 (−19.21, 20.48)	**SW + HRT**	**25.94%**	
36.97 (14.02, 59.82)	26.31 (13.58, 38.81)	23.67 (6.09, 41.46)	16.54 (−1.93, 34.73)	14.53 (−3.68, 32.62)	5.85 (−4.51, 16.24)	5.04 (−10.15, 20.31)	1.93 (−9.67, 13.56)	1.39 (−15.95, 18.66)	0.71 (−16.58, 17.94)	**PK + HRT**	**22.61%**
37.95 (14.97, 60.82)	27.32 (14.33, 40.13)	24.69 (6.73, 42.54)	19.60 (4.12, 35.33)	15.54 (−2.77, 33.63)	6.84 (−3.82, 17.50)	5.98 (−9.25, 21.31)	2.93 (−8.85, 14.67)	2.39 (−15.17, 19.81)	1.65 (−15.79, 19.16)	0.99 (−13.41, 15.36)	**XFZY + HRT**
41.80 (21.23, 62.41)	28.71 (5.49, 51.93)	28.48 (13.88, 43.07)	26.33 (11.90, 40.91)	19.30 (4.23, 34.49)	10.65 (8.12, 13.30)	9.81 (−1.43, 21.17)	6.73 (0.97, 12.45)	6.19 (−7.97, 20.21)	5.46 (−8.59, 19.55)	4.80 (−5.28, 14.85)	3.82 (−6.54, 14.14)

(Continued)

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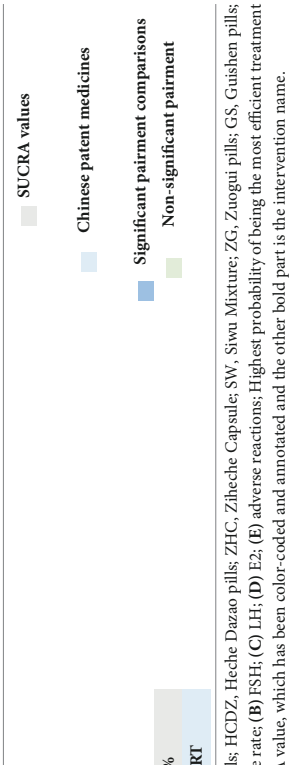
TABLE 1 (Continued)



HRT, hormone replacement therapy; YR, Fuke Yangrong Capsule; LWDH, Liuwei Dihuang Pills; XFZY, Xuefu Zhuyu Capsule; PK, Peikun pills; HCDZ, Heche Dazao pills; ZHC, Zihecheche Capsule; SW, Siwu Mixture; ZG, Zuogui pills; GS, Guishen pills; ZSYT, Zishen Yutai pills; HS, Huanshao Capsule; KT, Kuntai Capsule. SUCRA, surface under the cumulative ranking. **(A)** Total clinical response rate; **(B)** FSH; **(C)** LH; **(D)** E2; **(E)** adverse reactions; Highest probability of being the most efficient treatment (With high SUCRA values) and Lowest probability of being the most efficient treatment (With low SUCRA values). The bold part is the SUCRA value, which has been color-coded and annotated and the other bold part is the intervention name.

studies were located outside the funnel chart, which provides evidence for small-sample effects in the study network (see in Supplementary Figure 2). None of the five outcome indicators in this study exhibited a closed loop, so there is no necessary to do a consistency test.

Secondary outcome

A total of 59 studies involving 5,415 patents reported FSH. Only KT + HRT (MD = −6.78, 95% CrI = −8.47, −5.09), YR + HRT (MD = −5.59, 95% CrI = −10.48, −0.92), and LWDH + HRT (MD = −5.57, 95% CrI = −9.69, −1.48) had a significant benefit compared with HRT, and other Chinese patent medicines combined with HRT had no statistical differences. According to the SUCRA value, XFZY + HRT was ranked first (SUCRA = 72.90%) (see Table 1).

There are 51 studies involving 4,629 patents reported LH. Only GS + HRT (MD = −5.53, 95% CrI = −11.05, −0.03), LWDH + HRT (MD = −4.17, 95% CrI = −6.65, −1.7), KT + HRT (MD = −3.87, 95% CrI = −4.95, −2.72), and YR + HRT (MD = −3.03, 95% CrI = −6.27, −0.17) had a significant benefit compared with HRT, and other Chinese patent medicines combined with HRT had no statistical differences. According to the SUCRA value, GS + HRT was ranked first (SUCRA = 72.18%) (see Table 1).

A total of 58 studies involving 5,315 patents reported E_2. ZHC + HRT (MD = 41.8; 95% CrI = 21.23, 62.41; SUCRA = 95.95%) had the highest rate of impact on POF patients in terms of the other 11 Chinese patent medicines combined with HRT. Next, the second was YR + HRT (MD = 28.71, 95% CrI = 5.49, 51.93, SUCRA = 86.84%), and ZG + HRT (MD = 28.48, 95% CrI = 13.88, 43.07, SUCRA = 81.97%) was third (see Table 1).

A total of 13 studies involving 1,168 patents reported the adverse reactions. Only KT + HRT had a significant benefit compared with HRT. KT + HRT (OR = 0.44, 95% CrI = 0.21, 0.85, SUCRA = 81.86%) shows the best, and ZHC + HRT (OR = 0.45, 95% CrI = 0.03, 4.3, SUCRA = 70.18%) was as follows. The comparison-correction funnel plot of all secondary outcome is shown in Supplementary Figure 2.

Discussion

Our study has adopted a Bayesian NMA which involves 64 RCTs to evaluate the effectiveness of 12 Chinese patent medicines combined with HRT in POF patients. In terms of primary outcome, ZG + HRT appears to be the most promising way to help patients with POF improve their clinically integrated outcomes.

In recent years, with the development of social economy, women's increasing mental stress and poor living habits have

led to the gradual rejuvenation of patients with POF, which is a devastating diagnosis for women of childbearing age, because it indicates a decline in fertility (1, 86). As a first-line treatment widely used in patients with POF, HRT can improve the clinical symptoms caused by estrogen deficiency in patients, but it is not insisted on by patients because it cannot restore ovarian function and the treatment time is too long (recommended until natural menopause) (5). At present, in addition to hormone therapy, there are still complementary therapies, such as inositol, vitamin D, and traditional Chinese medicine. Inositol has been found to be a natural endogenous compound, which includes D-Chiro-Inositol (DCI) and Myo-inositol (MI), of which MI is the second messenger of insulin and FSH, which can improve insulin resistance (87), correct the FSH/LH ratio, and promote ovulation (88); DCI can improve the quality of oocytes and blastocysts, which has a potential role in improving fertility (89). Similarly, the fact that vitamin D receptors are present in women's central and peripheral reproductive organs, tissues, and cells suggests that vitamin D plays a key role in fertility. Vitamin D supplementation promotes oocyte development, improves embryo quality, and increases endometrial tolerance (90); in addition, inositol can reduce fasting insulin, blood total cholesterol, blood triglycerol levels, thereby reducing diabetes, dyslipidemia, and cardiovascular disease risk, vitamin D supplementation can also reduce the risk of osteoporosis, and no adverse reactions have been shown, which is undoubtedly a significant advantage for perimenopausal women (91).

In addition, for patients of reproductive age with POF, how to protect fertility at a young age is a key concern. Vitrification of oocytes is an effective technique for fertility protection that allows women to preserve gametes for future fertility in advance. One prospective study found no statistically significant difference in pregnancy and clinical pregnancy rates per cycle between vitrified oocytes and sibling fresh oocytes in closed systems (92). Another prospective study found that open and closed vitrification protocols were equally effective for sibling oocyte cycles when performing blastocyst embryo transfers (93). Thus, vitrification of oocytes offers women the possibility of delaying fertility until the end of treatment or finding a suitable time for fertility. However, the psychological support given during the treatment of infertile patients is also a point that cannot be ignored. POF can cause depression and anxiety disorders in most women. In terms of fertility, studies have found that due to gender differences, infertile couples, although they are seriously affected by infertility diagnosis, in terms of behavior, relationship, social, emotional, and cognitive aspects, regardless of the way they are conceived, women are more likely than men to "seek social support" (94, 95). Therefore, understanding the sources and changes of psychological stress in female patients and providing patients with specific psychotherapy can benefit them in terms of interventions and outcomes of infertility treatment.

Proprietary Chinese medicine has always been a hot topic in Chinese medical research and is widely used in the clinical frontline, especially by gynecologists and fertility center doctors in general hospitals. Based on this purpose, we conducted a study of proprietary Chinese medicine combined with HRT in the treatment of POF, trying to obtain relatively objective evidence, hoping to give full play to the advantages of the three combination of the two, gradually restore ovarian function, to shorten the treatment time, and look for the best efficacy of proprietary Chinese medicine combined with HRT to provide an evidence base. The essence of POF is considered to be the depletion of follicles, and experimental studies have found that Zuogui pills may improve the therapeutic effect of chemotherapy-induced POF by inhibiting the pathway of mitochondrial-dependent apoptosis, laying an experimental foundation for Zuogui pills as a reasonable treatment choice for POF (96). A meta-analysis study also shows that ZG + HRT is more therapeutic and safer than HRT alone (97). Combined with the findings of this study, in the primary outcome, total clinical response rate, ZG + HRT (OR = 3.92; 95% CrI = 0.86, 23.84) is the best in all treatments. Therefore, we can cautiously think that ZG + HRT seems to be the most effective proprietary Chinese medicine in terms of improving total clinical response rate.

This study showed that XFZY + HRT (SUCRA = 72.90%) worked best at reducing FSH levels, but it has no statistical differences. Some scholars found that the ovarian artery peak systolic velocity in POF patients was negatively correlated with FSH (98). Modern pharmacological studies have found that the extract of Honghai–Taoren drug pair can promote blood circulation by affecting hemodynamics, plasma coagulation, and platelet aggregation (99, 100). XFTZ Capsule is composed of a variety of traditional Chinese medicines for promoting blood circulation and removing blood stasis, including Honghai and Taoren. So we infer that XFZY Capsule may reduce FSH levels by improving hemodynamics and improving ovarian blood supply, but this inference lacks experimental research. For the outcome of E_2, ZHC + HRT (MD = 41.8; 95% CrI = 21.23, 62.41; SUCRA = 95.95%) was best in all treatments. The composition of Ziheche Capsule is Ziheche. Modern pharmacological studies have found that Ziheche contains a large number of hormones, including gonadotropin, corticotropin-releasing hormone, thyrotropin, prolactin, a variety of LHs, and erythropoietin, which can directly stimulate ovarian tissue and promote endometrial hyperplasia (101). Therefore, we can cautiously conclude that ZHC + HRT has the best effect in improving the E_2 level. However, outcome measures for many interventions were not statistically significant, so more long-term and high-quality, large-sample, multi-center RCTs are needed in future to further confirm this.

Innovation and limitations of this study

In this study, for the first time, a NMA was used to classify and analyze the relevant efficacy indicators of proprietary

Chinese patient medicines combined with HRT in the treatment of POF. Giving full play to the advantages of proprietary Chinese patient medicines, it provides evidence-based evidence for reasonable and targeted drugs in clinical practice. In addition, this study searched seven databases and finally included 64 RCTs involving 5,675 POF patients, with a large sample size and many sources of evidence.

At the same time, this study has certain limitations: (1) There are differences in the number of studies included in different interventions. For example, there are 42 studies involving Kuntai capsule, and only one study involving Zuogui pills, Guishen pills, Zishen Yutai pills, Huanshao capsule, and Siwu mixture. The difference in number may affect the results of this study make an impact; (2) in this study, 12 proprietary Chinese patient medicines were indirectly compared with HRT, and there was no RCTs that directly compared the efficacy of proprietary Chinese patient medicines, and the evidence network graphs of the five outcome indicators did not form a closed loop, which affected the credibility and stability of the results to a certain extent. (3) In terms of literature quality evaluation, all studies were single-center, Chinese literature, and most of the random methods used the random number table method, and none of them mentioned the allocation concealment, the blinding method of subjects and outcome evaluators, and other sources of bias could not be judged. Therefore, we look forward to conducting more RCTs with large samples, multicenter, and high methodological quality in future to provide more robust and reliable evidence support for clinical drug use.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Author contributions

H-ZZ served as principal author, had full access to all the data in the study, took responsibility for the accuracy of the data analysis and the integrity of the data, and contributed to the draft of the manuscript. C-LB and M-YL contributed to the conception and design. X-LY, C-LB, and S-YZ contributed to data acquisition and interpretation. X-LY, M-YL, and S-BW contributed to revising of the article and final approval. All authors contributed to the article and approved the submitted version.

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We affirm that the work submitted for publication is original and has not been published other than as an abstract or preprint in any language or format and has not been submitted elsewhere for print or electronic publication consideration. We affirm that each person listed as authors participated in the work in a substantive manner, in accordance with ICMJE authorship guidelines, and is prepared to take public responsibility for it. All authors consent to the investigation of any improprieties that may be alleged regarding the work. Each author further releases and holds harmless the Endocrine Society from any claim or liability that may arise therefrom.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.1043390/full#supplementary-material

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Signaling pathway intervention in premature ovarian failure

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Premature ovarian failure (POF) is a multifactorial disease that refers to the occurrence of secondary amenorrhea, estrogen decrease, and gonadotropin increase in women under the age of 40. The prevalence of POF is increasing year by year, and the existing instances can be categorized as primary or secondary cases. This disease has adverse effects on both the physiology and psychology of women. Hormone replacement therapy is the recommended treatment for POF, and a multidisciplinary strategy is required to enhance the quality of life of patients. According to recent studies, the primary mechanism of POF is the depletion of ovarian reserve function as a result of increased primordial follicular activation or primordial follicular insufficiency. Therefore, understanding the processes of primordial follicle activation and associated pathways and exploring effective interventions are important for the treatment of POF.

KEYWORDS

premature ovarian failure, PI3K, mTOR, PTEN, signaling pathway

Introduction

Premature ovarian failure (POF), also known as premature menopause, is a condition in which a woman experiences amenorrhea before the age of 40 due to the cessation of ovarian function. POF can lead to infertility in women with physiological or psychological problems (1, 2). Genetic, immunological, metabolic, viral, and medicinal variables all have a role in the etiology of POF (3). Studies have shown that the daughters of POF patients are six times more likely to suffer from the disease than normal peers, and the treatment of diseases such as malignancies of the reproductive system and endometriosis often causes secondary POF (4). Abnormalities in some signaling pathways can also result in POF. To improve patients' quality of life and maintain their fertility, we should learn the processes of primordial follicle activation and oocyte death and explore the corresponding therapeutic prevention or therapy techniques (5). The purpose of this review is to provide an overview of the signaling pathways that play significant roles in follicular activation.

The ovary is an essential part of the female reproductive system that plays a crucial role in the growth and fertility of the reproductive system by producing mature oocytes and secreting many kinds of hormones (6). The primordial follicle, which begins to develop shortly after birth (7), is the reproductive unit of the mammalian ovary (8).

The primordial follicular pool, which is the only source of germ cells in female mammals, is formed by a vast number of primordial follicles that are distributed across the ovary's periphery (9). Females have about 10^6 primordial follicles at birth, but only a small percentage of them mature into oocytes; the remainder are either kept dormant or disappear. One of the factors that influence the length of a woman's reproductive life is the balance between the primordial follicle's dormant, active, and apoptotic stages (10). Ninety-nine percent of the primordial follicles in women die as they age, and menopause occurs when there are less than 10^3 primordial follicles left in the body (11). A woman is thought to have POF if this condition occurs before the age of 40.

Long-term hormone replacement therapy (HRT), which uses estrogen, progesterone, melatonin and other hormone to alleviate menopausal symptoms caused by POF, is the current preferred treatment (12, 13). HRT can be administered through a variety of methods, and the dose schedule is becoming more personalized (3). However, several clinical studies have revealed that people who receive HRT have a higher long-term risk of developing breast cancer, heart disease, stroke, and other disorders (14). In patients with reproductive malignancies, surgery can result in iatrogenic POF. Some early-stage patients may receive HRT while preserving fertility to prevent iatrogenic POF (15, 16). To preserve fertility, these patients can use drugs such as GnRH to promote ovulation and cryopreserve them before surgery, which is well developed (17, 18). In recent years, stem cells and their secreted cytokines or exosomes have been discovered to enhance the milieu of ovarian tissue, control inflammation, and encourage the growth of follicles. However, successful treatment has only been accomplished in laboratory animals (19).

Follicular activation and its pathways

Activation of primordial follicles determines oocyte growth and development, as well as the differentiation and proliferation of surrounding somatic and granulosa cells, both of which are essential for the ovulation process (11). To provide new perspectives on the treatment of POF, we need to comprehend the crucial factors affecting human follicular activation and the mechanisms that control follicular activation. We also need to explore the mechanisms that control awakening and the early development of dormant follicles to understand the process of follicular development and apoptosis and propose targeted interventions to promote oocyte maturation. This is a crucial step in identifying, treating, and preventing POF (20).

The primordial follicles are maintained in a dormant state by a certain inhibitory system, which is achieved by a combination of signaling pathways and molecular processes. Studies have shown that this system is necessary for the preservation of the dormant follicle pool (10).The current research on this system mainly focuses on follicle activation signaling pathways and related molecules, such as phosphatidylinositol 3 kinase (PI3K) and mammalian target of rapamycin (mTOR) (11). Loss of molecular function in any of these pathways has the potential to cause premature and irreversible activation of the primordial follicle pool, leading to depletion of dormant follicle reserves and POF (Figure 1). Further, the influence of the ovarian environment needs to be delineated. Some scholars have studied the effect of extracellular matrix on the activation of primordial follicles (11).

PI3K/PTEN/Akt/Foxo3 pathway

Although the mechanism of primordial follicle activation is not fully understood, evidence shows that the phosphatidylinositol 3-kinase/protein kinase B pathway (PI3K/Akt) plays a key role in this process. Cell motility, survival, differentiation, growth, and intracellular transport are all regulated by PI3K, which catalyzes the phosphorylation of phosphatidylinositol (21). Protein kinase B (PKB), also known as Akt, is a general term for three serine/threonine-specific protein kinases that play important roles in a variety of cellular processes, including cell apoptosis, proliferation, transcription, and migration (22). The PI3K/Akt pathway is composed of various signaling molecules, such as kinases, phosphatases, and transcription factors, that establish intracellular signaling cascades. It is a classic intracellular signaling pathway that not only regulates cell proliferation, survival, and migration but is also involved in the activation of primordial follicles in the ovary (23). Akt is a crucial kinase in primordial follicle activation; it has a variety of substrates that are expressed in both oocytes and granulosa cells of the human ovary, playing both direct and indirect roles in follicle activation (24). Among the various substrates of Akt, the Foxo3 protein was first found to control the activation pathway of primordial follicles, which is encoded by the Foxo3 gene. Experiments have shown that in mice deficient in the Foxo3 gene, all dormant follicles are prematurely activated in the pubertal ovary, and when the active Foxo3 gene is expressed in mouse oocytes, the development of oocytes and follicles is delayed; this means that normal expression of the Foxo3 gene can inhibit follicular development and maintain a dormant state of follicles (25). Goldbraikh et al. highlighted PI3K/Akt/Foxo3 signaling as the central pathway controlling growth and metabolism in all cells (26). The PI3K pathway can be inhibited by the deletion of the phosphatase and tensin homolog gene PTEN on human chromosome 10. The PTEN gene encodes a specific phospholipid phosphatase that selectively dephosphorylates phosphatidylinositol substrates and negatively regulates intracellular phosphatidylinositol triphosphate (PIP3) to generate diphosphate products (23). This inhibits cell growth, proliferation, and survival signaling pathways mediated by PI3K. This function is significant because

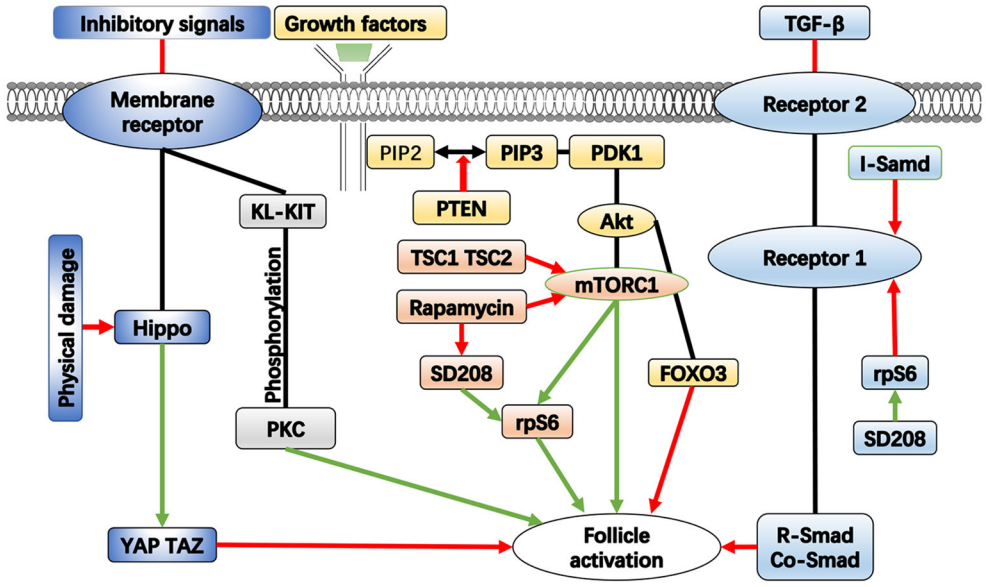

FIGURE 1
The important pathways in the activation of follicle. Green arrows represent enhancement, red arrows represent inhibition.

it suppresses the Akt signaling pathway, which is crucial for controlling the growth, survival, and migration of cells. Due to the above functions, PTEN can participate in the regulation of the cell cycle, prevent the rapid growth and division of cells, induce apoptosis, inhibit the adhesion of cells to the surroundings, and prevent the formation of new blood vessels. PTEN can also maintain the stability of cell genetic information. All of these functions help prevent the occurrence of malignant cell proliferation. As a result, numerous anticancer drugs were designed to target the PTEN gene because it was previously considered to be of great significance for the treatment of malignant tumors (27).

In the process of follicle development, PTEN has an inhibitory effect on the PI3K/Akt pathway, maintaining primordial follicles in a dormant state for a long time. When the inhibition of PTEN is removed, PI3K is activated in primordial follicles, converting phosphatidylinositol diphosphate (PIP2) to PIP3, thereby stimulating phosphatidylinositol-dependent kinase 1 (PDK1), and activating Akt. This causes the downstream Foxo3 protein to be phosphorylated, resulting in its loss of transcriptional activity and relocation from the nucleus to the cytoplasm for destruction, which activates primordial follicles (28). As a negative regulator of PI3K, PTEN can lead to inhibition of the PI3K signaling pathway. Studies have shown that when the expression of the PTEN gene is inhibited due to the enhanced PI3K signaling pathway in mammals, dormant primordial follicles are activated. When the PTEN gene is knocked out from the oocytes of mouse primordial follicles, overgrowth of primordial follicles and premature activation of the entire primordial follicle pool can be observed at puberty, which also leads to follicle depletion in early adulthood and POF in mice (29). Han et al. compared the granulosa cells of POF patients with those of healthy individuals and found that interleukin-4 (IL-4) levels were higher in POF patients. IL-4 can inhibit the growth of granulosa cells by activating PI3K/Akt pathway (30). Liu et al. found that exosomes from stem cells can enhance the expression of PI3K/Akt pathway, increase the proliferation rate of granulosa cells, and improve ovarian function in a POF mouse model (31). Li et al. (32) found that quercetin attenuated cyclophosphamide-induced follicle loss by preventing the phosphorylation of PI3K/Akt/Foxo3 pathway members and maintaining the anti-Müllerian hormone level through reduced apoptosis in growing follicles (32). These findings imply that the PI3K/PTEN/Akt/Foxo3 cascade in oocytes is essential for the activation of primordial follicles (10).

mTOR pathway

As a downstream molecule of the PI3K/Akt pathway, mammalian target of rapamycin (mTOR) signaling is a crucial intracellular signaling pathway that regulates the cell cycle and has received extensive attention (33). It is a member of the PI3K-related kinase family and is linked to other proteins. mTOR is the core component of two protein complexes known as mTOR protein complex 1 (mTORC1) and mTOR protein complex 2 (mTORC2), two protein complexes with different structures and functions. The two complexes have distinct downstream targets

that regulate distinct cellular activity. As a highly conserved serine/threonine kinase among these two complexes, mTOR not only regulates cell growth, proliferation, survival, migration, and other activities but also participates in the regulation of protein synthesis, cell autophagy, gene transcription, and other processes (34). As a core element of mTORC2, mTOR also acts as a tyrosine kinase that influences cell metabolism and survival. It not only integrates information from upstream molecules, such as insulin, growth hormones, amino acids, and other substances, but also perceives the nutrition, oxygen, and energy levels of the cell. This route is a major metabolic and physiological regulator of mammals and is crucial for the health of the liver, muscles, brain, and other organs (35). Previous research has shown that the mTOR pathway's overactivation plays a significant role in the development of cancer because of its impact on proteins. In lung cancer, prostate cancer, breast cancer, and other malignant tumors, the activity of mTOR has been found to be significantly increased (36).

The most significant of the various reasons for elevated mTOR activity is the mutation of the PTEN gene. Normally, PTEN inhibits mTOR signaling by blocking the activity of PI3K, an upstream effector of mTOR. When the inhibitory impact of PTEN is weakened, mTOR promotes cell cycle progression, boosts proliferation, and prevents autophagy (37). Zhang et al. (38) found that histone deacetylase 6 (HDAC6) may play an indispensable role in balancing the maintenance and activation of primordial follicles through mTOR signaling in mice. HDAC6 is a microtubule-associated deacetylase that predominantly functions in the cytoplasm by deacetylating various substrates to regulate cell migration and motility. The expression level of mTOR in the follicle and the activity of PI3K in the oocyte of the follicle can be simultaneously upregulated by inhibiting HDAC6, which can also promote the activation of limited primordial follicles (38). Studies have shown that during the transition from primordial to primary follicles, glycolysis in granulosa cells is enhanced and can increase the expression of the mTOR signaling pathway, thereby promoting the activation of primordial follicles (39).

As an inhibitor of mTOR, rapamycin can bind to the FKBP12 protein, an intracellular receptor. By directly interfering with the FKBP12 rapamycin-binding domain of mTOR, the FKBP12–rapamycin combination inhibits the activity of mTOR (40). Among the two mTOR complexes, only mTORC1 is responsive to rapamycin. In mammals, mTORC1 promotes preprotein synthesis, ribosome formation, and cell growth by activating ribosomal protein S6 (RPS6) and eukaryotic translation initiation factors 4E (4E-BPs), which in turn positively regulates cell growth and proliferation (41). The activity of mTORC1 is negatively regulated by a heterodimeric complex composed of two protein molecules. The two protein molecules are tuberous sclerosis complex 1 (tsc1) and tuberous sclerosis complex 2 (tsc2), which are the products of the tumor suppressor genes tsc1 and tsc2. Tsc1 keeps tsc2 from deterioration and ubiquitination to maintain its stability. They combine to generate a heterodimeric complex that prevents mTORC1 from being activated (42). Similar to the absence of PTEN in mouse oocytes, when tsc1 and tsc2 are specifically deleted, premature activation of primordial follicles occurs in mouse ovaries, resulting in an overall activation of primordial follicles around puberty, causing follicular depletion in early adulthood (43). This finding implies that excessive activation of mTORC1 signaling accelerates the activation of primordial follicles, and rapamycin, an inhibitor of mTORC1, can inhibit the development of primordial follicles and maintain the size of the follicular pool by blocking this signaling pathway. After treating POF mice with electroacupuncture therapy, He et al. (44) found that this method can promote the expression of mTOR pathway, induce the proliferation of ovarian cells, and restore the estrous cycle in mice (44). Shi et al. (45) designed a new biomaterial that moderately inhibits the mTOR pathway and prevents premature follicle activation, thereby delaying ovarian aging (45).

Hippo pathway

The Hippo signaling pathway is composed of a series of conserved kinases and is an important signaling pathway regulator. It can control the size of an organ by regulating cell proliferation and apoptosis, and has the ability to inhibit cell growth (46). In mammals, the membrane protein receptor is upstream of the Hippo signaling pathway and is a receptor for extracellular growth inhibition signals. Once the extracellular growth inhibition signal is activated, it initiates a series of kinase cascade phosphorylation reactions, eventually resulting in the phosphorylation of the downstream transcriptional co-activators YAP and TAZ. The cytoskeletal proteins will bind to the phosphorylated transcriptional co-activators, forcing them to remain in the cytoplasm and decrease the activity of the nucleus, accomplishing regulation of organ growth and volume (47).

Hippo signaling is also impacted by changes in cellular connections, shape, and polarity. Intercellular tension in organs is altered when organs are physically damaged, accompanied by inhibition of the Hippo signaling pathway accumulation of co-activators in the nucleus. This series of modifications affects organ size and controls cell proliferation. Cell proliferation, survival, and growth are promoted when Hippo signaling is inhibited (48).

The Hippo signaling system has been demonstrated in mouse models to play a function in controlling cell proliferation and death by preserving organ volume at the ideal size by inhibiting cell growth (49). Genes connected to the Hippo signaling system are expressed in follicles at different stages of mice and human ovaries. Fragmentation of the ovary temporarily stimulates follicle development and reduces the

phosphorylation of YAP. The disruption of mouse ovaries promotes dynamic changes in actin and disturbs the Hippo signaling pathway, increasing the growth and maturation of follicles and oocytes (50). A recent study demonstrated that mechanical effects caused by internal or external forces can affect follicular activation *via* the Hippo and Akt pathways, involving signaling pathways such YAP, TAZ, PTEN, mTOR, and Foxo3.

Notch pathway

The Notch signaling pathway is a widely distributed and evolutionarily conserved pathway that controls numerous cellular activities, including cell proliferation, migration, differentiation, and death in both healthy and unhealthy conditions (51). Certain physiological processes in the ovary associated with the Notch pathway are crucial for female reproduction and directly affect female fertility. This pathway consists mainly of ligands and receptors. The receptors are unidirectional transmembrane proteins with numerous intricate structural domains, and extracellular domains of receptors are important for delivering signaling transmitters to cells nearby (52). There are four main types of Notch receptors that control the expression of downstream target genes by interacting with nuclear transcription factors. These receptors are encoded by different genes and have various structural features (53). Their ligands mainly include functional Notch ligands, the Delta-like ligand family of unidirectional integral membrane proteins, and these ligands are specifically distributed and expressed in adult organs (54).

In addition to many mitotic cycles, the Drosophila oocyte maturation process also includes an endoreplication step in which genomic DNA is duplicated without cell division (55). The transition from mitosis to endoreplication is regulated by the Notch pathway (56). In mice, the Notch pathway is expressed temporally and spatially specifically in both germ cells and somatic cells, and inhibition of Notch signaling prevents mouse oocytes from entering meiosis (57). Granulosa cells surround the oocyte and create primordial follicles in the early postnatal period; this is an important step in the development of mammalian follicles. Granulosa cells express the Notch2 receptor gene, which is necessary for the development of follicles (58). Notch2 suppression in granulosa cells reduces oocyte apoptosis, which in turn decreases fertility in mice. Notch2 mutant animals also experienced a drop in the number of primordial follicles. During the maturation of primordial follicles into mature follicles, the volume of oocytes increases, granulocytes proliferate and differentiate, and Notch signaling can promote cell proliferation and regulate ovarian hormone release and ovulation processes (59). When Notch signaling is disrupted, granulosa cell growth is restrained (60).

In a clinical trial, patients with POF were discovered to have mutations in the Notch2 gene, indicating that these mutations may be connected to the emergence of POF (61). In a mouse model of POF, growth hormone was discovered to activate the Notch1 signaling pathway, upregulate Notch1 expression, raise plasma estradiol levels, reduce follicle-stimulating hormone concentrations, alleviate symptoms, and promote ovarian maturation (62). These findings may provide a new target for the treatment of POF.

KL–KIT pathway

The tyrosine kinase receptor KIT and its ligand cytokine KL mediate signaling between granulosa cells and oocytes and are closely associated with female fertility (63). Follicular development is significantly influenced by the interchange of materials and bidirectional signaling between somatic cells and oocytes. Oocytes play a critical function in the differentiation and proliferation of granulosa cells, and granular cells can provide growth factors to oocytes. KIT, encoded by the c-kit gene, is a tyrosine kinase type III receptor expressed on the surface of hematopoietic stem cells and others. KIT consists of extracellular, transmembrane, proximal, and intracellular structural domains. Five immunoglobulin-like domains make up the extracellular structural domain, while a variable length sequence divides the domain into distal and proximal ends (64). KIT is expressed on the oocyte surface during the whole follicular development process in humans, mice, and rats (65). KL is the ligand of Kit, also known as stem cell factor, and is a growth factor that exists in the form of a monomer. KL is expressed in fibroblasts and endothelial cells and promotes proliferation, migration, differentiation, and survival of germ cells (66). In general, granulosa cells and ovarian epithelial cells generate KL, and follicular membrane cells and oocytes express KIT. KL binds to KIT and forms a dimer, which activates KIT's intrinsic tyrosine kinase activity, causes it to be phosphorylated, and then activates signal transduction molecules in cells. These effects have huge impacts on the production of primordial germ cells in the ovary, the activation of primordial follicles, the survival and growth of oocytes, the proliferation of granulosa cells, and the maintenance of meiosis (67).

The KL–KIT interaction promotes directed migration of primordial germ cells. KL has been shown to support germ cell proliferation *in vitro* culture assays, and the activation of KIT has been found to inhibit germ cell apoptosis by Sakata et al. (68) However, the precise mechanism of action of this pathway is still unknown (68).

This signaling pathway can also play a role in basal follicle activation and early follicular development. After injecting mice with a function-blocking antibody to KIT, Yoshida et al. found that recruitment and growth of primary follicles and proliferation of granules were disturbed (69). Parrott et al. (70) treated the ovaries of 4-day-old rats with culture medium supplemented with KIT blocking antibody, and established

a control group at the same time. After 2 weeks of *in vitro* culture, they noticed a decrease in the number of latent primordial follicles in the experimental group and some spontaneous activation of primordial follicles, which supported the involvement of endogenous KL. The proportion of primordial follicles was reduced, whereas the proportion of developing follicles increased when exogenous KL was added to the culture media. Parrott thought KL could activate primordial follicles in mice, but this process is likely to require additional factors (70). A similar study was done by Carlsson et al. using human follicle cells, but they did not find a stimulating effect of KL on the transformation of primordial follicles into primary follicles. They hypothesized that this discrepancy may be caused by species-related differences in rodents and humans. Carlsson et al. (71) posited that KIT and KL are expressed early in follicular development in the human ovary and can control follicle survival during this period, and blocking KIT can induce follicular atresia. However, unlike rodents, exogenous KL cannot improve human follicle survival (71). Jin et al. (72) suggested that KL could promote early follicle development through the mediation of protein kinase C. It could also inhibit oocyte apoptosis by upregulating the anti-apoptotic protein Bcl-2 and downregulating the pro-apoptotic factor Bax (72).

TGF-β/Smad pathway

Transforming growth factor beta (TGF-β) is a multifunctional cytokine that belongs to the transforming growth factor superfamily, which includes three different isoforms of transforming growth factors (TGF-β 1–3) and many other signaling molecules (73). TGF-β is a doublet composed of two structurally related subunits connected by disulfide bonds. It can be activated by altering the ionic strength or by hydrolysis and excision actions of proteases, forming a serine/threonine kinase complexes with other factors, and attaching to the TGF-β receptor (74). Type 1 receptor (TGF R1) and type 2 receptor (TGF R2) make up the TGF β receptor, and TGF R1 can be blocked by SD208. After binding to TGF-β, the TGF R2 phosphorylates and activates the TGF R1, resulting in a series of signaling responses (75). These responses activate downstream substrates and regulatory molecules, which in turn stimulate the expression of target genes and regulate the activation, proliferation, and differentiation of many cell types as well as the self-renewal of stem cells, thus playing crucial roles in embryonic development and tissue homeostasis (76).

The Smads protein is a key downstream medium of TGF-β signaling, which is a group of structurally similar proteins that are the main signal transduction factors of the TGF-β pathway and are essential for regulating cell growth and development. There are three different isoforms of Smads: combinatorial Co-Smads, inhibitory I-Smads, and receptor-regulated R-Smads (77). The intracellular kinase structure of TGF-R1 phosphorylates R-Smads, exposing nuclear input sequences and building a complex with Co-Smads that can relocate to the nucleus and bind to target genes (78). I-Smads TGF-β signals through a variety of mechanisms, including blocking the binding of R-Smads to TGF R1 and Co-Smads, reducing TGF R1 expression, and obstructing transcription in the nucleus (79). TGF-β slows cell cycle progression in adult cells and stops cells from entering the G1/S phase transition, leading to the induction of apoptosis. The Smad signaling pathway plays a role in the regulation of this phenomenon; this situation is found in epithelial cells of numerous organs (80). Exhaustion of Co-Smads results in endocrine disturbance and increased follicular atresia in the ovary, and overexpression of I-Smads greatly increases the rate of follicular apoptosis (81, 82). Zhu et al. (83) found that thymopentin has a significant therapeutic effect on POF by stimulating Smad signaling, reducing cellular stress damage and inflammatory factors (83).

TGF-β is crucial for the growth of mouse follicles and gonadal tissues. Zheng et al. conducted *in vitro* mouse ovary culture experiments to further understand the role of TGF-β, in which the control group received no treatment, while experimental groups 1 and 2 received TGF R1 and SD208, respectively. The number and shape of follicles were assessed by sectioning the ovaries after 7 days of culture. It was found that the growth of primordial follicles and oocytes in ovaries treated with TGF R1 was inhibited, the number was decreased, and the proliferation of granulosa cells was significantly weakened. By contrast, in the ovaries treated with SD208, oocytes grew faster, had larger cell diameters, a higher percentage of activated cells, and more granulosa. After TGF R1 expression was significantly reduced by si-RNA, as seen by PCR and western blot, it was observed that the proportion of developing follicles in the ovaries increased, the growth was accelerated, and the volume became larger. Therefore, the researchers came to the conclusion that the TGF-β signaling pathway is crucial for maintaining the dormancy of the primordial follicular pool and that the mechanism of action of SD208 may involve activating the ribosomal protein s6 (RPS6) signaling pathway in mouse ovaries, thereby producing an inhibitory effect to the TGF-β signaling pathway (84).

Further, research has shown that progesterone and luteinizing hormone can increase the survival of preovulatory follicles. In a rat model, luteinizing hormone prevented follicular apoptosis by increasing the level of insulin-like growth factor 1 (85). Progesterone acts on nuclear and membrane receptors to prevent granulosa cell death (86). FSH can promote the secretion of progesterone and increase the expression of the luteinizing hormone receptor, and the TGF-β pathway can enhance this process, thereby indirectly improving the survival rate of follicles (87).

JAK/STAT pathway

The JANUS kinase-signal transducer and the activator of transcription (JAK/STAT) pathway is considered to be one of the most important signaling pathways in cells. It allows extracellular chemical signals to pass through the cell membrane and be delivered to the DNA promoter in the nucleus to control the expression of the corresponding genes. Several growth factors and cytokines, including interferon, interleukin, and colony-stimulating hormone, are involved in this route, which is related to a number of bodily processes, including immunological adaption, tissue repair, inflammatory response, and apoptosis (88).

The pathway is highly conserved in evolution and consists of JAK, STAT, and the receptor–ligand complex. JAK is a non-acceptor tyrosine kinase that is a subgroup of tyrosine kinases that catalyzes the transfer of phosphate groups from nucleoside triphosphate donors to tyrosine subunits in proteins, regulating cell growth, differentiation, proliferation, death, and migration (89). JAK has two similar phosphate structure transfer domains, one of which has a kinase function, while the other has inhibitory effects on the kinase activity of the first domain. A family of intracellular transcription factors called STAT proteins regulates the proliferation, differentiation, and death of cells (90).

When a ligand binds to the receptor in this pathway, the receptor undergoes a conformational change, so that the two domains in JAK are close to each other and phosphorylation occurs, resulting in conformational change that further activates STAT. The activated STAT signal transfers into the nucleus and regulates the transcription of specific genes (91). Zhang et al. (92) investigated the transcriptomic profile of human granulosa cells and found that the JAK/STAT signaling pathway was more strongly expressed and the expression of JAK1 was upregulated in granulosa cells at the primordial follicle stage, suggesting that this pathway may mediate the transition from primordial to primary follicles (92). Ernst et al. (93) performed similar experiments, sequencing RNA samples from human primordial and primary follicles and discovered that the expression of JAK/STAT pathway was significantly downregulated during the transformation from human primordial to primary follicles, indicating that the pathway's expression starts to weaken after completing the role of mediating follicle maturation (93).

MAPK pathway

The mitogen-activated protein kinase (MAPK) signaling pathway can transmit signals from the extracellular matrix to the nucleus, which is important for cellular communication. MAPK is a group of serine/threonine protein kinases that can be stimulated by extracellular signals such as cytokines and neurotransmitters to regulate cell growth, proliferation, apoptosis, and other activities (94). The core of this pathway is tertiary, which consists of the MAPK kinase activator, MAPK kinase, and MAPK. As a key mechanism for eukaryotic signal transmission, it governs physiological and pathological processes, as well as gene expression (95). After the cell surface receptor binds to mitogen, it stimulates the Ras protein to convert GDP into GTP, then activates the MAPK pathway and intracellular transcription factors. During this process, MAPK is phosphorylated (96).

The MAPK pathway is involved in regulating the processes of meiosis, cytoplasmic maturation, nuclear membrane formation, chromatin condensation, and spindle assembly. It can be triggered to promote the resumption and progress of meiosis. Salamone et al. compared prepubertal calf and adult bovine oocytes and found that calf oocytes lacked developmental capacity. They found that calf oocytes contained higher concentrations of MAPK after comparing the levels of the two. This demonstrates that MAPK can regulate oocyte activation by influencing cytoplasmic maturation (97).

Goudet et al. (98) discovered that after equine oocytes were cultured*in vitro*, the majority of cells were unable to complete meiosis, and MAPK remained in a non-phosphorylated form; however, in mature equine oocytes, MAPK was phosphorylated and possibly had kinase activity (98). Even though the biochemical mechanism is unclear, it seems that the inability of oocytes to complete meiosis may be caused by unphosphorylated MAPK.

To further clarify the role of the MAPK pathway, Zhang et al. (99) cultured porcine oocytes with U0126, an inhibitor of the MAPK pathway, and observed the process of meiosis and the expression of related genes. They discovered that U0126 could prevent the resumption of meiosis. Some mRNAs in the cells displayed high levels of expression; however, these mRNAs experienced a decline in oocytes that completed meiosis normally. This implies that adequate MAPK expression contributes to normal oocyte development, while normal expression of the MAPK pathway promotes the above mechanisms (99). Furthermore, after transferring U0126-treated oocytes to a drug-free medium, the maturation ability of the oocytes was restored, indicating that the blocking effect of U0126 is reversible.

Multi-pathway collaboration

Activators of the PI3K signaling pathway can stimulate primordial follicles in ovarian tissue to treat POF. PTEN inhibitors and PI3K activators can regulate the PI3K/Akt signaling pathway in follicles, and can activate quiescent follicles in patients with POF to develop into preovulatory follicles (100). Ovarian rupture can influence the Hippo signaling system and stimulate the growth of secondary follicles. Patients with POF may benefit from a combination of these two methods. Using activators of Akt or the PI3K pathway can simulate resting

follicles in ovaries, and then inhibit Hippo signaling through ovarian fragmentation, thereby stimulating follicle growth and obtaining mature egg cells (101). Sun et al. (102) found in mice that the use of gonadotropin-releasing hormone (GnRH) agonist can promote the expression of PI3K/Akt signaling pathway, reduce the occurrence of ovarian atresia, and improve ovarian reserve function (102). Li et al. (103) found that Notch, insulin, and other pathways also interact with Foxo3 to make it act as a guardian of the ovarian follicle pool in mammals and a potential determinant of the onset of menopause (103).

By activating the ribosomal protein s6 (RPS6) signaling pathway, which is a downstream signal of mTORC1, the TGF-pathway inhibitor SD208 can induce follicular growth and break the dormant state of follicles (84). Rapamycin has an inhibitory effect on the mTOR pathway. After the stimulatory effect of SD208 on oocyte growth was observed, rapamycin was found to partially inhibit this stimulatory effect and reduce the number of growing follicles. In addition, the TGF-β signaling pathway can activate the PI3K/Akt pathway in many cells, induce phosphorylation of Akt and Foxo3 (104), and activate the mTORC1 signal *via* the PI3K/Akt/TSC2 pathway (105), but these effects may not involve regulating the growth of primordial follicles. *In vitro* activation (IVA) techniques have been investigated in recent years to mobilize residual follicles in POF patients. Initially, IVA disrupted Hippo signaling by disrupting the ovaries, and then the fragments were cultured *in vitro* with an Akt stimulant for 2 days before being transplanted back into the patient. This method has now been developed into a one-step procedure that does not rely on drugs to obtain fertile, mature follicles. When antral follicles develop to the preovulatory stage, mature oocytes are extracted, and embryos are transferred after *in vitro* fertilization with sperm. IVA has been performed in patients with POF, and the follicles can grow and fertilize successfully (106). To date, about 20 live births have been reported using this technique, which is regarded as the most hopeful method to reproduce genetic offspring in patients with POF. Thus, efforts should be made to clarify the mechanism of action and guarantee the safety of this treatment before it is used extensively in the clinic. In a clinical trial, Suada et al. (107) used autologous growth factors to activate damaged ovaries *in vitro*, then transplanted them into patients, and measured hormone levels in patients. They found that there was a correlation between improved hormone levels and the volume of transplanted tissue (107). Further, interference with the Hippo pathway should be done with caution, since dysregulation of it is linked to the development of cancer.

The process of transformation from primordial follicles into mature follicles is complex. There are a number of intercellular and intracellular signaling pathways that interact in various ways to form a complicated network that can respond to many stimuli, but the precise mechanism remains to be further studied.

Despite these new advances, regulating primordial follicle activation and controlling oocyte development *in vitro* are still different for patients with POF. Moreover, current studies are mainly carried out on animal models; thus, due to the differences between human and animal models, the research findings may have limitations.

Signaling pathway and stem cells

Some signaling pathways may be related to stem cells. The KL-KIT and TGFβ-Smad and other pathways discussed earlier can regulate the self-renewal of stem cells through different mechanisms and play a role in embryonic development and tissue homeostasis. In recent years, stem cell transplantation has become a hotspot in the treatment of POF. The mechanism by which stem cells improve ovarian function is related to the cytokines or exosomes secreted by them, which regulate immunity and promote follicle development while improving the microenvironment of ovarian tissue, and are related to various signaling pathways.

Stem cells are a subtype of cells that maintain an undifferentiated form in both embryonic and adult tissues and can undergo self-renewal and differentiation (108). By participating in the repair of organ damage, stem cells in differentiated organs promote the restoration of organ function. Stem cells can be categorized as embryonic stem cells (ESC), mesenchymal stem cells (MSC), induced pluripotent stem cells (iPSC), spermatogonia stem cells (SSC), ovarian stem cells (OSC), etc. Stem cell therapy can promote ovulation in patients with POF. There are many studies focusing on different protocols for stem cell isolation, purification, and culture for the treatment of POF (108). Different types of stem cells have been used in recent studies to treat POF (Table 1). Studies have shown that human ESCs play an important role in the repair and functional recovery of endometrial damage, and ESCs have become an important tool for cell therapy (109). MSCs can colonize injured ovaries and secrete and release a range of cytokines to restore ovarian function. According to several studies, MSCs may be used to treat infertility brought on by ovarian and endometrial dysfunction (110). Researchers applied bone marrow mesenchymal stem cells (BMSC) therapy in a rabbit model of ovarian dysfunction induced by cyclophosphamide, and they observed that it restored the function and structure of follicles by decreasing gonadotropin levels and increasing estrogen and vascular endothelial growth factor levels (112). Mohamed et al. (113) transplanted BMSC into mice with POF and observed an increase in ovarian volume and weight in mice, which accommodated the development of follicles (113). Liu et al. (114) found that in mice with POF, human menstrual blood mesenchymal stem cells (hMensSCs) can promote the increase in follicle number and restoration of ovarian function, as

TABLE 1 The applications of stem cells in the treatment of POF.

Type of stem cell	Function	Authors name	Year	References
ESCs	Repair endometrial damage	Wang et al.	2019	(109)
MSCs	Produce cytokines, regulate inflammation	Trounson et al.	2017	(110)
	Secret exosomes to regulate the PI3K/Akt/mTOR pathway, promote ovarian angiogenesis, induce apoptosis of cells	Qu et al.	2022	(111)
BMSCs	Restore function and structure of follicles and increase estrogen and vascular endothelial growth factor levels	Somia et al.	2013	(112)
	Restore ovarian hormone production and reactivate folliculogenesis	Mohamed et al.	2018	(113)
hMensSCs	Increase ovarian weight, normal follicles number, AMH and estrogen level	Liu et al.	2014	(114)
hUCMSCs	Increase ovarian reserve function	Li et al.	2017	(115)
	Increase the activity of the PI3K/Akt signaling in dormant oocytes	Mi et al.	2022	(116)
iPSCs	Differentiate into ovarian epithelioid cells, increase estrogen levels and ovarian weight, decrease atretic follicles	Liu et al.	2013	(117)
OSCs	Induce the generation of follicle	White et al.	2012	(118)
ADMSCs	Protect primordial follicles from direct death, maintain quiescence through modulation of the PI3K/Akt pathway	Cacciottola et al.	2021	(119)

well as the secretion of estrogen and anti-Mullerian hormone (AMH) (114). A study revealed that the ovarian reserve function of rats' was improved and the number of follicles rose after receiving transplantation human umbilical cord mesenchymal stem cells (hUCMSCs) (115). The hepatocyte growth factor, which is secreted from hUCMSCs, can increase the activity of the PI3K/Akt signaling in dormant oocytes (116). By transplanting hUCMSCs into mice with POF, Lv et al. (120) found that multiple hUCMSCs transplantations had a better effect on the recovery of ovarian function than single hUCMSCs transplantation (120). iPSCs can be differentiated into hormone-sensitive ovarian epithelioid cells, and when these cells are transplanted into a mouse with POF, an increase in estrogen levels and ovarian weight and a decrease in atretic follicles were observed in mice (117). White et al. (121) isolated OSCs from the human ovarian cortex and transplanted them into immunocompromised mice. They found that the cells could induce the generation of mouse follicles and be used to treat POF (121). Vo et al. (122) suggested that this approach, which uses stem cells to generate new oocytes, is particularly useful in patients with POF who have no residual follicles after chemotherapy or radiotherapy (122). Cacciottola et al. (119) demonstrated that adipose tissue-derived stem cells (ADMSCs) exert positive effects on the ovarian reserve, not only by protecting primordial follicles from direct death but also by maintaining their quiescence through modulation of the PI3K/Akt pathway (119). Furthermore, MSCs can release a variety of bioactive molecules that can regulate inflammation and other immune responses, promote endometrial regeneration, and help restore fertility (123, 124). Qu et al. (111) used exosomes secreted by MSCs to treat POF in rats and found that exosomes could regulate the PI3K/Akt/mTOR pathway, promote ovarian angiogenesis, and induce apoptosis of cells (111). Zhao et al. mentioned in a review that exosomes might represent a new treatment method for enhancing decreased fertility in women with POF (125).

Although the effect of stem cell therapy has been shown in some animals, its clinical application is still severely limited because of the intrusiveness of stem cell collection and possible ethical and immune rejection issues. Further research is therefore needed to explore stem cells that are simple to collect, non-invasive and safe during collection, low in immunogenicity, and do not involve ethical concerns.

Summary

POF has significant physiological and psychological impacts on women; thus, it is critical to reveal more knowledge about its pathogenic mechanisms and available treatments. It is necessary to have a better understanding of the mechanisms related to follicular pool depletion and to master methods of intervening in ovarian aging. The activation of primordial follicles is a complex but coordinated process that is regulated by multiple factors and pathways. In this review, we focused on describing the roles of PI3K/Akt/Foxo3 signaling pathway, mTOR signaling pathway, etc. in primordial follicle activation, and also discussed other mechanisms that regulate follicle dormancy and activation. Based on our current understanding of follicle activation, the development of drugs that regulate

the activation of primordial follicles and target some signaling pathways may provide new possibilities for the treatment of POF. Further studies can also be conducted on other potential regulators that could help provide patients with a more personalized treatment plan. In recent years, many different types of stem cells have been found to have therapeutic effects on POF. For the protection of female fertility, stem cells can be considered treatment options to restore female fertility. In addition, psychological problems caused by impaired fertility also play a certain role in the treatment of POF patients, and the psychological state of patients should also be paid attention to in the treatment of POF (126). Studies have suggested that infertility problems caused by POF often plague the whole family, and while paying attention and treatment to female members of the family, we should also pay attention to the physical and psychological problems of male members (127).

However, there is still much unknown information about the above approaches, and some measures are still limited to the experimental level due to ethical or technical issues. Thus, further research is required. With the advancement of science, technology, and experimental-level strategies, as well as interventions for improving the quality of life of patients with POF through multidisciplinary therapy, we hope to obtain evidence of the safety and efficacy of these methods through large-scale clinical trials.

Author contributions

XB conceived the study and wrote the manuscript. SW conceived the study, organized, and edited the text. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Three-dimensional ultrasound assessment of risk factors for cystocele and Green classification in primipara

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Background and aims: The present study aimed to analyze the effects of factors on cystocele and the Green classification.

Materials and methods: We conducted a cross-sectional study on 357 primiparous women examined at our hospital from January 2019 to May 2021. The following data were recorded: maternal characteristics, neonatal characteristics, and factors of childbirth. It was added to the multivariate logistic regression model to determine the independent predictors of the cystocele and the Green classification.

Results: A total of 242 women had cystocele, including 71 women with Green type I cystocele, 134 women with Green type II cystocele, and 37 women with Green type III cystocele. In multivariate logistic regression analysis, body mass index (BMI) at delivery was associated with cystocele, while BMI at delivery and the second stage of labor (SSL) > 1 h were independently with the distance from the symphysis pubis to the bladder neck (SPBN) abnormal ($P < 0.05$). BMI at examination was associated with the large retrovesical angle (RVA) ($P < 0.05$). BMI at delivery and the fetal right occiput anterior position (ROA) were independently associated with the distance from the symphysis pubis to the posterior wall of the bladder (SPBP) abnormal ($P < 0.05$), while epidural anesthesia (EDA) was the protective factor ($P < 0.05$).

Conclusion: Primipara women should strive to avoid exposure to modifiable risk factors such as controlling weight during pregnancy, reducing weight after delivery, and shortening SSL to reduce the occurrence of cystocele.

KEYWORDS

ultrasound, vaginal delivery, cystocele, Green classification, pelvic floor dysfunction

Introduction

Pelvic floor dysfunction (PFD) refers to a group of disorders caused by structural defects or degradation, damage, and dysfunction of the pelvic floor support system that severely affects the quality of life. Multiple symptoms of PFD may include stress urinary incontinence (SUI), pelvic organ prolapse (POP), chronic pelvic pain, constipation, and sexual abnormalities (1). Vaginal delivery (VD) significantly increases the possibility of POP in women (2), and it is the most critical epidemiological risk factor for PFD. Most of the damage to a woman's pelvic floor is likely to occur during the first vaginal birth (3, 4). At present, most studies focus mainly on levator ani muscle (LAM) injury, but there are fewer studies on cystocele and the Green classification. Cystocele is the anterior vaginal wall prolapse accompanied by prolapse of the bladder wall, and it is the most common type of POP and the most prone to recur after surgery (5, 6). The current imaging classification of cystocele is proposed by Green. Cystocele has different clinical manifestations according to the Green's classification (6). Radiological cystocele type (Green classification) can be distinguished both clinically and on ultrasound, and agreement between methods as well as inter-observer agreement for the clinical diagnosis is moderate to good (7).

In this study, We aim to use three-dimensional ultrasound to diagnosis cystocele and its classification, and analyzed the effects of maternal characteristics, fetal characteristics, and delivery factors on cystocele and its classification. We hypothesized that BMI after 3 months of vaginal delivery will be effect of cystocele and the Green classification.

We used three-dimensional ultrasound to detect cystocele and its type and analyzed the effects of maternal, fetal, and delivery factors on cystocele occurrence and its classification. The objective was to reduce pelvic floor damage in primipara women during childbirth.

Materials and methods

Study design

This was a cross-sectional study of healthy pregnant Chinese women. Details on maternal characteristics and delivery outcomes were obtained from the medical records of the our hospital from January 2019 to May 2021.

The inclusion criteria were nulliparous women with maternal age of ≥18 years, and time of ultrasonography was 42 to 90 days after delivery. Exclusion criteria were delivery at <28 gestational weeks and pre-existing diseases/conditions that are likely to pre-dispose to PFD. This included previous bladder/bowel diseases and chronic kidney disease. Participants did not perform any rehabilitation exercises. The Institutional Review Board approved the study protocol.

Clinical data

The hospital medical record system was checked, and the following details were recorded: maternal characteristics (include maternal age, body mass index (BMI) at delivery, BMI at ultrasound examination, gestational age, hypertension, diabetes), neonatal characteristics (include fetal height, head circumference, chest circumference, birth weight), and factors of childbirth (SSL, episiotomy, perineal tear, forceps use, vacuum use, and fetal orientation).

Ultrasonographic data

An ultrasonographic evaluation was performed by a single examiner with more than 5 years of experience in obstetric ultrasound and with specific training in 3/4D imaging. Women were examined with *trans*-perineal ultrasound using the GE Voluson E8 system with a 3D/4D RIC 5–9-D probe with an acquisition angle of 180°.

Before the examination, the subject emptied the bladder (urine volume < 50 ml) and rectum and assumed the lithotomy position. The probe was wrapped in a condom and placed above the labia minora. The probe was placed close to the lower edge of the pubic symphysis for a clear display of the midsagittal section of the pelvic floor structure and the position and shape of the bladder were observed under the resting state and Valsalva. Valsalva movement requirements: the duration lasts more than 5 s, while the levator ani muscle hiatus is observed to be dilated, and the pelvic organs are displaced to the dorsal caudal side. The lower edge of the symphysis pubis (SP) was used as the reference level to analyzed the following parameters: the retrovesical angle (RVA), urethral inclination angle, distance from the inferior margin of the symphysis pubis to the bladder neck (SPBN), and the distance from the inferior margin of the symphysis pubis to the posterior wall of the bladder (SPBP) at rest and Valsalva. The urethral rotation angle (URA) was also calculated.

A cystocele was diagnosed on ultrasound if any part of the bladder below the symphysis pubis (8). According to Green classification, the following types of cystocele were diagnosed: Green I cystocele (RVA ≥ 140°, URA < 45°), Green II cystocele (RVA ≥ 140°, URA between 45° and 120°, and Green III (RVA < 140°, the lowest point of the bladder reaching below the symphysis pubis) (9). In Green III cystocele, the lowest point of the bladder is often the posterior wall of the bladder, unlike the bladder neck in other types.

Statistical analyses

Statistical analyses were performed using SPSS v. 20 (SPSS, Chicago, IL, USA). The normality of data was assessed using the Shapiro Wilk method. Normally and non-normally

TABLE 1 Analysis of factors associated with cystocele, symphysis pubis to the bladder neck (SPBN), and urethral rotation angle (URA).

Variables	Cystocele		*P*	SPBN		*P*	URA		*P*
	No cystocele	Cystocele		Normal	Abnormal		Normal	Abnormal	
Maternal characteristics									
Maternal age (years)*	27 (25.25∼29)	28 (26∼30)	0.268[a]	27 (25∼29)	28 (26∼30)	0.177[a]	27 (26∼29)	28 (25∼30)	0.626[a]
Gestational age (weeks)*	39.5 (39∼40.2)	39.5 (39∼40.3)	0.912[b]	39.5 (39∼40.2)	39.5 (39∼40.3)	0.681[b]	39.5 (39∼40.3)	39.5 (39∼40.3)	0.685[b]
Gestational diabetes, *n* (%)	8 (2.2)	20 (5.6)	0.946[b]	5 (1.4)	23 (6.4)	0.644 [b]	11 (3.1)	17 (4.8)	0.560[b]
Hypertension, *n* (%)	1 (0.3)	3 (0.8)	1.000[d]	1 (0.3)	3 (0.8)	1.000 [e]	1 (0.3)	3 (0.8)	0.776[d]
BMI at delivery (kg/m^2)*	24.82 (23.19∼26.77)	26.24 (24.61∼28.2)	**<0.001**[a]	24.82 (23.36∼26.77)	26.13 (24.54∼28.02)	**0.002**[a]	25.65 (23.63∼27.68)	26.15 (24.54∼27.77)	0.130[a]
BMI at examination (kg/m^2)*	21.9 (20.69∼24.15)	23.53 (21.88∼25.49)	**<0.001**[a]	21.9 (20.69∼24.21)	23.44 (21.59∼25.39)	**0.001**[a]	22.58 (20.96∼24.8)	23.44 (21.7∼25.27)	**0.047**[a]
Neonatal characteristics									
Fetal height (cm)*	50 (50∼50)	50 (50∼51)	0.091[a]	50 (50∼50)	50 (50∼51)	0.141[a]	50 (50∼51)	50 (50∼50)	0.740[a]
Head circumference (cm)*	34 (33∼34)	34 (33∼34)	0.829[a]	34 (33∼34)	34 (33∼34)	0.424[a]	34 (33∼34)	34 (33∼34)	0.590[a]
Chest circumference (cm)*	32 (32∼33)	32 (32∼33)	0.738[a]	32 (32∼33)	32 (32∼33)	0.358[a]	32 (32∼33)	32 (32∼33)	0.657[a]
Birth weight (g)#	3216.15 ± 352.28	3328.42 ± 355.47	**0.007**[c]	3220.39 ± 343.96	3316.09 ± 359.23	**0.038**[c]	3315.85 ± 367.66	3279.55 ± 349.61	0.341[c]
Delivery characteristics									
SSL < 1 h, *n* (%)	88 (24.6)	157 (44)	**0.048**	64 (17.9)	181 (50.7)	< **0.001**[f]	113 (31.7)	132 (37)	0.581
SSL > 1 h, *n* (%)	21 (5.9)	74 (20.7)		7 (2)	88 (24.6)		38 (10.6)	57 (16)	
SSL > 2 h, *n* (%)	6 (1.7)	11 (3.1)		5 (1.4)	12 (3.4)		8 (2.2)	9 (2.5)	
EDA, *n* (%)	45 (12.6)	123 (34.5)	0.358[b]	32 (9)	136 (38.1)	0.329[b]	77 (21.6)	91 (25.5)	0.642[b]
Episiotomy, *n* (%)	27 (7.6)	66 (18.5)	0.980[b]	20 (5.6)	73 (20.4)	0.953[b]	39 (10.9)	54 (15.1)	0.557[b]
Second-degree vaginal tear, *n* (%)	62 (17.4)	151 (42.3)	0.990[b]	46 (12.9)	167 (46.8)	0.863[b]	101 (28.3)	112 (31.4)	0.183[b]
Forceps and vacuum assisted delivery, *n* (%)	1 (0.3)	3 (0.8)	1.000[d]	1 (0.3)	3 (0.8)	1.000[e]	2 (0.6)	2 (0.6)	1.000[d]
Fetal position			0.160[b]			**0.035**[b]			0.092[b]
LOA, *n* (%)	71 (19.9)	181 (50.7)		52 (14.6)	200 (56)		115 (32.2)	137 (38.4)	
ROA, *n* (%)	29 (8.1)	52 (14.6)		23 (6.4)	58 (16.2)		37 (10.4)	44 (12.3)	
Others, *n* (%)	4 (1.1)	20 (5.6)		1 (0.3)	23 (6.4)		7 (2)	17 (4.8)	

BMI, body mass index; SSL, second stage of labor; EDA, Epidural anesthesia; LOA, left occiput anterior; ROA, right occiput anterior; SPBN, symphysis pubis to the bladder neck; URA, urethral rotation angle. Bold values are $P < 0.05$ and are statistically significant.

*Median (Inter-Quartile Range).

#Mean ± Standard Error.

[a]Mann-Whitney *U* test.

[b]Chi-square test.

[c]Student's *t*-test.

[d]Continuity Correction.

[e]Fisher's exact test.

[f]Likelihood ratio test.

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TABLE 2 Analysis of factors associated with retrovesical angle (RVA) and symphysis pubis to the posterior wall of the bladder (SPBP).

Variables	RVA	Abnormal	*P*	SPBP	Abnormal	*P*
	Normal			Normal		
Maternal characteristics						
Maternal age (years)*	27 (25∼29)	28 (26∼30)	0.664[a]	27.5 (26∼29)	28 (25∼30)	0.724[a]
Gestational age (weeks)*	39.5 (39∼40.25)	39.6 (39∼40.3)	0.732[b]	39.5 (39∼40.3)	39.6 (39∼40.3)	0.341[b]
Gestational diabetes, *n* (%)	8 (2.2)	20 (5.6)	0.622[b]	21 (5.9)	7 (2)	0.788[b]
Hypertension, *n* (%)	0 (0)	4 (1.1)	0.385[c]	4 (1.1)	0 (0)	0.507[c]
BMI at delivery (kg/m^2)*	25.89 (23.88∼27.24)	26.04 (24.44∼28.03)	0.094[a]	25.71 (23.86∼27.71)	26.35 (24.79∼27.84)	**0.030**[a]
BMI at examination (kg/m^2)*	22.45 (20.7∼24.41)	23.43 (21.68∼25.44)	**0.012**[a]	23.14 (21.22∼25.24)	23.42 (21.6∼25.11)	0.374[a]
Neonatal characteristics						
Fetal height (cm)*	34 (33∼34)	34 (33∼34)	0.050[a]	50 (50∼51)	50 (50∼50)	0.937[a]
Head circumference (cm)*	34 (33∼34)	34 (33∼34)	0.413[a]	34 (33∼34)	34 (33∼34)	0.968[a]
Chest circumference (cm)*	32 (32∼33)	32 (32∼33)	0.777[a]	32 (32∼33)	32 (32∼33)	0.498[a]
Birth weight (g)*	3,250 (3,005∼3,500)	3,330 (3,100∼3,547.5)	0.054[a]	3,300 (3,080∼3,512.5)	3,330 (3,020∼3,545)	0.423[a]
Delivery characteristics						
SSL < 1 h, *n* (%)	88 (24.6)	157 (44)	0.164	183 (51.3)	62 (17.4)	0.381[d]
SSL > 1 h, *n* (%)	24 (6.7)	71 (19.9)		64 (17.9)	31 (8.7)	
SSL > 2 h, *n* (%)	5 (1.4)	12 (3.4)		13 (3.6)	4 (1.1)	
EDA, *n* (%)	50 (14)	118 (33.1)	0.253[b]	131 (36.7)	37 (10.4)	**0.039**[b]
Episiotomy, *n* (%)	30 (8.4)	63 (17.6)	0.902[b]	65 (18.2)	28 (7.8)	0.459[b]
Second-degree vaginal tear, *n* (%)	69 (19.3)	144 (40.3)	0.853[b]	160 (44.8)	53 (14.8)	0.237[b]
Forceps-assisted and vacuum-assisted delivery, *n* (%)	2 (0.6)	2 (0.6)	0.839[c]	2 (0.6)	2 (0.6)	0.640[c]
Fetal position			0.476[b]			**0.046**[b]
LOA, *n* (%)	79 (22.1)	173 (48.5)		193 (54.1)	59 (16.5)	
ROA, *n* (%)	31 (8.7)	50 (14)		52 (14.6)	29 (8.1)	
Others, *n* (%)	7 (2)	17 (4.8)		15 (4.2)	9 (2.5)	

Bold values are $P < 0.05$ and are statistically significant.
*Median (IQR).
#Mean ± SD.
[a]Mann-Whitney *U* test.
[b]Chi-square test.
[c]Continuity Correction.
[d]Likelihood ratio test.

distributed continuous data were expressed as mean ± standard deviation and quartile, respectively. Normally and non-normally distributed continuous data were analyzed by Student's *t*-test and Mann-Whitney *U* test, respectively. Categorical variables were analyzed by the chi-square test. $P < 0.05$ was considered to be statistically significant. Logistic regression analysis was used to demonstrate independent risk factors for cystocele while controlling for potential confounding factors.

Results

A total of 357 women who had vaginal delivery were enrolled in this study. Of these, 242 (67.8%) women had cystocele, including 71 (19.9%) women with Green type I cystocele, 134 (37.5%) women with Green type II cystocele, and 37 (10.4%) women with Green type III cystocele. Moreover, 281 (78.7%) women had abnormal SPBN, 198 (55.5%) women had increased URA, 240 (67.2%) women had open RVA, and 97 (27.2%) women had abnormal SPBP.

The results for the hospital parameters were as follows: episiotomy: 93 (26.1%) women; second-degree perineal tear: 213 (59.3%) women; epidural anesthesia: 18 (47.1%) women, diabetes: 28 (7.8%) women; hypertension: 4 (1.1%) women; assisted labor: 4 (1.1%) women [including 1 (0.3%) woman with forceps-assisted delivery and 3 (0.8%) women with vacuum-assisted delivery]. The following results were noted for fetal orientation: fetal left occiput anterior (LOA) position in 252 (70.6%) women, fetal right occiput anterior (ROA) position in 81 (22.7%) women, and other fetal positions in 24 (6.72%) women [including occipital posterior (OP) position in 11 (3.1%) women and occipital bone is directly in front of the pubic symphysis position in 13 (3.6%) women].

In the univariate analysis, BMI at delivery, BMI at the examination, and birth weight in patients with cystocele were greater than those in the normal group, and the SSL in these patients was also had an impact on the cystocele group. BMI at delivery, BMI at the examination, and birth weight were greater in the abnormal SPBN group than in the normal group. SSL and

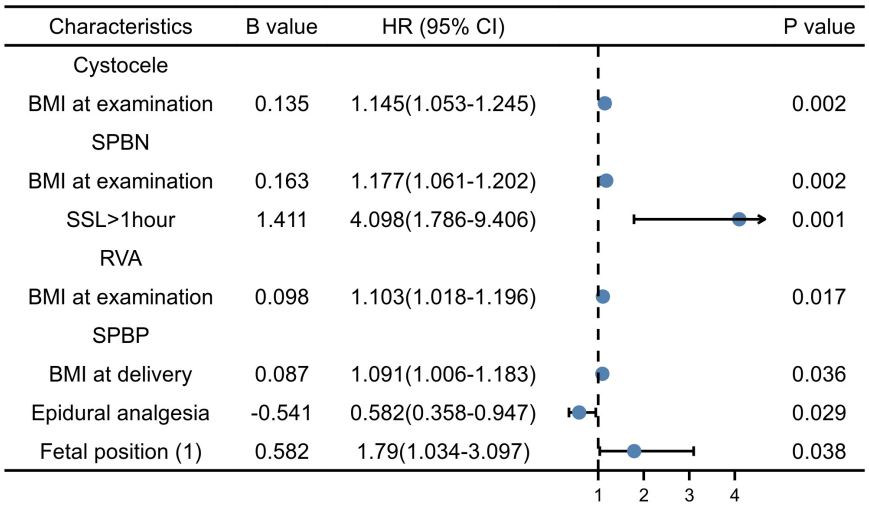

FIGURE 1
Multiple factor analysis of cystocele, symphysis pubis to the bladder neck (SPBN), retrovesical angle (RVA), and symphysis pubis to the posterior wall of the bladder (SPBP).

different fetal positions also had an impact on SPBN (Table 1). BMI at delivery with increased URA was greater than that in the normal group. BMI at delivery, BMI at the examination, and birth weight were greater in patients with large RVA than in the normal group. The BMI of patients with abnormal SPBP was also higher than that of the normal group. Epidural anesthesia (EDA) and fetal position were found to affect SPBP (Table 2).

According to the results of the univariate analysis, the above factors were entered into the multivariate logistic regression model (forward, conditional). BMI at delivery was an independent risk factor of cystocele (OR = 1.145, 95% CI: 1.053∼1.245); BMI at delivery (OR = 1.18, 95% CI: 1.065∼1.307) was independent risk factors of SPBN ($P < 0.05$). The SSL > 1 h compared to the SSL < 1 h (OR = 4.10, 95% CI: 1.786∼1.562) was the risk factors of SPBN ($P < 0.05$). BMI at examination (OR = 1.103, 95% CI: 1.018∼1.196) was the risk factor for the large RVA ($P < 0.05$). BMI at delivery (OR = 1.091, 95% CI: 1.006∼1.183) and the fetal ROA position compared to the LOA were the risk factors of SPBP ($P < 0.05$), while EDA (OR = 0.582, 95% CI: 0.358∼0.947) was the protective factor ($P < 0.05$). In the logistic regression analysis, all factors had no significant association with URA ($P > 0.05$) (Figure 1).

Discussion

Mechanical injury to the pelvic floor support system, denervation, ischemia and reperfusion injury, and defective soft tissue remodeling are some of the underlying mechanisms of injury for the development of PFDs (10). During pregnancy and after VD, pelvic organ support changes, and area of levator hiatus (HA) increases; thus, indicating a decrease in pelvic organ support (11, 12), This may increase the risk of PFD. The present study found that many factors influence the development of maternal cystocele during delivery.

Maternal and neonatal characteristics

Similar to previous studies, we found that the increase in BMI at delivery was significantly associated with abnormal SPBP (OR = 1.091, 95% CI: 1.006∼1.183). Not only does BMI during pregnancy increase the risk of pelvic floor disease, but BMI after delivery also affects the occurrence of cystocele. The increase in BMI at examination was significantly associated with abnormal SPBN (OR = 1.18, 95% CI: 1.065∼1.307) and open RVA (OR = 1.103, 95% CI: 1.018∼1.196). An obvious potential explanation for the increased prevalence of cystocele in obese women may be the long-term increase in intra-abdominal pressure in these individuals. Noblett et al. (13) found a strong correlation between BMI and intra-abdominal pressure, and between BMI and intravesical pressure, with Pearson's correlation coefficient of 0.76 and 0.71, respectively. The chronic increased pressure may lead to pelvic floor muscle fatigue and/or a chronic stretch on the pudendal nerve, which in turn may lead to pelvic floor muscle weakness. Although the decrease in pelvic muscle strength after VD will lead to POP, the correlation between them will weaken after a reduction in BMI (14). For primipara women, early weight loss can also help to reduce cystocele.

In other studies on the pelvic floor, age was found to be an important factor for cystocele (15). In the present study, no significant association was found between age and Green's classification of cystocele. This may be because all the primipara women in this study were young. In addition, there were fewer cases of gestational diabetes mellitus and gestational

hypertension in this study, neither hypertension nor GDM showed a statistically significant association with cystocele.

The most important risk factor for avulsion injuries during natural delivery is the birth weight (16). The increase in birth weight has an important effect on the time of labor and subsequently has a series of effects on the pelvic floor muscles. In our study, it had no significant in the multivariate binary logistic analysis on cystocele, only appeared as a significant variable in the Mann-Whitney *U* test.

Delivery characteristics

Prolonged labor may exceed the stretch limit of soft tissues, resulting in an imbalance in the repair and degradation process. When used for a long time, stress may cause temporary or permanent physical and/or functional damage through hypoxia, ischemia, and other harmful processes, leading to PFD (17). We found that SSL > 1 h was 4.10 times higher than SSL < 1 h for primary maternal of abnormal SPBN. Reports on the effects of episiotomy and perineal tear on pelvic floor function are not completely consistent (18, 19). But most studies believe that the use of episiotomy during vacuum-assisted delivery or forceps-assisted delivery of primipara women can reduce perineal tear or OASIS (20, 21). We did not confirm that episiotomy and tear have a significant effect on cystocele, which is consistent with the report of Ruan et al. (22). This may be because of the low episiotomy rate or the short observation period, or it may be because of only first-and second-degree tears in our study. Studies have shown that third-degree perineal tear is a significant risk factor for postpartum PFD (23).

Schiessl B (24) found that EDA can prolong SSL, thereby increasing the risk of UI; however, in our study we think that EDA is a protective factor of SPBP (OR = 0.582, 95% CI: 0.358∼0.947). The EDA effect could be explained by the resulting muscle relaxation. It is plausible that active pushing in labor distends and compresses the pelvic floor more forcefully, resulting in neuromuscular or vascular injury. Intrapartum epidural analgesia may be beneficial by preventing premature pushing. Another potential explanation may be the degree of levator relaxation in women with dense epidurals because a paralyzed muscle is less likely to suffer trauma, given a certain degree of distension (25).

The occipital position (OP) is the most common malposition with a prevalence of 5–13% at delivery (26). It increases the risk of LAM injury (27). In the present study, there is no difference in statistics due to the low numbers of OP. However, to our surprise, the results showed that ROA was more prone to SPBP abnormalities than LOA (OR = 1.79, 95% CI: 1.034∼3.097).

Strengths and limitations

All women underwent delivery in the same hospital following similar obstetric approaches. Moreover, all parturients had complete delivery information, which largely reduced the recall bias. Previous studies on postpartum pelvic floor injury mostly focused on factors such as birth weight, weight gain during pregnancy, and the labor process. In our study, we found that not only BMI at delivery has an important effect on cystocele in primipara women, but BMI within 42∼90 days postpartum is also an independent risk factor for abnormal SPBP and open RVA. Early postpartum weight loss can reduce pelvic floor injury.

The limitations of our study are a small sample size and relatively inadequate data. In this study, all primipara women were younger, with less degree of hypertension and diabetes. However, in future research, we will collect more samples for a more comprehensive analysis.

Conclusion

During delivery, the pelvic floor muscles of pregnant women will continue to be affected by mechanical injury and physiological changes after delivery. Some potential risk factors are uncontrollable, such as maternal age, fetal position, and fetal weight. However, according to the currently available data, efforts should be made to avoid exposure to modifiable risk factors, such as controlling weight during pregnancy, reducing weight after delivery, shortening the SSL, and reducing the occurrence of cystocele.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

WY: project development, manuscript writing, data analysis, and data collection. QM: project development, data analysis, and manuscript editing. WX: data collection and manuscript editing. YZ: data analysis. JW: project development, data collection, and manuscript editing. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Short interpregnancy interval can lead to adverse pregnancy outcomes: A meta-analysis

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Background: The evidence of some previous papers was insufficient in studying the causal association between interpregnancy interval (IPI) and adverse pregnancy outcomes. In addition, more literature have been updated worldwide during the last 10 years.

Methods: English and Chinese articles published from January 1980 to August 2021 in the databases of PubMed, Cochrane Library, Ovid, Embase, China Biology Medicine disc (CBM), and China National Knowledge Infrastructure (CNKI) were searched. Then following the inclusion and exclusion criteria, we screened the articles. Utilizing the Newcastle–Ottawa Scale (NOS), we evaluated the quality of the included articles. The literature information extraction table was set up in Excel, and the meta-analysis was performed with Stata 16.0 software (Texas, USA).

Results: A total of 41 articles were included in the meta-analysis, and NOS scores were four to eight. The short IPI after delivery was the risk factor of preterm birth (pooled odds ratio 1.49, 95% confidence interval 1.42–1.57), very preterm birth (pooled OR: 1.82, 95% CI: 1.55–2.14), low birth weight (pooled OR: 1.33, 95% CI: 1.24–1.43), and small for gestational age (pooled OR: 1.14, 95% CI: 1.07–1.21), offspring death (pooled OR: 1.60, 95% CI: 1.51–1.69), NICU (pooled OR: 1.26, 95% CI: 1.01–1.57), and congenital abnormality (pooled OR: 1.10, 95% CI: 1.05–1.16), while was not the risk factor of gestational hypertension (pooled OR: 0.95, 95% CI: 0.93–0.98) or gestational diabetes (pooled OR: 1.06, 95% CI: 0.93–1.20).

Conclusion: Short IPI (IPI < 6 months) can lead to adverse perinatal outcomes, while it is not a risk factor for gestational diabetes and

gestational hypertension. Therefore, more high-quality studies covering more comprehensive indicators of maternal and perinatal pregnancy outcomes are needed to ameliorate the pregnancy policy for women of childbearing age.

KEYWORDS

interpregnancy interval, adverse pregnancy outcomes, preterm birth, gestational hypertension, gestational diabetes

Introduction

Interpregnancy interval (IPI) is the period between the previous delivery and the following pregnancy. Previous studies have found that the length of IPI was related to adverse perinatal outcomes, such as preterm birth, low birth weight, small for gestational age, and stillbirth (1). In particular, 18–23 months IPI could promote maternal and fetal outcomes (2–4), while short IPI (IPI less than 6 months) was significantly associated with an increased risk of preterm birth, low birth weight, and small for gestational age (1). Therefore, WHO recommended that the IPI after delivery should be more than 24 months (5).

Previous meta-analyses have explored the relationship between short IPI and adverse pregnancy outcomes (6, 7). However, the included literature varies in its quality, for example, the analyzed studies were cross-sectional studies, and the birth interval (the time interval between two live births) rather than the IPI was measured. Thus, the evidence of meta-analysis was insufficient in studying the causal association between IPI and adverse pregnancy outcomes (8). A systematic review (7) found that short IPIs in high-resource settings may be associated with an increased risk of maternal obesity and gestational diabetes, as well as a reduced risk of preeclampsia in the next pregnancy. However, most pregnancy outcomes from the systematic review were evaluated in a single study, and the supportive evidence of associations is insufficient, and there were few studies focused on the influence of short IPIs on maternal morbidity and mortality.

The most recent meta-analysis on the effects of short gestation intervals on fetal and maternal outcomes was published in 2012 (6), which was 10 years old and lacked research from some countries, such as China. Previous systematic reviews and meta-analyses have examined the influence of short IPIs on perinatal mortality outcomes, but the researchers compared the IPI < 6 months with IPI ≥ 6 months and calculated the combined effect values, so these researches could limit the results (9). Through meta-analysis, this study discussed the influence of short IPI on adverse pregnancy outcomes and provided a basis and guidance for women of childbearing age to choose the appropriate IPI. Choosing an appropriate IPI is vital to protect mothers and babies, perfect the quality of birth population, and reduce the occurrence of birth defects.

Materials and methods

Search methods

English and Chinese articles were searched in the databases of PubMed, Cochrane Library, Ovid, Embase, China Biology Medicine disc (CBM), and China National Knowledge Infrastructure (CNKI). The search formula was: ("birth intervals" [Mesh] OR "interpregnancy interval" [Text Word] OR "birth interval" [Text Word] OR "interbirth interval" [Text Word] OR "pregnancy spacing" [Text Word] OR "pregnancy interval" [Text Word] OR "birth spacing" [Text Word]) AND ("pregnancy outcome" [Mesh] OR "infant, low birth weight" [Mesh] OR "premature birth" [Mesh] OR "infant, small for gestational age" [Mesh] OR "fetal growth retardation" [Mesh] OR "intensive care units, neonatal" [Mesh] OR "fetal death" [Mesh] OR "stillbirth" [Mesh] OR "perinatal death" [Mesh] OR "fetal mortality" [Mesh] OR "perinatal mortality" [Mesh] OR "infant mortality" [Mesh] OR "congenital abnormalities" [Mesh] OR "diabetes, gestational" [Mesh] OR "hypertension, pregnancy-induced" [Mesh] OR "pre-eclampsia" [Mesh] OR "hypertensive disorders" [Text Word] OR "maternal morbidity" [Text Word] OR "maternal mortality" [Mesh] OR "maternal death" [Mesh] OR "uterine rupture" [Mesh] OR "abruptio placentae" [Mesh] OR "placenta previa" [Mesh] OR "obesity" [Mesh] OR "dystocia" [Mesh]). The search ranged from Chinese articles to English articles published from January 1980 to August 2021.

Criteria for inclusion and exclusion of literature

The inclusion and exclusion criteria were determined according to PECO (Population, Exposure, Control and Outcome).

Inclusion criteria

1) Cohort study and case–control study-based population.

2) At least one birth and second pregnancy.

3) The definition of IPI was the interval from the last delivery to the beginning of the next pregnancy (the last menstrual date).

4) Defined short IPI (IPI < 6 months) and reference IPI. Previous studies have found a J-shaped relationship between IPI and adverse pregnancy outcomes (2, 4). Therefore, upper and lower limits must be clearly defined with reference IPI to reduce bias caused by the misclassification of IPI.

5) At least one pregnancy outcome analyzed.

6) IPI, a grouping variable, the OR, RR, and 95% confidence interval (CI) of association between different IPIs and pregnancy outcomes were reported.

Exclusion criteria

1) Experimental study and cross-sectional study.

2) Analyzed birth interval without IPI.

3) IPI was a quantitative continuous variable.

4) Summary, abstract.

5) OR, RR, and 95% CI were not reported, or the statistics above cannot be calculated according to the original data.

6) Excluded the studies only about inter-pregnancy interval after unnatural pregnancy, preterm birth, stillbirth, and pregnancy loss or termination. Considering the use of assisted reproductive technology, and the adverse outcomes of the previous pregnancy, such as preterm birth, stillbirth, and termination of pregnancy, may be caused by maternal health status, genetic-related and other factors, which may also affect the subsequent pregnancy interval and the pregnancy outcomes. So, in those studies, there was potential for confounding bias, and the external validity was low.

Literature screening

The first and second authors read and screened the titles and abstracts of the retrieved articles and excluded irrelevant articles by following the inclusion criteria and exclusion criteria preliminarily.

Literature evaluation

The first and second authors evaluated the contained studies independently, using Newcastle–Ottawa Scale (NOS) (10) and excluded the studies with NOS score $\leq$ 4. The main studies included in the assessment were selection of subjects, measurement of exposure factors, inter-group comparability, and follow-up. For studies with inconsistent evaluation results by two screeners, judgment was made through mutual consultation.

Statistical analysis

Data extraction

Excel was used to establish the extraction table for literature information; extraction contents include study implementers, study site, study subjects, sample size, observation period, exposure measurements, outcome indicators, study results, and controlled confounding factors. When combining effect values, we used the method of Hamling et al. (11) to convert OR value or RR value, because different reference groups may be selected in multivariate analysis of different studies. Stata 16.0 software (Texas, USA) was applied to analyze the included studies statistically.

Heterogeneity test

The heterogeneity of the study was tested by I^2 test. If $P > 0.05$ and $I^2 < 50\%$, it meant that the study was homogeneous and applied the fixed effects model; If $P < 0.05$, or $I^2 > 50\%$, it meant that the study was heterogeneous, and further subgroup analysis was made by factors such as study area, study type, and the outcome of the previous pregnancy. If the heterogeneity still existed after removing the study that had a great influence on the merger effect, the random-effects model was adopted.

Sensitivity analysis

Three sensitivity analyses were performed. First, after each study was removed from the meta-analysis of pregnancy outcomes, the combined effect value was recalculated. Second, the combined effect value was recalculated after the references with NOS score $\leq$ 4 were excluded. Third, for meta-analyses with no more than four references included, both random-effects model and fixed effects model were used. Before and after sensitivity analysis, if the confidence interval of the combined statistics changes from $P > 0.05$ to $P < 0.05$ or from $P < 0.05$ to $P > 0.05$, or the variation range of the combined OR value exceeds 10%, it indicates that the deleted references are outliers with important influence.

Publication bias

Egger's test was used to evaluate publication bias. If $P > 0.05$, the risk of publication bias was considered to be low.

Assessment of evidence quality

The GRADE (The Grades of Recommendation, Assessment, Development, and Evaluation) (12) system was used to evaluate the quality of evidence. The risk of bias, inconsistencies, inaccuracies, indirection, and the bias of reporting were investigated.

Results

Literature characteristics

Through searching the database, 1,499 English articles and 16 Chinese articles were searched, and six articles were supplemented by systematic review and meta-analysis. After the exclusion of duplicates, 1,390 articles remained. After subsequently reading the title and abstract, the researchers excluded 1,324 articles. Finally, a total of 41 articles (1–3, 13–50) were included (**Supplementary Table 2**) in the meta-analysis after further reading the full text. **Supplementary Table 1** showed the characteristics of the excluded studies. Among the 25 articles excluded, four were excluded because of incomplete data, five for the IPI after adverse outcomes of the last pregnancy, and 16 for IPI grouping data did not meet inclusion criteria. Literature screening process is shown in **Figure 1**. Most of the studies included in the meta-analysis were in European and American countries: the United States (22), Canada (6), United Kingdom (1), the Netherlands (3), Sweden (1), Israel (1), China (1), Australia (4), and multi-countries (2). The NOS scores of the 41 articles included in the meta-analysis ranged from four to eight, and the characteristics of the articles are shown in **Supplementary Table 2**.

When analyzing the association between different IPIs and pregnancy outcomes, three studies (14, 25, 38) did not use 18–23 months as the reference group. Therefore, the data of the reference group were transformed when the effect values were combined.

Among the included studies, the Lieberman et al. (13) study and the Lang et al. (14) study were the same population with different outcomes. The Schummers et al. (37) study and the Schummers et al. (47) study were the same database and population. The Tessema et al. (48) study and the Marinovich et al. (49) study were the same database and population. The vast majority of studies were based on population-based birth registration information systems. In the analysis of the association between IPI and pregnancy outcome, the following covariables were included in the studies: maternal age (39 articles); mother's sociodemographic characteristics, such as race/ethnicity (29 articles), educational level (23 articles), marital status (18 articles), and economic status (12 articles), smoking before or during pregnancy (26 articles), drinking alcohol before or during pregnancy (10 articles), parity (17 articles), and prenatal care (11 articles). Sixteen studies controlled the outcome of a previous pregnancy, such as preterm birth.

Based on the inclusion and exclusion criteria, nine pregnancy outcomes were included in the meta-analysis, including preterm birth, very preterm birth, low birth weight, small for gestational age infants, offspring death, neonatal intensive care (NICU), congenital abnormality, gestational hypertension, and gestational diabetes. In light of pregnancy outcomes, the heterogeneity test and publication bias test of the studies included in the meta-analysis were carried out, as shown in **Table 1**. The result of Egger's test of each outcome index was $P > 0.05$, which suggested that the publication bias of studies included in the meta-analysis was small.

The influence of short interpregnancy interval on the adverse outcomes of perinatal infants

The influence of short interpregnancy interval on preterm birth

A total of 23 studies (2, 14–16, 19–21, 24, 25, 27, 29–31, 34–36, 38, 41–43, 47–49) met the inclusion criteria, most of which were in North America, two of which covered four countries: the United States, Australia, Finland, and Norway (48, 49), two were in Asia, three were in Europe, and two were in Australia. Although the Tessema et al. (48) study and the Marinovich et al. (49) study were derived from the same database at the same time period, considering that the Marinovich et al. (49) study focused on the effect of pregnancy interval after full-term pregnancy on preterm birth; therefore, the Marinovich et al. (49) study was preferentially selected for the meta-analysis. At last, 22 articles (2, 14–16, 19–21, 24, 25, 27, 29–31, 34–36, 38, 41–43, 47, 49) with 28 research data were selected for meta-analysis. The definition of preterm birth was that the gestational age at delivery was less than 37 weeks, but one article (16) defined preterm birth as 33–36 weeks. The results showed that short IPI (IPI < 6 months) after delivery was a risk factor for preterm birth (pooled OR: 1.49, 95% CI: 1.42–1.57; **Figure 2**). After each study was removed seriatim from the sensitivity analysis, the pooled OR values varied from 1.48 to 1.52, the minimum 95% CI lower limit of 1.40, and the maximum 95% CI upper limit of 1.59. After removing the matched data (the data about with-in mother comparisons) of the Hanley et al. (36) study and the Ball et al. (24) study, the pooled OR changed a little (1.53, 95% CI: 1.46–1.60). Similarly, we removed the study with NOS score of 4 (15) (pooled OR: 1.52, 95% CI: 1.45–1.59). When we removed the study with women under 20 years old (27), it didn't change the relationship between short IPI and preterm birth (pooled OR: 1.49, 95% CI: 1.41–1.56; $I^2 = 92.0\%$, $P < 0.001$).

The influence of short interpregnancy interval on very preterm birth

Eleven articles (16, 19, 25, 27, 29, 31, 35, 38, 42, 43, 47) (13 research data) classified preterm birth according to gestational age at delivery. When analyzing very preterm birth, a meta-analysis found that four studies only provided the upper limit of very preterm birth, including one article that defined very preterm birth as less than 32 weeks (27), one less than 33 weeks (19), and two less than 34 weeks (31, 35). Five studies defined the range of very preterm birth as 28–34 weeks of gestational age at

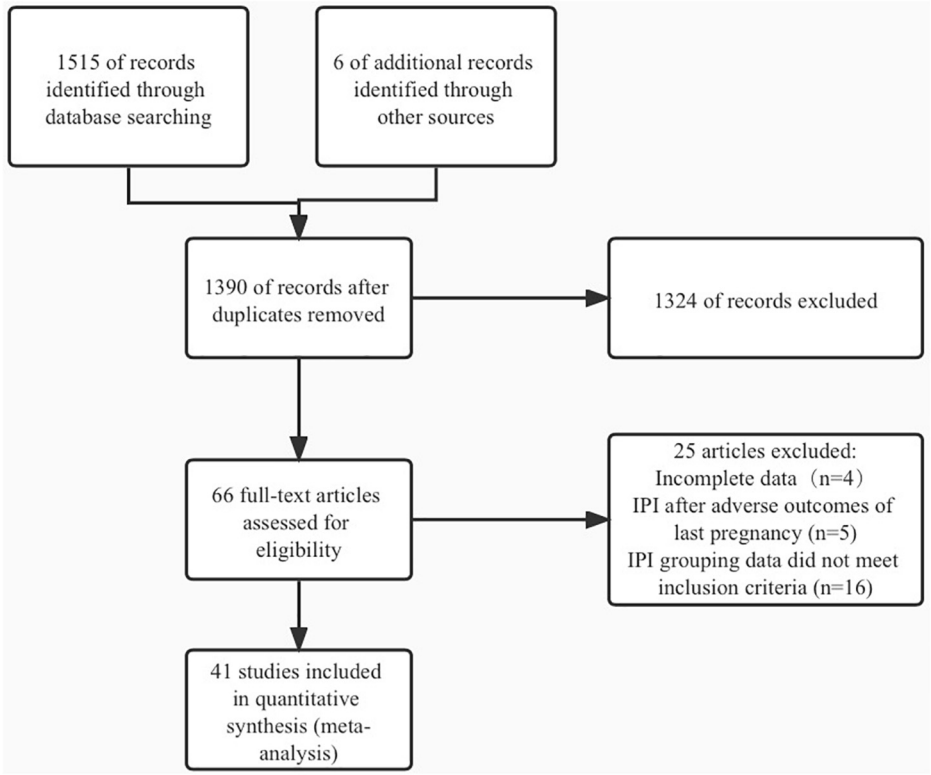

FIGURE 1
Literature screening process.

TABLE 1 Heterogeneity test and publication bias test of the meta-analysis of various pregnancy outcomes.

Maternal and infant outcome	Number of articles	Heterogeneity test		Egger's test		Effect model	Quality evidence
		I^2	*P* of Cochran's *Q*-test	*t*	*P*		
Preterm birth	28	92.0%	<0.001	−1.77	0.088	Random-effects model	⊕⊕⊖⊖
Very preterm birth	13	89.0%	<0.001	0.74	0.476	Random-effects model	⊕⊖⊖⊖
Low birth weight	18	84.7%	<0.001	−1.72	0.105	Random-effects model	⊕⊕⊖⊖
Small for gestational age infants	23	83.8%	<0.001	−0.88	0.386	Random-effects model	⊕⊕⊖⊖
Offspring death	6	0	0.719	0.60	0.583	Fixed effects model	⊕⊖⊖⊖
NICU	4	90.3%	<0.001	−0.97	0.511	Random-effects model	⊕⊖⊖⊖
Congenital abnormality	4	0	0.458	−0.47	0.683	Fixed effects model	⊕⊖⊖⊖
Gestational hypertension	6	2.7%	0.399	−0.49	0.652	Fixed effects model	⊕⊖⊖⊖
Gestational diabetes	6	92.8%	<0.001	2.64	0.058	Random-effects model	⊕⊖⊖⊖

delivery (25), 24–31 weeks (29), 24–32 weeks (16), 26–32 weeks (38), and 28–32 weeks (42), respectively. One study (43) did not define very preterm birth but reported a subgroup analysis of preterm births between 24 and 31 gestational weeks, and one (47) studied the preterm birth before 28 gestational weeks, both of which were included in the meta-analysis. Meta-analysis exhibited that short IPI (IPI < 6 months) was the risk factor for very preterm birth (pooled OR: 1.82, 95% CI: 1.55–2.14; Figure 3). After removing each study seriatim, the pooled OR value varied from 1.74 to 1.89, with the minimum 95% CI lower limit of 1.49 and the maximum 95% CI upper limit of 2.23. After removing the study of Nerlander et al. (27), the

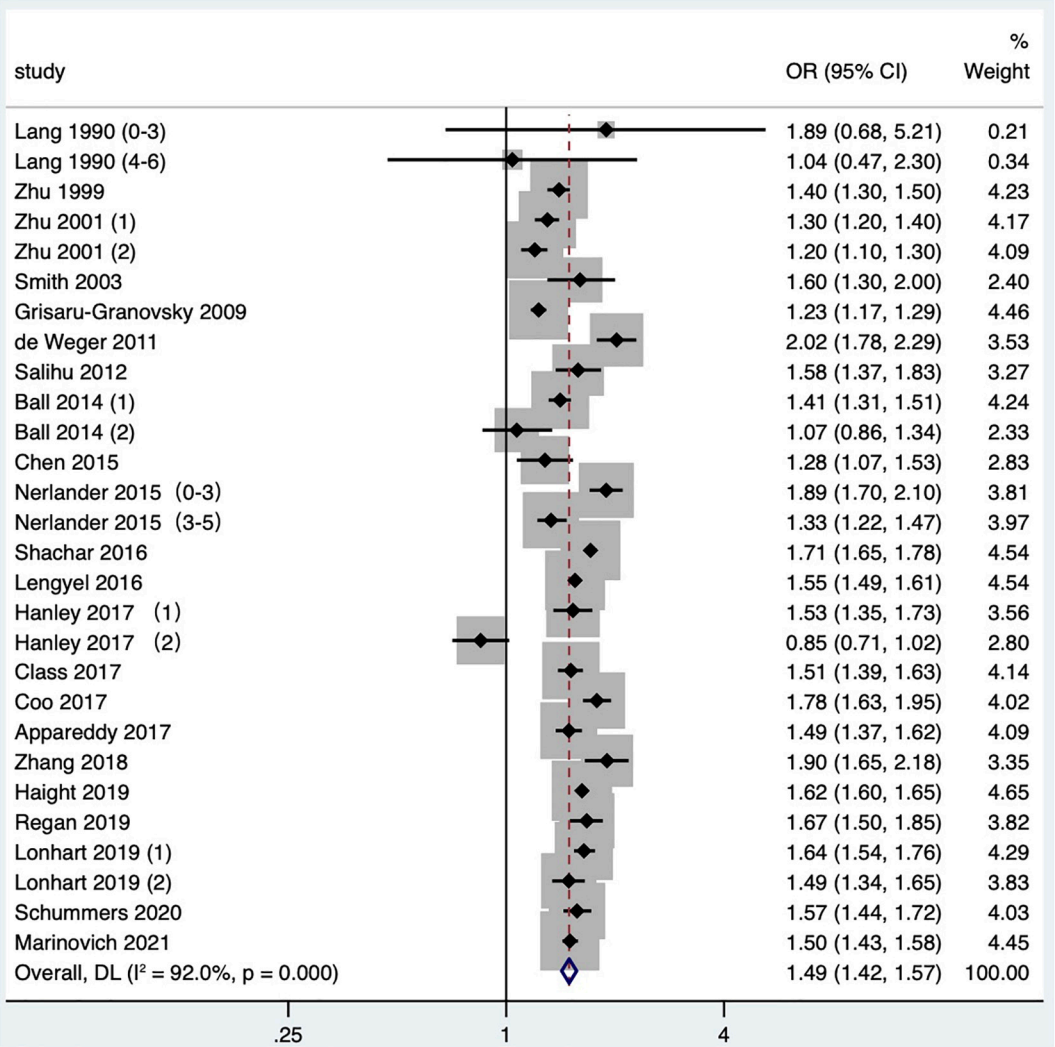

FIGURE 2
Meta-analysis forest map of the effect of short IPI on preterm birth. Lang et al. (14) (0–3) referred to the IPI of 0–3 months vs. 18–23 months; Lang et al. (14) (4–6) referred to the IPI of 4–6 months vs. 18–23 months; Nerlander et al. (27) (0–3) referred to the IPI of 0–3 months vs. 18–23 months; Nerlander et al. (27) (3–5) referred to the IPI of 3–5 months vs. 18–23 months; Zhu et al. (15) (1) was white data, Zhu et al. (15) (2) was black data; Hanley et al. (36) (1) was non-matching data, and Hanley et al. (36) (2) was within-mother matching data; Ball et al. (24) (1) was non-matching data, Ball et al. (24) (2) was within-mother matching data; Lonhart et al. (43) (1) was non-Hispanic whites data, and Lonhart et al. (43) (2) was non-Hispanic blacks data.

relationship between short IPI and very preterm birth didn't change (pooled OR: 1.78, 95% CI: 1.49–2.14; I^2 = 90.0%, $P < 0.001$).

The influence of short interpregnancy interval on low birth weight

Twelve articles (2, 3, 15, 20, 21, 24, 25, 31, 34–36, 42) with 18 research data were included in the meta-analysis. Low birth weight is defined as birthweight < 2,500 g. The data reported by Zhu and Le (3) were divided into four groups of IPI according to parity. Hanley et al. (36) and Ball et al. (24) reported the comparison of non-matching among mothers and the comparison of matching between two consecutive IPIs of the same mother, respectively. The results showed that the short IPI after delivery (IPI < 6 months) was the risk factor for low birth weight (pooled OR: 1.33, 95% CI: 1.24–1.43; **Figure 4**). After removing each study seriatim, the pooled OR value varied from 1.31 to 1.38, with the minimum 95% CI lower limit of 1.22 and the maximum 95% CI upper limit of 1.46. After removing the matched data in two studies (24, 36), the odds ratio changed a little (pooled OR: 1.40, 95% CI: 1.33–1.47). In the study of Zhu and Le (3), removing the IPI after the second birth had little effect on the odds ratio (pooled OR: 1.33, 95% CI: 1.23–1.45; I^2 = 86.4%, $P < 0.001$), which was the same as removing

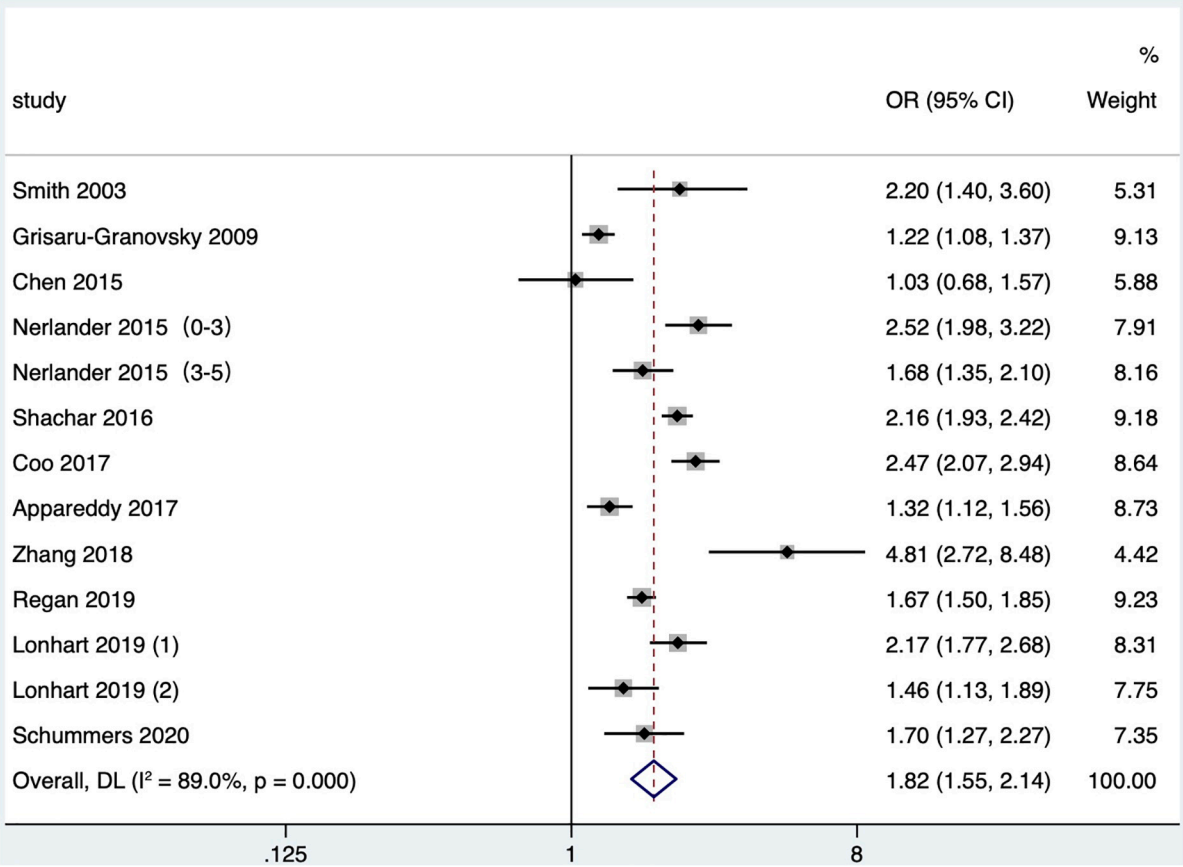

FIGURE 3
Meta-analysis forest map of the effect of short IPI on very preterm birth. Nerlander et al. (27) (0–3) referred to the IPI of 0–3 months vs. 18–23 months; Nerlander et al. (27) (3–5) referred to the IPI of 3–5 months vs. 18–23 months; Lonhart et al. (43) (1) was non-Hispanic whites data, and Lonhart et al. (43) (2) was non-Hispanic blacks data.

the study with NOS score of 4 (15) (pooled OR: 1.31, 95% CI: 1.21–1.42; $I^2 = 85.1\%$, $P < 0.001$).

The effect of short interpregnancy interval on small-for-gestational-age

Eighteen articles (2, 13, 15, 16, 18–21, 23–26, 34–36, 38, 42, 47, 48) (23 research data) were contained in the meta-analysis. Data from the Schummers et al. (37) study were not reported in the main findings, so only the data from the Schummers et al. (47) study were included in the quantitative synthesis. For the definition of small for gestational age, eight articles (2, 24, 35, 36, 38, 42, 47, 48) were birthweight less than 10th percentile of sex and gestational age-specific birthweight based on given standards; three articles (18, 20, 23) were birthweight less than 10th percentile of a given sex, parity, and gestational age; one article (15) was birthweight less than 10th percentile of a given gestational age, race, sex, and parity; one article (34) was that the birthweight was more than two standard deviations below the average weight of a given gestational age, and one article (16) was birthweight less than fifth percentile among the birthweight of live births; four articles (13, 19, 21, 25) were birthweight less than 10th percentile of a given gestational age. The result showed that the short IPI (IPI < 6 months) was a risk factor for small for gestational age infants (pooled OR: 1.14, 95% CI: 1.07–1.21; **Figure 5**). After removing each study seriatim, the pooled OR value varied from 1.12 to 1.15, with the minimum 95% CI lower limit of 1.06 and the maximum 95% CI upper limit of 1.22. Removing the matching data (24, 36, 48) had little effect on the odds ratio (pooled OR: 1.16, 95% CI: 1.09–1.23), which was the same as removing the study with NOS score of 4 (15) (pooled OR: 1.11, 95% CI: 1.05–1.18; $I^2 = 75.1\%$, $P < 0.001$).

The effect of short interpregnancy interval on offspring death

Nine studies (16, 19, 22, 25, 31, 32, 40, 44, 47) met the literature screening criteria, and the outcome indicators of fetal/infant death included stillbirth (fetal death at gestational age ≥ 20 weeks) (40), perinatal death (16, 25, 47), neonatal death (19, 22, 44), and infant death (22, 31, 32, 44). Schummers et al. (47) studied perinatal mortality including stillbirth and neonatal

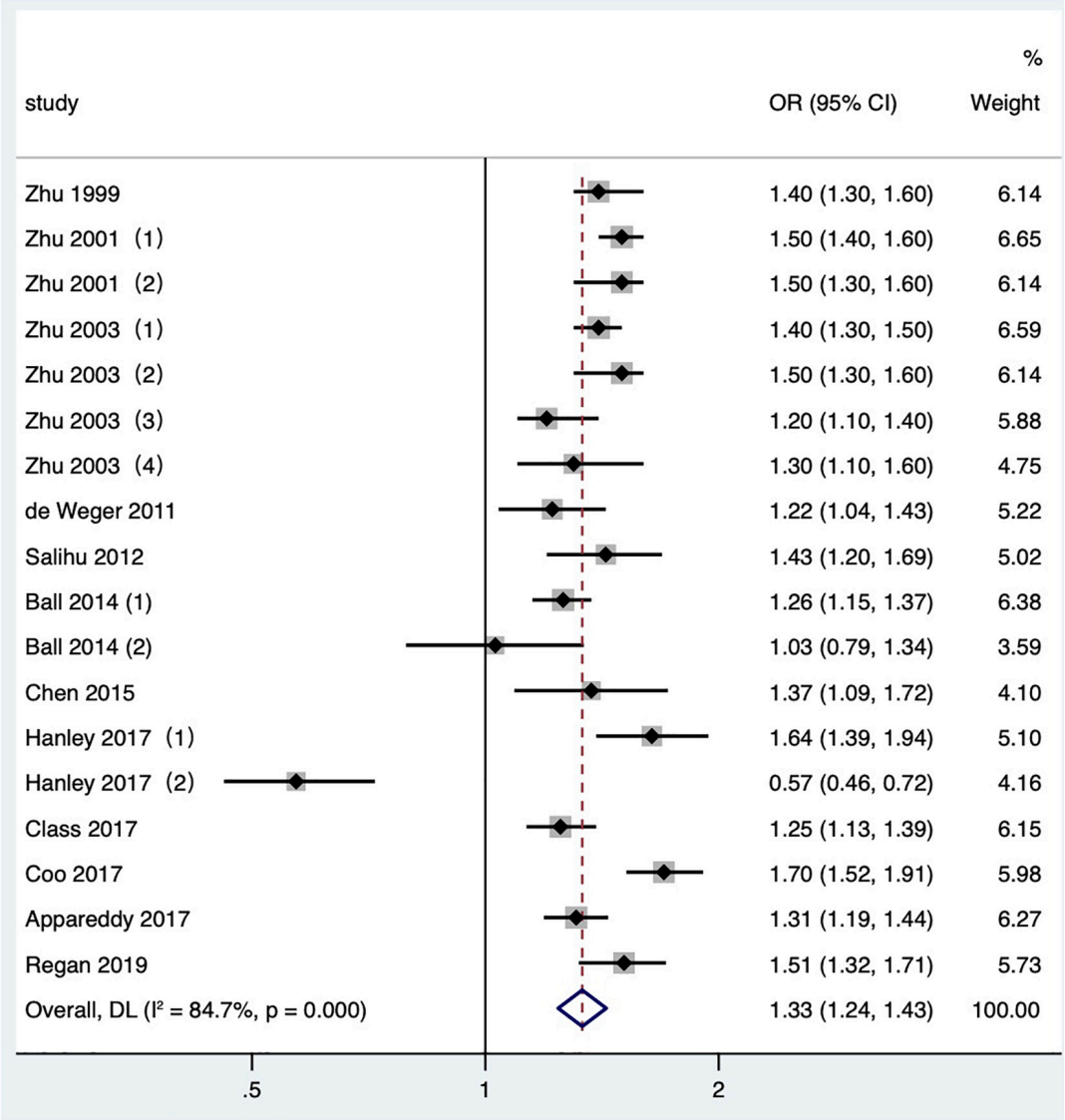

FIGURE 4
Meta-analysis forest map of the effect of short IPI on low birth weight. Zhu et al. (15) (1) was white data, Zhu et al. (15) (2) was black data; Zhu and Le (3) (1) studied the IPI between the first pregnancy and the second pregnancy; Zhu and Le (3) (2) studied the IPI between the second and third pregnancy; Zhu and Le (3) (3) studied the IPI between the third and fourth pregnancy; Zhu and Le (3) (4) studied the IPI between the fourth and fifth pregnancy; Ball et al. (24) (1) was non-matching data, Ball et al. (24) (2) was within-mother matching data; Hanley et al. (36) (1) was non-matching data, and Hanley et al. (36) (2) was within-mother matching data.

death within 28 days after birth. Data on the relationship between IPI and offspring death in one study (25) were not available and not included in the quantitative combination. As the cause of infant death within 1-year-old may be affected by a variety of confounding factors, so the study data with infant death as the outcome indicator were not included in the quantitative combination. Finally, six (16, 19, 22, 40, 44, 47) studies were included in the meta-analysis, including two case–control studies (22, 40) and four cohort studies (16, 19, 44, 47). Meta-analysis with a fixed effects model showed that short IPI (IPI < 6 months) was a risk factor for fetal/infant death (pooled OR: 1.60, 95% CI: 1.51–1.69; Figure 6). After removing each study one by one, the pooled OR values ranged from 1.57 to 1.60, with the minimum 95% CI lower limit of 1.32 and the maximum 95% CI upper limit of 1.87.

The influence of short interpregnancy interval on neonatal intensive care

Four studies (25, 26, 31, 36) met the literature inclusion criteria, all of which were published after 2010 in North America, and the outcome indicator was NICU admission. Data

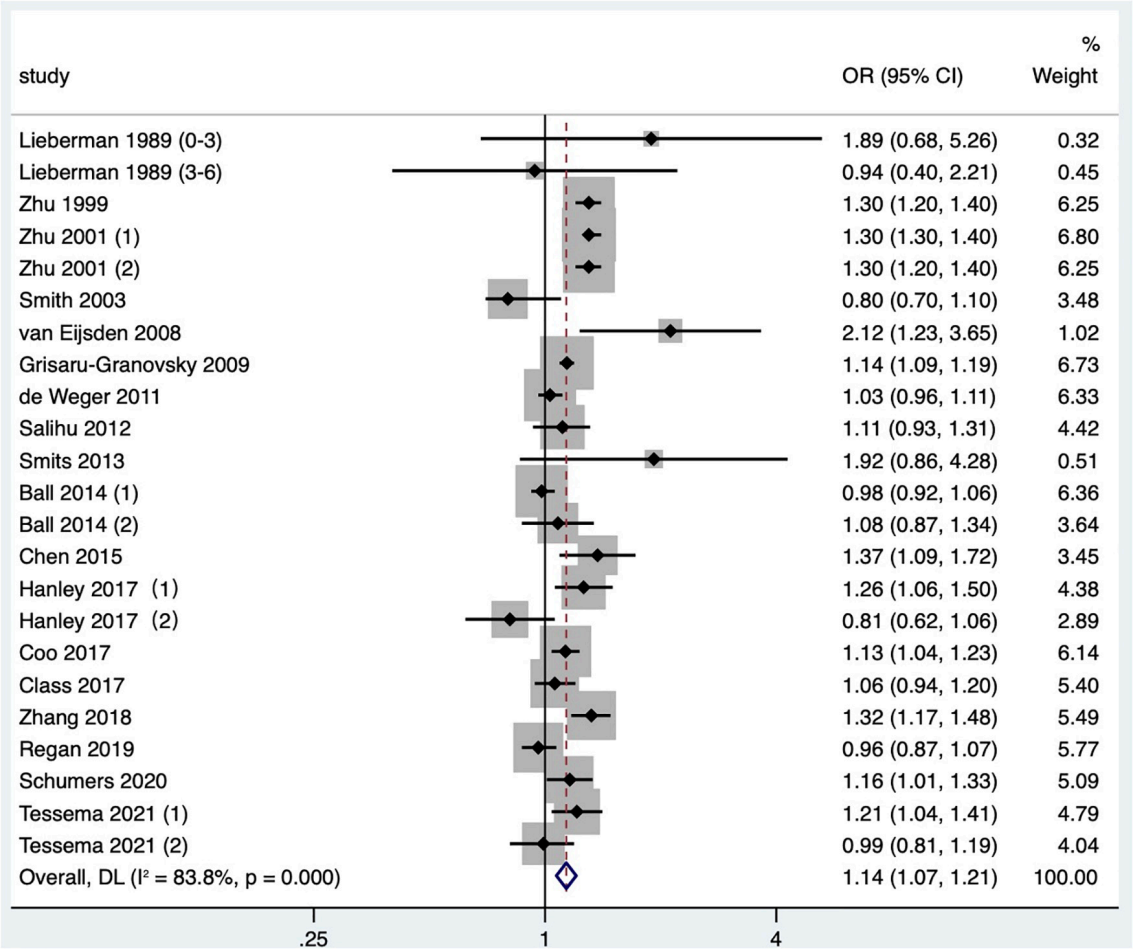

FIGURE 5
Meta-analysis forest map of the effects of short IPI on small for gestational age infants. Lieberman et al. (13) (0–3) referred to the IPI of 0–3 months vs. 18–23 months; Lieberman et al. (13) (3–6) referred to the IPI of 3–6 months vs. 18–23 months; Zhu et al. (15) (1) was white data, Zhu et al. (15) (2) was black data; Ball et al. (24) (1) was non-matching data, and Ball et al. (24) (2) was within-mother matching data; Hanley et al. (36) (1) was non-matching data, and Hanley et al. (36) (2) was intra-maternal matching data; Tessema et al. (48) (1) was non-matching data, and Tessema et al. (48) (2) was within-mother matching data.

on the NICU admission in one study (25) were not available and not included in the quantitative combination. One study (26) only reported the number of NICU admission cases in different IPI groups, so we combined the effect values after calculating crude OR values according to the number. One study (36) reported the comparison between mothers and the comparison of matching data between two consecutive IPIs of the same mother, respectively. At last, three articles (26, 31, 36) with four research data were selected in our meta-analysis. Short IPI (IPI < 6 months) was the risk factor for NICU (pooled OR: 1.26, 95% CI: 1.01–1.57; **Figure 7**). After removing each study seriatim, the pooled OR values varied from 1.14 to 1.32, with the minimum 95% CI lower limit of 0.97 and the maximum 95% CI upper limit of 1.68. After removing the matching data of Hanley et al. (36), the pooled OR value changed little (pooled OR: 1.29, 95% CI: 1.01–1.65), but short IPI was no longer being a risk factor for NICU after removing the non-matching data (pooled OR: 1.28, 95% CI: 0.97–1.67).

Influence of short interpregnancy interval on congenital abnormality

Four studies (1, 19, 28, 33) met the literature inclusion criteria. The outcome indicator was the congenital abnormality of offspring. In one article, the outcome indicator was major congenital malformation (19), one was congenital abnormality (33) and two were birth defects (1, 28), and the diagnostic criteria were based on ICD-9-CM 740 to 759 or ICD-10-CA Q00-Q99. Short IPI was a risk factor for congenital abnormality (pooled OR: 1.10, 95% CI: 1.05–1.16; **Figure 8**). After removing each study seriatim, the pooled OR values varied from 1.08 to 1.12, with the minimum 95% CI lower limit of 1.02 and the maximum 95% CI upper limit of 1.18.

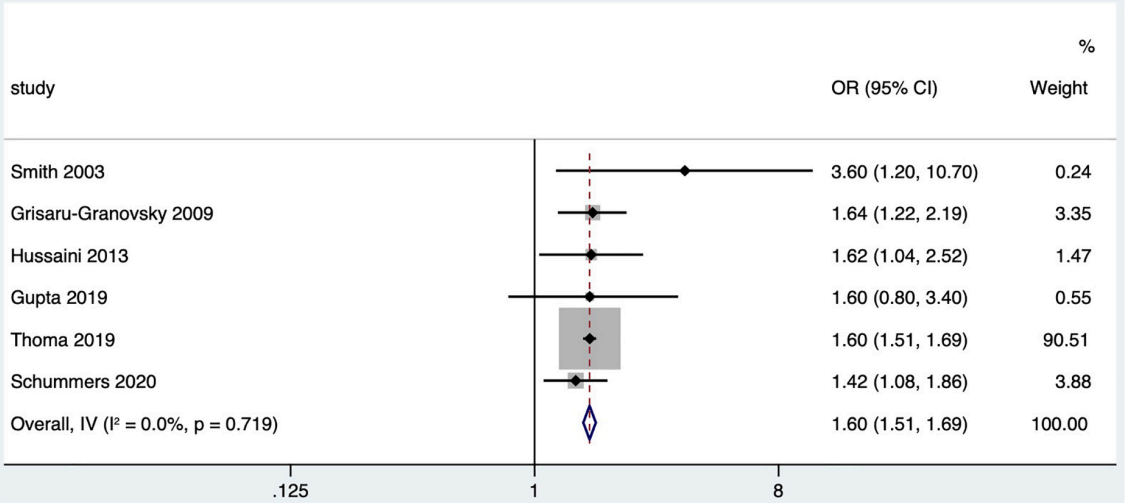

FIGURE 6
Meta-analysis forest map of the influence of short IPI on offspring death.

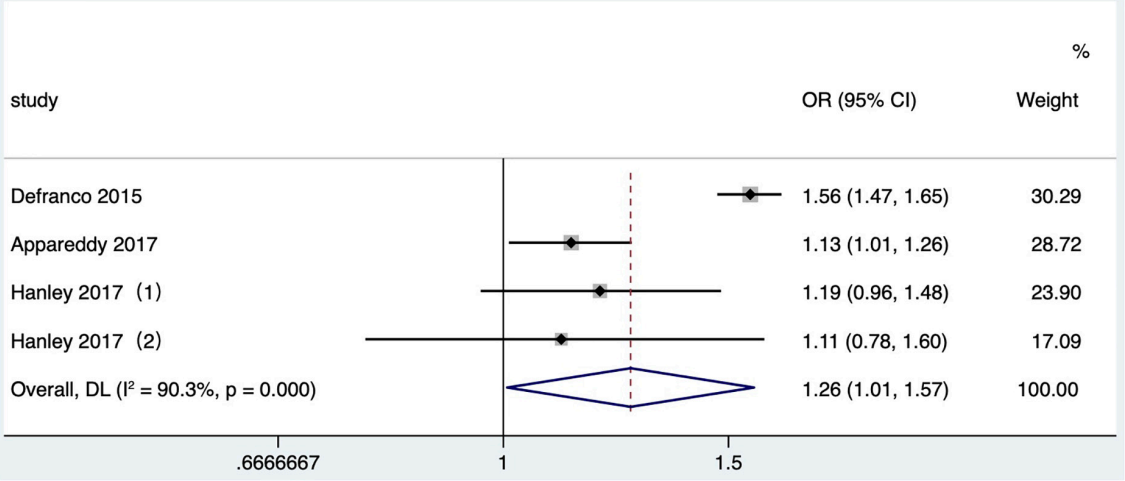

FIGURE 7
Meta-analysis forest map of the influence of short IPI on NICU. Hanley et al. (36) (1) was non-matching data, and Hanley et al. (36) (2) was within-mother matching data.

Subgroup analysis of adverse perinatal outcomes caused by short interpregnancy interval

Subgroup analysis of preterm birth according to study publication time showed a pooled OR of 1.30 (95% CI: 1.22–1.39) for studies published before 2010, while a pooled OR of 1.55 (95% CI: 1.48–1.62) after 2010, and there was a statistically significant difference found in the pooled OR among subgroups ($P < 0.001$; **Table 2** and **Figure 9**). Subgroup analysis of SGA according to study area found that short IPI (IPI < 6 months) was a risk factor for SGA in North America (pooled OR: 1.20, 95% CI: 1.13–1.28), while in other studies, short IPI (IPI < 6 months) was not a risk factor for SGA (pooled OR: 1.07, 95% CI: 0.99–1.16; **Table 2** and **Figure 10**).

Short interpregnancy interval on adverse maternal outcomes

The effect of short interpregnancy interval on gestational diabetes

Four articles (26, 36, 39, 41) (six research data) published after 2010 were included in the meta-analysis. Gestational diabetes was determined based on ICD-9 or ICD-10 in 2 studies

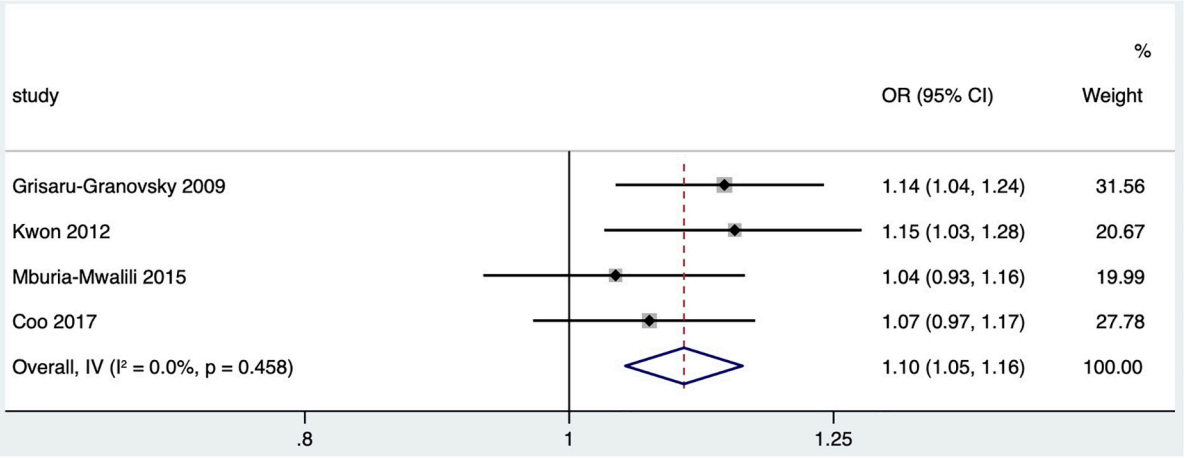

FIGURE 8
Meta-analysis forest map of the influence of short IPI on congenital abnormality.

(36, 39) and referred to maternal diabetes information recorded in birth certificates in the other studies (26, 41). The crude OR was calculated and included in a quantitative combination based on the numbers of maternal gestational diabetes in different IPI subgroups reported in one study (26), as the adjusted OR values were not reported. The result was demonstrated that short IPI (IPI < 6 months) was not a risk factor for gestational diabetes (pooled OR: 1.06, 95% CI: 0.93–1.20; **Figure 11**). According to the research site, no significant difference was found in the pooled OR between subgroups (**Table 2**). After removing each study one by one, the pooled OR values varied from 0.98 to 1.11, with the minimum 95% CI lower limit of 0.89 and the maximum 95% CI upper limit of 1.29. There was no significant effect on the pooled OR (pooled OR: 1.06, 95% CI: 0.91–1.24) after excluding the data of with-in mother matching of the two studies (36, 39).

The effect of short interpregnancy interval on gestational hypertension

Four articles (26, 36, 41, 46) (six research data) published after 2010 in North America were included in the meta-analysis. In two studies (26, 41), the outcome indicator was gestational hypertension based on data recorded in birth certificates, one study (36) was eclampsia or preeclampsia, and one study (46) was hypertensive disorders, which included preeclampsia and gestational hypertension without proteinuria, based on ICD-9 or ICD-10. The crude OR was calculated and included in a quantitative combination based on the numbers of maternal gestational hypertension in different IPI subgroups reported only in one study (26), as the adjusted OR values were not reported. The meta-analysis used a fixed effects model. The results manifested that short IPI (IPI < 6 months) was not a risk factor for gestational hypertension (pooled OR: 0.95, 95% CI: 0.93–0.98; **Figure 12**). After removing each study detail by detail, the pooled OR values varied from 0.95 to 0.97, with the minimum 95% CI lower limit of 0.92 and the maximum 95% CI upper limit of 1.02. The pooled OR value changed a little (pooled OR: 0.95, 95% CI: 0.93–0.98) after excluding the data of within-mother matching of the Hanley et al. (36) study.

Effect of short interpregnancy interval on uterine rupture

Two studies (17, 45) met the inclusion criteria. One study (17) was based on 17 hospitals in the United States, which limited the outcome of previous pregnancy to cesarean. The result showed that short IPI (IPI < 6 months) was associated with a higher risk of uterine rupture compared with IPI of 18–60 months (OR: 3.05, 95% CI: 1.36–6.87). De Silva and Thoma (45) found that short IPI after live birth was associated with a higher risk of uterine rupture compared with the reference IPI group (OR: 2.78, 95% CI: 2.29–3.39). Due to the obvious differences between the research populations and the reference group, the quantitative synthesis was not performed.

Influence of short interpregnancy interval on premature rupture of membranes

One study (31) met the inclusion criteria, but the original paper only reported the incidence of premature rupture of membranes in different IPI groups, and did not report the OR value. So it was not included in the multivariate statistical model for analysis.

Impact of short interpregnancy interval on maternal morbidity

Five studies (17, 37, 41, 45, 50) have reported the association between short IPI and comprehensive indicators of maternal morbidity. Stamilio et al. (17) defined the main incidence indicators as any one or more of the following symptoms: uterine rupture, bladder, ureter or intestinal injury, and

TABLE 2 Subgroup analysis of adverse pregnancy outcomes caused by short IPI.

Hierarchical variable	Preterm birth		Very preterm birth		Low birth weight		Small for gestational-age infants		Offspring death		NICU		Congenital abnormality		Gestational diabetes		Gestational hypertension	
	n	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)
Publication time																		
<2010	7	1.30 (1.22∼1.39)[abc]	2	1.56 (0.88∼2.77) [ab]	7	1.41 (1.34∼1.49) [ab]	8	1.22 (1.12∼1.33)[ab]	2	1.73 (1.30∼2.29)	0	∼	1	1.14 (1.04∼1.24)	0	∼	0	∼
≥2010	21	1.55 (1.48∼1.62) [abc]	11	1.86 (1.60∼2.19) [ab]	11	1.27 (1.12∼1.44) [ab]	15	1.10 (1.03∼1.17)[ab]	4	1.59 (1.51∼1.68)	4	1.26 (1.01∼1.57)	3	1.08 (1.02∼1.15)	6	1.06 (0.93∼1.20)[a]	6	0.95 (0.93∼0.98)
Research site																		
North America	20	1.48 (1.41∼1.56) [ab]	9	1.81 (1.51∼2.15)[ab]	13	1.36 (1.24∼1.48) [ab]	13	1.20 (1.13∼1.28)[abc]	4	1.59 (1.51∼1.68)	4	1.26 (1.01∼1.57)	3	1.08 (1.02∼1.15)	4	1.13 (0.94∼1.35)[ab]	4	0.95 (0.93∼0.97)
Other areas	8	1.53 (1.34∼1.74) [ab]	4	1.91 (1.37∼2.68)[ab]	5	1.28 (1.16∼1.40) [ab]	10	1.07 (0.99∼1.16)[abc]	2	1.73 (1.30∼2.29)	0	∼	1	1.14 (1.04∼1.24)	2	0.96 (0.89∼1.13)	2	0.99 (0.92∼1.06)
Type of research																		
Cohort	28	1.49 (1.42∼1.57)	13	1.82 (1.55∼2.14)	18	1.33 (1.24∼1.43)	23	1.14 (1.07∼1.21)	4	1.60 (1.51∼1.69)	4	1.26 (1.01∼1.57)	3	1.09 (1.03∼1.15)	6	1.06 (0.93∼1.20)[a]	6	0.95 (0.93∼0.98)
Case–control	0	∼	0	∼	0	∼	0	∼	2	1.61 (1.11∼2.36)	0	∼	1	1.15 (1.03∼1.28)	0		0	∼
Research design																		
Non-matched	26	1.53 (1.46∼1.60)[abc]	13	1.82 (1.55∼2.14)	16	1.40 (1.33∼1.47) [abc]	20	1.16 (1.09∼1.23)[abc]	6	1.60 (1.51∼1.69)	3	1.29 (1.01∼1.65)[ab]	4	1.10 (1.05∼1.16)	4	1.06 (0.91∼1.24)[ab]	4	0.95 (0.93∼0.98)
Matched	2	0.94 (0.75∼1.18)[abc]	0	∼	2	0.76 (0.43∼1.36) [abc]	3	0.97 (0.84∼1.13)[c]	0	∼	1	1.11 (0.78∼1.59)	0	∼	2	1.07 (0.71∼1.63)[ab]	2	0.99 (0.89∼1.10)

[a] $I^2 > 50\%$ in subgroup;
[b] $P < 0.05$ in the heterogeneity test within subgroup;
[c] $P < 0.05$ in the heterogeneity test between subgroups.

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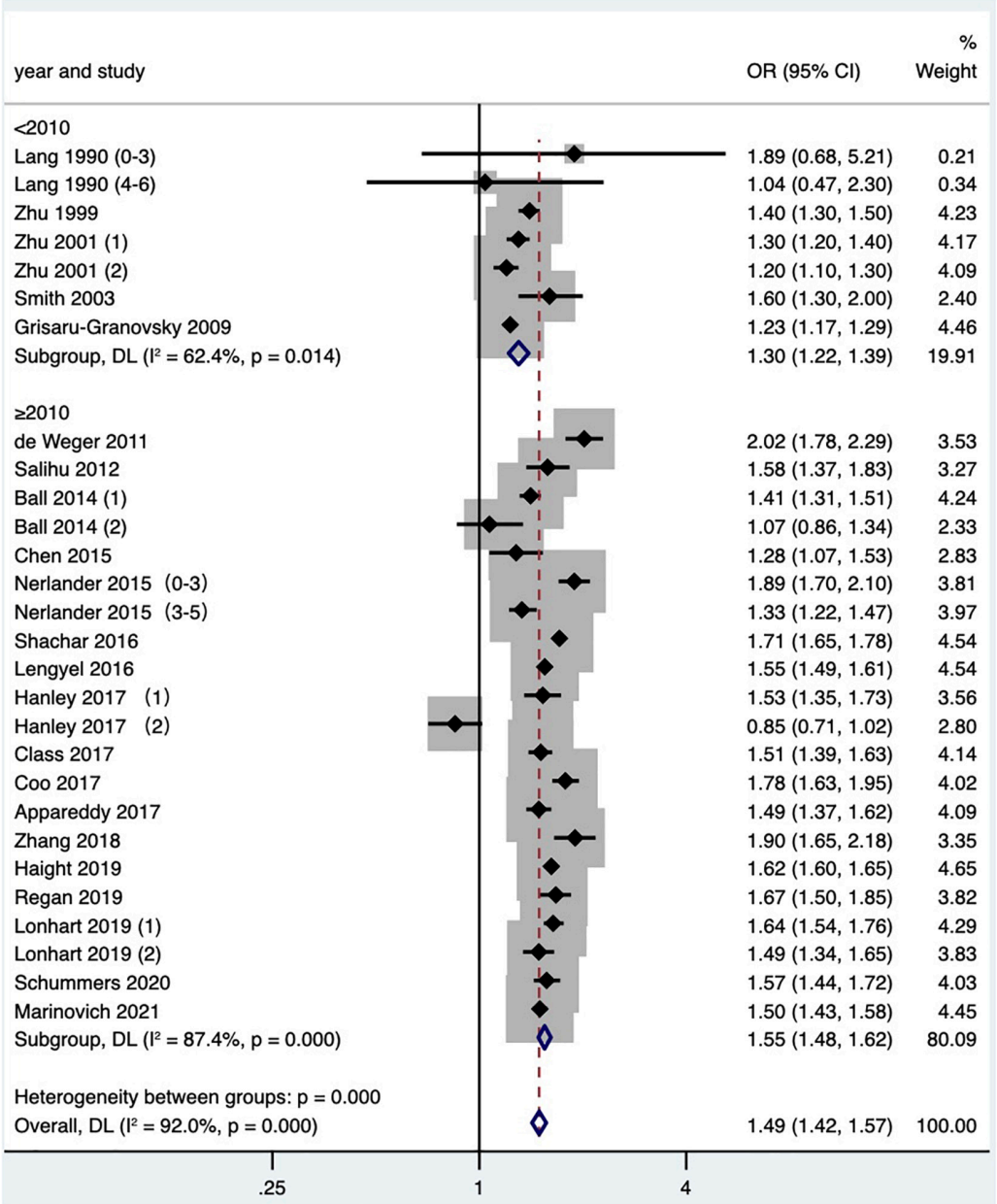

FIGURE 9
Subgroup analysis of the effect of short IPI on preterm birth. Lang et al. (14) (0–3) referred to the IPI of 0–3 months vs. 18–23 months; Lang et al. (14) (4–6) referred to the IPI of 4–6 months vs. 18–23 months; Zhu et al. (15) (1) was white data, Zhu et al. (15) (2) was black data; Nerlander et al. (27) (0–3) referred to the IPI of 0–3 months; Nerlander et al. (27) (3–5) referred to the IPI of 3–5 months; Ball et al. (24) (1) was non-matching data, and Ball et al. (24) (2) was within-mother matching data; Hanley et al. (36) (1) was non-matching data, and Hanley et al. (36) (2) was within-mother matching data; Lonhart et al. (43) (1) was non-Hispanic whites data, and Lonhart et al. (43) (2) was non-Hispanic blacks data.

uterine artery tear. Haight et al. (41) defined the indicator of maternal morbidity during delivery and hospitalization, as any combination of maternal transfusion, perineal laceration, uterine rupture, unplanned hysterectomy, and intensive care admission. The outcome indicators in the Schummers et al. (37) study were maternal mortality or severe maternal morbidity defined as mechanical ventilation, admission to an intensive care unit, organ failure, maternal transfusion, unplanned postpartum surgery, or death, which covered pregnancy up to 42 days postpartum. De Silva and Thoma (45) analyzed severe maternal morbidity, which included maternal transfusion, admission to intensive care unit, ruptured uterus, and third- or fourth-degree perineal laceration. Liu et al. (50) analyzed severe maternal morbidity with about 21 indicators of ICD-9. Due

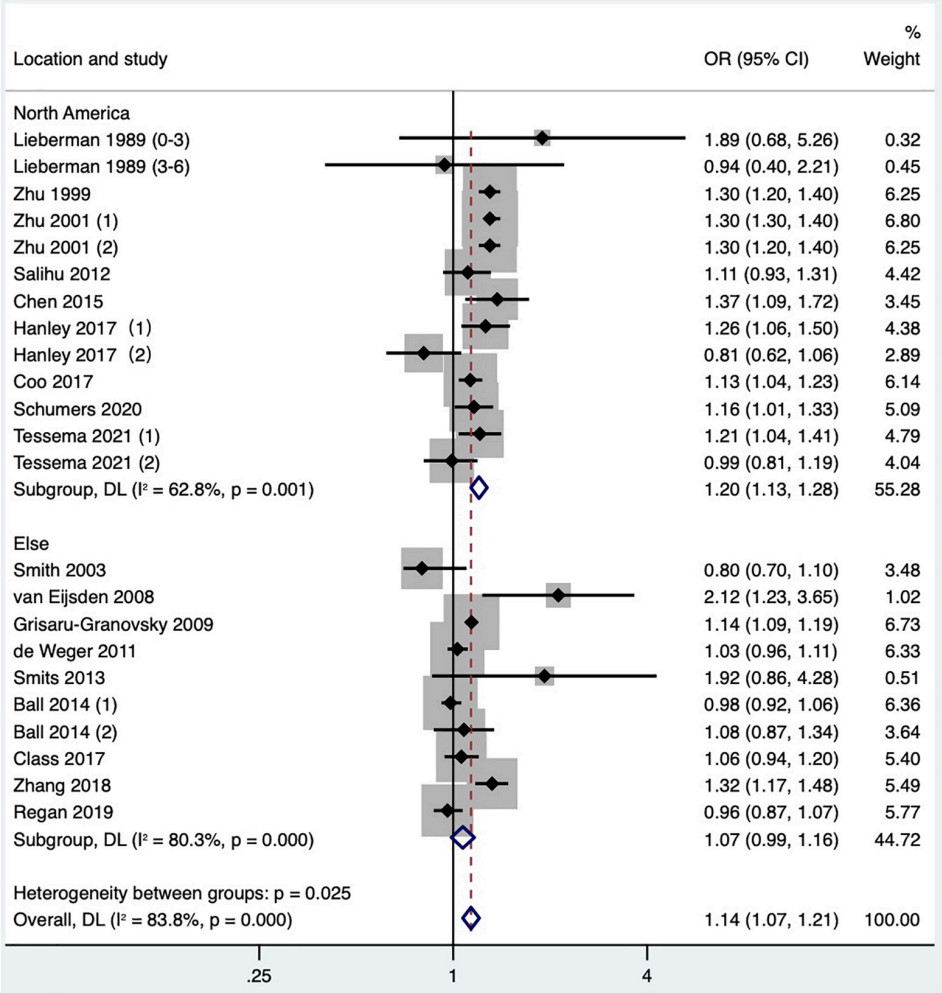

FIGURE 10
Subgroup analysis of the effect of short IPI on SGA. Lieberman et al. (13) (0–3) referred to the IPI of 0–3 months vs. 18–23 months; Lieberman et al. (13) (3–6) referred to the IPI of 3–6 months vs. 18–23 months; Zhu et al. (15) (1) was white data, Zhu et al. (15) (2) was black data; Hanley et al. (36) (1) was non-matching data, and Hanley et al. (36) (2) was within-mother matching data; Ball et al. (24) (1) was non-matching data, and Ball et al. (24) (2) was within-mother matching data; Tessema et al. (48) (1) was non-matching data, and Tessema et al. (48) (2) was within-mother matching data.

to methodological heterogeneity in the indicators' definition of maternal morbidity in these studies, quantitative combinations were not included.

Discussion

This study revealed that short IPI could lead to an increased risk of adverse pregnancy outcomes. Compared with the IPI of 18–23 months, the IPI less than 6 months after delivery was the risk factor of adverse perinatal outcomes in the next pregnancy and increased the risks of preterm birth, very preterm birth, low birth weight, small for gestational age infants, offspring death, congenital abnormality, and NICU. The previous meta-analysis showed that short IPI was associated with increased risk of preterm birth, extreme preterm birth, low birth weight, and small for gestational age (4, 6, 51), and short IPI after live birth was related to perinatal death (9), which was accordance with our research results. In recent years, it has been found that the mechanism of adverse pregnancy outcomes caused by short IPI after delivery could be attributed to the comprehensive influence of factors. The most common explanation was the maternal depletion hypothesis (52). During the short IPI, the essential nutrients for pregnancy may have not fully recovered to the previous prepregnancy levels, such as insufficient reserves for folic acid (18, 53–55) and iron (56), which may cause intrauterine growth retardation and fetal death. Other studies found that the incomplete healing of the uterine after cesarean section (17) and increased levels of the proteins-associated

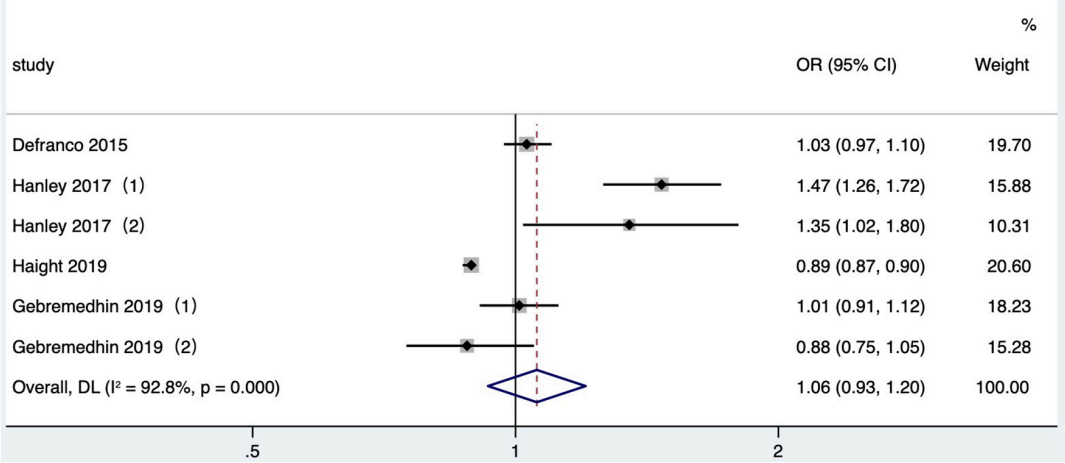

FIGURE 11
Meta-analysis forest map of the effect of short IPI on gestational diabetes. Hanley et al. (36) (1) was non-matching data, and Hanley et al. (36) (2) was within-mother matching data; Gebremedhin et al. (39) (1) was non-matching data, and Gebremedhin et al. (39) (2) was within-mother matching data.

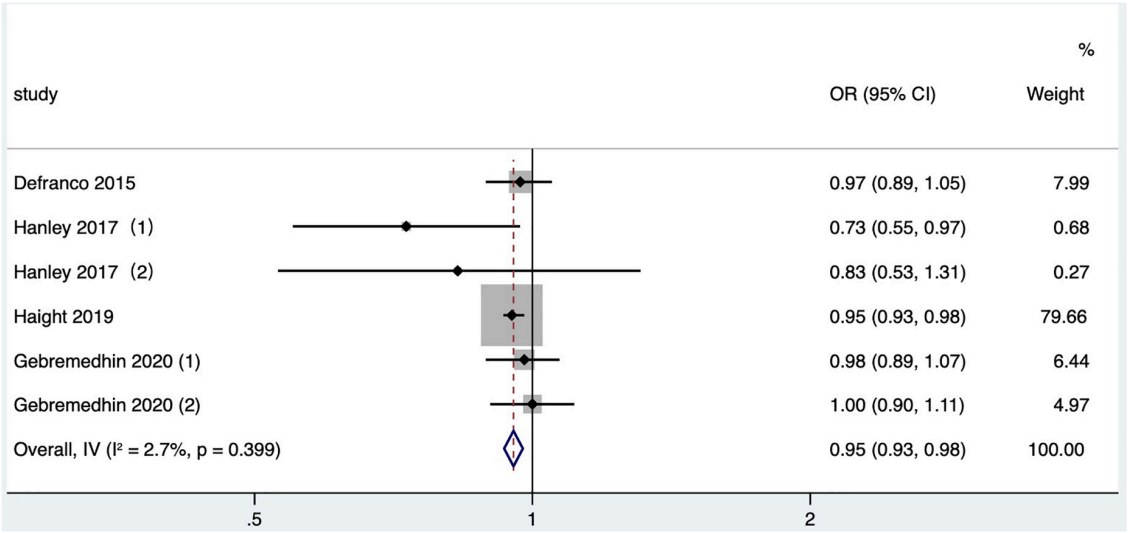

FIGURE 12
Meta-analysis forest map of the effect of short IPI on gestational hypertension. Hanley et al. (36) (1) was non-matching data, and Hanley et al. (36) (2) was within-mother matching data; Gebremedhin et al. (46) (1) was non-matching data, and Gebremedhin et al. (46) (2) was within-mother matching data.

contraction (16) may cause premature delivery. The breast-feeding status after delivery may affect the mothers' nutrients reservation, and the competition among siblings (57) may also affect fetal health. In addition, the women with short IPI didn't have enough time to lose weight and were more likely to be obese at beginning of the subsequent pregnancy (36), which may also affect the fetal health in the subsequent pregnancy. However, due to the lack of comparative analysis on the maternal physical conditions at different period, including prepregnancy, pregnancy, postpartum, and lactation period, we need more studies to find out the biological mechanism of short IPI and adverse perinatal outcomes.

This study concluded that short IPI was a risk factor for NICU and congenital abnormality. NICU, as one of the comprehensive indicators of neonatal postpartum outcomes, might be caused by preterm birth, low birth weight, small for gestational age infants, and other causes, which reflected the severity of the impact of short IPI on the overall health status of neonates. There are few studies involving offspring death and NICU, and the measurement methods of indicators

are inconsistent. However, given the very important clinical significance of offspring death and NICU, even though the strength of evidence provided by this meta-analysis was extremely low, it can still provide valuable information for clinical intervention trials. In our analysis, the included studies had given different definitions of congenital abnormality, and the categories of diseases involved were not completely consistent. As our meta-analysis gave an overview of short IPI and congenital abnormality, it may need more researches and data to find out the mechanism of congenital abnormality induced by short IPI.

This study found that short IPI was not related to the increased risk of gestational diabetes in mothers, which was different from the results of a systematic review published by Hutcheon et al. (7). The possible reason was that Hutcheon et al. included one study in high-resource settings, while this meta-analysis included more studies and had a wider coverage. This study showed that short IPI was not a risk factor for gestational hypertension and may lead to a reduced risk. Wainstock et al. believed that women who suffered from preterm birth, perinatal mortality, gestational diabetes, and other adverse outcomes rather than preeclampsia in the first pregnancy were likely to suffer from primary preeclampsia in the next pregnancy (58). There were many factors causing gestational hypertension and eclampsia/preeclampsia in pregnant women, especially the genetic susceptibility to the mother (59), as well as the first pregnancy with preeclampsia and the second pregnancy with preeclampsia relapse (60); therefore, the relationship between IPI and gestational hypertension might be influenced by the mixed adverse outcomes of the first pregnancy. However, few literature were included in the meta-analysis, and these literature did not effectively control the key factors, such as whether the previous pregnancy was accompanied by gestational diabetes (39, 41) and gestational hypertension (41). Therefore, the evidence strength of the association between short IPI and adverse maternal pregnancy outcomes in this meta-analysis was extremely low.

The advantages of this study were as follows.

The studies included in the meta-analysis were cohort studies and case–control studies, which provided valuable information for exploring the etiological relationship between IPI and pregnancy outcomes. Most of the research data came from the birth registration system, obstetric medical records, and other medical and health management data, which covered the vast majority of the target population in the research area, so the researches had high external validity.

The included studies controlled at least one confounding factor, especially maternal age, which ensures a higher quality of research. We used Egger's test to evaluate the included studies and found no statistical risks of publication bias. It is worth mentioning that Egger's test has high research significance for the meta-analysis with more than 10 articles included, but has low efficacy for the meta-analysis with less than 10 articles, such as our meta-analysis for infant death, NICU admission, congenital abnormality, gestational hypertension, and gestational diabetes.

The exposure factor (short IPI) defined by our meta-analysis was less than 6 months, and the reference group was 18–23 months or the wider group including 18–23 months, which ensured the comparability of the exposure factors of various studies (4, 61).

As we defined the exposure indicator as IPI, we checked the definition of the time interval between two consecutive pregnancies in the original articles and excluded the articles about birth interval. Adverse pregnancy outcomes between two live births would be omitted if the birth interval was only analyzed, including maternal death before 20 weeks of pregnancy (8) and adverse maternal and fetal situations after 20 weeks of pregnancy, such as maternal complications, late abortion ($\geq$ 20 weeks of pregnancy, $<$ 28 weeks of pregnancy), and stillbirth, which would lead to selection bias and measurement bias. Therefore, this study made clear that using IPI, rather than birth interval, made our research conclusion more accurate and was more conducive to the development of IPI-related consulting services for the target population in practical work.

This study also had the following limitations.

The original studies included in this analysis were at risk of bias in design. Because the majority of included studies were cohort studies, the quality of research design, implementation, data collection, and other links was highly required. The research sites were concentrated in the United States and Europe, while there were few studies in Africa and Asia. Due to the influence of regional economic development and medical level, the regions with a high medical level in which the research was conducted, there were differences in the population of the studies included in the meta-analysis. Most of the studies included in the meta-analysis were located in high-income countries, which made hard to compare the relationship between IPI and adverse pregnancy outcomes based on the different socioeconomic development levels. In addition, most research information came from birth registration system and perinatal system. The recorded information based on live births may ignore key fetal outcome events such as pregnancy termination and pregnancy loss between two live births, resulting in longer IPI recorded in the system than in the actual situation. Therefore, selection bias and misclassification bias may still exist in the original study even if pregnancy interval rather than birth interval was considered in this meta-analysis. China gradually launched the two-child policy at the end of 2015, and adverse events such as pregnancy termination and abortion may exist before the second live birth. Therefore, researchers did not include the data of people with pregnancy termination or pregnancy loss between two live births in the study of Zhang et al. (38) in the meta-analysis. This may

abnegate useful information about adverse pregnancy outcomes associated with short IPI.

The studies with within-mother matched analysis were included in this meta-analysis (36, 39). That is, the associations between IPI and pregnancy outcome of the same mother were compared, and the research population was limited to women with at least three consecutive singleton births (at least two IPIs), which was different from other studies. Subgroup analysis found that in the unmatched group, the OR values of short IPI on preterm birth, low birth weight, and small for gestational age infants were 1.53 (95% CI: 1.46–1.60), 1.40 (95% CI: 1.33–1.47), and 1.16 (95% CI: 1.09–1.23), respectively, and the OR values in the matched group were 0.94 (95% CI: 0.75–1.18), 0.76 (95% CI: 0.43–1.36), and 0.97 (95% CI: 0.84–1.13), respectively. Short IPI was not a risk factor for preterm birth, low birth weight, and small for gestational age infants in the study of matched design, which suggested that within-mother matched study designs may attenuate the association of short IPI with adverse pregnancy outcomes. Since the within-mother (matched) analysis narrowed the study populations to women with three or more pregnancies, while other non-matched data focused on the women with two consecutive pregnancies, the difference in study population may not only be related to IPI but also affect the pregnancy outcomes. The selection bias and confounding bias of matched data cannot be ignored. However, the inclusion of these data in the meta-analysis did not result in a significant change in the pooled OR value. The matched design controlled the time-invariant factors such as maternal genetic characteristics and lifestyle. Therefore, we believe that the study using within-mother (matched) comparison method would not affect the support for association in this meta-analysis, and the results could provide clinical guidance for pregnant women with high parities. On the contrary, if the data of within-mother (matched) was discarded, the evidence support of the meta-analysis may be reduced.

We only conducted the meta-analysis for two maternal outcomes in our study. Because the literature available for the analysis was limited, only gestational diabetes and gestational hypertension were considered, and a quantitative combination of effect values was not conducted for adverse maternal outcomes such as obesity, dystocia, placental abruption, uterine rupture, and premature rupture of membranes in the meta-analysis, the influence of short IPI on adverse maternal outcomes could not be comprehensively analyzed.

Different studies had different definitions of pregnancy outcomes. For example, very preterm birth meant that the gestational age was less than 31 to less than 34 weeks at delivery, and there were differences in the definition of SGA. Therefore, the quality assurance of the results of meta-analysis on very premature birth and SGA was affected. In addition, maternal morbidity was mentioned as an outcome indicator in five studies with different definitions of morbidity, so a quantitative combination of effect values was not performed.

The grouping of IPI was defined in this meta-analysis when determining the inclusion criteria of literature, but different studies were inconsistent. Therefore, abandoning the studies that did not meet the inclusion criteria in the quantitative combination may lose the available research information (62–72).

This study did not conduct a meta-analysis on the related factors affecting the IPI, such as maternal age, parity, pregnancy intention, and social and economic status, which were important confounding factors of short IPI and adverse pregnancy outcomes. The association between short IPI and adverse pregnancy outcomes may be influenced by confounding factors.

In this analysis, the heterogeneity of the studies was prominent. Even with subgroup analyses based on the publication time, study site, study type, and matched data or not, the heterogeneity remained high. Heterogeneity mainly came from research methods, including data collection methods and data analysis methods. The original study we analyzed mainly obtained data through birth registration records, death records, and hospital records. As birth registration records may ignore miscarriage and stillbirth information between pregnancies, it may overestimate pregnancy interval and underestimate the incidence of adverse pregnancy outcomes. Data analysis methods, especially matching methods, as mentioned above, narrowed the research subjects and lead to low external validity of each study.

In a word, short IPI (IPI $<$ 6 months) can lead to adverse perinatal outcomes, while it was not a risk factor for gestational diabetes and gestational hypertension. However, the evidence strength of the association between short IPI and maternal pregnancy outcomes was very low in this study. Therefore, we need more high-quality studies covering more comprehensive indicators of maternal and perinatal pregnancy outcomes, especially from the populous country in Asia (China). We suggest that future research use unified, comparable IPI groups, give full consideration to the key influencing factors such as the social demographic characteristics of pregnant women, pre-pregnancy and pregnancy behavior, and previous pregnancy outcomes, in order to adequately explore the causality short IPI and adverse pregnancy outcomes, and provide a reference for further intervention trial, which is also our next research plan to provide a relevant basis for WHO policies.

Conclusion

Short IPI (IPI $<$ 6 months) can lead to adverse perinatal outcomes, while it is not a risk factor for gestational diabetes and gestational hypertension. Therefore, more high-quality studies covering more comprehensive indicators of maternal and perinatal pregnancy outcomes are needed to ameliorate the pregnancy policy for women of childbearing age.

Data availability statement

The original contributions presented in the study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author/s.

Author contributions

YW, CZ, XL, and YL designed the manuscript. YW and CZ drafted the protocol with input from YC, DT, and LY. YW, CZ, YC, DT, and LY extracted and analyzed the data. YW and CZ screened the literature. YL and XL revised and ensured the quality of the manuscript. All authors contributed to manuscript revision reviewed, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.922053/full#supplementary-material

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Inconsistency between non-invasive prenatal testing (NIPT) and conventional prenatal diagnosis due to confined placental and fetal mosaicism: Two case reports

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We aimed to identify the causes of inconsistent results between non-invasive prenatal testing (NIPT) and invasive testing methods for trisomy 21. In the first case, NIPT was performed at 11 weeks of pregnancy, and the result showed a high risk of trisomy 21 [fetal fraction (FF) = 6.98%, 21 chromosome Z-score = 3.6]. The patient underwent quantitative fluorescent (QF)-PCR and karyotyping at 14 + 0 weeks of pregnancy through CVS showing mosaicism of 47, XX, + 21[11] and 46, XX [39] in karyotyping. The patient underwent amniocentesis at 15 + 6 weeks, showing a normal pattern in QF-PCR and 46, XX karyotyping in long term culture. The second case underwent NIPT at 16 + 5 weeks of pregnancy (FF = 7.52%, 21 chromosome Z-score = 2.503). She underwent an invasive test at 19 weeks through amniotic fluid sampling. As a result, trisomy 21 was detected by QF-PCR, and mosaicism of XX, +21[22]/46, XX [4] was identified by karyotyping. Despite significant advances in fetal chromosome analysis using NIPT, invasive testing is still needed as placenta-derived DNA does not reflect 100% fetal genetic information. Placental mosaicism can be detected by NIPT, but more research is needed to increase its sensitivity. Therefore, if the NIPT result is positive, an invasive test can confirm the result, and continuous monitoring is required even if the NIPT result is negative.

KEYWORDS

placenta-derived cell free DNA, whole genome sequencing, non-invasive prenatal testing, confined placental mosaicism, mass parallel sequencing

1 Introduction

Prenatal screening and diagnosis of fetal chromosomal aneuploidy have become common among pregnant women (1–3). These screening methods, such as fetal ultrasound and maternal serum biomarker screening, have detection rates of 60–90% and a false positive rate of 5% (1, 4). If these tests show a high risk of fetal chromosomal aneuploidy, pregnant women are recommended to undergo invasive diagnostic tests such as chorionic villus sampling (CVS) at 12–13 weeks of gestation and amniocentesis at 15–16 weeks of gestation (1, 2). Although these tests are valuable diagnostic tools because of their high accuracy, they are associated with a risk of miscarriage between 0.5 and 1.0% (5, 6). Since the discovery of placenta-derived cell-free DNA (cfDNA) in the peripheral blood of pregnant women in the late 1980s, various attempts have been made to use it for prenatal genetic screening (7–10).

Therefore, detection of fetal chromosomal aneuploidy using cfDNA is expected to be an alternative to invasive tests. Many clinical studies have successfully applied mass parallel sequencing (MPS) of maternal cfDNA using whole genome sequencing or target sequencing methods. (11, 12). A meta-analysis of the clinical validation and implementation of the non-invasive prenatal testing (NIPT) method revealed a high sensitivity and specificity (92–99%) for trisomy 21, 18, and 13 (4, 13, 14). NIPT has been recently recommended by several professional societies, such as the International Society for Ultrasound in Obstetrics and Gynecology (ISUOG) and the American College of Obstetricians and Gynecologists (ACOG), International Society of Prenatal Diagnosis (ISPD), and the Royal College of Obstetricians and Gynecologists (RCOG).

Although the genetic information of placental tissues is known to representative of the fetus in most cases, mosaicism is observed (mostly confined to the placenta) in 1–2% of karyotypes (15). Therefore, the proportion of inconsistent results due to confined placental mosaicism (CPM) observed between CVS and amniocentesis is similar to that of NIPT and amniocentesis.

Non-invasive prenatal testing is a next-generation technology with great potential as a screening tool for pregnant women. However, it is important to emphasize that NIPT is only a screening tool, not a diagnostic of fetal aneuploidy. Therefore, if the NIPT result is positive, an invasive test is required to confirm the result, and continuous observation is required even if the NIPT result is negative.

In this study, we report two cases of discrepancy between NIPT and invasive diagnostic methods and their follow-up studies for more accurate prenatal genetic counseling on NIPT results. The z-scores of two patients were showed values that did not belong to the low-risk group and the high-risk group.

2 Materials and methods

2.1 Participants

Patient 1: The 30-year old patient had a history of four pregnancies including this pregnancy and three spontaneous abortions; 47, XY, +21 in the first pregnancy, 45, X in the second pregnancy, and conjoined twin in the third pregnancy. She was naturally pregnant and nuchal translucency (NT) was 1.0 mm on ultrasonography at 15 + 6 weeks of gestation. No ultrasound abnormalities were identified.

Patient 2: The 37-year old patient had a history of five pregnancies, including this pregnancy and four spontaneous abortions; She was pregnant through *in vitro* fertilization (IVF) and nuchal translucency (NT) was 1.2 mm on ultrasonography at 16 + 5 weeks of gestation. No ultrasound abnormalities were identified.

We obtained written informed consent for participation in the study from 2 patients, and the study was approved by the institutional review board of the CHA Gangnam Medical Center, CHA University, Seoul, Korea (Approval number: GCI-2022-04-015). The studies for 1,653 data were approved by the institutional review board of the CHA Gangnam Medical Center, CHA University, Seoul, Korea (approval number: GCI-20-11).

2.2 Sample preparation and sequencing

Approximately 10 ml of maternal peripheral blood samples were collected in Cell-Free DNA BCT^{TM} tubes (Streck, Omaha, NE, USA) and stored at room temperature until further processing. After centrifuging the whole blood samples at 1,200 × *g* for 10 min at 4°C, plasma was separated from the maternal cells and transferred to microcentrifuge tubes. Samples were centrifuged at 16,000 × *g* for 10 min and the supernatant was separated from residual cells, transferred to new tubes and stored at −20°C until required for analysis. For each sample, plasma cfDNA was extracted from 1 mL of plasma using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany). The cfDNA was used to for library preparation using the Ion Plus Fragment Library kit (Thermo Fisher, Waltham, CA, USA) according to the manufacturer's instructions. DNA libraries were analyzed using the Ion S5^{TM} XL System (Life Technologies, Singapore) with an average 0.3× sequencing coverage depth. A total of 12 cfDNA samples were loaded onto an Ion 540^{TM} Chip Kit (version 2.0; Life Technologies, CA, USA). The raw reads of each sample were above 5 million, and the rates of uniquely mapped reads was above 65.0%.

2.3 Data and statistical analyses

Raw reads obtained from the Ion Torrent Suite software (version 5.16.1) were trimmed and filtered using Picard with default parameters. The sequence fragments were aligned and mapped to the human reference genome sequence (hg19) using the Burrows-Wheeler Aligner (BWA). The effect of GC bias was reduced and normalized using LOESS regression. The z-score for each chromosome in each sample was calculated using the mean mapped reads and the standard deviation (SD). Standard formulas for binomial distributions were used to calculate the positive predictive value (PPV) and negative predictive value (NPV). Data were analyzed using Wilson's interval method and MedCalc version 12.1.4 (MedCalc Software Ltd., Ostend, Belgium). Samples with a fetal fraction (FF) less than 4.0% were described as no-calls and re-sampled or rejected according to the FF value. The aneuploidy of chromosome 21 was assessed according to the z-score value and identified with one of the following groups: $\geq$3.5 = high risk, $\geq$ 2.5 = intermediate risk, and between −2.5 and 2.5 = low risk.

2.4 DNA extraction

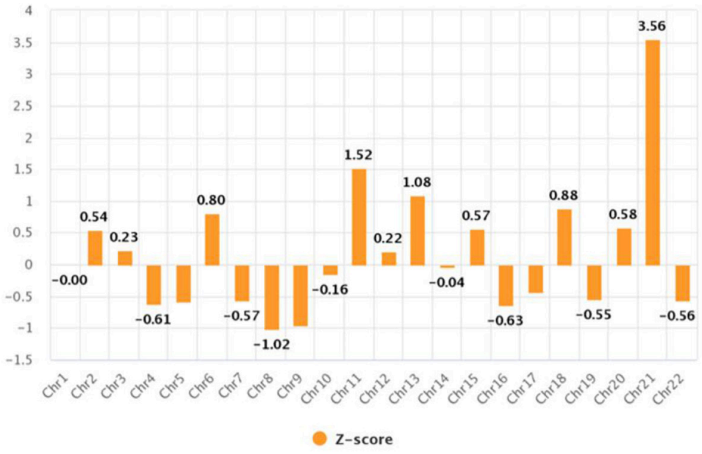
Genomic DNA was extracted from the amniocytes, placental tissue, and parental blood samples; 1.5 ml of amniotic fluid using InstaGene^{TM} Matrix (Bio-Rad Laboratories, Inc., CA, USA), 200 μl of peripheral blood using QuickGene DNA blood kit (Kurabo, Osaka, Japan), and 1 mg of placental tissue using QuickGene DNA tissue kit (Kurabo) according to the manufacturer's instructions.

2.5 QF-PCR and UPD test

DNA (10 ng) was amplified using Elucigene QST*R Plus v2 or QST*R-21 (Delta Diagnostics, Manchester, UK) according to the manufacturer's instructions. The PCR products were analyzed using ABI 3500 (Applied Biosystems, CA, USA) and GeneMapper software (Applied Biosystems). Short tandem repeat (STR) markers, such as the 7 informative markers D21S11 (21q21.1), D21S1437 (21q21.1), D21S1409 (21q21.1), D21S1442 (21q21.3), D21S1435 (21q21.3), D21S1411 (21q22.3), and D21S1446 (21q22.3), were used to perform polymorphic marker analysis on chromosome 21 region to exclude uniparental disomy (UPD).

2.6 Cytogenetic analysis

Amniocytes were grown in Chang Medium® *In Situ* (Irvine Scientific, Santa Ana, CA, USA) using the *in situ* coverslip culture method. GTG-banded metaphase chromosomes were obtained from 15 colonies and analyzed using CytoVision version 3.6 (Applied Imaging, Thunderland, UK). The results



GC(%)	Y_FF(%)	S1_FF(%)	S2_FF(%)	Z-score	PFF
40.97	Under 1%	6.98	9.53	3.56	4.39

FIGURE 1
Non- invasive prenatal testing (NIPT) data in case 1.

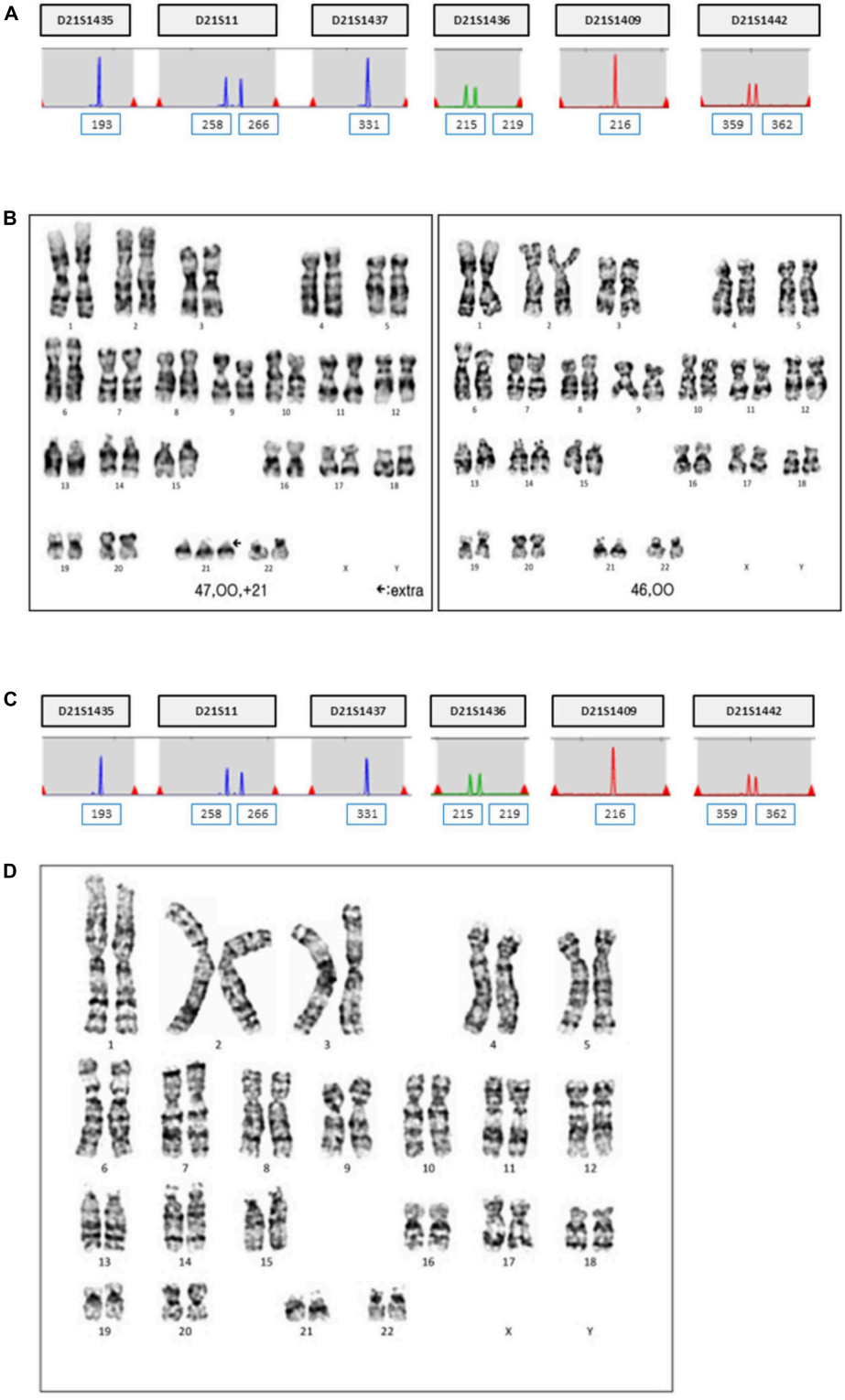


FIGURE 2
QF-PCR and conventional karyotyping results in case 1. **(A)** QF-PCR analysis for chromosome 21 of uncultured chorionic villi. **(B)** Conventional karyotype analysis of uncultured chorionic villi; 47, XX, +21[11]/46, XX [39]. **(C)** QF-PCR result for chromosome 21 of uncultured amniocytes. **(D)** Conventional karyotype analysis of cultured amniocytes; 46, XX.

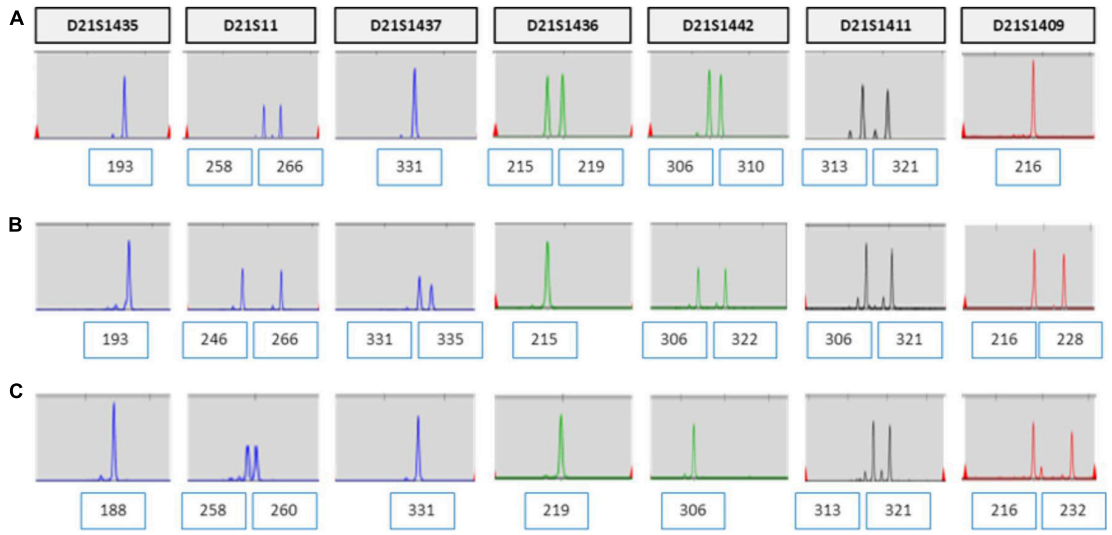


FIGURE 3
UPD test for chromosome 21 using QF-PCR in case 1. **(A)** Fetus allele in uncultured amniocytes; UPD of chromosome 21 was not detected. **(B)** Maternal allele in peripheral blood. **(C)** Paternal allele in peripheral blood.

were interpreted according to the International System for Human Cytogenetic Nomenclature, 2020.

3 Results

3.1 Case 1

The patient was 30 years old and had a history of four pregnancies including this pregnancy and three spontaneous abortions: 47, XY, +21 in the first pregnancy, 45, X in the second pregnancy, and conjoined twin in the third pregnancy. She and her partner requested prenatal fetal screening for aneuploidy. After adequate genetic counseling, NIPT was performed at 11 + 0 weeks of pregnancy, and the result showed a high risk of trisomy 21 (FF = 6.98%, 21 chromosome Z-score = 3.6) (Figure 1). The patient underwent QF-PCR and karyotyping at 14 + 0 weeks of pregnancy through CVS and exhibited a normal pattern in QF-PCR (Figure 2A) and mosaicism of 47, XX, +21[11] and 46, XX [39] (Figure 2B). Owing to the discrepancy between the NIPT and CVS results, the patient underwent amniocentesis at 15 + 6 weeks, and showed a normal pattern in QF-PCR (Figure 2C) and 46, XX karyotyping in long-term culture (Figure 2D).

Short tandem repeat marker tests for chromosome 21 were performed on the parental and amniotic fluid samples to rule out the possibility of UPD. UPD of chromosome 21 was not detected in the amniotic fluid sample (Figure 3). She continued her pregnancy and gave birth to a baby with labor induction at 38 + 5 weeks. After delivery, we sampled 7 × 1 cm^3 positions of the placenta and checked chromosome 21 using QF-PCR (Figures 4A–G). Chromosome 21 was normal in cord blood and the placenta region close to the fetus. However, trisomy 21 was identified in the placental region close to the mother, including in the amniotic membrane (Figures 4A–G).

3.2 Case 2

The patient was 37 years old and had a history of five pregnancies, including four spontaneous abortions. She was transferred from another hospital, and the cause of her previous miscarriage could not be confirmed. She underwent NIPT at 12 + 5 weeks of pregnancy, and the results showed no-call data because the FF was relatively low (3.86%). Re-sampling was performed at 16 + 5 weeks of pregnancy, and the results showed a intermediate risk of trisomy 21 (FF = 7.52%, 21 chromosome Z-score = 2.503) (Figure 5A). We repeated the experiment on this sample to exclude a false positive result, and the results again showed a intermediate risk of trisomy 21 again (FF = 7.02%, 21 chromosome Z-score = 2.62) (Figure 5B). We performed an invasive test at 19 weeks of pregnancy through amniotic fluid sampling, followed by QF-PCR and karyotyping. Trisomy 21 was detected by QF-PCR (Figure 6A), and mosaicism of 47, XX, + 21[22]/46, XX [4] was detected by karyotyping (Figure 6B). The patient was counseled with these results and decided to terminate the pregnancy. As the source of cell-free fetal DNA in maternal blood has been shown to be placental in origin, placental biopsies were obtained after abortion. Samples were collected from 7 × 1 cm^3 positions of the placenta (Figure 7). QF-PCR in one of the samples showed three of the samples showed trisomy 21 (Figures 7A, D, F)

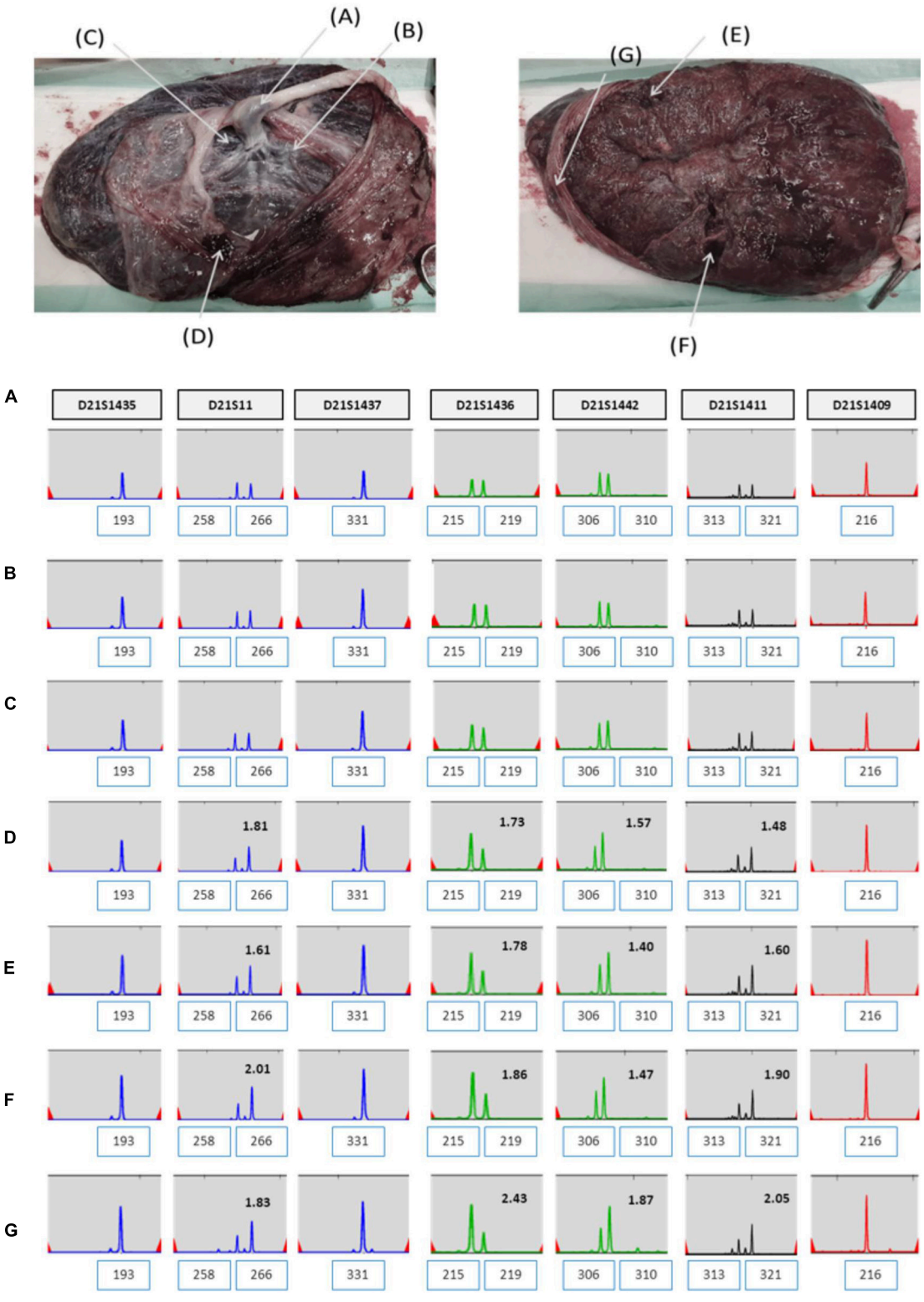
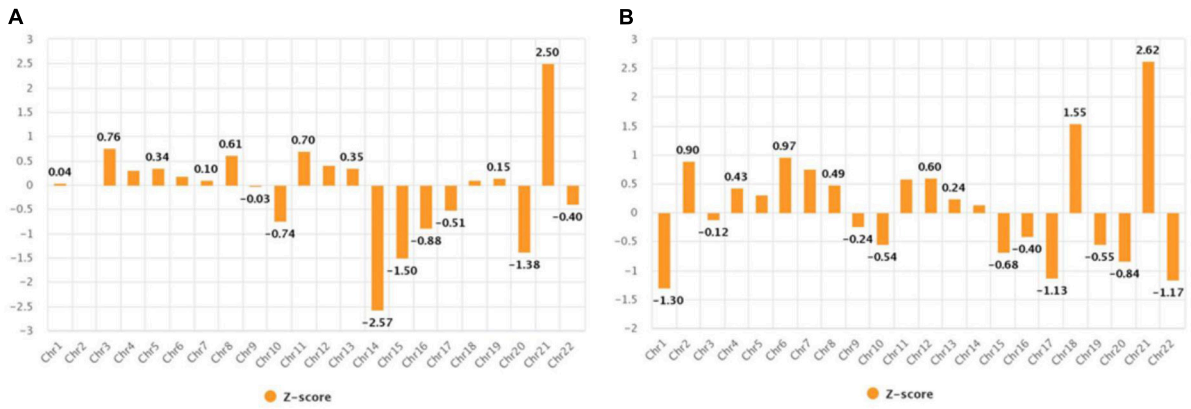


FIGURE 4
(A–G) QF-PCR result for chromosome 21 in each location of placenta in case 1 **(A)** cord blood, **(B)** chorion, **(C)** villus parenchyma (fetal side section), **(D)** villus parenchyma (middle section), **(E)** villus parenchyma (maternal side section I), **(F)** villus parenchyma (maternal side section II), **(G)** amnion.



	GC(%)	Y_FF(%)	S1_FF(%)	S2_FF(%)	Z-score	PFF
(A)	40.9	Under 1%	7.52	10.45	2.503	3.078
(B)	41.0	Under 1%	7.02	9.58	2.620	3.265

FIGURE 5
Non- invasive prenatal testing (NIPT) data in case 2 **(A)** 1st trial data **(B)** re-test data.

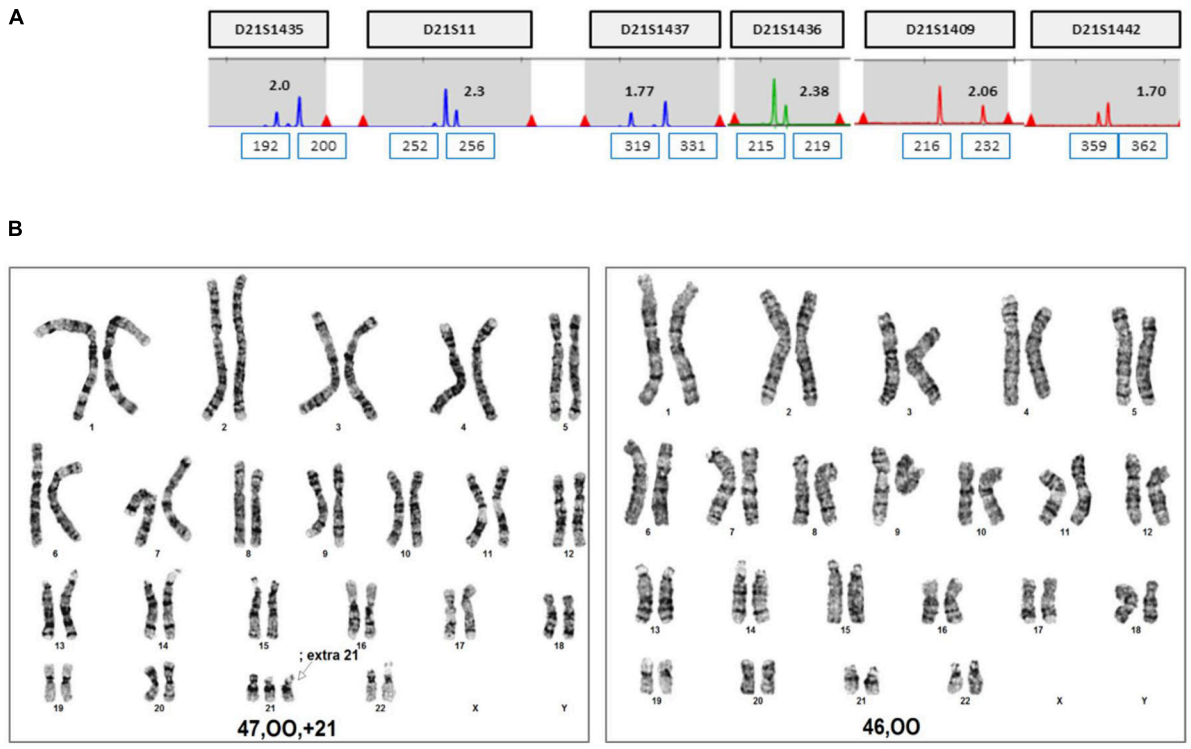
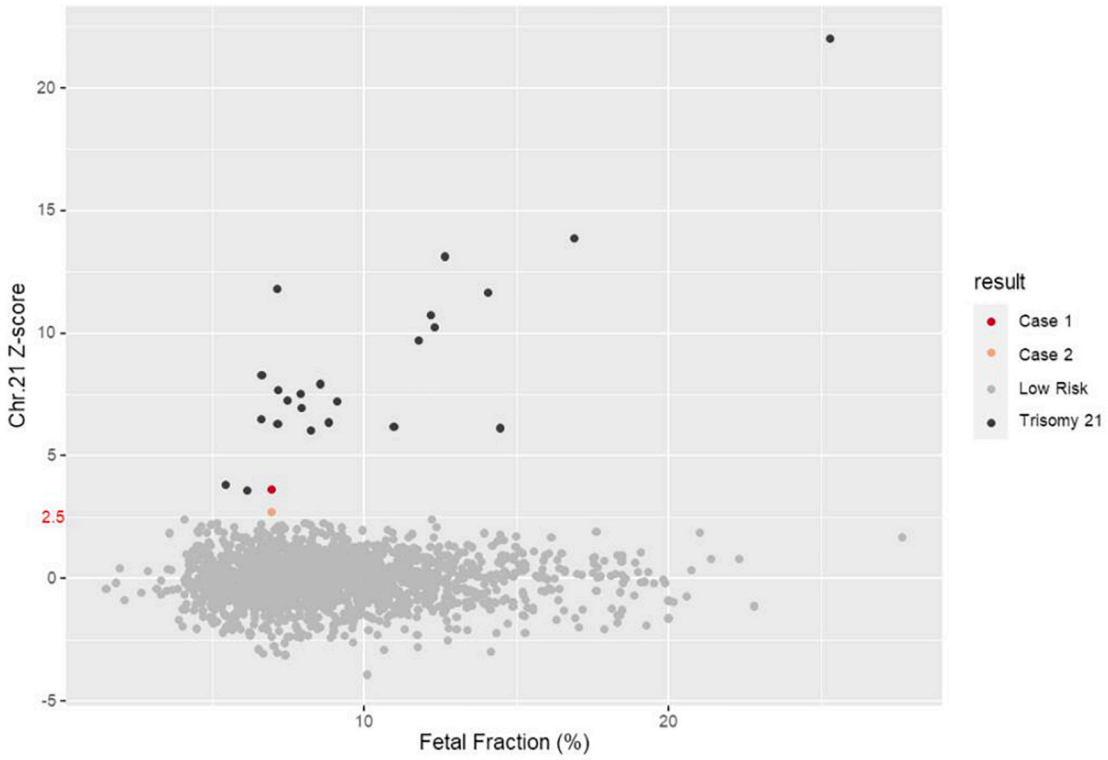


FIGURE 6
QF-PCR and conventional karyotyping results in case 2. **(A)** QF-PCR result for chromosome 21 of uncultured amniocytes. **(B)** Conventional karyotype analysis of cultured amniocytes; 47, OO, +21[22]/46, OO [4].

FIGURE 7
(A–G) QF-PCR result for chromosome 21 sampling each location of placenta in case 2 **(A)** cord blood, **(B)** chorion, **(C)** villus parenchyma (fetal side section), **(D)** villus parenchyma (middle section), **(E)** villus parenchyma (maternal side section I), **(F)** villus parenchyma (maternal side section II), **(G)** amnion.



Result	Case 1	Case 2	Low Risk (N=1628)	Trisomy21 (N=23)	*p*
FF (%)	6.98	7.27	9.02 ± 3.31	10.21 ± 4.49	0.305
Z-score of chr.21	3.56	2.56	-0.03 ± 0.97	8.74 ± 3.96	< 0.001

FIGURE 8
Z-score for chromosome 21 of 1,653 data from Jan 2019 to December 2020.

and the remaining four samples showed a mosaic pattern (**Figures 7B, C, E, G**).

3.3 Comparison of z-score values for chromosome 21 between two cases and 1,651 data

We summarized the z-scores of chromosome 21 and FF values of total 1,653 including two cases recently acquired in our laboratory. As shown **Figure 8**, a z-score of 2.5 lies value on the boundary line that separates the low-risk from the intermediate-risk group. The mean FF% of 1,628 low-risk patients was 9.02 ± 3.31, and the z-score value for chromosome 21 was −0.03 ± 0.97. The mean FF% of 23 high-risk group for chromosome 21 was 10.21 ± 4.49 and the z-score was a value of 8.74 ± 3.96. The FF% of the two patients was not significantly different from the other groups, but the z-score showed values that did not belong to the low-risk group and the high-risk group.

4 Discussion

Here, we reported two cases of discrepancy between NIPT and invasive tests. In case 1, thorough examinations continued until the patient gave birth, even if trisomy 21 was confined to the placenta. After delivery, the baby weighed 2,760 g, and chromosomal abnormalities, including that in chromosome 21, were not observed. In case 2, the z-score values (2.503 and

2.62) were much lower than FF values (7.52 and 7.02%) in the NIPT results. Hence, it was expected to detect normal or low level of mosaicism pattern, not trisomy 21 in QF-PCR. In contrast, the QF-PCR results of the placental tissue confirmed that the allele pattern differed depending on the location of the placental tissue, and genetic discrepancies existed between the fetus and placenta.

Although there is no way to measure the exact actual chromosomal mosaicism rate, the percentage of mosaic trisomy 21 was evaluated by QF-PCR in the placenta after birth. The rates of trisomy 21 in the placenta of case 1 and 2 was 57.1 and 42.9%, respectively. However, in CVS, it was 22 and 84.6%, respectively. So, our data showed that the level of mosaicism detected by CVS does not always reflect the level present in placenta.

These two patients had a similar experience of several spontaneous abortions, and the results at this pregnancy showed that there was placental mosaicism. Prior reports have suggested that the placental mosaicism influences fetal development and CPM is more likely when placental insufficiency occurs in advanced maternal age (3–5). However, most cases of CPM are undiagnosed and are difficult to identify. Furthermore, since the patient in case 1 was 30 years old, which is a relatively young age, further studies at the molecular genetic level are required to determine the other putative factors apart from age.

Cell-free DNA-based prenatal screening, also known as non-invasive prenatal testing (NIPT), shows excellent sensitivity and specificity for detecting trisomy 21 compared to other screening methods (detection rate is greater than 99%, and the false-positive rate is less than 0.1%) (6). However, at the same time, extremely low but consistent false-negative cases have been reported (7–9). A low trisomic fraction relative to FF may suggest CPM or complete trisomy in fetuses with normal placental cells (10). Mitotic CPM occurs in normal diploid zygotes, and errors after conjugation occur in placental cell lineages, which usually lead to local areas of placental trisomy and low-level mosaicism. In contrast, meiotic CPM occurs in trisomic zygotes, wherein a trisomy rescue event occurs at the beginning of fetal development. In these cases, the fetus is usually diploid, and the placenta is mosaic or completely aneuploid. However, a risk of mosaicism in the fetus depends on the timing of the loss of trisomy in the embryonic cell lineage (5). There may also be a risk of fetal UPD after trisomy rescue, depending on the origin of the missing chromosome.

Fetal cfDNA in maternal peripheral blood originates from trophoblasts and is mainly composed of placental DNA (11–13). NIPT is widely used as an alternative to ultrasonography or invasive fetal testing. However, discrepancies in genetic information between placental and fetal tissues may affect the NIPT outcome, leading to inaccurate results. False-positive NIPT results have been consistently reported and have become concern in recent years (8, 14–16). Additionally, the mosaic condition of the placenta may reduce the measurement accuracy and lead to false-negative results. Therefore, the level of mosaicism is a vital factor in NIPT. Given the influence of the placenta on NIPT, the results should be interpreted in conjunction with various clinical tests based on comprehensive background information.

If the Z-score values of the boundary between low risk and intermediate risk are obtained from the NIPT test results, it is necessary to check whether the Z-score values are consistent even after repeated experiments. If so, a confirmation invasive test is necessary, considering the possibility of mosaicism. We suggest that this approach is optimal for obtaining accurate results and exclude false positives and false negatives. These two cases reaffirm the importance of complementary verification testing following NIPT.

Data availability statement

The datasets presented in this article are not readily available to protect patient confidentiality and privacy. All data generated or analyzed during this study are included in this article.

Ethics statement

We obtained written informed consent for participation in the study from 2 patients, and the study was approved by the Institutional Review Board of the CHA Gangnam Medical Center, CHA University, Seoul, Republic of Korea (Approval number: GCI-2022-04-015). The studies for 1,653 data were approved by the Institutional Review Board of the CHA Gangnam Medical Center, CHA University, Seoul, Republic of Korea (approval number: GCI-20-11). Written informed consent was obtained form the 2 patients for the disclosure of data such as publication of articles about the results.

Author contributions

SS was obtained by funding for this study. SS and DC conceptualized and designed the manuscript. KK and SK drafted the manuscript. SK and SR collected the samples and performed obstetric work-up. JP and HK analyzed the data and interpreted the findings. HJ, MG, SY, and SB performed the experiments. All authors contributed to the article and approved the submitted version.

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Conflict of interest

KK, JP, HK, HJ, MG, SY, SB, DC, and SS were employed by CHA Biotech Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Effect of interval between oocyte retrieval and resuscitation embryo transfer on pregnancy outcomes

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Objectives: Resuscitation transfer of embryos after elective cryopreservation has been widely applied in *in vitro* fertilization-embryo transfer (IVF-ET) therapy for human infertility or sterility owing to higher embryo implantation rates. This method separates oocyte retrieval from embryo transfer. The optimal time for frozen embryo transfer (FET) remains unknown. Therefore, this study mainly compares the advantages and disadvantages of delayed FET and immediate FET through retrospective analysis.

Methods: We analyzed real world data of patients who underwent resuscitation transplantation between October 2019 and July 2021 at the Reproductive Center of Chengdu Jinjiang Hospital for Women's and Children's Health. Propensity score matching was applied to control potential confounding factors. A total of 5,549 patients who received at least one FET were analyzed. Patients undergoing transplantation within 60 days of oocyte retrieval were included in the immediate FET group (n = 1,265) and those undergoing transplantation > 60 days after retrieval were included in the delayed FET group (n = 4,284).

Results: Live birth rates between the two groups were comparable (45.25% vs. 45.76%, p = 0.757). Moreover, no difference was observed in the rates of biochemical pregnancy (64.50% vs. 66.80%), clinical pregnancy (55.24% vs. 56.83%), ectopic pregnancy (1.47% vs. 1.39%), early miscarriage (14.41% vs. 16.20%), late miscarriage (2.21% vs. 2.09%), singleton premature delivery (16.67% vs. 18.29%), and neonatal deformity (1.97% vs. 1.80%). After stratifying the patients based on the type of embryo transferred, number of embryos transferred, FET protocol, and good prognosis criteria, live birth rates remained comparable between the two groups (p > 0.05).

Conclusion: Pregnancy outcomes were comparable between the immediate and delayed FET groups.

KEYWORDS

immediate frozen embryo transfer, delayed frozen embryo transfer, live birth rate, clinical pregnancy, pregnancy outcome

1. Introduction

Controlled ovarian hyperstimulation (COH) is one of key processes in assisted reproductive technology (ART) therapy. COH may lead to ovarian hyperstimulation syndrome (OHSS), cause endometrial synchronicity and other adverse factors that could reduce pregnancy rates, such as elevated progesterone and tubal effusion. Nearly half of the patients receiving COH undergo resuscitation transplantation after embryo freezing (1–4). Patients undergoing *in vitro* fertilization (IVF) are eager to get pregnant with shortened interval between egg retrieval and transplantation. However, there is no uniform regulation regarding the timing of post-COH resuscitation transplantation (5, 6). However, since the ovaries are enlarged during COH and the risk of ovarian torsion are increased, physicians of reproductive medicine tend to start embryo resuscitation and transplantation preparations until at least the second menstrual cycle.

In addition, more and more young cancer patients accept fertility preservation services at present since they receive certain types of cancer surgery which lead to removal of organs needed for a pregnancy, and certain therapy might increase hormone levels or cause damage to a female's eggs (7). Previous studies have not come to a definitive conclusion on how immediate or delayed resuscitation will benefit pregnancy rates (8, 9). Additionally, many of the retrospective studies on this issue were conducted in years ago, and the results of these studies may be biased against changes in the existing COH protocols (1, 10, 11). Therefore, owing to the advantage of large data volumes at our hospital, we retrospectively analyzed the clinical data of patients who underwent COH and resuscitation transplantation from October 2019 to August 2021. We compared clinical data from first, second, and successive menstrual cycle resuscitations thereafter involving full embryo freezing.

2. Materials and methods

2.1. Patients

This retrospective cohort study reviewed the data of patients who underwent IVF/intracytoplasmic sperm injection (ICSI) between October 2019 and July 2021 at the Reproductive Center of Chengdu Jinjiang Hospital for Women's and Children's Health, Sichuan, China. This study was approved by the Ethics Committee of the Chengdu Jinjiang Hospital for Women's and Children's Health. And all methods were performed in accordance with the relevant guidelines and regulations. The informed consent was obtained from all participants of the study.

Patients aged 20–38 years who underwent IVF/ICSI cycles, patients who froze all embryos, and patients who subsequently received FET after failure of fresh embryo transplantation were included in the study. Further, patients who underwent rescue ICSI cycles, with an endometrial thickness of <8 mm before embryo transfer, and patients with endometriosis, genital malformation, or uterine abnormality were excluded.

2.2. COH protocols

Four ovulation stimulation protocols were used. (I) The antagonist protocol, involving ovulation induction with follicle stimulating hormone (FSH) (Gonal-F, Merck Serono, Puregon, Organon) from days 2–5 of menstruation, followed by gonadotropin releasing hormone antagonist (GnRH-a) (Cetrorelix, Merck Serono, or Orgalutran Organon) at a daily dose of 0.25 mg, which was commenced when the largest follicle exceeded 12–14 mm. (II) The long ovulation stimulation protocol, involving 3.75 mg GnRH-a (Triptorelin, Ferring) injections on the 2nd–5th day of menstruation and FSH ovulation induction after 28–30 days. (III) The luteal phase improvement long protocol, involving 0.1 mg GnRH-a (Triptorelin, Ferring) injection during the luteal phase followed by FSH administration starting on the 3rd day of menstruation to initiate ovulation. (IV) The micro stimulation protocol, involving administration of FSH or Menotrophins (Lebaode, LiZhu) on the 2nd–3rd day of menstruation was used to promote egg excretion. Urinary human chorionic gonadotropin 2000–6000 and GnRH-a 0.2 mg or recombinant human choriogonadotropin alfa (AiZe, Merck Serono, Darmstadt, Germany) were then injected when the target follicle was 18–20 mm. Egg retrieval was carried out 36–38 h after triggering, and 90 mg/d of crinone (Merck Serono, Darmstadt, Germany) and 20 mg/d of dydrogesterone (Abbott Biologicals, Beijing, China) were administered as luteal support after egg retrieval. All frozen embryos were vitrified with open system.

2.3. Endometrial preparation

Patients who underwent transplantation within 60 days of oocyte retrieval were included in the immediate FET group and those who underwent transplantation > 60 days after retrieval were included in the delayed FET group. The endometrial preparation program included natural cycle, hormone replacement cycle, ovulation-promoting cycle, and down-regulation cycle. The natural cycle was determined by monitoring follicular development using transvaginal ultrasonography and hormone levels. For the hormone replacement cycle, on days 2–4 of the menstrual cycle, 4 mg estradiol valerate tablets (Progynova, Berlin, Germany) were administered daily for 10 days. For the ovulation-promoting cycle, on days 2–4 of menstruation, 2.5–5 mg of letrozole, 20–40 mg of tamoxifen, or 50 mg of clomiphene was administered for 5 days. The down-regulation cycle involved injection of 3.75 mg GnRH-a (Triptorelin, Ferring) on days 2–3 of menstruation.

Luteal transformation was achieved with 90 mg/d of crinone or 600 mg/d of soft capsule progesterone (Cyndea Pharma, S.L.) and 40 mg/d of dydrogesterone, after the endometrium reached 8 mm thickness. Cleavage-stage embryos or blastocysts were transferred 3–5 days after transformation. Luteal support was provided after embryo transfer.

2.4. Outcomes

The primary outcome was the live birth rate, defined as the delivery of a living baby at ≥28 weeks of pregnancy after the first embryo transfer. The secondary outcomes were the rates of biochemical pregnancy, clinical pregnancy, ectopic pregnancy, early miscarriage, late miscarriage, singleton premature delivery, and neonatal deformity.

2.5. Statistical analysis

Propensity score matching (PSM) was used to make the baseline characteristics between the immediate and delayed FET groups balanced and comparable. The variables for PSM included female age, FSH, progesterone, fertilization method, COS protocol, FET protocol, the number of top-quality embryos transferred, and the type of embryo transferred. The (1:3) nearest neighbor caliper matching method without replacement was used to match data between the two groups, and the caliper was 0.05. The Kolmogorov–Smirnov test and the Shapiro–Wilk test were used to test the normality of the variables. Continuous variables are presented as mean ± SD or median (IQR). Categorical variables are presented as numbers and percentages. Continuous variables were compared between the groups using the *t*-test or Mann–Whitney *U* test. Categorical variables were compared using the Chi-square test. Multiple logistic regression models, odds ratio (OR), and 95% confidence intervals (CI) were calculated after adjusting for confounders. A *P*-value < 0.05 was considered statistically significant. All analyses were performed using SPSS software, version 25.0 and R software version 3.3.0.

3. Results

3.1. Demographics

All demographic data are shown in Table 1. A total of 5,549 patients who received at least one FET were analyzed in the study. The immediate FET group consisted of 1,265 patients, and the delayed FET group consisted of 4,284 patients. The interval between oocyte retrieval and embryo recovery was significantly shorter in the immediate FET group than in the delayed FET group [days: 34 (30–56) vs. 83 (67–111)]. Additionally, there were significant differences in maternal age, basal FSH, basal P, fertilization method, COS protocol, FET protocol, the number of top-quality embryos transferred, and the type of embryo transferred between the two groups ($p < 0.05$). After PSM, a total of 1,231 immediate FET patients were successfully matched to 3,280 delayed FET patients. The interval between oocyte retrieval and embryo recovery was still significantly shorter in the immediate FET group than in the delayed FET group [days: 30 (27, 33) vs. 69 (59, 94)]. After PSM, there were no significant differences in maternal age, basal FSH, basal progesterone, fertilization method, FET protocol, and number of top-quality embryos transferred between the two groups ($p > 0.05$). However, the COS protocol and type of embryo transferred were significantly different between the two groups ($p < 0.05$).

3.2. Pregnancy outcomes

Before PSM, the live birth rate was not significantly different between the immediate FET and the delayed FET groups (45.22 vs. 45.38%, $p = 0.920$) (Table 2). Additionally, after PSM the live birth rates between the groups remained comparable (45.25 vs. 45.76%, $p = 0.757$) (Table 2). Moreover, no significant differences were observed in the rates of biochemical pregnancy (64.50 vs. 66.80%), clinical pregnancy (55.24 vs. 56.83%), ectopic pregnancy (1.47 vs. 1.39%), early miscarriage (14.41 vs. 16.20%), late miscarriage (2.21 vs. 2.09%), singleton premature delivery (16.67 vs. 18.29%), and neonatal deformity (1.97 vs. 1.80%) between the immediate and delayed FET groups, respectively.

The COS protocol and type of embryo transferred are displayed in Table 3. The interval between oocyte retrieval and embryo recovery had no significant effect on pregnancy outcomes. The findings in Table 3 after PSM are consistent with the results from multivariate regression analysis adjusted for potential confounding factors, including maternal age, basal

TABLE 1 Comparison of baseline data between the groups before and after propensity matching.

	Before PSM			After PSM		
	Immediate FET group	Delayed FET group	*P*-value	Immediate FET group	Delayed FET group	*P*-value
No.	1,265	4,284		1,231	3,280	
Interval days	34 (30, 56)	83 (67, 111)	<0.001	30 (27, 33)	69 (59, 94)	<0.001
Age	30 (27, 33)	31 (28, 33)	<0.001	30 (27, 33)	30 (28, 33)	0.846
BMI	21.67 (19.73, 24.00)	21.48 (19.82, 23.81)	0.469	21.52 (19.83, 23.88)	21.60 (19.71, 23.81)	0.384
Infertility years	3 (1, 4)	3 (2, 5)	0.055	3 (2, 5)	3 (2, 5)	0.152
FSH	7.42 (6.28, 8.75)	7.20 (6.21, 8.40)	<0.001	7.31 (6.26, 8.65)	7.25 (6.26, 8.45)	0.061
LH	4.52 (3.29, 6.17)	4.43 (3.23, 6.14)	0.294	4.52 (3.31, 6.34)	4.54 (3.34, 6.35)	0.484
E2	45.00 (34.00, 58.00)	44.0 (34.00, 57.00)	0.204	44 (33, 57)	43 (33, 56)	0.259
P	0.59 (0.40, 0.89)	0.57 (0.38, 0.83)	0.008	0.60 (0.40, 0.88)	0.58 (0.39, 0.85)	0.210
AFC	18 (11, 26)	89 (12, 26)	0.136	19 (12, 27)	19 (13, 27)	0.075
AMH	4.29 (2.32, 6.76)	4.37 (2.59, 6.63)	0.132	4.49 (2.61, 7.03)	4.61 (2.74, 6.94)	0.095
FET day endometrial thickness	9.00 (8.50, 10.50)	9.50 (8.50, 10.50)	0.464	9.5 (8.5, 10.5)	9.5 (8.5, 10.5)	0.949
Types of infertility			0.246			0.748
Primary	666 (52.65%)	2176 (50.79%)		645 (52.40%)	1701 (51.86%)	
Secondary	599 (47.35%)	2108 (49.21%)		586 (47.60%)	1579 (48.14%)	
Fertilization method			0.049			0.733
IVF	1051 (83.08%)	3454(80.63%)		1022 (83.02%)	2709 (82.59%)	
ICSI	214(16.92%)	830 (19.37%)		209 (16.98%)	571 (17.41%)	
COS protocol			<0.001			0.001
Antagonist protocol	775 (59.17%)	2535 (61.26%)		774 (62.88%)	2093 (62.88%)	
Long protocol	262 (20.71%)	1063 (24.81%)		262 (21.28%)	753 (22.96%)	
Luteal phase improvement long protocol	55 (4.35%)	256 (5.98%)		55 (4.47%)	158 (4.82%)	
PPOS	131 (10.36%)	169 (3.94%)		98(7.96%)	154 (4.70%)	
Others	42 (3.32%)	261 (6.09%)		42(3.41%)	122 (3.72%)	
Endometrial preparation program			<0.001			0.863
HRT	1141 (90.20%)	3418 (79.79%)		1109 (90.09%)	2935 (89.48%)	
DRC	16 (1.26%)	324 (7.56%)		16 (1.30%)	53 (1.62%)	
OPC	37 (2.92%)	251 (5.86%)		37 (3.01%)	105 (3.20%)	
NC	71 (5.61%)	291 (6.79%)		69 (5.61%)	187 (5.70%)	
NET			0.091			0.496
1	436 (34.47%)	1588 (37.07%)		422 (34.28%)	1160 (35.37%)	
2	829 (65.53%)	2696 (65.53%)		809 (65.72%)	2120 (64.63%)	
HQEN			<0.001			0.449
0	363 (28.70%)	1521 (35.50%)		360 (29.24%)	1023 (31.19%)	
1	529 (41.82%)	1622 (37.80%)		515 (41.84%)	1330 (40.55%)	
2	373 (29.49%)	1141 (26.63%)		356 (28.92%)	927 (28.26%)	
ET			<0.001			<0.001
Cleaved-embryo	240 (18.97%)	458 (10.69)		212 (17.22%)	410 (12.50%)	
Blastocyst	1025 (81.03%)	3826 (89.31%)		1019 (82.78%)	2870 (87.50%)	

BMI, body mass index; HRT, hormone replacement cycle; DRC, down-regulation cycle; OPC, ovulation-promoting cycle; NC, natural cycle; NET, number of embryos transferred; HQEN, high-quality embryos number; ET, embryo transfer type.

FSH, basal P, fertilization method, COS protocol, FET protocol, number of top-quality embryos transferred, and type of embryo transferred.

3.3. Live birth outcomes by stratification analysis

To compare the live birth rates between the immediate and delayed FET groups in patients with different characteristics, we carried out further analysis by stratifying the patients according to the type of embryo transferred, number of embryos transferred, FET protocol, and criteria for good prognosis (AMH > 1.1 ng/ml and AFC > 8) (**Tables 4**, **5**). After PSM, live birth rates were comparable among the groups for each stratification ($p > 0.05$). Additionally, after adjusting for the COS protocol and type of embryo transferred, multivariate logistic analysis on the four stratified groups revealed no significant correlation between oocyte retrieval and embryo recovery with live birth rates (**Tables 4**, **5**).

4. Discussion

Patients undergoing embryo transplantation during fresh cycles have elevated estrogen and progesterone levels and unpredictable OHSS may occur. Although, there are some studies suggest that the administration of a rescue double GnRH antagonist dose at 1 day before hCG trigger may represent a safe alternative preventive strategy for preventing early OHSS without affecting the reproductive outcomes (12). However, more studies are pointing to embryo transfer in resuscitation cycles to improve clinical pregnancy rates and live birth rates and to reduce the occurrence of OHSS (13, 14). There are many randomized controlled trials comparing the advantages and disadvantages of conventional whole embryo freezing and fresh cycle transfer strategies (15). Although there is no favorable evidence to prove that whole embryo freezing has better advantages, clinicians are increasingly using this method (16). A recent review mentioned that in the long-term follow-up of newborns, it was found that the perinatal incidence rate and neonatal congenital malformation rate of patients

TABLE 2 Comparison of pregnancy outcomes between the immediate and delayed groups before and after PSM.

	Before PSM			After PSM		
	Immediate FET group	Delayed FET group	*P*-value	Immediate FET group	Delayed FET group	*P*-value
LBR	45.22% (572/1,265)	45.38% (1,944/4,284)	0.920	45.25% (557/1,231)	45.76% (1,501/3,280)	0.757
BPR	64.51% (816/1,265)	66.59% (2,849/4,284)	0.187	64.50% (794/1,231)	66.80% (2,191/3,280)	0.146
LPR	55.26% (699/1,265)	56.47% (2,419/4,284)	0.446	55.24% (680/1,231)	56.83% (1,864/3,280)	0.338
EPR	1.43% (10/699)	1.28% (31/2,419)	0.761	1.47% (10/680)	1.39% (26/1,864)	0.886
EMR	14.59% (102/699)	16.62% (402/2,419)	0.200	14.41% (98/680)	16.20% (302/1,864)	0.272
LMR	2.15% (15/699)	1.90% (46/2,419)	0.681	2.21% (15/680)	2.09% (39/1,864)	0.860
SPDR	16.27% (69/424)	17.01% (234/1,376)	0.725	16.67% (69/414)	18.29% (193/1,055)	0.464
NDR	1.92% (11/572)	1.49% (29/1,944)	0.469	1.97% (11/557)	1.80% (27/1,501)	0.792

LBR, live birth rate; BPR, biochemical pregnancy rate; PR, clinical pregnancy rate; EPR, ectopic pregnancy rate; EMR, early miscarriage rate; LMR, late miscarriage rate; SPDR, singleton premature delivery rate; NDR, neonatal deformity rate; PSM, propensity score matching.

TABLE 3 Multivariate logistic regression analysis of pregnancy outcomes for immediate FET and delayed FET.

	Before PSM		After PSM	
	Adjusted OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
LB	1.033 (0.907, 1.178)	0.622	1.016 (0.889, 1.160)	0.819
BP	0.972 (0.848, 1.115)	0.687	0.940 (0.818, 1.080)	0.383
CP	0.994 (0.872, 1.133)	0.926	0.971 (0.850, 1.110)	0.668
EP	0.930 (0.443, 1.955)	0.849	0.958 (0.456, 2.011)	0.910
EM	0.873 (0.684, 1.114)	0.274	0.854 (0.666, 1.095)	0.212
LM	1.037 (0.565, 1.901)	0.907	1.043 (0.569, 1.909)	0.892
SPD	0.993 (0.734, 1.343)	0.962	0.911 (0.673, 1.234)	0.547
NDR	1.187 (0.577, 2.442)	0.641	1.093 (0.536, 2.228)	0.806

LR, live birth; BP, biochemical pregnancy; CP, clinical pregnancy; EP, ectopic pregnancy; EM, early miscarriage; LM, late miscarriage; SPD; singleton premature delivery; NDR, neonatal deformity rate; PSM, propensity score matching.

TABLE 4 Stratified analysis comparing the live birth rate between the immediate FET and delayed FET groups.

	Before PSM			After PSM		
	Immediate FET group	Delayed FET group	*P*-value	Immediate FET group	Delayed FET group	*P*-value
ETT						
Cleaved	37.50% (90/240)	32.75% (150/458)	0.210	36.79% (78/212)	30.98% (127/410)	0.144
Blastocyst	47.02% (482/1,025)	46.89% (1,794/3,826)	0.939	47.01% (479/1,019)	47.87% (1,374/2,870)	0.634
NT						
1	34.86% (152/436)	34.13% (542/1,588)	0.776	34.60% (146/422)	33.71% (391/1,160)	0.741
2	50.66% (420/829)	52.00% (1,402/2,696)	0.500	50.80% (411/809)	52.36% (1,110/2,120)	0.451
EPP						
HRT	45.49% (519/1,141)	45.49% (1,555/3,418)	0.996	45.63% (506/1,109)	45.79% (1,344/2,935)	0.925
DRC	56.25% (9/16)	43.52% (141/324)	0.317	56.25% (9/16)	32.08% (17/53)	0.080
OC	45.95% (17/37)	45.82% (115/251)	0.988	45.95% (17/37)	48.57% (51/105)	0.783
NC	38.03% (27/71)	45.70% (133/291)	0.243	36.23% (25/69)	47.59% (89/187)	0.105
AMH > 1.1 and AFC > 8	47.30% (491/1,038)	46.59% (1,755/3,767)	0.683	47.29% (489/1,034)	47.36% (1,375/2,903)	0.968

ETT, embryo transfer type; NT, number of embryos transferred; EPP, endometrial preparation program; HRT, hormone replacement cycle; DRC, down-regulation cycle; OC, ovulation-promoting cycle; NC, natural cycle.

TABLE 5 Multivariate logistic regression analysis of live birth rates for immediate FET and delayed FET.

	Before PSM		After PSM	
	Adjusted OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
ETT				
Cleaved	1.321 (0.932, 1.873)	0.118	1.339 (0.941, 1.905)	0.105
Blastocyst	0.991 (0.861, 1.142)	0.905	0.969 (0.840, 1.119)	0.671
NT				
1	1.088 (0.863, 1.373)	0.476	1.078 (0.850, 1.366)	0.537
2	1.003 (0.854, 1.179)	0.968	0.981 (0.832, 1.156)	0.821
EPP				
HRT	1.179 (1.036, 1.342)	0.577	1.027 (0.893, 1.181)	0.712
DRC	2.091 (0.710, 6.155)	0.181	2.934 (0.817, 10.533)	0.099
OC	1.141 (0.543, 2.398)	0.727	0.956 (0.424, 2.157)	0.913
NC	0.645 (0.362, 1.147)	0.135	0.607 (0.335, 1.101)	0.101
AMH > 1.1 and AFC > 8	1.041 (0.904, 1.200)	0.575	1.014 (0.879, 1.170)	0.852

ETT, embryo transfer type; NT, number of embryos transferred; EPP, endometrial preparation program; HRT, hormone replacement cycle; DRC, down-regulation cycle; OC, ovulation-promoting cycle; NC, natural cycle.

undergoing FET were similar to those of patients undergoing fresh embryo transfer, and in some specific nervous systems, newborns who had frozen embryo pregnancy even had better cognitive function. Therefore, it is suggested that frozen embryo resuscitation transfer is a safe and reliable choice in clinical practice (17).

If whole embryo freezing is selected despite the risks of OHSS and increase of progesterone during COH, it is suggested that the preparation for resuscitation and transplantation can start immediately. However, patients may need to prolong the time before FET, owing to several reasons, such as endometrial abnormalities and hydrosalpinx. Of note, the optimal time to perform resuscitation and transfer after embryo freezing is yet unclear. Existing studies are primarily based on retrospective analysis, and high quality prospective randomized controlled studies are needed.

In previous retrospective studies, they found that immediate FET had higher live birth rates than delayed FET (18, 19). However, other studies concluded that immediate and delayed FET did not differ in clinical pregnancy and live birth rates (20–22). In those studies, the use of natural cycles in endometrial preparation protocols was excluded from the cycles due to ovulatory disturbances because of the impact of COH. Our study included natural cycle endometrial preparation, which means data is more comprehensive; however, the immediate FET group had fewer natural cycles than the delayed FET group, that might due to the patients in the immediate FET group have ovulation disorders and were not suitable for the natural cycle scheme. In the immediate FET group, the hormone replacement cycle was not affected by ovarian cysts. Additionally, hormone replacement can inhibit the growth of follicles, thereby reducing physiological cysts after egg retrieval. It is also the preferred option. We did not record the cycles that were canceled due to ovulation disorders. Our study concluded that immediate FET and delayed FET showed comparable live birth rates for different embryo types, embryo numbers, and different endometrial preparation protocols, which is consistent with the findings of other studies (1, 6). Therefore, there is no evidence to support the necessity for routine delay of at least one menstrual cycle after IVF/ICSI before FET.

However, patients with breast cancer or leukemia need fertility preservation before tumor therapy, embryo or egg freezing is their main choice for fertility preservation and they have to delayed FET. A retrospective study showed that 43% of breast cancer patients decided to preserve their fertility (7), and another study shows that whether performing COH before, or ART following anticancer treatment in young women with breast cancer does not seem to be associated with detrimental prognostic effect in terms of breast cancer recurrence, mortality or event-free survival (23). Therefore, the delayed resuscitation is also a good choice for tumor patients. Egg freezing is similar to embryo freezing, since 2013, egg cryopreservation has no longer been considered experimental by the American, clinical application faces some ethical challenges (24, 25), such as the effect on tumor recurrence of patients. For some patients, such as patients with endometrial cancer, there have been some studies on molecular level to find out whether the patients are suitable for fertility preservation and whether these treatments have an impact on the prognosis of patients (26). These studies are very meaningful for guiding our treatment.

Now the embryo freezing has been well development and utilized in IVF therapy or fertility preservation, and the frozen embryo is mainly carried out through open and closed carriers. Some scholars believe that the closed carrier for embryo freezing can reduce the risk of sample pollution, and the closed vitrification system may be the future development trend (27). However, although it is theoretically believed that there is a risk of sample contamination in the freezing of open carriers, there is no such report in the world at present, and some studies believe that whether open vitrification system or closed vitrification system have no significant impact on the freezing outcome of embryos (28). We have to admit that embryo freezing technology has helped countless infertile couples. In the future, whether for fertility preservation or for reducing OHSS risk, embryo freezing may be an increasing choice for clinicians. Therefore, perhaps we need more prospective research to explore which is safer, open, or closed vitrification system?

In conclusion, our study further confirmed patients undergoing immediate FET and delayed FET had comparable live birth rates and other pregnancy outcomes with a large amount of patients' population. However, we failed to record reasons from patients why they chose immediate FET or delayed FET. And therefore, further high quality, randomized, controlled trials are needed to obtain more accurate results and conclusions.

Data availability statement

The original contributions presented in this study are included in this article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Chengdu Jinjiang Hospital for Women's and Children's Health. The patients/participants provided their written informed consent to participate in this study.

Author contributions

QW and M-XC designed the study and were responsible for the conception of the study and for manuscript drafting. X-JW, LT, H-JY, X-YL, Z-HZ, X-JT, and Y-BD contributed to the manuscript drafting and statistical analysis. YL and MX contributed to the revision and final approval of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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TSC2/PKD1 contiguous deletion syndrome in a pregnant woman: A case report

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TSC2/PKD1 contiguous gene deletion syndrome is a disease caused by the deletions of the *TSC2* and *PKD1* genes. This is a rare contiguous genomic disease with clinical manifestations of tuberous sclerosis and polycystic kidney disease. To our knowledge, this case report is the first known case of TSC2/PKD1 contiguous gene deletions in a pregnant woman. The patient had multiple renal cysts, angiomyolipoma, hypomelanotic macules, shagreen patch, subependymal giant cell astrocytoma, multiple cortical tubers, and subependymal nodules. The patient underwent genetic testing. To exclude genetic defects in the fetus, prenatal fetal genetic testing was performed after obtaining the patient's consent. We found an increasing trend in the size of renal cysts and renal angiomyolipomas in patients with polycystic kidney with tuberous sclerosis during pregnancy. Through enhanced clinical monitoring of patients and prenatal genetic testing of the fetus, timely and effective clinical intervention for the mother may be achieved, thus obtaining the best possible outcome for both mother and fetus.

KEYWORDS

TSC2/PKD1 contiguous gene deletions, pregnant woman, renal angiomyolipoma, tuberous sclerosis, polycystic kidney disease, prenatal diagnosis

1. Introduction

The TSC2/PKD1 contiguous gene deletion syndrome (MIM# 600273), also known as polycystic kidney disease with tuberous sclerosis (PKDTS), is a contiguous gene deletion syndrome involving the chromosome 16p13.3 on *PKD1* and *TSC2* genes. PKDTS was first reported by Brook-Carter et al. (1). Mutations in the *TSC2* gene cause approximately 69% of all tuberous sclerosis complex (TSC) cases (2). TSC is a multisystemic genetic disorder with an incidence of 1 per 6,000–10,000 live births (3). Its clinical presentation is diverse and varies from person to person, with the main symptoms being malformations of multiple organ systems including the brain, skin, heart, kidneys, and lungs. Complications of TSC include epilepsy, learning difficulties, behavioral problems, and renal failure. Almost all patients with TSC have skin lesions such as depigmentation, facial angiofibromas, shagreen patch, brown fibrous plaques, and nail fibromas (4). The main clinical features of autosomal dominant polycystic kidney disease (ADPKD) are renal cysts, hepatic cysts, intracranial aneurysms, and heart valve lesions. A common complication of ADPKD is kidney stones, with end-stage renal disease being its most serious complication and the leading cause of mortality (5). The most common renal lesions are renal cysts and vascular smooth muscle lipomas in patients with TSC. A review of the relevant literature reveals that PKDTS is broadly an

overlay of symptoms of both TSC and polycystic kidney disease (PKD), in addition to the clinical renal manifestations. PKDTS exhibits more severe polycystic kidney growth with early renal impairment and early end-stage renal failure than TSC and ADPKD (6). To the best of our knowledge, there are no definitive clinical guidelines for the diagnosis and treatment of PKDTS to date.

2. Case report

A 21-year-old pregnant woman was followed-up by an obstetrician and gynecologist for bilateral cystic nephropathy and angiomyolipoma since the 13th week of pregnancy. Upon admission, the patient's urine protein was negative and renal function was poor [serum uric acid (UA), 572.74 μmol/L; serum creatinine (Scr), 302.98 μmol/L; urea, 22.48 mmol/L]. At 16 weeks, the urine protein was still negative but serum UA had increased (598.91 μmol/L); Scr (291.62 μmol/L); and urea (15.23 mmol/L) levels had decreased. At 23 weeks, the patient's Scr was increased (332.15 μmol/L), the UA was 594.80 μmol/L, and urea was 18.29 mmol/L. At 30 weeks, the patient had an increase in UA, Scr, and urea (UA, 644.39 μmol/L; Scr, 343.46 μmol/L; and urea, 29.8 mmol/L). The characteristics of both TSC and PKD suggest dual pathogenesis; thus, we clinically suspected PKDTS. During the patient's hospitalization, we carried out multi-disciplinary treatment and informed the patient of the possible risks during pregnancy, including complications such as end-stage renal failure, ruptured renal angiomyolipoma (RAML), and bleeding. The patient was also informed of the possible need for renal replacement therapy as the disease progressed and the possible need for super-selective intra-arterial embolization for RAML. We recommended termination of the pregnancy, but the patient refused termination and opted for conservative treatment. We conducted molecular analysis to delineate the underlying genetic etiology.

3. Physical examinations and findings

A soft tissue mass could be seen on the cranial top near the forehead (Figure 1A). Hypomelanotic macules were found in multiple locations throughout the body (Figures 1B, C). Multiple masses could be palpated on the left side of the abdomen (Figure 1C), and a shagreen patch was seen on the face and lower back (Figures 1B, D).

4. Ultrasonography findings

Bilateral polycystic renal lesions (maximum cystic diameter: right 30 × 27 mm, left 25 × 24 mm) and multiple solid masses (maximum solid diameter: right 26 × 25 mm, left 54 × 38 mm) were seen on ultrasonography during the 13th week of pregnancy. We conducted a follow-up observation for about 3 months from the patient's 13th week of pregnancy, in which MRI showed a trend of increasing size of renal cysts and RAML s. Bilateral polycystic renal lesions (maximum cystic diameter: right 42 × 32 mm, left 55 × 35 mm) and multiple solid masses (maximum solid diameter: right 41 × 29 mm, left 75 × 60 mm) were seen on ultrasonography at the 24th week of pregnancy. Bilateral polycystic renal lesions (maximum cystic diameter: right 42 × 32 mm, left 55 × 35 mm) and multiple solid masses (maximum solid diameter: right 46 × 32 mm, left 59 × 47 mm) were seen on ultrasonography at the 30th week of pregnancy. The multiple solid masses were thought to be RAML (Figure 2).

5. MRI findings

At 13 weeks of pregnancy, MRI of the head revealed a nodule in the anterior horn of the ventricle, multiple dysplasia dislocation-like nodules in the cortex and subcortex of both cerebral hemispheres, and subependymal giant cell astrocytoma (Figure 3A). At 16 weeks of pregnancy, multiple cysts in the liver and kidney and multiple angiomyolipoma were found upon MRI of the upper abdomen. A large angiomyolipoma in the left abdominal cavity with a maximum cross-sectional area of 75.4 × 180.4 mm (Figure 3B) was noted. At 30 weeks of pregnancy, another MRI revealed an increased angiomyolipoma with a maximum cross-sectional area of 96 × 182 mm.

6. Impression

The patient had bilateral multiple renal cysts, vascular smooth muscle lipoma, and subependymal giant cell astrocytoma (Figures 2, 3). To further clarify the diagnosis, the patient and her parents agreed to undergo whole exome sequencing and multiple ligation-dependent probe amplification (MLPA). To exclude genetic defects in the fetus, the patient also underwent prenatal fetal genetic testing after informed consent.

7. Results

Whole exome sequencing of the patient's parents did not reveal pathogenic or potentially pathogenic variants. Whole exome sequencing of the patient revealed a 37.14-kB deletion in the p13.3 region of chromosome 16, including a large heterozygous deletion of *TSC2* exons 11–42 and *PKD1* exons 33–46. No detectable deletions or mutations were found in the exons of the *TSC2* and *PKD1* genes of the fetus. The MLPA molecular study confirmed the presence of deletion of *TSC2* gene exons 10–42 and PKD1 gene exons 24–46 in the maternal samples and confirmed the presence of consecutive deletions in the *TSC2/PKD1* gene (Figure 4). There were no abnormalities in the fetal samples.

8. Discussion

Polycystic kidney disease with tuberous sclerosis is a rare, contiguous genomic disease. To date, no more than 90 cases have been identified worldwide; moreover, to our knowledge, no reports

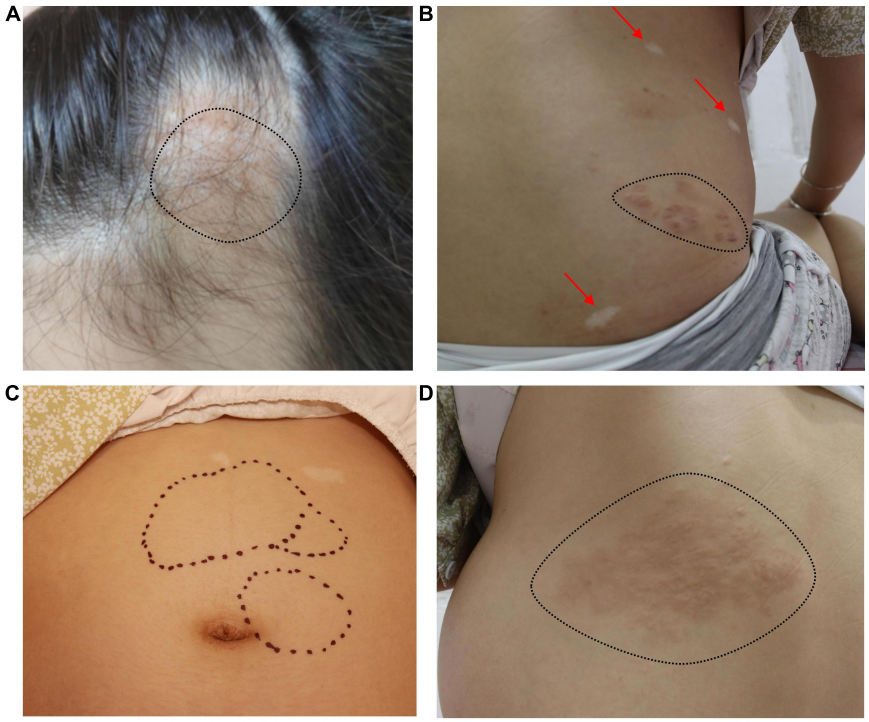

FIGURE 1
(A) A soft tissue mass can be seen on the left cranial top near the forehead (black dotted line). **(B)** Hypomelanotic macules in multiple locations throughout the body (red arrow). Shagreen patch (black dotted line). **(C)** Multiple masses can be palpated on the left side of the abdomen (black dotted line) and hypomelanotic macules can be seen in the upper abdomen. **(D)** Shagreen patch (black dotted line).

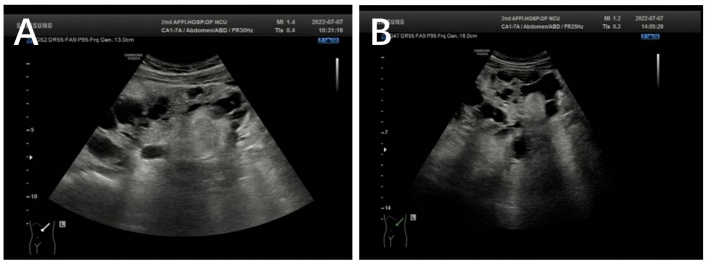

FIGURE 2
(A) Left polycystic renal lesions (maximum cystic diameter: 25 × 24 mm) and multiple solid masses (maximum solid diameter: 54 × 38 mm) were seen on ultrasonography at the 13th week of pregnancy. **(B)** Left polycystic renal lesions (maximum cystic diameter: 55 × 35 mm) and multiple solid masses (maximum solid diameter: 75 × 60 mm) were seen on ultrasonography at the 24th week of pregnancy.

of PKDTS in pregnant women have yet been reported. Herein, we have reported the first case of PKDTS in pregnancy presenting with hypomelanotic macules, shagreen patch, subependymal giant cell astrocytoma, angiomyolipomas, bilateral multiple renal cysts, and multiple hepatic cysts. The patient did not present with neuropsychiatric disorders such as epilepsy, and had no abnormalities upon neurological examination. The *PKD1* gene is located 60 bp downstream of *TSC2* in a tail-to-tail orientation. Approximately 2–3% patients with large *TSC2* genomic deletions have a collateral gene deletion, namely the TSC2/PKD1 contiguous gene syndrome (7).

Tuberous sclerosis complex is caused by inactivating mutations in the *TSC1* and *TSC2* genes, which encode the hamartin and tuberin proteins. *TSC1* and *TSC2* are located at positions 9q34 and 16p13.3, respectively (4). These two proteins are widely expressed in normal tissues and combine to form a complex that negatively regulates the mechanistic target of rapamycin (mTOR) complex 1 (mTORC1). mTORC1 activity is important in healthy cells for supporting cell proliferation and differentiation. Tuberin plays a role in the cell cycle (8). mTORC1 plays a role in regulating the bioenergetic and metabolic requirements of cell proliferation and differentiation (4, 9). mTORC1 regulates mTOR-S6K and GTPase activating proteins. mTORC1 plays a central role in the regulation of cell growth and division. When mutations in the *TSC1* and/or *TSC2* genes result in deletion of the complex, they cause aberrant activation of mTORC1, which in turn promotes lipid, nucleotide, and protein synthesis and inhibits cellular autophagy (10–12). Genetic test results can be used as an independent diagnostic

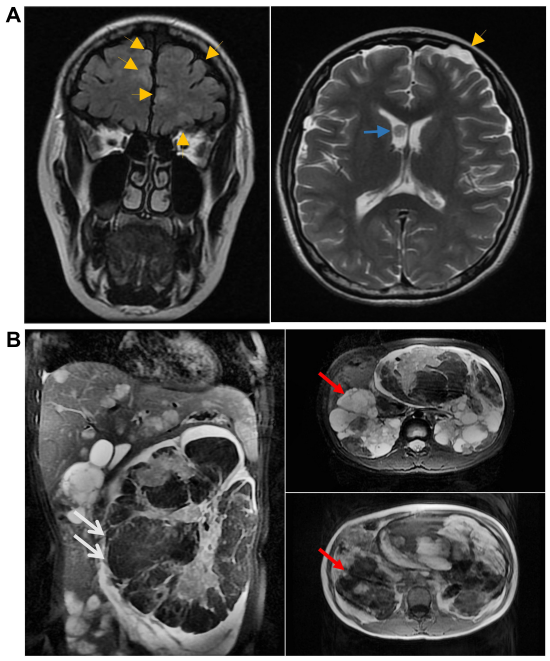

FIGURE 3
(A) Head MRI at 13 weeks of pregnancy showing nodules in the anterior horn of the ventricle (blue arrow). The MRI also showed multiple cortical tubercles (yellow arrow). Multiple lesions were also seen in the subcortical and periventricular areas. **(B)** At 16 weeks of pregnancy, multiple cysts in the liver and kidney and multiple angiomyolipoma were found upon MRI of the upper abdomen. White arrows point to renal vascular smooth muscle lipoma, red arrows point to renal cysts.

criterion for TSC, and approximately 70% TSC patients with mutations in the *TSC2* gene can be identified by genetic testing (13). The failure of routine genetic testing to identify pathogenic variants in the *TSC1* or *TSC2* genes does not exclude the diagnosis of TSC, and patients require long-term clinical monitoring and management for a definitive diagnosis. Patients with TSC can be diagnosed according to clinical and genetic diagnostic criteria (14). It is considered a definite diagnosis if one or two major and two minor features are present, and as suspected diagnosis, if one major or more than two minor features are present (13, 14). Our patient presented with five major features, namely hypomelanotic macules, shagreen patch, subependymal giant cell astrocytoma, multiple cortical tubercles, and angiomyolipomas, and two minor features, namely multiple renal cysts and non-renal hamartomas. Therefore, this patient was definitively diagnosed with TSC by clinical criteria and whole exome sequencing. Imaging by ultrasound and MRI and mutation analysis by prenatal genetic testing may be effective in diagnosing fetuses with TSC and is of high clinical value for early therapeutic intervention in fetuses, particularly in families wherein the child or parents are known to carry the mutation (3).

The most common cause of ADPKD is mutations in the *PKD1* and *PKD2* genes encoding polycystin 1 (PC1) and polycystin 2 (PC2), respectively. *PKD1* and *PKD2* are located at positions 16p13.3 and 4q21, respectively. *PKD1* mutations are responsible for approximately 80% of ADPKD (5). *PKD1* and *PKD2* genes encode for PC1 and PC2 that are localized in the primary cilia. They transmit information from the external environment into the cell. When the level of functional PC1 or PC2 falls below a critical threshold, the likelihood of cyst formation is greatly increased (15). Inhibition of cyst growth and maintenance of renal function are critical in the current treatment of patients with ADPKD. As the disease progresses, the patient's renal function may decline progressively, requiring enhanced clinical monitoring and management, and even renal replacement therapy if necessary. Ultrasound is now the preferred radiological diagnostic method for ADPKD. MRI and CT are also effective in detecting polycystic kidneys in patients, with greater sensitivity and specificity. Our patient had multiple renal cysts on both ultrasound and MRI with a tendency to increase in size (Figure 2). ADPKD is usually inherited, but new mutations without a family history occur in approximately 10% of cases. A more comprehensive clinical examination for cystic kidney disease is required in patients with a negative family history. In patients aged 16–40 years, the corresponding number of diagnostic cysts detected by MRI is defined as more than 10 to be diagnosed as ADPKD (16). The patient's diagnosis of PKD was confirmed by ultrasound, MRI and whole exome sequencing.

The *PKD1* and *TSC2* genes are both located on chromosome 16p13.3, and our patient was diagnosed with PKDTS after genetic testing revealed a contiguous gene fragment deletion at this locus. The renal manifestations of PKDTS are dominated by cysts, and the early onset, size, and number of cysts are the main features (17). There are currently no definitive treatment options for PKDTS for clinical use. Patients' kidneys often enter end-stage renal disease very early, mostly in childhood and adolescence and, to a lesser extent, in early adulthood; hence, many patients are already in end-stage renal disease at diagnosis and require renal replacement therapy (5, 18). Renal transplantation is likely to remain the preferred renal replacement therapy option at present, but reports of renal transplantation in patients with PKDTS are scarce, so immunosuppressive therapy may be one of the main treatment options in the future. The use of mTORC1 inhibitors such as sirolimus (also known as rapamycin) has become one of the important therapeutic measures for patients with TSC, but more clinical studies are needed to better understand the efficacy of these drugs in patients with PKDTS during pregnancy and determine whether they have any adverse effects on the fetus. From this case report, we found that the burden on the kidneys of patients with PKDTS during pregnancy may be further increased and renal function further decreased, allowing patients to rapidly progress to end-stage renal disease. Therefore, nephrotoxic drugs should be avoided in patients with PKDTS, especially if they are pregnant. Notably, total kidney volume is considered in PKD not only as a marker of disease progression but also as a test of treatment efficacy (19).

In this case, the large abdominal mass detected by ultrasound was diagnosed as RAML with blood supply from the left renal artery (Figure 2). RAML is a rare benign renal tumor. The tumor tissue in RAML comprises blood vessels, smooth muscle, and fat and is one of the main symptoms commonly seen in patients with typical TSC (4). Patients with RAML during pregnancy may experience fatal complications such as rupture and hypovolemic shock due to bleeding. It is now generally accepted that the main factor for RAML rupture is the elevated expression of estrogen and progesterone receptors in the tumor (20). Moreover, increased maternal circulation and abdominal pressure during pregnancy play an important

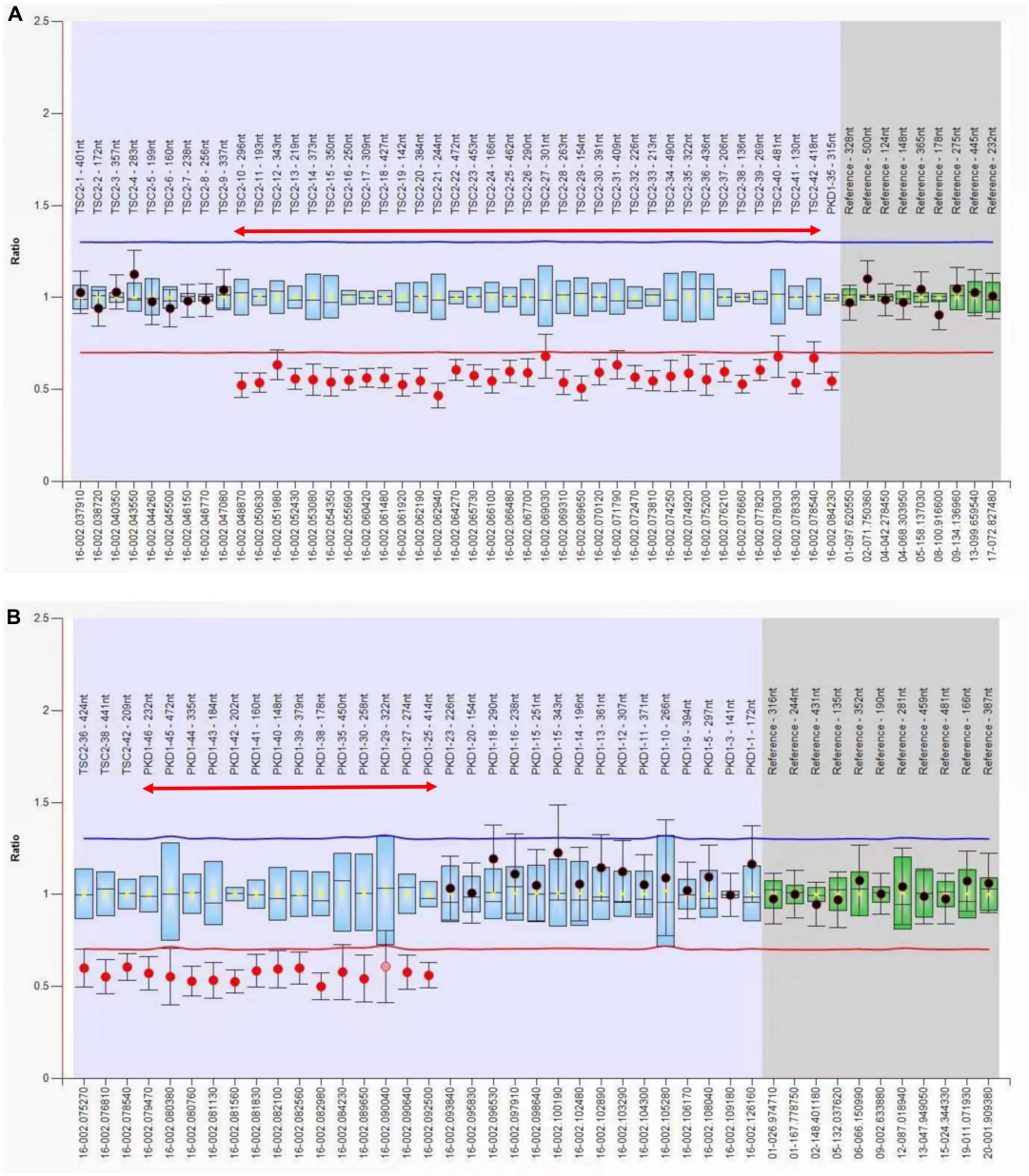

FIGURE 4
Multiple ligation-dependent probe amplification (MLPA) analysis of the *TSC2* and *PKD1* genes. **(A)** Exon 10–42 deletion of the *TSC2* gene (red two-way arrow). **(B)** Exon 24–46 deletion of the *PKD1* gene (red two-way arrow). The presence of TSC2/PKD1 contiguous gene deletions was confirmed by MPLA molecular studies on the patient.

role in the rupture of RAML (21). In conclusion, RAML in pregnant patients tend to grow and rupture as the pregnancy advances. By reviewing clinical guidelines and relevant reports, the treatment of pregnant patients with RAML should be individualized according to the patient's hemodynamic status and fetal maturity. Patients with RAML can be treated conservatively when they are hemodynamically stable and should be managed with intensive clinical monitoring and follow-up. When acute rupture or hemodynamic instability is present, either emergency surgery or selective arterial embolization (SAE) can be an option, but in cases of acute ruptured hemorrhage, SAE is often chosen (20, 22).

In summary, patients with PKDTS often have symptoms of both TSC and ADPKD, and the symptoms are typically more severe than those of TSC or ADPKD alone. In pregnant patients with PKDTS, the focus should be on the patient's renal manifestations, including multiple renal cysts and RAML. Enhanced clinical monitoring and follow-up with an individualized treatment plan is essential to improve the patient's renal function and obtain the desired pregnancy outcome. We have found through clinical monitoring and follow-up that patients with PKDTS during pregnancy have enlarged renal cysts and RAML. To date, no progressive deterioration in renal function has occurred during conservative treatment of pregnant women with

PKDTS. Confirmation of whether PKDTS patients have accelerated progression to end-stage renal disease during pregnancy and whether fatal complications such as ruptured RAML bleeding may ultimately lead to adverse pregnancy outcomes requires more clinical data. Our patient's hemodynamic status and fetal maturity were fully assessed and a conservative treatment plan was decided upon after prenatal genetic testing ruled out fetal genetic defects to achieve the best possible outcome for both mother and fetus. We monitored the patient until 31 weeks of gestation. The fetal growth and development were good, and the patient's vital signs were stable. No abnormal results in genetic testing of fetal samples.

9. Conclusion

There are currently no diagnostic criteria and clinical guidelines for PKDTS. There is a considerable lack of experience in the management of this disease, which is both a clinical challenge and risk for accurate management of such patients. To the best of our knowledge, this is the first case report to describe the clinical presentation and characteristics of PKDTS in a pregnant patient. We have focused on the main clinical features of patients with PKDTS in pregnancy, particularly the renal manifestations. We found an increasing trend in the size of renal cysts and RAMLs in patients with PKDTS during pregnancy. Through enhanced clinical monitoring of patients and prenatal genetic testing of the fetus, timely and effective clinical intervention for the mother may be achieved, thus obtaining the best possible outcome for both the mother and fetus.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

XH designed this study. SH and YX conducted the data collection and analysis. SH and KX drafted the manuscript which was checked by LZ and XH. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Fallopian tubal histogenesis of ovarian endometriosis—A study of folate receptor-alpha expression

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Background: Ovary is a common organ site involved by endometriosis. We previously found that fallopian tube may contribute to the histogenesis of ovarian endometriosis. The finding was novel and requires further studies. We addressed this issue by examining a differentially expressed gene folate receptor alpha (*FOLR1*) and its protein (FRA) in this study.

Results: A total of 144 tissue samples were studied. These included 32-paired tubal-endometrial-ovarian endometriosis samples (n =96), 18 samples of ovarian endometriosis without corresponding fallopian tube or endometrium, and 30 ovarian tissue samples with ovarian surface epithelia but without endometriosis. Multiple comparisons among groups of ovarian endometriosis, normal fallopian tube and benign endometrium were performed. *FOLR1* was highly expressed in the epithelia of fallopian tube and ovarian endometriosis, with paired endometrial samples showing a significantly lower level of expression. Similar differential studies for FRA protein were performed through Western blot and immunohistochemistry (IHC). The expression of folate receptor alpha at both mRNA and protein levels in the tissues (fallopian tube or ovarian endometriosis vs. the endometrium) were significantly different (p <0.001). All ovarian surface mesothelial epithelia showed negative expression of FRA by IHC.

Conclusion: The results further support that the fallopian tube may contribute to the development of ovarian endometriosis. Understanding the tubal contribution to ovarian endometriosis should ultimately contribute to ongoing investigative efforts aimed at identifying alternative ways to prevent and treat endometriosis. High level of FRA expression in the fallopian tube and endometriosis might be considered as potential tissue sites for targeted therapy.

KEYWORDS

ovarian endometriosis, fallopian tube, tubal origin of endometriosis, biomarkers of endometriosis, FRA

Background

Endometriosis, one of the most common gynecologic disorders, is a bewildering and debilitating disease that affects millions of women in the world. Endometriosis, defined as the presence of endometrial glands and stroma in extrauterine sites. Endometriosis can be divided into several types based on the anatomic locations. Peritoneal endometriosis is typically located in the pelvic surface of peritoneum or on the ovary. Ovarian endometrioma or endometriotic cyst describes the lesion in the ovary when endometriosis forming a cystic structure. Deep infiltrating endometriosis mostly represents a solid mass comprised of endometriotic tissue with local adipose and fibromuscular tissue within pelvic structures between the rectum and the vagina. The ovarian endometriosis is the most common. Clinical symptoms of endometriosis are numerous and may include dysmenorrhea, dyspareunia, menorrhagia, dyschezia, pelvic and abdominal pain, infertility, and symptoms from gastrointestinal tract.

Pathogenesis of endometriosis remains unclear. The most popular hypothesis is the retrograde menstruation theory, which was originally proposed by Sampson (1) in 1927. Although it is popular, it remains controversial for a variety of reasons. Retrograde menstruation is thought to occur in up to 90% of reproductive age women (2), but only 6–10% of these women have clinical symptoms related to endometriosis (2). Additionally, the theory falls flat to explain the presence of endometriosis outside the peritoneal cavity. The coelomic metaplasia hypothesis proposed by Meyer (3) states that endometriosis may originate from mesothelial cells through a metaplastic process, although how the metaplasia happens is ambiguous. Initial endometriosis, which our group described in 2005 (4), represents a spectrum of the earliest morphologically identifiable changes of endometriosis within the ovary. At that time, we assumed that the morphologic transitions from ovarian surface epithelia (OSE) or ovarian epithelial inclusions (OEI) to initial endometriosis lesions represented a metaplastic process from OSE as discussed in 2005 (4). However, nowadays we realize that the majority of epithelia attached on the ovarian surface and OEIs are derived from the fallopian tubal epithelia. This was found when we studied the origin of OEIs and the low-grade serous carcinoma from the fallopian tube epithelia vs. ovarian surface mesothelia in 2011 (5). We are far from understanding the molecular mechanism of pathogenesis of endometriosis. More recently, Koninckx et al. proposed a novel wholistic genetic/epigenetic theory of the pathogenesis of endometriosis (6). Based on this theory, the set of genetic and epigenetic incidents transmitted at birth could explain many aspects of the endometriosis including hereditary, predisposition, immunology, and placentation. To develop cystic ovarian endometriosis, Koninckx et al. believed that a series of additional transmissible genetic/epigenetic incidents are required to occur in a cellular level, including stem or stem-like cells (6). Interestingly, this genetic/epigenetic theory is compatible with the most, if not all, the observations made on endometriosis including subtle microscopic lesions we described as "initial endometriosis" (4).

The fallopian tube, particularly tubal fimbriated end, has recently caught great attention in studies of adnexal pathology and the origin of ovarian serous cancers. This is mainly because most investigators understand that the majority ovarian serous cancers are actually derived from the fallopian tube, not the ovary as was historically believed (7–9). Similarly, in our study of the cellular origins of OEIs, we found that the majority OEIs (also called endosalpingiosis) display a phenotype suggesting they are derived directly from tubal epithelial cells rather than from ovarian surface mesothelial cells (5, 10). Indeed, microscopic examination of resected ovarian and tubal tissues frequently shows various stages of the following sequence: tubal epithelia mostly from fimbriated end are frequently adherent on the ovarian surface, accompanied by invagination of morphologically similar epithelia onto ovarian surface, then invaginate into the cortex forming OEIs or endosalpingiosis (5, 7). From those findings, we believe that the formation of ovarian initial endometriosis occurs through the conversion of tubal-like epithelia within the ovarian cortex into endometriosis-like tissue, rather than the conversion of ovarian surface mesothelial cells into endometriosis-like tissue. The potential for tubal mucosal epithelia to convert into endometriosis-like tissue is also supported by the phenomenon of "endometrialization" of the proximal end of the fallopian tube after a tubal ligation procedure (11, 12). Based in part on the aforementioned morphological findings, we first proposed the hypothesis that tubal epithelia contribute to the formation of ovarian endometriosis in 2014 and 2015 (10, 13).

The biomarker FRA (folate receptor alpha), the product of the *FOLR1* gene, has previously been shown to be differentially expressed between the fallopian tube and the endometrium (14, 15). We have also noticed that *FOLR1* was differentially expressed in our previous gene expression array study (10). We did not study *FOLR1* and FRA in detail at that time mainly because the antibody against FRA was not available. It is interesting to know if FRA is expressed in the ovarian endometriosis. Understanding the level of FRA expression in ovarian endometriosis may help understanding cellular lineage of endometriosis and potential target therapy. In this study, we aimed to investigate FRA expression in paired tissue samples of human ovarian endometriosis and normal tubal and endometrial tissue samples to further examine the cellular origin of the ovarian endometriosis.

Results

Multiple differentially expressed genes identified in the fallopian tube and the endometrium

A total of 4,114 and 3,451 genes were identified from the tubal and endometrial samples, respectively. The gene expression profiles of the paired tubal and endometrial samples were compared using a Volcano Plot. The threshold for a differentially expressed genes between the tubal and endometrium was set at ≥2.0-fold level. There were 1796 genes identified with more than two-fold differential expressions between the two tissue samples. All differentially expressed genes were further scrutinized and some representative genes were listed and studied previously (10).

Compared with the endometrial samples, the tubes showed a total of 911 upregulated genes. These included genes that were ≥50-fold (n=8), ≥20-fold (n=28), and ≥2-fold (n=875) comparatively upregulated in the fallopian tube. There were no genes with more than

Abbreviations: FOLR1, Folate receptor alpha gene; FRA, Folate receptor alpha protein; GPI, Glycosylphosphatidylinositol; IHC, Immunohistochemistry; OEI, Ovarian epithelial inclusions; OSE, Ovarian surface epithelia.

50-fold upregulation in the endometrial sample relative to the tubal tissue. *FOLR1* (gene bank accessing number NM_016729), which was not listed previously and represented another most differentially expressed gene, showed 55.6-fold increment in the tubal sample compared to the gene expression level at the endometrium ($p = 0.012831689$).

FOLR1 gene detection in the fallopian tube, endometrium, and ovarian endometriosis by real-time PCR

Real-time PCR was used to examine the level of *FOLR1* expression in 15 paired fresh tissue samples (total of 45 samples, including 15 each of the fallopian tube, endometrium, and ovarian endometriosis specimens). *FOLR1* was highly expressed in the fallopian tube compared with the paired endometrium, with fold increment ranging from 9 to 35 (average fold change = 18.25, $p < 0.001$). Similarly, the *FOLR1* gene was highly expressed in the samples of ovarian endometriosis compared with the paired endometrium, with fold increment ranging from 3 to 20 (average fold change = 11.18, $p < 0.001$). However, although there were some expression level differences between the fallopian tube and ovarian endometriosis samples, the difference did not reach statistical significance ($p = 0.125$). The gene expression level from the 15-paired cases, together with pooled data, is shown in Figure 1. Among these 15-paired samples, 12 pairs (from 1 to 12) were in the proliferative phase and 3 (13 to 15) were in secretory phase of the menstrual cycle (Figure 1). The gene expression levels of the two menstrual phases were compared and no significant differences ($p > 0.10$) in the organ-matched samples were found (data not shown).

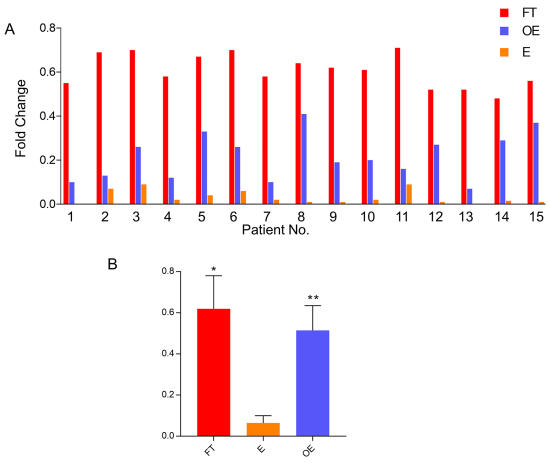

FIGURE 1
FOLR1 gene expression level in paired tubal, endometrial, and ovarian endometriosis samples. Comparisons of mRNA expression levels in the fallopian tube, the endometrium, and the foci of endometriosis by quantitative PCR in 15 individual paired cases **(A)** and the pooled data **(B)**. *FOLR1* levels in fallopian tube (FT) and ovarian endometriosis (OE) were similar, but both were higher than the observed *FOLR1* level in the endometrium (E) [*$p = 0.176$ (FT vs. OE) and **$p = 0.0018$ (E vs. OE)]. FT: fallopian tube; E: endometrium; OE: ovarian endometriosis.

FRA protein expression in the fallopian tube, endometrium, and ovarian endometriosis by Western blot

FRA protein level examination for the 15-paired samples was performed by Western blot. FRA protein expression was significantly higher in the fallopian tube and ovarian endometriosis samples than that in the endometrium, with an average fold of increment of 12.36 ($p = 0.001$) and 9.72 ($p = 0.022$), respectively. As expected, there were no significant differences between the fallopian tube and ovarian endometriosis samples ($p > 0.10$). These results were compatible with the findings from real-time PCR validation, indicating that *FOLR1* or FRA do not change significantly at the transcriptional and post-transcriptional levels. Western blots of 6 representative paired samples and pooled data of FRA protein expression are shown in Figure 2.

FRA expression in tissue sections of the fallopian tube, endometrium, ovarian endometriosis and ovarian surface epithelia by immunohistochemistry

A total of 114 tissue samples were analyzed with anti-FRA antibodies by IHC. These tissue sections represented the 32 paired tissue sections of the fallopian tube, endometrium, and ovarian endometriosis and additional 18 samples of ovarian endometriosis. All 32 normal fallopian tube samples showed strong and diffuse FRA expression as expected. The observed staining pattern was mostly intense cytoplasmic, with some extending to the luminal border of tubal epithelia. The staining score ranged from 185 to 300 with an average of 228 for the tubes. There were minor differences in FRA staining between tubal secretory and ciliated cells, with the latter showing stronger staining intensity on the luminal border than the former. However, these staining differences were not statistically significant (data not shown). In addition, compared with the menstrual phases, proliferative versus secretory, we did not observe a significant difference of FRA staining pattern or intensity in the tubal and endometrial epithelia.

FRA expression in 32-paired and 18-non-paired ovarian endometriosis samples were also highly positive, with IHC scores ranging from 94 to 300 with an average of 160. The majority of the epithelial cells were positive for FRA stain. Among all the 50 cases with endometriosis, there were 38 cases showing staining intensity score 3, 8 score 2, and 4 score 1. There were no significant differences in FRA expression scores between the paired and non-paired endometriosis. FRA expression was negative in all Pax-8 negative OSE as well as in all Pax-8 negative OEIs (surface epithelium derived ovarian inclusions) from the 30 ovarian sections without endometriosis examined (data not shown). These findings suggest that mesothelial cell derived structures are negative for FRA, while tubal cell generated endosalpingiosis is positive for FRA.

In the eutopic endometrial tissue sections, FRA expression was largely absent, with IHC scores that ranged from 0 to 84 (average 42). Typically, only focal areas of endometrial glands were positive with weak intensity at the luminal apical borders of the epithelial cells. Among the 32 endometrial samples, 26 were in proliferative and 6 in secretory phase; Occasional weak staining was seen in samples of both

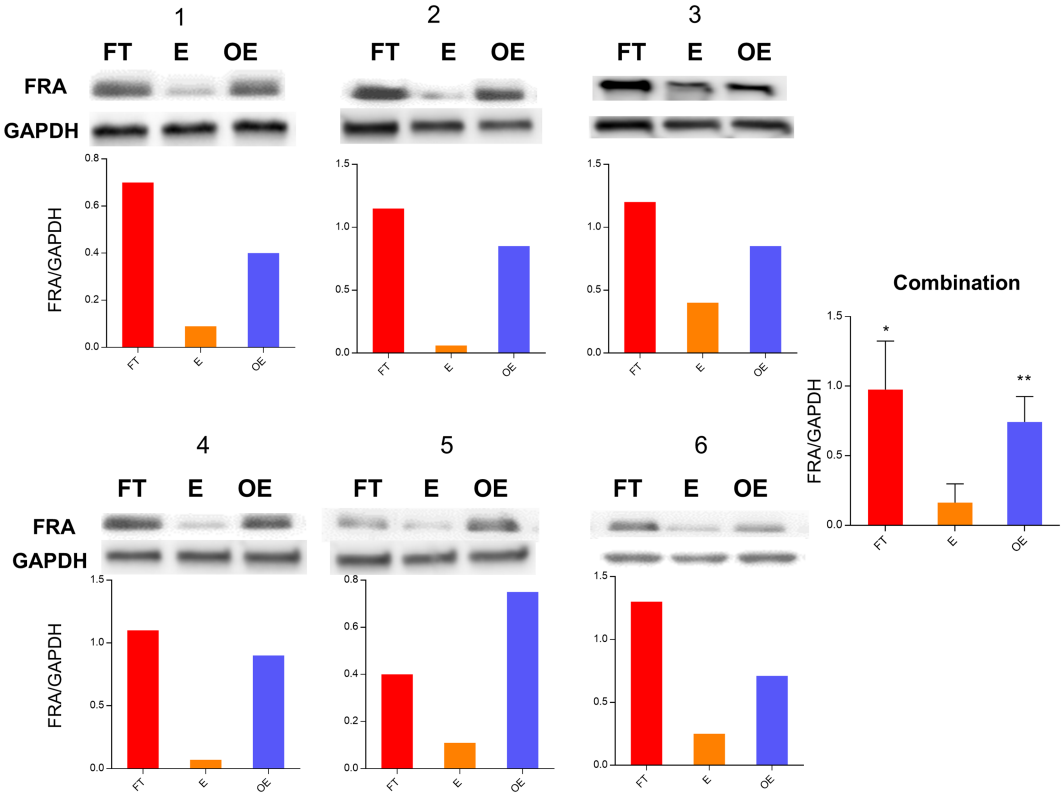

FIGURE 2
FRA protein expression level in paired tubal, endometrial, and ovarian endometriosis samples. Comparisons of protein expression levels of the fallopian tube (FT), the endometrium (E), and the foci of ovarian endometriosis (OE) by Western blot analyses. The figure shows the data from 6 paired samples with number labeled on the top of each panel. The FRA and GAPDH control bands are presented on the top, while the ratio of FRA/GAPDH is illustrated in the bottom for all 6 paired samples. The pooled data are summarized on the right side of the figure. Similar to the gene expression levels, FRA protein was significantly higher in the fallopian tube and the ovarian endometriosis samples than that in the endometrium [*p=0.103 (FT vs. OE) and **p=0.001 (E vs. OE)].

proliferative and secretory endometria with no clearly discernible differences in staining frequency or intensity.

Compared with the endometrium, both tubal and ovarian endometriosis samples showed significantly higher FRA IHC scores with $p=0.00051$ and $p=0.00174$, respectively. Interestingly, the difference of FRA expression between the fallopian tube and ovarian endometriosis also reached a statistical significance ($p=0.05$) with the expression was higher in the tubal samples. The data is summarized in Figure 3, and representative IHC pictures of the FRA expression are presented in Figure 4. Stromal cells and blood vessels were all negative for FRA expression in all samples examined.

Discussion

Endometriosis remains a serious problem for reproductive-age women. The etiology and pathogenesis of endometriosis have been the subject of numerous investigations, although answers remain unclear (4, 10, 13, 16, 17). Starting in 2005, our group described the earliest morphologic changes of endometriosis in the ovary, which we named "initial endometriosis" (4). That study, which was largely based on light microscopic observations, provided morphologic evidence that retrograde menstruation may not explain how the initial endometriosis forms either on the ovarian surface or within the ovarian cortex. That study indirectly provided some supportive evidence that ovarian endometriosis may be derived from the fallopian tube. Morphologically, foci of initial endometriosis show gradual transitions from a typical example of endosalpingiosis (tubal type epithelia with ovarian stroma but without appreciable vascular changes) to areas with a classic appearance of endometriosis (endometrioid epithelia associated with endometrioid stromal cells and increased density of microcapillary vessels) (4). Such morphologic transitions suggest that the foci of ovarian endometriosis started from the site within the ovary, rather than being deposited from retrograde menstrual endometrial tissue.

From what being presented, we questioned the hypothesis of retrograde menstruation and proposed that tubal cells are a plausible tissue source for ovarian endometriosis (18). Recent studies of the tubal origin of ovarian serous carcinomas (5, 19, 20) have highlighted many previously under-recognized biologic and physiologic properties of the fallopian tube. *In vivo*, the fallopian tube is in close spatial proximity to the ovary (5, 8, 19, 21, 22), tubal epithelia are easily sloughed from the tubal mucosae (19, 23), and the majority of the endosalpingiosis or OEIs are originated from the fallopian tube. Moreover, endosalpingiosis or OEIs are readily observed in ovarian cortex in many ovarian endometriosis (5). It is likely therefore that the relationship between endosalpingiosis and initial endometriosis represents a trans-differentiation process from the former to the

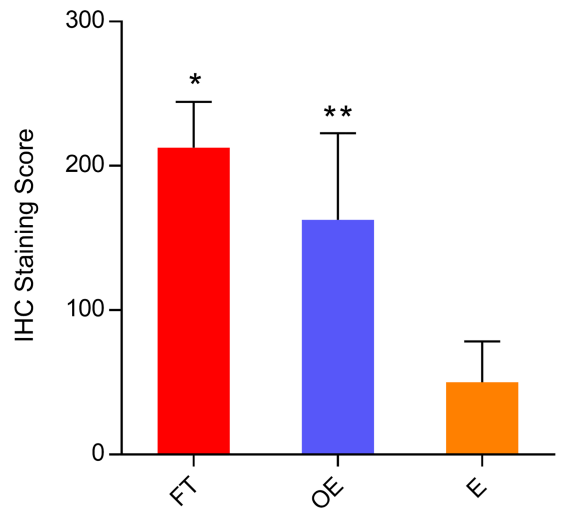

FIGURE 3
FRA protein expression levels by immunohistochemical scores in paired tubal, endometrial, and ovarian endometriosis samples. Comparisons of FRA protein expression levels in the fallopian tube, the endometrium, and the lesions of endometriosis by IHC scores in 32 paired and 18 endometriosis cases (pooled data): FRA expression was significantly higher in the fallopian tube and ovarian endometriosis than that in the endometrium [*p=0.00051 (FT vs. E) and **p=0.00174 (OE vs. E)]. The FRA expression level in the fallopian tube was also higher than the FRA level in the ovarian endometriosis, with the difference just attaining statistical significance (p=0.05). FT: fallopian tube; E: endometrium; OE: ovarian endometriosis.

latter, although detailed underlying mechanisms remain to be clarified.

The current study used another biomarker, FRA (folate receptor alpha), which is highly differentially expressed in the tubal and endometrial tissues. FRA, the product of the *FOLR1* gene, is a glycosylphosphatidylinositol (GPI)-anchored protein that binds plasma folate (5-methyltetrahydrofolate) and transports it into the cell *via* endocytosis (24). Folate is essential for 1-carbon metabolism, transferring single carbon units in reactions involving purine and pyrimidine synthesis, DNA repair, and methylation of various biomolecules including DNA, proteins, phospholipids, and neurotransmitters (25, 26). Folate deficiency has been linked with dysregulation of these processes and, in some cases, is associated with an increased risk of developing cancer, including serous type epithelial ovarian tumors (15, 27–29). However, no specific data have been reported on the expression of FRA in normal fallopian tube relative to ovarian endometriosis or to evaluate what role, if any, FRA may play in the development of ovarian endometriosis.

Since *FOLR1* and its protein product FRA were highly differentially expressed in the tube and the eutopic endometrium, we assessed the tubal and endometrial samples with the FRA antibody on 50 patients with ovarian endometriosis (32 paired and 18 non-paired). We found that all fallopian tubal epithelia expressed FRA with strong intensity. In contrast, FRA expression in the eutopic endometrium was either negative or weakly and focally positive. Therefore, FRA may be considered a tubal-specific biomarker when compared to that of the endometrium. In our previous whole-genome expression microarray analysis (10), we identified that *FMO3* was also more specific for the fallopian tube and endometriosis, but not for the endometrium, although the relationship between *FOLR1* and *FMO3* is unclear. Among 18 non-matched ovarian endometriosis, 15 cases had strong or moderately membrane and intracellular staining. Only 3 (18%) samples showed a weak staining on apical luminal borders. Furthermore, Pax-8 positive epithelial cells on the ovarian surface and OEIs showed moderate to strong FRA expression, while Pax-8 negative epithelial cells on the ovarian surface and OEIs were negative for FRA, which is supportive of tubal origin of ovarian endometriosis. Morphologically ovarian surface like epithelia and OEIs have two origins with one originated from classic OSE (Pax-8 negative) and the other from fallopian tube (Pax-8 positive) (5). Original OSE negative for FRA expression suggests that ovarian endometriosis is unlikely derived from OSE through a metaplastic process. In short, our findings from this study further support the hypothesis that ovarian endometriosis is at least partially derived from the fallopian tube. In our previous study (10), we estimated that approximately 60% of ovarian endometriosis may be originated from the tubal epithelia based on the *FMO3* and *DMBT1* expression study. This is correlated to the number of OEIs from fallopian tube (5). The design of the current study does not allow such quantification; however, the findings bolster the argument that the majority of ovarian endometriosis comes from the fallopian tube rather than from retrograde menstrual endometrium.

Two essential conditions must be met to consider the fallopian tube to be the origin of endometriosis when present in the ovary. First, tubal cells must enter the ovary. Frequent detachment of tubal epithelia from fimbriated ends makes this possible. Tubal cells are easily retrieved by flushing the fallopian tube (19, 23). The process is further facilitated by juxtaposed spatial relationship between tubal fimbria and ovarian surface (21, 30). The rupture of ovarian surface caused by ovulation (31, 32) provides a favorable condition for tubal epithelia to implant onto the ovary then get into ovarian cortex. The latter process, from a morphologic perspective, has long been described as endosalpingiosis (33–35). Second, endosalpingiosis or OEI must transform itself into endometriosis. The latter probably occurs through trans-differentiation, a process that is commonly seen in the Müllerian system (36), although detailed molecular mechanism remains elucidated. Initial endometriosis within the ovary describes the morphologic transition of OEIs, with some glands of OEIs displaying the earliest morphologic changes of endometriosis in only half of the gland (4). Trans-differentiation from tubal epithelia is the most likely explanation for this morphologic observation, especially since transitional areas from normal-appearing tubal epithelia to endometrial like tissue are commonly present (19, 37). In summary, a graphic abstract is illustrated in Figure 5 to aid understanding to process of endometriosis formation within the ovary. We may conclude, therefore, endometriotic or endometrioid epithelial cells are likely originated from the tubal epithelia. However, by all means, so far we do not have solid scientific evidence that ovarian endometriosis are either derived from or not coming from the endometrial cells from retrograde menstruation. Further studies in this regard are needed.

FRA came into focus recently as an anticancer target after the successful development of drugs targeting intracellular folate metabolism and protein location in the cytoplasm or on cell membrane. Binding to FRA is one of several methods by which folate is taken up by cells; however, the receptor FRA is an attractive anticancer drug target owing to the overexpression of FRA in various types of cancers, including ovarian epithelial carcinomas (38).

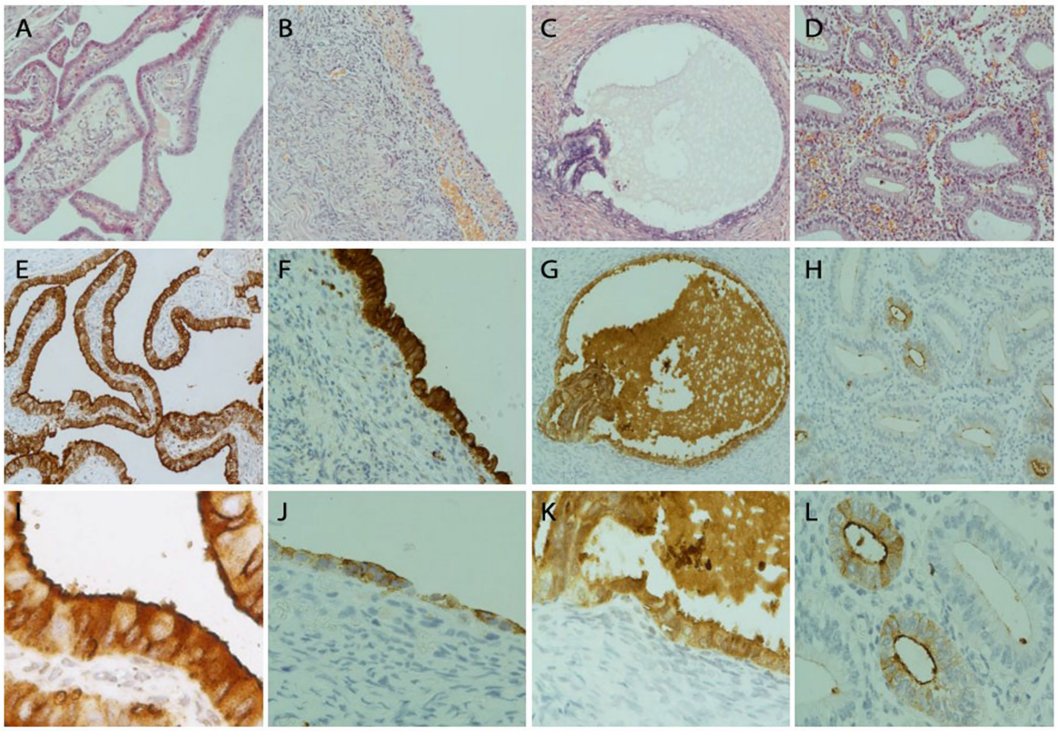

FIGURE 4
FRA protein detection by immunohistochemistry in fallopian tubal, ovarian endometriosis, endosalpingiosis, and endometrial tissue sections. Representative IHC stains of FRA showed strong diffuse FRA expression in the fallopian tube (left column, **A,E,I**), ovarian endometriosis (left mid, **B,F,J**), and ovarian endosalpingiosis (right mid, **C,G,K**), but only focal and weak staining was observed in a case of proliferative endometrium (right column, **D,H,L**). FRA staining was mainly cytoplasmic. Membranous stains can be seen when the staining intensity is moderate **(E)** and luminal stains are more prominent when stains become weak or moderate and magnified **(I,L)**. Histologic H&E sections are arranged on the top panel. FRA expression in the fallopian tube is illustrated in panels **(E,I)**, where **I** (200x) represents the magnification of part of **E** (100x). FRA expression in 2 ovarian endometriosis cases, with one showing high (**F**, 100x) and one showing intermediate (**J**, 100x) FRA expression levels. FRA expression in endosalpingiosis is shown in panels **G** (100x) and **K** (200x). Focal glandular with a mainly luminal pattern is seen in an endometrial sample (**H**, 100x; **L**, 200x).

Treatment of endometriosis is typically either hormonal therapy or surgical excisions. Interestingly to note that expression of FRA was detected in 94.4% of endometriosis samples from 18 patients in a targeted intraoperative imaging study (39) Considering FRA is highly expressed in ovarian endometriosis, targeted therapy against FRA deserves more exploratory studies. In addition, the finding of FRA highly expressed in the fallopian tubal epithelial cells may suggest an alternative way to prevent tubal derived ovarian serous carcinomas as well as possible ovarian endometriosis. The novel findings of this study have provided further evidence supporting our previously proffered theory of the tubal contributions for ovarian endometriosis. Although our findings remain preliminary, they might provide an alternative way of thinking about the etiology and pathogenesis of endometriosis that might aid the prevention and early treatment of ovarian endometriosis.

Conclusion

The folate receptor alpha gene *FOLR1* identified through a differential gene array analysis is highly expressed in ovarian endometriosis and the fallopian tube epithelial cells. However, the expression level of *FOLR1* and its protein FRA is significantly lower in the endometrium and ovarian surface epithelia. These novel findings further support that the fallopian tube is likely the cellular source of ovarian endometriosis. Understanding the tubal contribution to ovarian endometriosis should ultimately contribute to ongoing investigative efforts aimed at identifying alternative ways to prevent and treat endometriosis.

Materials and methods

Tissue specimens

Fresh tissue samples, including normal fallopian tube fimbria and corresponding endometrium were obtained from pathology specimens within 30 min of their surgical resections as described previously (10). A total of 114 formalin fixed and paraffin-embedded tissues were obtained from Henan Provincial People's Hospital, Zhengzhou, China. Among 114 samples, 96 were derived from 32 patients with each patient contributing 1 ovarian endometriosis, 1 endometrium and 1 fallopian tube sample and 18 patients each contributing one sample of ovarian endometriosis only. In all 50 ovarian endometriosis samples studied, 42 were endometriomas or endometriotic cyst, 6 were endometriosis foci

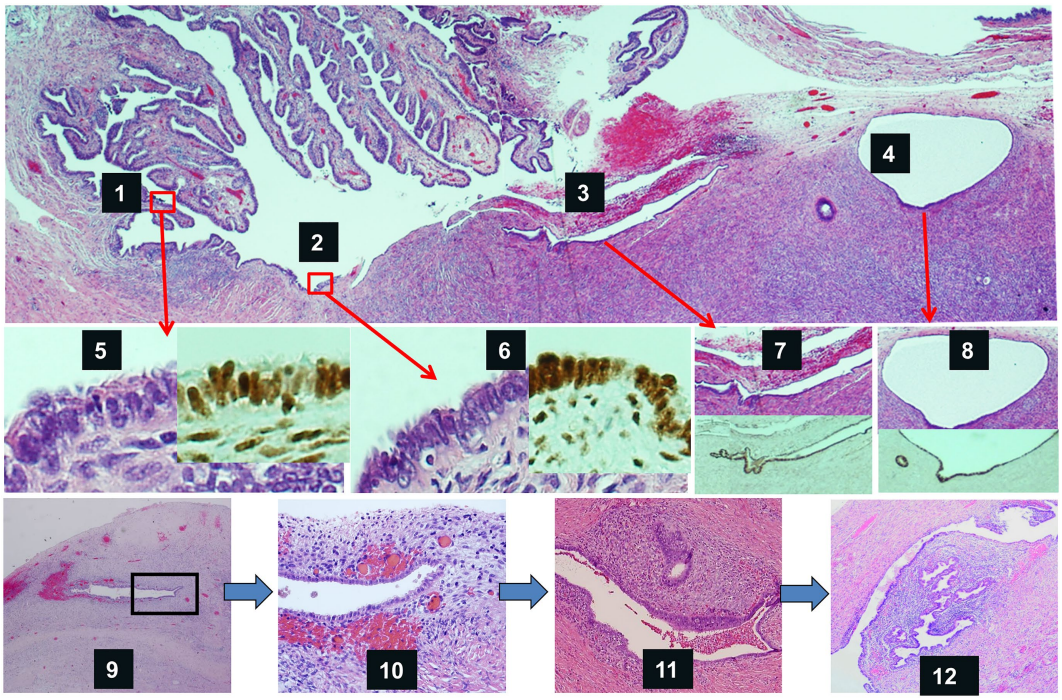

FIGURE 5
Graphic abstract of the process of ovarian endometriosis developed from tubal epithelia. Top panel shows tubal fimbriated end adherent to the ovary. Tubal epithelia (1) spreads to the ovarian surface (2), invaginates onto ovary through adhesions (3), and forms endosalpingiosis or ovarian epithelial inclusions (4). Mid-panel shows magnified morphology as well as Pax-8 IHC stains of tubal epithelia (5), epithelia on the ovarian surface (6), epithelia within ovarian surface adhesions (7), and endosalpingiosis (8). Low-panel shows endometriosis develops from initial endometriosis within the ovary. Endosalpingiosis like gland start to have endometrioid stroma and increased micro capillary vessels (9), more apparent in a magnified view (10), endometriosis without evidence of bleeding (11), and typical appearance of endometriosis in the ovary (12).

within the ovarian parenchyma without cyst or mass formation, and 2 were endometriosis on the ovarian surface. Among the 32-paired samples, 15 had fresh tissue samples available in addition to the formalin-fixed paraffin sections. The remaining 18 patients had ovarian endometriosis from formalin-fixed tissues only. In addition, 30 ovarian tissue sections containing Pax-8 negative OSE were included for FRA IHC stains. The representativeness of the ovarian endometriosis foci (n = 15) for quantitative PCR and Western blot analysis were confirmed by examining the corresponding frozen sections under the microscope. Foci of endometriosis, comprised predominantly of epithelial and stromal tissue, were manually dissected by removing non-endometriotic ovarian tissue.

Patients' age ranged from 22 to 50 years (mean 34.5). The reasons for total hysterectomy and bilateral salpingo-oophorectomy were prolonged ovarian endometriosis or benign gynecologic disorders including leiomyomata and benign ovarian cysts. No patient received hormonal treatment in 12 months prior to the surgery. Pathologic diagnosis of ovarian endometriosis were confirmed by two pathologists (RZ and WZ). Endometriosis, tubal mucosa and endometrium with both glandular epithelia and stroma were confirmed to be present under microscope. Determination of menstrual cycle phase (proliferative or secretory) for the 15-paired cases with both fresh and formalin-fixed tissues was made by examining hysterectomy specimens microscopically. Patients with a history of malignancy or tubal ligation were excluded. The research protocol was approved by the institutional review board of Henan Provincial People's Hospital at Zhengzhou, Henan Province, China.

Microarray and data analysis

In order to identify tissue-specific biomarkers, we compared the gene expression profiles between the fallopian tube and the endometrium from patients without evidence of endometriosis by gene array analysis (10). The endometriosis samples were manually dissected after microscopic confirmation. Three pairs of fresh samples of the fallopian tube fimbria and corresponding endometrial specimens from 3 reproductive-age women were collected and sent to Kang Chen Bio-Tech (Shanghai, China) to perform whole-genome expression microarray analysis using the Agilent array platform (Agilent Technologies, Palo Alto, CA, United States), as previously described (10). To limit potential confounding interference, endometrial samples showing tubal metaplasia were excluded from the study. Total RNA from three pairs of hand-dissected epithelial samples under routine microscopy were prepared by using TRIzol (Invitrogen, Gaithersburg, MD, United States), further quantified by the NanoDrop 1,000, and RNA integrity was confirmed by standard denaturing agarose gel electrophoresis. The Human Gene Expression Array was manufactured by Agilent with a cluster of 41,000 genes and their corresponding transcripts and they are present in those public domain annotations.

Array hybridization and sample labeling were done according to the protocol provided by Agilent Technologies (Palo Alto, CA, United States) as described elsewhere (40). Median normalization and subsequent data processing were analyzed by using the GeneSpring GX software (version 11). Highly differentially expressed genes were selected for further analysis after normalization of the raw data. Differentially expressed genes were identified through fold change filtering. Hierarchical clustering analyses were carried out also by using the Agilent GeneSpring GX software. Analysis of gene ontology and pathway studies were performed by using the standard enrichment computation method. A gene was considered to be highly differentially expressed between the tubal fimbria and the endometrium if there was a ≥2-fold difference in its expression level between or among the tissue categories, and the *p*-value less than 0.05 was considered significant.

Validation of microarray data by real-time PCR

Multiple differentially expressed genes were verified and published previously (10). In the current study, we examined another highly differentiated gene *FOLR1* to further test the possibility of tubal contribution of ovarian endometriosis.

To verify the gene expression data obtained from the microarray, real-time PCR was performed on *FOLR1* gene, using total RNAs from 15-paired tubal fimbria and corresponding endometrial samples. Among the 15-paired specimens, 12 were in the proliferative phase and the remaining 3 were secretory endometria, which were determined based on endometrial morphology. The *FOLR1* gene was selected in this study because it was 56-fold more expressed in the tubal samples compared with the endometria by gene array study. In addition, the antibody against corresponding protein FRA suitable for immunohistochemistry on FFPE tissue samples was recently available. FRA represents one of the glycosylphosphatidylinositol (GPI)-anchored receptors that bind plasma folate (5-methyltetrahydrofolate) with high affinity ($K_D \sim 1$ nM), and transport it into the cell *via* endocytosis (24). Together with folate receptor beta, gamma, and delta, FRA lies in tandem on chromosome 11q13 (24), although much less is known about those other family members. FRA has been shown to be expressed in normal placenta, fallopian tube, kidney, lung, breast and choroid plexus (15).

GAPDH was used as internal control. Real-time PCR was done on 15-paired ovarian endometriosis, tubal, and endometrial samples. Primers were designed using Primer 3 software and the sequences were as follows.

FOLR1: F5′-CCCGAGGACAAGTTGCATGA-3′
R5′-TCCACAGTGGTTCCAGTTGAATCTA-3′
GAPDH: F5′-AGCAAGAGCACAAAGAGGAAGAG-3′
R5′-TCTACATGGCAACTGTGAGGAG-3′

The protocol of real-time PCR was described elsewhere (41). Data analysis was executed with StepOnePlus™ Real-Time PCR System software, version 2.2 (Applied Biosystem, Hercules, CA). Comparative Ct method (ΔΔCt) was used to obtain relative quantification of the gene expression levels. Quantification of the amplified products were normalized against GAPDH (ΔCt). Paired two-tailed t test was used to compare relative mRNA expression levels of the studied tissue samples. Statistical significance was defined as a p value < 0.05.

Western blot analysis

Monoclonal antibodies against FRA, 1:500 dilution, was obtained from Dr. Daniel O'Shannessy (Department of Diagnostics Development, Morphotek Inc., Exton, Pennsylvania). All samples mentioned above were subsequently evaluated for protein expression by Western blot, as described elsewhere (42). GAPDH antibody (Abcam, United States) was used as the loading control.

Immunohistochemistry

Among many differentially expressed genes, folate receptor-alpha (FRA) was one of the most highly differentially expressed. Immunohistochemistry (IHC) stain with antibodies against FRA, mentioned above, was performed as described previously (15). Normal fallopian tube samples served as a positive control since it is ubiquitously expressed in tubal epithelial cells (15). Negative controls were carried out by replacing primary antibodies with class-matched mouse and rabbit IgGs on parallel sections. The subcellular staining localization for FRA was both cytoplasmic and membranous. Rabbit monoclonal anti-PAX8 antibodies were purchased from Abcam (Boston, United States). Positive and negative controls for PAX8 IHC were the same as anti-RFA.

IHC stained slides were evaluated and scored by counting at least 500 epithelial cells independently by two pathologists (RZ and WZ). Positive FRA expression was defined as discrete membrane and intracellular (mainly cytoplasmic) brown color with at least weak intensity within the epithelial cells. IHC scoring criteria have been described previously (15). Briefly, the staining intensity was scored as 0 if negative, 1 if with weak intensity, 2 with moderate intensity, and 3 if strong and intensely stained. Tissues with score 3 staining showed an intensely positive pattern that was readily identifiable at low magnification (4× objective) under light microscope. Sometimes a complete circumferential staining was seen. Score 2 was only visible at the 10× objective level and it was typically localized to the apical luminal or occasionally to the lateral cell border. In contrast, score 1 stain was generally limited to the luminal borders, or required 20× to 40× objectives to confirm. Percentage of the positive cells at each intensity was calculated for each case. The final score was summarized by multiplying the staining intensity and the percentage of the positive cells for each sample (10).

Statistical analysis

Multiple comparisons among categories of ovarian endometriosis, benign fallopian tube and endometrial tissues were carried out by using Mann–Whitney U test and SPSS statistical software program version 13.0 (SPSS, Chicago, IL, United States) when appropriate. p value < 0.05 was considered statistically significant.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The research protocol was approved by the institutional review board of Henan Provincial People's Hospital at Zhengzhou, Henan Province, China. The current study does not require patient consent to take part in this study since only residual tissues in the paraffin blocks were used after clinical diagnosis and management were completed.

Author contributions

WZ, YiW, and YuW conceived the study design and experiments. QL, YuW, RZ, ZY, JZ, and YaW carried out experiments and data analysis. QL and WL performed data analysis. QL, YiW, WZ, RZ, and OF wrote the manuscript. YiW, YuW, RZ, and WL provided the majority of cases with relevant clinical information. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Effectiveness of small-angle episiotomy on incisional laceration rate, suturing time, and incisional bleeding in primigravida: A meta-analysis

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Objective: To investigate the effect of small-angle lateral perineal incision on postoperative perineal rehabilitation in primiparous women.

Method: The Cochrane Library, PubMed, Embase, CINAHL, CNKI, WanFang, VIP, and the Chinese Biomedical Literature Database were searched for randomized controlled trials (RCTs) on the effect of small-angle episiotomy on postoperative maternal perineal wound rehabilitation in puerpera until April 3, 2022. Two researchers independently performed literature screening, data extraction and evaluation of risk of bias in the included literature, and statistical analysis of the data was performed using RevMan 5.4 and Stata 12.0 software.

Result: A total of 25 RCTs were included, with a total sample of 6,366 cases. Meta-analysis results showed that the use of small-angle episiotomy reduced incisional tearing [*OR*=0.32, 95% *CI* (0.26, 0.39)], shortened incisional suture time [*MD*=−4.58min, 95% *CI* (−6.02, −3.14)] and reduced incisional bleeding [*MD*=−19.08mL, 95% *CI* (−19.53, −18.63)], with statistically significant differences (all $p<0.05$). There was no significant difference in the rate of severe laceration between the two groups [*OR*=2.32, 95% *CI* (0.70, 7.70), $p>0.05$].

Conclusion: The use of a small-angle episiotomy during vaginal delivery can reduce the incision tear rate without increasing the incidence of severe perineal laceration, while shortening the incisional suturing time and reducing incisional bleeding. It can be used clinically according to birth canal conditions of the maternal, the intrauterine condition of the fetus and maternal needs.

Systematic Review Registration: PROSPERO International Prospective Register of Systematic Reviews [CRD42022369698]; [https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=369698].

KEYWORDS

episiotomy, angle, primigravida, meta-analysis, randomized controlled trial

1. Introduction

A lateral episiotomy is to cut the perineum at 45° (60°~70° for a highly dilated perineum) from the midline of the posterior perineal coalition to one side, with a length of 4~5 cm (1). In 1920 De Lee first recommended episiotomy as a way to protect the pelvic floor from lacerations and to protect the fetal head from trauma during vaginal delivery (2, 3). For many years, episiotomy was considered to help prevent more extensive vaginal tears during labor and to heal better than natural lacerations (2, 4). Results from two European centres have shown that episiotomy can significantly reduce the number of genital lacerations, especially in the case of vaginal deliveries in advanced maternal age, higher parity occipitoposterior presentation and fetal macrosomia (5). It has been suggested that for every 6° increase in perineal incision angle from the midline, the risk of third-degree perineal tears is relatively reduced by 50% (6). It has also been suggested that narrower incision angles that are too close to the anal sphincter may increase the risk of obstetric anal sphincter injuries (OASIS) (7). When the incision angle is less than 15° or greater than 60°, the risk of severe perineal tears is nine times higher than that of 15°~60° (8). The laceration condition is closely related to the healing, pain, and infection of the lateral incision wound. Based on clinical experience, some domestic researchers have proposed a small-angle (15°~30°) lateral perineal incision, which reduces both the angle of the lateral incision and the length of the incision to a certain extent, and uses the recovery of the perineal wound after delivery as an important indication to assess the effectiveness of this procedure. In this study, we collected randomized controlled studies on small-angle episiotomy from home and abroad, aiming to evaluate the clinical effects of small-angle episiotomy through an evidence-based approach and provide an evidence-based basis for the selection of the angle of lateral incision during vaginal delivery.

2. Materials and methods

2.1. Literature search strategy

This review followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines (9). The protocol was registered in the PROSPERO database (CRD42022369698) (10).

PubMed, Embase, The Cochrane Library, CINAHL, China National Knowledge Infrastructure (CNKI), Wanfang, VIP and China Biomedical Literature Database were searched for RCTs on the effect of small-angle episiotomy on maternal prognosis, using the combination of subject headings and free words. English search terms include: episiotomy, perineotomy, angle, mediolateral, lateral, etc. The retrieval time limit was from the establishment of the database to April 2022.

2.2. Inclusion and exclusion criteria

2.2.1. Inclusion criteria

(I) Study type: Randomized controlled trials (RCTs). (II) Participants: parturients who underwent lateral episiotomy. (III) Interventions: the intervention group used modified small-angle (15°~30°) episiotomy; The control group received conventional episiotomy (45°, 60°~70° when perineal height distension). (IV) Outcomes: including at least one of the following outcome indicators: perineal laceration during labor, incision suture time, incision bleeding.

2.2.2. Exclusion criteria

(I) Literatures not in Chinese and English; (II) Literatures for which the full text cannot be obtained or repeated publications; (III) Literatures with incomplete data or without reporting the above outcome measures.

2.3. Literature screening and data extraction

Two researchers (Zhang JY and Xiao L) independently conducted literature screening and data extraction according to the inclusion and exclusion criteria, and then checked each other. In case of any disagreement, it was resolved through discussion or consulting a third researcher to decide. The data extraction mainly included: (I) basic information of the included studies (e.g., title, first author, publication year, etc.); (II) baseline information of the study subjects (e.g., sample size, age, gestational age, etc.); (III) detailed information of the interventions (e.g., lateral incision angle, incision length, suture method, etc.); (IV) outcome indicators of interest and outcome measurement data; (V) key elements of risk of bias evaluation.

2.4. Literature quality evaluation

Two researchers independently evaluated the risk of bias of the included RCTS according to the RCT risk assessment tool (11) recommended by the Cochrane Systematic Reviews Manual 5.1.0, and cross-checked the results.

2.5. Statistical analysis

Meta-analysis was performed using RevMan5.4 software. Odds ratio (*OR*) was used as the effect analysis statistic for dichotomous variables. Mean difference (*MD*) was used as effect analysis statistic for continuous variables, and 95% confidence intervals (95%*CI*) was given for estimation of all outcome indicators. The χ^2 test and I^2 were used to quantitatively determine the magnitude of heterogeneity. If $p > 0.10$ and $I^2 < 50\%$, it indicates that the heterogeneity among the results of each study is acceptable, and meta-analysis was performed using a fixed-effects model (test level $\alpha = 0.05$); If $p \leq 0.10$ and $I^2 \geq 50\%$ indicated that there was significant statistical heterogeneity among the results of each study, meta-analysis was performed using a random-effects model. Subgroup analysis was selected to find sources of heterogeneity. Egger linear regression in Stata 12.0 software was used to test for publication bias, and the presence of publication bias was suggested if $p < 0.05$.

3. Results

3.1. Literature screening process and results

A preliminary search was conducted to obtain 11,060 literature articles. After layer-by-layer screening, 25 RCTs (12–36) were finally

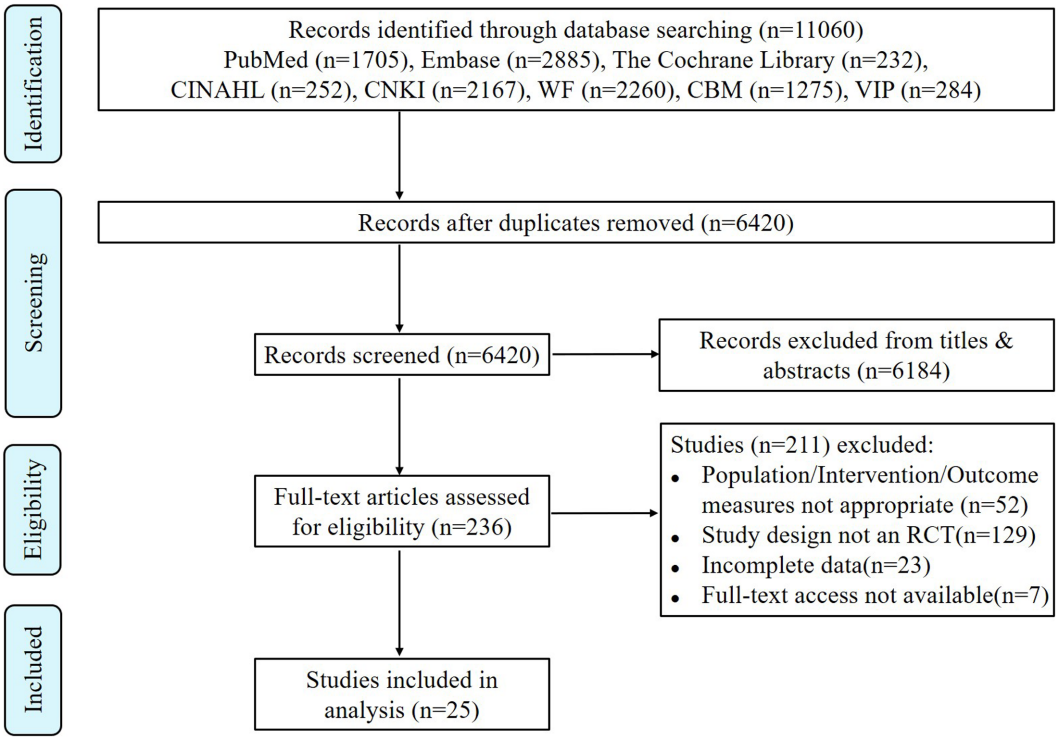

FIGURE 1
Flow chart of literature screening.

included, with a total of 6,366 parturients. The literature screening process and results were shown in Figure 1.

3.2. Basic characteristics of included studies

The basic characteristics of the included studies were shown in Table 1. The sample sizes ranged from 50 to 678 cases. The lateral incision angles in the Chinese literature were the comparison between small-angles (15° ~ 30°) and conventional angles (45°), and the lateral incision angles in the English literature were the comparison between 40° and 60° at crowning, i.e., 22.5° versus 45° at non-crowning (37, 38). Eleven studies (13, 15, 19, 25–28, 30, 33–35) had the same incision length in both groups, two studies (20, 29) did not describe the incision length accurately, one (22) stated only the incision length in the test group, and the remaining 13 studies had an incision length approximately 1–3 cm shorter in the test group than in the control group.

3.3. Risk of bias evaluation results

The evaluation results of the risk of bias of the included studies were shown in Figure 2. According to the risk of bias evaluation criteria recommended by the Cochrane Assist Network, of the 25 included studies, four studies used the randomized number table method (18, 30, 33, 35), two studies (12, 26) used a computerized randomization system for grouping, and the remaining papers only mentioned "randomized" but did not describe the specific randomization method. None of the included studies had missing data, but most of them did not give information about the allocation concealment method and the use of blinded methods.

3.4. Meta-analysis results

3.4.1. Incidence of perineal laceration

Nine RCTs (18, 19, 21, 22, 25, 27, 28, 33, 36) reported the effect of lateral incision angle on the rate of lateral incision laceration, including a total of 2,470 primiparas. Fixed-effect model analysis showed a statistically significant difference in the incisional laceration rate in the small-angle perineal lateralization group compared with the conventional perineal lateralization group [$OR = 0.32$, 95% CI (0.26, 0.39), $p < 0.00001$], as shown in Figure 3A. Three RCTs (12, 17, 18) reported the effect of lateralization angle on the incidence of severe laceration (perineal third- and fourth-degree laceration) that included a total of 830 parturients. As two of these studies had an incidence of 0 in both groups, a test for heterogeneity could not be performed. EL-Din et al. (12) showed that there was no statistically significant difference between the test and control groups in the rate of severe laceration [$OR = 2.32$, 95% CI (0.70, 7.70), $p > 0.05$], as shown in Figure 3B.

3.4.2. Duration of incisional suturing

A total of 12 RCTs (13, 14, 16, 22–24, 26, 30, 31, 34–36) with 2,972 primiparas were included. Meta-analysis of the random-effects model showed that the incision suture time was significantly lower in the

TABLE 1 Basic characteristics of the included studies.

Rank	Study	Sample		Intervention		Suturing method	Age/year	Gestational week/W	Fetal weight	Outcome
		T	C	T	C					
1	El-Din A S 2014	165	165	40°, 20 ~ 55 mm	60°，28 ~ 56 mm	The episiotomy wound was repaired by continuous simple suturing using 2/0 slowly absorbable polyglactin 910 stitches. The skin was repaired using the subcuticular technique.	T:20.87 ± 3.01 C:21.29 ± 3.38	–	T:2951.85 ± 239.98 C:2985.89 ± 266.87	①
2	Chen YH 2009	341	337	15 ~ 30°, 2 ~ 3 cm	45°(60 ~ 70°when the perineum is highly inflated)，4 ~ 5 cm	Vaginal and perineal wounds were closed with interrupted sutures using absorbable threads. The skin was closed using a continuous intradermal suture technique.	23 ~ 35	–	<3,500 g	②
3	Gu XH 2009	300	300	15 ~ 30°, 2 ~ 3 cm	45°(60 ~ 70°when the perineum is highly inflated), 4 ~ 5 cm	Vaginal and perineal wounds were closed with interrupted sutures using absorbable threads. The skin was closed using a continuous intradermal suture technique.	–	–	<3,500 g	②
4	Huang Y 2014	200	200	25 ~ 30°, 3 ~ 4 cm	45°，4 ~ 5 cm	The episiotomy wound was closed layer by layer using absorbable sutures.	20 ~ 32	38 ~ 42	3,250 ± 150 g	①
5	Jin AY 2016	50	50	30°, 1.7 ~ 3 cm	45°，4 ~ 5 cm	The vaginal mucosal and muscular wounds were closed with interrupted absorbable sutures. The skin was closed with embedding sutures.	T:26.660 ± 3.121 C:26.180 ± 3.102	–	<4,000 g	①③
6	Li HX 2010	44	60	15 ~ 30°	45°	The vaginal wound was closed with continuous sutures using absorbable sutures. The subcutaneous tissues and muscles were closed with interrupted sutures. The skin was closed with continuous intradermal suturing.	21 ~ 32	–	3,000 ~ 4,000 g	③
7	Li LJ 2011	100	100	20 ~ 30°, 3 cm	45°，4 cm	The vaginal wound was closed with continuous sutures using absorbable sutures. The subcutaneous tissues and muscles were closed with interrupted sutures. The skin was closed with continuous mattress stitching.	–	–	–	①
8	Ling CL 2013	50	50	20 ~ 30°, 2 ~ 3 cm	45°(60°when the perineum is highly inflated)，4 ~ 5 cm	The vaginal mucosa was closed with continuous sutures using absorbable thread. The perineal musculature was closed with interrupted sutures. The skin was closed with continuous intracutaneous sutures.	Average age: 25	–	–	②③
9	Ni XL 2009	120	80	25 ~ 30°, 3 cm	45°, 3 cm	The vaginal mucosa was closed with continuous locking sutures with absorbable thread, the perineal muscle was closed intermittently and the skin was closed with interrupted mattress sutures with silk thread.	Average age: 24	–	≥3,500 g	①
10	Song QX 2007	110	116	25 ~ 30°, 3 cm	45°, 3 cm	–	21 ~ 32	–	≥3,400 g	①
11	Wu SR 2014	150	150	30°	45°	–	T:21 ~ 36 C:22 ~ 37	T:34 ~ 43 C:34 ~ 42	–	③

(Continued)

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TABLE 1 (Continued)

Rank	Study	Sample		Intervention		Suturing method	Age/year	Gestational week/W	Fetal weight	Outcome
		T	C	T	C					
12	Xie FY 2016	50	50	30°, 3 ~ 5 cm	45°, 3 ~ 5 cm	The vaginal mucosa, muscle layer and skin were closed with absorbable sutures.	T:26.09 ± 3.45 C:25.95 ± 3.27	T:37 ~ 41 C:37 ~ 40	–	②③
13	Yin YM 2015	58	58	25 ~ 30°, 2 ~ 3 cm	45°, 4 ~ 5 cm	The episiotomy wound was sutured with the same suture material and method.	T:27.6 ± 2.9 C:27.2 ± 2.4	T:39.3 ± 1.2 C:39.2 ± 1.5	–	②③
14	Zhang C 2012	200	200	20 ~ 30°, 3 cm	45°, 4 cm	The vagina was closed with continuous sutures using absorbable thread. The subcutaneous tissues and muscles were closed with interrupted sutures. The skin was closed with intradermal sutures.	20 ~ 34	–	2,000 ~ 4,000 g	③
15	Zhang XY 2015	149	149	25 ~ 30°, 3 ~ 4 cm	45°, 3 ~ 4 cm	The vaginal mucosa layer was closed with interrupted sutures, the perineal muscle layer was closed with interrupted mattress sutures with No.0 suture, and the skin was closed with continuous intracutaneous sutures.	26.42 ± 5.94	39.78 ± 2.16	2,600 ~ 4,100 g	①
16	Zhang XD 2017	45	45	30°, 3 ~ 5 cm	45°, 3 ~ 5 cm	The episiotomy wound was closed sequentially using absorbable sutures.	T:29.43 ± 1.44 C:29.68 ± 1.36	–	–	②③
17	Zhang XM 2015	170	170	30°, 3 ~ 5 cm	45°, 3 ~ 5 cm	The vaginal mucosa, muscle layer and skin were closed in sequence using absorbable sutures.	24 ~ 33	37 ~ 41	3,500 ~ 4,000 g	②③
18	Zhou YX 2013	86	83	30°, 3 ~ 4 cm	45°, 4 ~ 5 cm	The skin was cosmetically closed subcutaneously using absorbable threads.	T:25.33 ± 4.58 C:25.97 ± 3.16	T:37.17 ± 2.17 C:38.21 ± 2.52	T:3510 ± 750 g C:3310 ± 620 g	①②③
19	Cai F 2021	44	44	30°, 3 ~ 5 cm	45°, 3 ~ 5 cm	The episiotomy wound was closed with absorbable surgical sutures。	T:30.25 ± 2.25 C:30.26 ± 2.26	T:40.25 ± 0.13 C:40.27 ± 0.15	T:3520 ± 430 C:3530 ± 470	②③
20	Ding YQ 2013	45	45	15 ~ 30°, 3 ~ 4 cm	45°, 3 ~ 4 cm	The episiotomy wound was closed layer by layer using absorbable surgical sutures.	18 ~ 35	37 ~ 41	3,250 ± 450 g	③
21	Jin SF 2005	100	100	25 ~ 30°, 3 ~ 5 cm	45°, 3 ~ 5 cm	–	23 ~ 30	–	≥3,300 g	①
22	Li CX 2009	293	270	30°， 3 ~ 4 cm	45°	Intradermal suturing with absorbable thread.	–	–	–	①②
23	Liu GL 2007	32	78	25 ~ 30°, 2 ~ 4 cm	45°, 4 ~ 5 cm	The vagina was closed with continuous sutures using absorbable threads, the subcutaneous tissues and muscles were closed with interrupted sutures and the skin was closed with continuous intradermal sutures.	23 ~ 32	–	3,300 ~ 4,000 g	②③
24	Si WX 2021	25	25	30°, 3 ~ 5 cm	45°, 3 ~ 5 cm	The vaginal mucosa and muscular skin tissue were sutured with absorbable threads.	T:26.9 ± 1.3 C:26.7 ± 1.2	–	–	②③
25	Wang JX 2015	257	257	25 ~ 30°, 3 cm	45°, 3 cm	The labial frenulum was sutured in the mattress suturing. The perineal muscle layer was sutured in the intermittent mattress with No. 1 suture.	T:29.91 ± 6.24 C:26.86 ± 5.32	T:39.58 ± 2.32 C:39.87 ± 2.19	2,600 ~ 4,100 g	①

Note: T: Test group; C: Control group; –: Unclear; Outcome: ① perineal tears, ② incision suture time, ③ incision bleeding volume.

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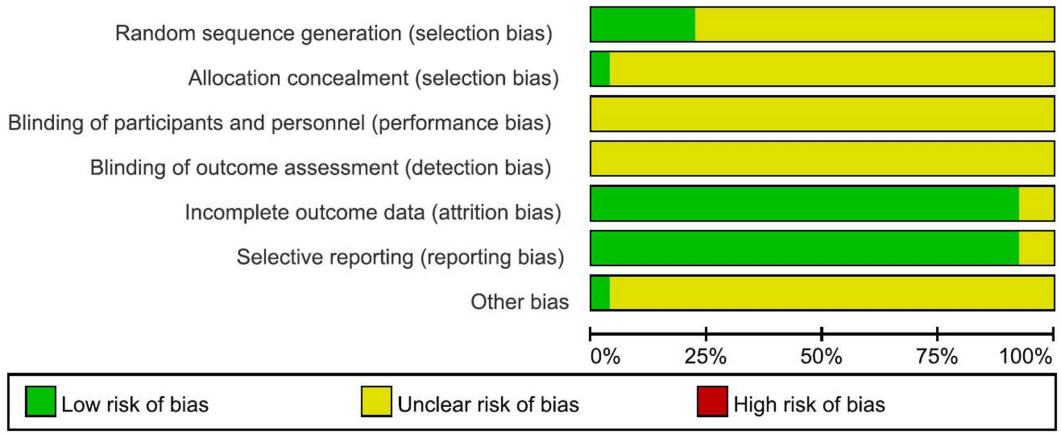

FIGURE 2
Risk of bias evaluation results.

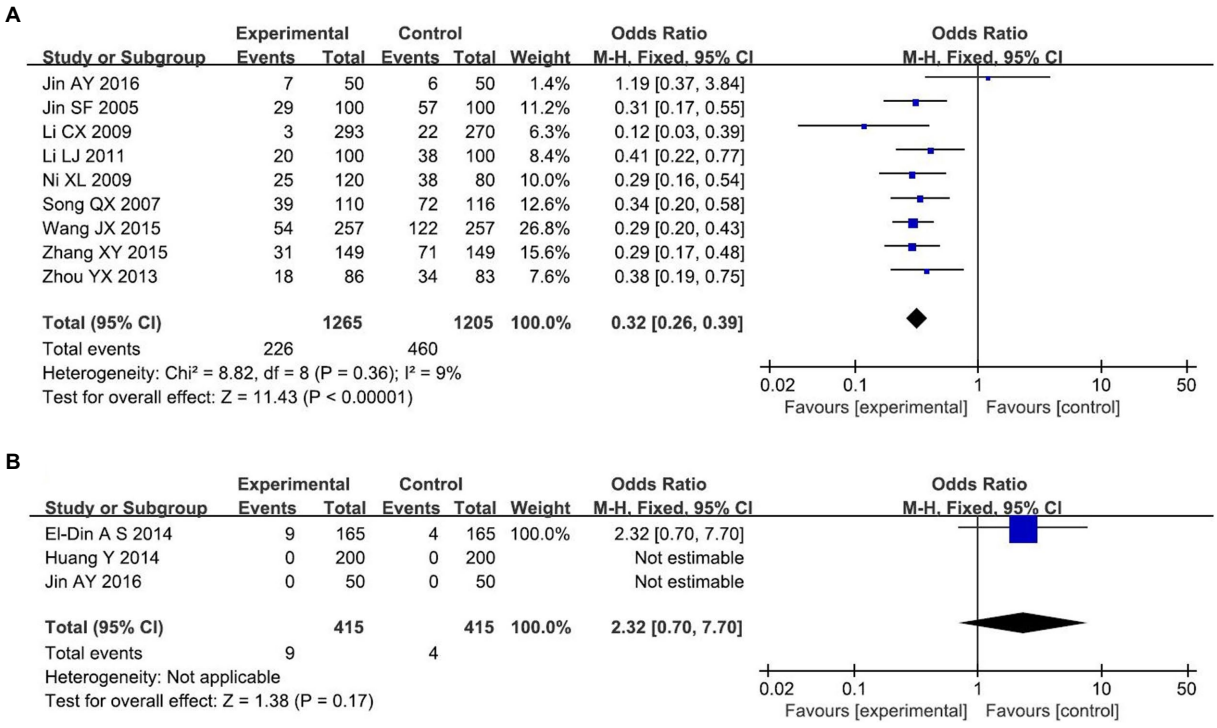

FIGURE 3
Perineal laceration **(A)** Laceration rate. **(B)** Severe laceration rate.

small-angle group than in the conventional group [$MD = -4.58$ min, 95% CI (-6.02, -3.14), $p < 0.00001$], with statistically significant differences between the two groups, as shown in Figure 4. Subgroup analysis was conducted according to whether the incision length of the two groups was the same. Five studies had the same incision length in both groups (all 3 ~ 5 cm), with no statistical heterogeneity between studies ($I^2 = 8\%$, $p = 0.36$).so meta-analysis using fixed-effect model showed that the incision suture time in the test group was lower than that in the control group [$MD = -2.32$ min, 95%CI (-2.44, -2.20), $p < 0.00001$], and the difference was statistically significant. The incision suture time in other small-angle lateral perineal incisions with different incision lengths was also shorter than that in the control group. As can be seen from Table 2, it is clear that incision length is a source of heterogeneity.

3.4.3. Incisional bleeding volume

A total of 11 RCTs (13, 18, 20, 23, 24, 26, 30, 31, 34–36) reported the effect of lateral incision angle on incisional bleeding volume, including 1,319 primigravida. Meta-analysis using a random-effects model showed that incisional bleeding was significantly lower in the small-angle group than in the conventional group [$MD = -19.08$ ml, 95% CI (-19.53, -18.63), $p < 0.00001$], with statistically significant differences between the two groups, as shown in Figure 5. The results

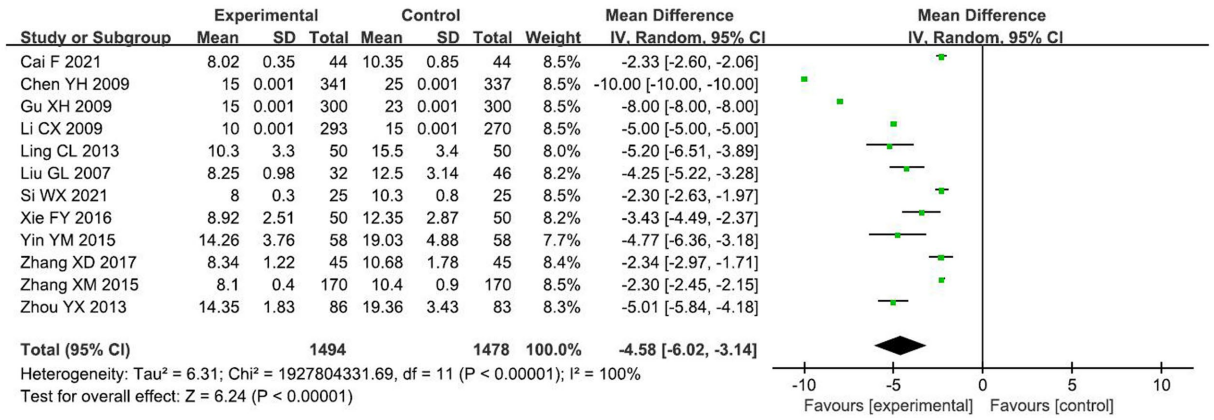

FIGURE 4
Comparison of incision suture time between the two groups.

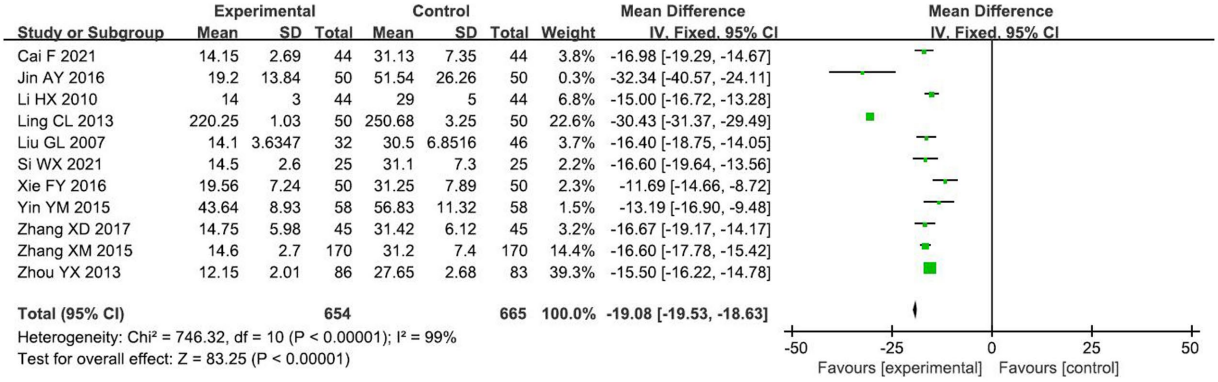

FIGURE 5
Comparison of incisional bleeding between the two groups.

TABLE 2 Results of subgroup analysis comparing the suture time in the two groups.

Outcome indicator	Number of included studies	Heterogeneity test		Effect model	Results of Meta-analysis	
		I^2	*P*		*MD* (95%*CI*)	*P*
Comparison of suture time when the incision lengths were the same in both groups	5	8%	0.36	Fixed	−2.32 (−2.44, −2.20)	<0.00001
Comparison of suture time when the incision lengths were different between the two groups	6	100%	<0.00001	Random	−6.36 (−7.56, −5.17)	<0.00001
Comparison of suture time when the length of the incision in one of the two groups was unclear	1	–	–	–	−5.00 (−5.00, −5.00)	<0.00001

of the subgroup analysis are shown in Table 3: there were four studies with the same incision length between the two groups, and there was no statistical heterogeneity among the studies ($I^2 = 0\%$, $p = 0.99$), so meta-analysis using fixed-effects model showed that the incisional bleeding volume in the test group was lower than that in the control group [*MD* = −16.67ml, 95% *CI* (−3.18, −2.70), $p < 0.00001$], and the difference was statistically significant; the incisional bleeding volume in other small-angle lateral perineal incisions with different incision lengths were also shorter than those of the control group. From Table 3, it is clear that incision length is a source of heterogeneity.

3.4.4. Incisional bleeding >50mL

A total of 3 studies (15, 29, 32) with 790 primigravida were included. The incidence of incisional bleeding >50 mL was found to be lower in the test group than in the control group using a fixed-effect model analysis [*OR* = 0.19, 95% *CI* (0.08, 0.46), $p < 0.00001$],

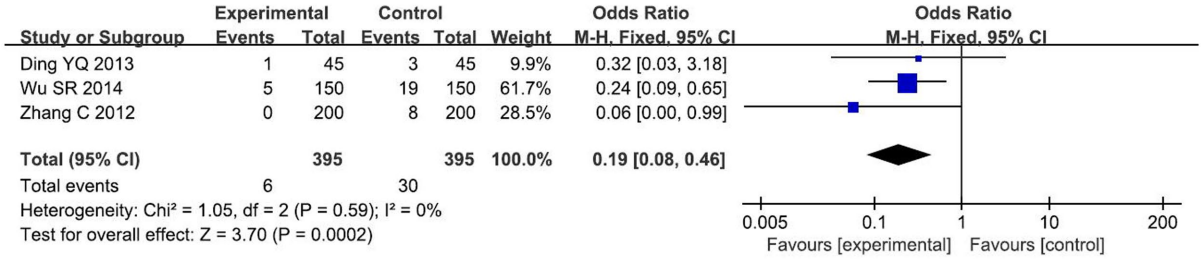

FIGURE 6
Comparison of incisional bleeding >50mL between the two groups.

TABLE 3 Results of subgroup analysis comparing incisional bleeding in the two groups.

Outcome indicator	Number of included studies	Heterogeneity test		Effect model	Results of Meta-analysis	
		I^2	*P*		*OR/MD* (95%*CI*)	*P*
Comparison of incisional bleeding when incision lengths was the same in both groups	4	0%	0.99	Fixed	−16.67 (−17.60, −15.75)	<0.00001
Comparison of incisional bleeding when the incision lengths were different between the two groups	7	99%	<0.00001	Random	−19.82 (−20.33, −19.30)	<0.00001

and the difference between the two groups was statistically significant, as shown in Figure 6.

3.5. Publication bias test

The Egger test in Stata 12.0 software was used to evaluate the publication bias for incisional suture time and incisional bleeding, and the *p* values were greater than 0.05 and the 95% confidence interval included 0, showing that there was no publication bias in the results, as shown in Table 4.

4. Discussion

4.1. Small-angle perineal perineotomy reduces the incidence of perineal lacerations and does not increase the incidence of third- or fourth-degree perineal lacerations

The present Meta-analysis showed that small-angle perineal perineotomy did not increase the risk of third- and fourth-degree perineal lacerations, which was basically consistent with the findings of previous studies (39). Episiotomy is intended to prevent severe perineal tears (e.g., OASIS) that may result during transvaginal delivery (40). Some studies have reported that the incidence of OASIS ranges from 0.25 to 7.31% in women who deliver vaginally, and this delivery complication can have a significant impact on maternal health and may lead to a range of problems such as anal incontinence, urinary incontinence, wound infection, perineal pain, sexual dysfunction, and postpartum depression, and a high proportion (42%) of wound complications required further specialist treatment (41–45). This analysis showed a lower rate of tearing with small-angle lateral perineotomy and no substantial difference in the rate of third- or fourth-degree perineal tears. EL-Din et al. (12) found that there was no statistically significant difference in the incidence of third- or fourth-degree lacerations with 40° compared with 60° episiotomy ($p > 0.05$). The reason for this may be that with a larger angle lateral incision, some of the bulbocavernosus and anal raphe muscles will be directly sheared, resulting in a less elastic lateral incision, while the tissue of the inner vaginal wall is more extended and torn due to the larger incision (46). Limited by the small number of included studies, this conclusion needs further confirmation by more studies.

4.2. Small-angle episiotomy facilitates rapid closure of the incision in a short time, which can not only shorten the duration of maternal pain and discomfort, but also reduce the degree of postoperative pain

The current study showed that the small-angle lateral episiotomy shortened the suturing time by about 4 min compared with that used in the traditional lateral episiotomy. In the case of greater heterogeneity, the heterogeneity was significantly reduced after removing the studies with different incision lengths in the test and control groups, indicating that the source of heterogeneity may be associated to the length of the incision, which is related to the shortening of the incision length on the one hand and the thickness of the incised tissue on the other hand. The modified lateral episiotomy requires less muscle tissue and vascular tissue to be incised, demonstrating less bleeding, facilitating the recovery of anatomical structures and it is easy to suture, thus significantly shortening the surgical suturing time. In addition, suturing technique is also a factor affecting suture time (47), which certainly includes the suturing skills of the doctor and obstetrician at the time of suturing, and suturing skills can also have a direct impact on episiotomy, while most of the included studies did not specify the suturing method and

TABLE 4 Egger's test.

Outcomes	Bias	95%*CI*		*P* > |t|
Suturing time	339.3275	−9971.052	10649.71	0.943
Bleeding volume	2.180309	−9.223097	13.58371	0.676

technique for each layer of tissue, which needs to be further explored in depth in future studies. Future studies need further more comprehensive and in-depth comparisons and studies according to incision length and suturing technique to improve the evidence supporting the effect of episiotomy angle on suture time. The reduction in suture time also correspondingly shortened maternal discomfort during suturing, and the small-angle episiotomy can reduce the incidence of pain in the lateral incision 24 to 72 h postoperatively (39). Postpartum perineal pain has been reported in 92 to 100% of all women, and perineal pain associated with episiotomy or perineal tearing persists in 10% of women, which not only affects the quality of life, but also the persistence of pain may be a cause of postpartum depression (48, 49). Because the small-angle episiotomy only partially cuts the tendon close to the bulbocavernosus muscle, the incision is shallower and shorter, causing less damage to the tissue and correspondingly less postpartum pain. It was also found in this analysis that most of the studies did not specify the assessment method when measuring bleeding, which was not conducive to further comprehensive evaluation by the investigators. It is recommended that future researchers should specify the time of measurement when reporting outcome indicators, so as to provide a reliable basis for evidence-based studies.

4.3. A note on the application of small-angle episiotomy

Episiotomy, a widely used invasive procedure in obstetrics, is conditional and complex to perform (50). Major scientific groups, notably the World Health Organization, have explicitly cautioned against routine episiotomy and have reported frequent use of episiotomy without consent because of the additional risks associated with episiotomy, such as infection as well as vaginal discomfort, among others (51, 52). This, along with other controversial and poorly regulated techniques such as hip pressure, has much to do with the definition of obstetric violence (53). These aspects certainly need to be weighed against any benefits associated with episiotomy. Unlike conventional episiotomy, elective episiotomy can avoid the above-mentioned risks to a certain extent (54). Therefore, we are not advocating the routine performance of episiotomy here. In other words, episiotomy should be performed selectively based on clinical judgment and maternal or fetal indications, and must be restricted to those with good reasons (55). On this basis, when elective episiotomy must be performed, we recommend performing it at a small angle to circumvent problems such as perineal laceration and excessive bleeding during vaginal delivery in order to facilitate maternal postoperative perineal recovery. In conclusion, episiotomy remains a common practice, although its use is controversial (56). We need to weigh the risks and benefits of this procedure in a comprehensive manner and use it selectively. It also requires more researchers to further develop high-quality studies in this field to address these controversial issues and promote standardization and science in clinical application.

Limitations of this study: (1) this study is a secondary study, and some of the evidence is limited by the quality of the original studies included; (2) this study only included literature in Chinese and English, and did not involve literature searches in the remaining languages, which limited the extrapolation of the study findings. Future studies could expand the language range to include more high-quality studies to further evaluate the clinical outcomes of small-angle episiotomy.

In conclusion, small-angle episiotomy is beneficial for reducing the incisional tear rate, shortening the incisional suture time, reducing the incidence of incisional infection and incisional pain, and promoting good healing of the perineal incision, which is more beneficial for maternal postoperative recovery. However, due to the limitation of the quality and quantity of included studies, the above findings need to be confirmed by more high-quality studies.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

YZ, JZ, LZ, and JT conceptualized the study, drafted the protocol for the meta-analysis. JZ and LX searched the academic databases and identified the eligible trials. JZ and LZ extracted the data and wrote the initial draft of the manuscript. LZ and LX performed quality assessment. JZ conducted the meta-analysis. YZ, JZ, and LZ interpreted the results. YZ, JZ, LZ, LX, and JT conducted critical review of the manuscript. YZ undertook the post revision and proofreading of the article. FW supervised and reviewed a series of revisions to the article after submission. All authors approved the final version of the manuscript for submission.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The cut-off value for HOMA-IR discriminating the insulin resistance based on the SHBG level in women with polycystic ovary syndrome

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Introduction: The study aimed to estimate the cut-off value for homeostatic model assessment for insulin resistance (HOMA-IR) discriminating the insulin resistance based on the sex hormones binding globulin (SHBG) level in women with polycystic ovary syndrome (PCOS).

Materials and methods: Data from medical records of 854 Caucasian women diagnosed with PCOS were analyzed. Anthropometric data, fasting plasma glucose, insulin and SHBG levels were measured. HOMA-IR was calculated with a standard formula. The cut-off value was calculated using receiver-operating characteristics.

Results: Circulating SHBG levels below the normal range (26.1 nmol/L) were found in 25.4% of study participants. This subgroup had a significantly higher BMI, fasting glucose and insulin concentrations and HOMA-IR values. Empirical optimal cut-off values for HOMA-IR corresponding to low SHBG levels was ≥2.1 [area under the curve (AUC) 0.73, accuracy 0.65, sensitivity 72.3%, specificity 63.1%, positive predictive value (PPV) 40.0%, negative predictive value (NPV) 87.0%].

Conclusions: Our study suggests that the cut-off point for HOMA-IR discriminating the insulin resistance based on the SHBG level, in young Caucasian women with polycystic ovary syndrome is 2.1, and is consistent with the cut-off value adopted by the European Group for the Study of Insulin Resistance (above 2.0).

KEYWORDS

polycystic ovary syndrome, SHBG, HOMA-IR, cut-off value, receiver-operating characteristic

Introduction

Sex hormone binding globulin (SHBG) is a homodimer glycoprotein with a high affinity and specificity for androgens and estrogens (1). It is produced mainly in the liver and its synthesis is regulated mostly by circulating sex hormones and hyperinsulinemia compensating insulin resistance (2–4). Thus, SHBG may be a useful marker of the severity of hepatic insulin resistance and fatty liver that is linked to hepatic insulin resistance. Numerous previously published studies demonstrated that low circulating SHBG levels may serve as a surrogate marker of fatty liver (5–7). It has also been shown that SHBG levels were inversely proportional to the severity of fatty liver, insulin levels and homeostatic model assessment for insulin resistance (HOMA-IR) values (8). Moreover, the expression of *SHBG* mRNA correlated negatively with the accumulation of triglycerides in hepatocytes (9). A meta-analysis confirmed these observations, showing that low SHBG levels correlate with non-alcoholic fatty liver disease (NAFLD) in both women and men (10). One of the consequences of hepatic insulin resistance in NAFLD is increased gluconeogenesis resulting in the impaired fasting glucose level. Concurrently, the lower SHBG level is the predictor of type 2 diabetes (11). During a 5 years follow-up, men with the lowest SHBG levels had a four-fold higher risk of type 2 diabetes (12). This finding was corroborated by a meta-analysis of 13 prospective, observational studies (13). In a large cohort study including 42,034 women, a higher risk of type 2 diabetes was associated with SHBG levels $<$ 50 nmol/L (14). The role of SHBG in type 2 diabetes development is supported by experimental studies performed with the insulin-resistant human trophoblast cells (HTR8-SVneo cell line) characterized by low expression of *SHBG*, *GLUT-3* and *GLUT-4* (glucose transporters type 3 and 4) as well as high expression of *GLUT-1*. Notably, overexpression of SHBG inhibited levels of *GLUT-1* mRNA and promoted the expression of *GLUT-3* and *GLUT-4*. This finding suggests that SHBG may affect glucose metabolism and induce insulin resistance by regulating the activity of glucose transporters (15). In addition, incubation of macrophages and adipocytes with 20 nM SHBG significantly inhibited the synthesis of proinflammatory cytokines (monocyte chemoattractant protein-1, tumor necrosis factor and interleukin-6) induced by lipopolysaccharide treatment (16).

Polycystic ovary syndrome (PCOS) is defined as multiple endocrine and metabolic disturbances, among which the central position is ovarian dysfunction. Insulin resistance is one of the key factors in the pathogenesis of hormonal and metabolic disturbances observed in women with PCOS. However, it should be noted that insulin resistance is not a part of PCOS diagnosis. A gold standard for the assessment of insulin resistance is the hyperinsulinemic-euglycemic clamp technique. However, this method is very complicated and is not used in daily clinical practice. In clinical studies and daily practice, insulin resistance is assessed on the basis of a mathematical model named HOMA-IR, which probably reflects more hepatic than muscle insulin resistance (17). However, there is a lack of a clearly defined cut-off point for HOMA-IR related to insulin resistance. Among many of the proposed values for the general population, the value of 2.5 and above is most often used (18). Notwithstanding, studies performed in Caucasian and Thai women with PCOS suggested the HOMA-IR cut-off value of at least 2.0 (19, 20). Also, the European Group for the Study of Insulin Resistance uses the same cut-off point ($\geq$2.0) (21).

As mentioned above, compensatory hyperinsulinemia inhibits hepatic SHBG synthesis. Concordantly, we hypothesized that SHBG level may be a useful marker of the severity of hepatic insulin resistance. Contrary to the detectable cut-off point characterizing insulin resistance, the laboratory assays for SHBG have specified reference ranges and its lower limit may be used to establish a corresponding HOMA-IR cut-off point. Therefore, the aim of this study was to estimate the cut-off value for HOMA-IR discriminating the insulin resistance based on the SHBG level in women with PCOS.

Materials and methods

The retrospective study includes data from the medical records of 859 Caucasian women for the first time diagnosed with PCOS on the basis of the Rotterdam criteria (22), hospitalized at the Department of Gynecological Endocrinology from 2012 to 2019.

The inclusion criteria included age 18–30 years and diagnosis of PCOS. The exclusion criteria were: diagnosis of type 2 diabetes and other endocrinological disturbances, any pharmacological therapy, treatment of obesity in the past and currently and the lack of necessary data in the medical records.

The analyzed data set included: age, body mass, height and routine measurements of fasting glucose, insulin and SHBG levels, all performed in a single hospital laboratory using the same set of methods for all study subjects. Glucose concentration was measured using the colourimetric method (Roche reagents for Cobas e111). Insulin and SHBG levels were determined using the ECLIA method (Roche Diagnostic GmbH, Mannheim, Germany reagents for Cobas E411). Body mass index (BMI) and HOMA-IR values were calculated with standard formulas:

$$HOMA - IR = fasting\ serum\ insulin\ level(uIU/ml) \times fasting\ glucose\ level\ (mg/dL)/405.$$

As the retrospective analysis of patients' records does not meet the criteria of a medical experiment, the approval of the Bioethical Committee was not required.

Data analysis

Women with HOMA-IR values above 10 ($N = 5$)—data outliers, related to non-compliance and to the assessment of measured parameters in non-fasting subjects, were excluded from the analysis. The remaining women were divided according to the lower limit of the SHBG concentration laboratory's reference range for women aged 18–50 years ($<$26.1 nmol/L) into a subgroup with concentrations above

and below this limit [$N = 637$ (74.6%) and $N = 217$ (25.4%), respectively].

Statistical analysis

Statistical analysis was performed using STATISTICA 13.0 PL (TIBCO Software Inc., Palo Alto, CA, US), StataSE 13.0 (StataCorp LP, TX, US) and R software (23). Statistical significance was set at a p value below 0.05. All tests were two-tailed. Imputations were not done for missing data. Nominal and ordinal data were expressed as percentages. Interval data were expressed as median with lower and upper quartiles. The distribution of variables was evaluated by the W Shapiro-Wilk test and the quantile-quantile (Q-Q) plot. In order to compare two groups with SHBG $\geq$ 26.1 nmol/L and SHBG $<$ 26.1 nmol/L, the t-Student test for independent data or the U Mann-Whitney test was used, according to data distribution. The homogeneity of variances was assessed by the F Fisher-Snedecor test. The nominal and ordinal data were compared with the χ^2 test. Correlation between SHGB levels and other variables was assessed with the ρ Spearman rank correlation coefficient. Age adjustment was done with the Spearman rank partial correlation coefficient (package *ppcor* in R). In order to find a cut-off point discriminating the insulin resistance based on the SHBG level, parametric and non-parametric receiver-operating characteristic (ROC) curves were calculated with an area under the curve (AUC) and corresponding sensitivity, specificity, positive and negative predictive value as well as with accuracy of classification. In order to find an optimal, empirical cut-off point value for HOMA-IR, the Youden J statistic (index) was used.

Results

Study groups' characteristics' are listed in Table 1. Circulating SHBG levels below the reference lower limit of 26.1 nmol/L were found in 25.4% of study participants. This subgroup was characterized by a significantly higher BMI, fasting glucose and insulin concentrations as well HOMA-IR values. Obesity and impaired fasting glucose (IGF) were more frequently diagnosed in a subgroup with SHBG below 26.1 nmol/L (59.1% vs. 18.6%; $p < 0.001$ and 17.2% vs. 6.7%; $p < 0.001$, respectively). As expected, the median HOMA-IR value was significantly higher in a subgroup with low SHBG levels (2.8 vs. 1.7; $p < 0.001$). Figure 1 shows the ROC curve of HOMA-IR and SHBG levels below the lower limit of the laboratory reference range (<26.1 nmol/L). An empirical optimal cut-off, based on the Youden index, for HOMA-IR discriminating the insulin resistance, was ≥2.1 (Table 2). Subjects with HOMA-IR values below the established cut-off had a very low risk of having impaired fasting glucose (OR = 0.035; 95% CI: 0.013–0.097; $p < 0.001$) and decreased SHBG level (OR = 0.19; 95% CI: 0.13–0.27; $p < 0.001$) (Table 3). There was a moderate negative correlation between HOMA-IR values and SHBG levels (crude: $\rho = -0.50$; $p < 0.001$, age-adjusted: $\rho = -0.45$; $p < 0.001$), as well as positive with BMI values (crude: $\rho = -0.53$; $p < 0.001$, age-adjusted: $\rho = 0.60$; $p < 0.001$).

TABLE 1 Characteristics of the study group and subgroups.

	All $N = 854$	SHBG ≥ 26.1 $N = 637$ (74.6%)	SHBG < 26.1 $N = 217$ (25.4%)
Age (years)#	25 (22–29)	25 (22–29)	25 (21–29)
BMI (kg/m^2) #	26.6 (20.8–31)	22.9 (20.5–27.9)	31.3 (27.1–36.4)***
Overweight (*N*; %)	149 (17.4%)	104 (16.3%)	45 (20.9%)
Obesity (*N*; %)	246 (28.8%)	119 (18.6%)	127 (59.1%)***
Glucose (mg/dL)#	88.0 (83.0–93.0)	88.0 (83.0–92.0)	89.0 (84.0–95.0)**
Glucose ≥ 100 (mg/dL) (*N*; %)	80 (9.4%)	43 (6.7%)	37 (17.2%)***
Insulin (uIU/ml)#	8.9 (6.0–13.2)	7.7 (5.5–11.23)	13.0 (8.9–18.6)***
HOMA-IR#	1.9 (1.3–3.0)	1.7 (1.1–2.5)	2.5 (2.0–4.3)***
HOMA-IR ≥ 2.1 (*N*; %)	386 (45.4%)	235 (36.9%)	157 (72.3%)***
SHBG (nmol/L)#	39.1 (26.0–59.0)	48.1 (36.0–65.5)	19.3 (15.5–22.4)

#Median (lower quartile – upper quartile).
** $p < 0.01$; *** $p < 0.001$; the U Mann-Whitney test for interval data or χ^2 test for nominal data.

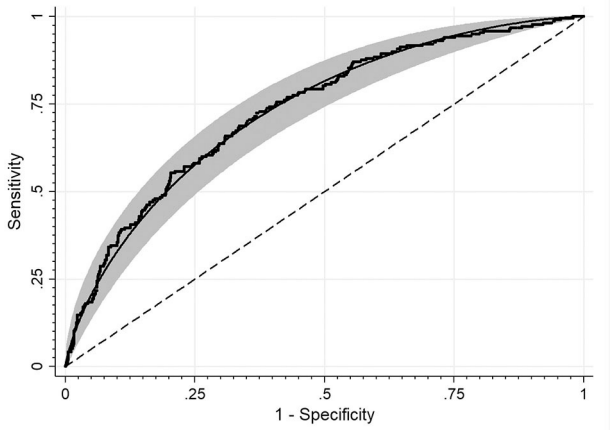

FIGURE 1
ROC curve for detecting HOMA-IR cut-off values discriminating the insulin resistance based on the SHBG level. The area under the curve: 0.73.

TABLE 2 Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of HOMA-IR ≥2.1 corresponding to low circulating SHBG levels (<26.1 nmol/L) in PCOS women.

Parameter	Percent (%)	95% CI
Sensitivity	72.3	65.8–78.1
Specificity	63.1	59.2–66.8
Positive predictive value	40.0	35.2–45.1
Negative predictive value	87.0	83.5–89.9

CI, confidence interval.

TABLE 3 Comparison between subjects with lower and higher HOMA-IR using the established cut-off value.

	HOMA ≥ 2.1 $N = 86$ (45.2%)	HOMA < 2.1 $N = 468$ (54.8%)	*p*
Age (years)*	25 (22–29)	25 (22–29)	0.85
BMI (kg/m^2)*	30.1 (24.6–35.7)	22.1 (20.1–24.7)	<0.001
Overweight (*N*; %)	76 (19.7)	73 (15.6)	<0.001
Obesity (*N*; %)	209 (54.2)	37 (7.9)	
Glucose (mg/dL)*	91.0 (86.0–97.0)	86.0 (81.0–90.0)	<0.001
Glucose ≥ 100 (mg/dL) (*N*; %)	76 (19.7)	4 (0.8)	<0.001
Insulin (uIU/dL)*	13.9 (11.2–18.6)	6.3 (4.7–7.7)	<0.001
SHBG (nmol/L)*	30.8 (20.5–43.9)	48.6 (33.4–67.7)	<0.001
SHBG < 26.1 (nmol/L) (*N*; %)	154 (39.9)	61 (13.1)	<0.001

*Median (lower quartile–upper quartile).

Discussion

To the best of our knowledge, this is the first study estimating the cut-off value for HOMA-IR discriminating the insulin resistance based on the SHBG level in women with PCOS.

It is established that HOMA-IR is a better measure of hepatic than muscle insulin resistance. In turn, compensatory hyperinsulinemia inhibits SHBG synthesis in the liver. In our study, 25.4% of women with PCOS had circulating SHBG levels below the adopted lower limit of the laboratory reference range (26.1 nmol/L). This subgroup was characterized by a significantly more frequent occurrence of overweight and obesity diagnosed based on BMI values, according to the Word Health Organization criteria (24), compared to the subgroup with normal SHBG levels. As expected, impaired fasting glucose was also significantly more prevalent in this subgroup, corresponding to a significantly higher median HOMA-IR value (2.9 vs. 1.7). These results, as well as the negative correlations between SHBG levels and HOMA-IR values or insulin levels, once again confirm that low SHBG levels are associated with the occurrence of insulin resistance. These correlations indicate that hyperinsulinemia and insulin resistance explain nearly 50% variability of SHBG concentrations. It is consistent with the results of a previous study analyzing the correlation between SHBG levels and insulin resistance in postmenopausal women (4). Among factors not included in our analysis was hyperandrogenemia exerting a suppressive effect on SHBG secretion, mostly in men (2, 3). However, a meta-analysis of 26 studies including 3,349 menopausal women showed that testosterone but not DHEA administration decreased SHBG levels (25). Thus, hyperandrogenemia potentially may modulate the associations between SHBG levels and hyperinsulinemia also in women with PCOS. However, estradiol/testosterone and estradiol/androstenedione indexes are quite similar in both women with PCOS and obesity and women with PCOS and normal-weight (26). Moreover, 12 months therapy with estrogens, which certainly affects the androgens/estrogens index, did not cause changes in insulin sensitivity in women with PCOS (27). These data suggest that at least the androgens/estrogens ratio has a much less important role than the changes in BMI/fat depot in the modulation of insulin resistance.

In our study, the empirically estimated HOMA-IR cut-off point discriminating the insulin resistance based on the SHBG level below the lower limit of the laboratory reference range (< 26.1 nmol/L) was 2.1. Thus, it is between the previously adopted cut-off points > 2.5 (28), > 2.0 (20, 21) and 1.67 (29). Of note, the HOMA-IR cut-off point determined in our study was characterized by quite high sensitivity but low specificity. Therefore, in many cases the low SHBG level would not allow for the diagnosis of insulin resistance but, on the other hand, the likelihood of false positive results is low. Therefore we do not recommend using SHBG level to diagnose insulin resistance. However, it should be noted that in our subgroup with SHBG levels below 26.1 mmol/L, the prevalence of impaired fasting plasma glucose was about three times more frequent than in a subgroup with SHBG 26.1 mmol/L and above.

Of note, the established HOMA-IR cut-off point in our study of 2.1 is very close to the value of 2.0 in Thai women with PCOS (20). This discrepancy indicates a tightening circle in the search for the optimal HOMA-IR cut-off point for diagnosis of insulin resistance in the population of young women with PCOS. In our study, subjects with HOMA-IR values below the established here cut-off value had a very low risk of impaired fasting glucose. These results are in accordance with a previously published study (17) suggesting that our HOMA-IR cut-off point is a good marker of hepatic insulin resistance. Of note, the cut-off point of 2.1 established in our study is similar to the value determined in 833 Chinese women diagnosed with PCOS and components of metabolic syndrome (30). In addition, the median SHBG concentration in this cohort was 27.9 nmol/L (lower quartile 18.8 nmol/L, upper quartile 45.5 nmol/L) (30), so it was close to the lower limit of the laboratory reference range used in our study.

There are some confounders that should be considered when analyzing HOMA-IR values and corresponding cut-off points discriminating the insulin resistance based on the SHBG level. Borai et al. (31) indicated that studies determining the cut-off points for insulin resistance indicators should refer to the method of insulin assessment, because its concentrations may significantly differ depending on the type of used kit. This may be the effect of several factors, such as variable specificity, different calibration settings, and different formulas used to convert insulin units, as demonstrated by a comparison of 11 insulin determination methods by Manley et al. (32). The same authors observed that the distribution of HOMA-IR values differed even twice, depending on the method of insulin assessment (33). This fact can significantly affect the HOMA-IR cut-off point value estimated in different studies. The results of our and other studies cause reflection or the use of only one parameter in the assessment of insulin resistance with no precisely defined cut-off point, which is associated with a high risk of not recognizing this disturbance. As mentioned above, HOMA-IR calculation is highly variable; therefore, requiring a wider analysis of insulin resistance based on various indicators, perhaps including SHBG. This approach

is also recommended by the authors of a study analyzing the advantages and disadvantages of various methods of insulin resistance assessment (33).

Our study has several limitations. The main limitation is its retrospective design. It also lacks hyperinsulinemic-euglycemic clamp, oral glucose tolerance test (OGTT), and HbA1c assessments, as well as body composition and visceral obesity (waist circumference) and fatty liver measures. However, the hyperinsulinemic-euglycemic clamp is still missing the reference values and, therefore, should not be used for the identification of subjects with hepatic insulin resistance. Moreover, both the hyperinsulinemic-euglycemic clamp and OGTT better characterize muscle insulin resistance, while HOMA-IR better assesses hepatic insulin resistance, which was the aim of our study (34). Another limitation is not taking into account hyperandrogenemia as a factor influencing SHBG synthesis. However, it has been previously shown that the contribution of SHBG to the variation in HOMA-IR is not dependent on estrogen and androgens levels in postmenopausal women (35). We hypothesize that this observation may also apply to premenopausal women, as recently published data show the similar predictive significance of SHBG levels for the development of insulin resistance in pre- and postmenopausal women (36).

The strength of our study relies on the large size of the study group and the inclusion of a homogenous cohort of young Caucasian women (between 20 and 30 years of age) with PCOS and a wide range of BMI. Of note, the established cut-off point for HOMA-IR may not be universal for all methods of insulin assessment. We think that the established here cut-off value for HOMA-IR, based on SHBG decline, could be useful for clinicians to identify women with PCOS that may benefit from the implementation of interventions such as an increase in physical activity and changes in eating habits to decrease visceral and liver fat accumulation and prevent the development of type 2 diabetes and cardiovascular disease.

Conclusions

Our study suggests that the cut-off point for HOMA-IR discriminating the insulin resistance based on the SHBG level in young Caucasian women with PCOS is 2.1 and is consistent with the cut-off value adopted by the European Group for the Study of Insulin Resistance (above 2.0).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the patients/participants or patients/participants legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

Concept and study design: AB-B, JC, and MO-G. Data collection: PK and PM. Analysis: AO and PC. Data interpretation and final approval and review: PM, MP-K, JC, and MO-G. Manuscript writing: AB-B and LM. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Effect of excessive gestational weight gain before and after 28 weeks on trial of labor after cesarean stratified by pre-pregnancy body mass index: a retrospective cohort study

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This study aimed to assess the effect of excessive gestational weight gain (GWG) before and after 28 weeks on the mode of delivery in women who attempted a trial of labor after cesarean (TOLAC), stratified by pre-pregnancy BMI. A retrospective analysis of the outcomes of eligible women who attempted trial of labor after cesarean (TOLAC) in a Chinese hospital from January 2016 to October 2022 was performed. GWG before and after 28 weeks was categorized as 'excessive' or 'non-excessive' based on the guideline of Institute of Medicine (IOM). Multivariable logistic regression analyses were used to estimate the effect of excessive GWG before and after 28 weeks on mode of delivery in women who underwent TOLAC, stratified by pre-pregnancy BMI. Of the 512 women who underwent term trial of labor, 71.1% achieved a vaginal birth. No correlation was found between excessive GWG before 28 weeks and the rate of vaginal birth after cesarean (VBAC). Among women with or without excessive GWG before 28 weeks, excessive GWG after 28 weeks was significantly associated with a reduced rate of VBAC. When stratified by pre-pregnancy BMI, women who had excessive gestational weight gain after 28 weeks gestation had lower rates of VBAC than those who did not, regardless of being underweight, normal or overweight (aOR 0.23, 95% CI 0.06–0.88; aOR 0.42, 95% CI 0.25, 0.70; and aOR 0.12, 95% CI 0.04–0.36; respectively). Excessive weight gain after 28 weeks of pregnancy was related to decreased rates of VBAC, irrespective of pre-pregnancy weight status and weight gain before 28 weeks.

KEYWORDS

trial of labor, weight gain, cesarean section, body mass index, pregnancy

1. Introduction

Women who have had a prior cesarean section can choose either vaginal birth after cesarean section (VBAC) or repeat cesarean section (RCS) for their subsequent deliveries. However, globally, most women with a previous cesarean opt for a repeat cesarean. The rate of trial of labor after cesarean (TOLAC) differs across countries, with lower rates in China

(10%) and the US (13%) and a higher rate in Germany (36.0–49.8%) (1–3). Although successful TOLAC is associated with lower overall morbidity rates than RCS (4), concerns about its safety and associated liability continues to limit its availability (5–8).

The factors that impact VBAC rates include onset of labor, previous vaginal birth or VBAC, multiple cesarean sections, body mass index (BMI), and interpregnancy interval (9–12). Gestational weight gain (GWG) is a potential risk factor for the global obesity crisis, and unlike most of the other factors, it can be modified during pregnancy (13, 14). Excessive GWG can lead to complications of a kind that cesarean section (CS), preeclampsia, gestational diabetes, large for gestational age babies (LGA), and macrosomia (15–17). Only a handful of studies have examined the link between GWG and VBAC, and these studies have yielded inconsistent results. Two studies from the USA and New Zealand indicated that excessive GWG could lower the chances of vaginal birth (18, 19), while another study from the USA detected no variation in TOLAC success rates with excessive GWG (20).

The Institute of Medicine (IOM) guideline proposed fitting GWG classified by pre-pregnancy BMI in 2009 (21). Maternal metabolic changes and GWG differ throughout pregnancy (22); therefore, some studies specifically examined the association between excessive GWG during the first or second trimester and pregnancy complications (16, 23, 24). However, the association between excessive GWG at different stages of pregnancy and VBAC remains unclear. This study aims to explore the relationship between weight gain before and after 28 weeks and VBAC stratified by pre-pregnancy BMI.

2. Participants and methods

2.1. Study setting

This was a retrospective study performed at the Fourth affiliated Hospital of Hebei Medical University, which is a comprehensive regional medical center located in northeast China that performs approximately 1,800 deliveries per year. Before week 8 of gestation, a specific electronic file was created for each pregnant woman at their first antenatal visit. They then received regular follow-up visits every 2–4 weeks until delivery. The study was approved by the Ethics Committee of the Fourth affiliated Hospital of Hebei medical university (2022KS010).

2.2. Eligibility criteria

The study population consisted of women who registered at their first prenatal visit before week 8 of gestation and attempted TOLAC and delivered between January 1, 2016 and October 31, 2022. We excluded women with malpresentation in the current pregnancy, previous uterine scar other than low transverse incision scar, multiple gestation, more than one prior cesarean delivery, placenta previa, delivery before week 37 (37 0/7 weeks), and incomplete information. We also omitted obese women (BMI > 30 kg/m2) from the study, as this group had an insufficient sample size for representation.

2.3. Data collection

For the analysis, the clinical characteristics of women attempting TOLAC were obtained from electronic files, including maternal age, maternal height, labor induction, gestational age at delivery, maternal hypertension or diabetes in pregnancy, previous vaginal birth or VBAC, pre-pregnancy weight, maternal weight at 28 weeks, maternal weight at delivery, and mode of delivery. Meanwhile, the weight of each participant was recorded at each visit; they wore light clothing without shoes during the measurement.

2.4. Determination of gestational weight gain

During the first antenatal care visit, each participant reported their pre-pregnancy weight. Maternal weight at 28 weeks was determined as the latest weight measured within 1 week around 28 weeks. Maternal weight at delivery was that measured within 2 days before the delivery date. By dividing the self-reported pre-pregnancy weight (25, 26) (in kilograms) of each participant by their height squared (in meters), the pre-pregnancy BMI was calculated (27). GWG was calculated as follows: GWG before 28 weeks = maternal weight at 28 weeks - pre-pregnancy weight; GWG after 28 weeks = maternal weight at delivery - maternal weight at 28 weeks.

We applied the recommendation of IOM for weight gain during pregnancy based on pre-pregnancy BMI (underweight: < 18.5 kg/m^2, normal: 18.5–24.9 kg/m^2, overweight: 25.0–29.9 kg/m^2) (21) (Table 1) to calculate the suitable range of gestational weight gain before and after 28 weeks (Table 2). Using this standard, we categorized women as having 'excessive GWG' if they exceeded the recommended upper limit for their BMI group, and 'non-excessive GWG' if they gained weight within or below the recommended range.

2.5. Data analysis

We evaluated participant characteristics across GWG before and after 28 weeks by frequency (percentage), and used the Chi-squared or Fisher exact tests to compare the distribution of differences. We analyzed continuous variables using the Student's t test. Predictors of VBAC success were evaluated using univariate logistic regression analyses. Then, we adjusted for potential confounders that were statistically significant in the univariate analysis (those with $p < 0.05$)

TABLE 1 Gestational weight gain recommended by the IOM guideline (21).

Pre-pregnancy weight category	Pre-pregnancy body index (kg/m^2)	First trimester GWG Range (kg)	Second and Third trimester rate of GWG (kg/week)
Underweight	<18.5	0.5–2.0	0.51 (0.44–0.58)
Normal	18.5–24.9	0.5–2.0	0.42 (0.35–0.50)
Overweight	25.0–29.9	0.5–2.0	0.28 (0.23–0.33)

GWG, gestational weight gain; IOM, Institute of Medicine.

TABLE 2 Gestational weight gain of included pregnant women according to IOM standard (21).

Pre-pregnancy weight category	Pre-pregnancy body index (kg/m^2)	Range of GWG before 28 weeks (kg)	Range of GWG after 28 weeks (kg)
Underweight	<18.5	6.66 ~ 10.12 (0.5 + 14 × 0.44 ~ 2 + 14 × 0.58)	(w-28) × (0.44 ~ 0.58)
Normal	18.5–24.9	5.04 ~ 8.72 (0.5 + 14 × 0.35 ~ 2 + 14 × 0.5)	(w-28) × (0.35 ~ 0.5)
Overweight	25.0–29.9	3.08 ~ 7.18 (0.5 + 14 × 0.23 ~ 2 + 14 × 0.33)	(w-28) × (0.23 ~ 0.33)

GWG, gestational weight gain; IOM, Institute of Medicine.

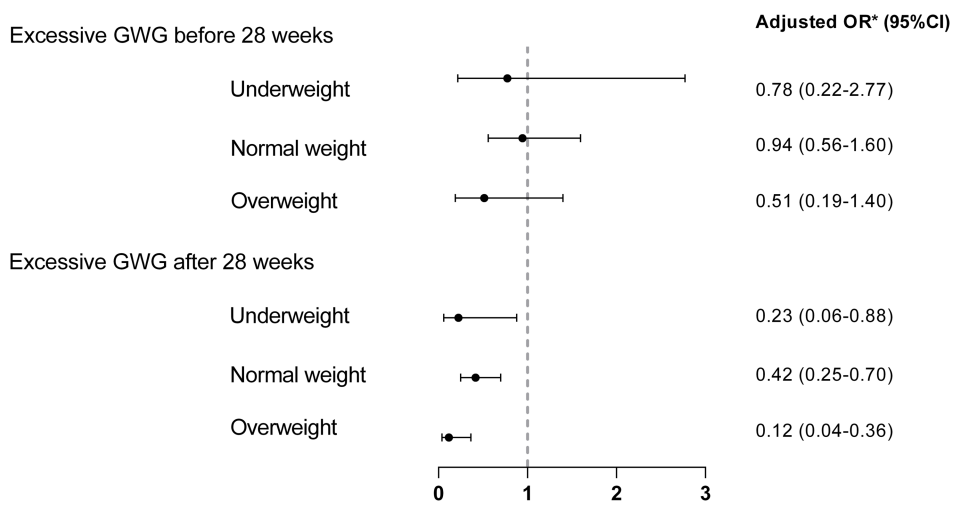

FIGURE 1
Association between excessive GWG before and after 28 weeks and VBAC stratified by pre-pregnancy BMI. Non-excessive GWG was used as the reference group. *Adjusted for induction of labor, hypertension in pregnancy, previous vaginal birth or VBAC.

and generated multivariable logistic regression models to investigate the association between gestational weight gain before and after 28 weeks and VBAC.

To understand how GWG before and after 28 weeks affects VBAC, a stratified analysis was performed to examine the relation between GWG after 28 weeks and the rate of VBAC in groups of GWG before 28 weeks (classified into excessive and non-excessive). The non-excessive GWG group was used as the reference group.

We also generated multivariable logistic regression models for the three subgroups (underweight, normal weight, and overweight) to further explore the relation between excessive gestational weight gain before and after 28 weeks and VBAC rate among women with different pre-pregnancy BMI categories, adjusting for the same confounders except pre-pregnancy BMI. The adjusted OR with 95% CI are shown in Figure 1. SPSS version 19.0 software was used for all statistical analyses, and GraphPad Prism version 6.0 was used to draw the figure. A two-tailed $p < 0.05$ indicated statistical significance for analyses.

3. Results

We examined 552 women who attempted TOLAC for eligibility during the study period. Thirty-one women who did not fulfil the eligibility criteria and 9 women were obese were excluded. In total, 512 (92.7%) women were included in the analysis. Nearly half of the 512 women who met the inclusion criteria exceeded the IOM recommendations for GWG after 28 weeks (45.5%), while 33.8% had excessive GWG before 28 weeks. The demographics of the participants are summarized in Table 3, stratified by GWG before and after 28 weeks. GWG before 28 weeks was not significantly influenced by hypertension or diabetes in pregnancy, maternal age, previous vaginal birth or VBAC, maternal height, gestational age at delivery, pre-pregnancy BMI, or induction of labor. However, GWG after 28 weeks was associated with pre-pregnancy BMI, hypertension in pregnancy, diabetes in pregnancy, and induction of labor. Pre-pregnancy overweight women had a higher likelihood of exceeding the IOM recommendations after 28 weeks ($p < 0.001$). Hypertension in pregnancy and induction of labor was more likely to occur in women with excessive GWG after 28 weeks ($p = 0.048$, $p < 0.001$, respectively), while women with diabetes in pregnancy tended not to exceed the IOM recommendations after 28 weeks ($p = 0.004$).

Among all women who attempted TOLAC, vaginal delivery occurred in 71.1%. The timing of weight measurement at 28 weeks did not significantly differ between the cesarean section and the vaginal delivery groups ($p = 0.588$). In the unadjusted model, women were

TABLE 3 Participant characteristics by gestational weight gain group.

Characteristics	GWG before 28 weeks			After 28 weeks		
	Not excessive (n = 339, 66.2%)	Excessive (n = 173. 33.8%)	p-value	Not excessive (n = 279, 54.5%)	Excessive (n = 233, 45.5%)	P-value
Age (y)			0.642			0.540
<35	261 (77.0)	130 (75.1)		216 (77.4)	175 (75.1)	
≥35	78 (23.0)	43 (24.9)		63 (22.6)	58 (24.9)	
Maternal height (cm)	162.36 ± 5.06	162.60 ± 5.06	0.610	162.18 ± 5.23	162.76 ± 4.83	0.193
Pre-pregnancy BMI (kg/m^2)			0.929			<0.001
<18.5	35 (10.3)	16 (9.2)		37 (13.3)	14 (6.0)	
18.5–24.9	250 (73.7)	129 (74.6)		218(78.1)	161 (69.1)	
25–29.9	54 (15.9)	28 (16.2)		24 (8.6)	58 (24.9)	
Gestational age of delivery			0.187			0.795
<40 weeks	237 (69.9)	111 (64.2)		191 (68.5)	157 (67.4)	
≥40 weeks	102 (30.1)	62 (35.8)		88 (31.5)	76 (32.6)	
Previous vaginal birth or VBAC			0.225			0.242
Yes	28 (8.3)	20 (11.6)		30 (10.8)	18 (7.7)	
No	311 (91.7)	153 (88.4)		249 (89.2)	215 (92.3)	
Hypertension in pregnancy			0.104			0.048
Yes	36 (10.6)	27 (15.6)		27 (9.7)	36 (15.5)	
No	303 (89.4)	146 (84.4)		252 (90.3)	197 (84.5)	
Diabetes in pregnancy			0.509			0.004
Yes	42 (12.4)	18 (10.4)		43 (15.4)	17 (7.3)	
No	297 (87.6)	155 (89.6)		236 (84.6)	216 (92.7)	
Induction of labor			0.292			<0.001
Yes	70 (20.6)	29 (16.8)		37 (13.3)	62 (26.6)	
No	269 (79.4)	144 (83.2)		242 (86.7)	171 (73.4)	

GWG, gestational week gain. BMI, body mass index. VBAC, vaginal birth after cesarean section.

more likely undergo a successful TOLAC if they were nonobese ($p < 0.001$), had a previous vaginal birth or VBAC ($p = 0.008$), had spontaneous labor ($p = 0.010$), and did not have hypertension in pregnancy ($p = 0.004$) (Table 4).

After adjusting for pre-pregnancy body mass index, induction of labor, previous vaginal birth or VBAC, hypertension in pregnancy, and other potential confounders, women who gained excessive weight after 28 weeks had significantly lower odds of vaginal birth than those who did not (Adjusted odds ratio [aOR] 0.31, 95% CI 0.20–0.48); however, excessive weight gain before 28 weeks had no significant effect on the VBAC rate (aOR 0.81, 95% CI 0.52–1.23) (Table 5).

Table 6 shows the association between GWG after 28 weeks and the VBAC rate stratified by GWG before 28 weeks. We found that among women with excessive or non-excessive GWG before 28 weeks, the non-excessive GWG after 28 weeks group had less risk of failed TOLAC than the excessive GWG after 28 weeks group (aOR 0.27, 95% CI 0.12–0.60; aOR 0.32, 95% CI 0.19–0.55; respectively).

The aORs from the multivariable logistic regression models stratified by pre-pregnancy BMI are shown in Figure 1. We found that excessive gestational weight gain after 28 weeks was associated with a lower success rate of TOLAC than non-excessive weight gain in underweight, normal weight, and overweight women (aOR 0.23, 95% CI 0.06–0.88; aOR 0.42, 95% CI 0.25–0.70; and aOR 0.12, 95% CI 0.04–0.36; respectively). Gestational weight gain before 28 weeks was not associated with VBAC in any BMI category in this study.

4. Discussion

Among Chinese women in this study, excessive GWG after 28 weeks was linked to a higher risk of failed TOLAC among women with or without excessive GWG before 28 weeks. Compared with non-excessive GWG, excessive GWG after 28 weeks also reduces the VBAC rate among underweight, normal, and overweight women.

We found that nearly half of the women gained more weight than recommended after 28 weeks (45.5%), which was higher than that before 28 weeks (33.8%), contrary to the findings of a previous study which showed the GWG between the second and third trimesters was approximately the same in the underweight, normal, and overweight groups (28). Overweight women overweight women had a higher rate of excessive weight gain after 28 weeks of gestation, and other studies have shown that overweight and obese women are more likely to gain

TABLE 4 Univariable analysis of association between maternal characteristics and TOLAC.

Variables	Vaginal birth (%) $n = 364$, 71.1%	Cesarean (%) $n = 148$, 28.9%	*P*-value
Maternal age (y)			
<35	283 (77.7)	108 (73.0)	0.249
≥35	81 (22.3)	40 (27.0)	
Maternal height (cm)	162.38 ± 5.12	162.47 ± 5.04	0.857
Pre-pregnancy BMI (kg/m2)			
<18.5	34 (9.3)	17 (11.5)	<0.001
18.5–24.9	297 (81.6)	82 (55.4)	
25–29.9	33 (9.1)	49 (33.1)	
Gestational age of weight measured (at 28 weeks)	28.20 ± 0.6	28.23 ± 0.61	0.588
Previous Vaginal birth or VBAC			
Yes	42 (11.5)	6 (4.1)	0.008
No	322 (88.5)	142 (95.9)	
Labor induction			
Yes	60 (16.5)	39 (26.4)	0.010
No	304 (83.5)	109 (73.6)	
Gestational age of delivery			
<40 weeks	243 (66.8)	105 (70.9)	0.357
≥40 weeks	121 (33.2)	43 (29.1)	
Hypertension in pregnancy			
Yes	35 (9.6)	28 (18.9)	0.004
No	329 (90.4)	120 (81.1)	
Diabetes in pregnancy			
Yes	45 (12.4)	15 (10.1)	0.477
No	319 (87.6)	133 (89.9)	

BMI, pre-pregnancy body mass index; TOLAC, trial of labor after cesarean; VBAC, vaginal birth after cesarean section.

too much weight during pregnancy than women with a normal BMI (20, 29). We also revealed that women who exceeded the IOM recommended weight gain after 28 weeks had a higher likelihood of having induced labor and gestational hypertension, which is consistent with previous studies (20, 23). Interestingly, we found that women with diabetes in pregnancy tended not to exceed the IOM recommendations for GWG after 28 weeks. This result is consistent with previous studies, Shi et al. also found that less than the recommended amount of weight gain by the IOM was observed in more than one-third of the pregnant women who had GDM (17). A potential explanation for this finding is that the women with GDM who received the diagnosis between the 24th and 28th week of pregnancy managed to lower their blood glucose levels and curb their excessive weight gain by following a healthy diet and engaging in physical activity (30). Moreover, women who were prone to GDM were more likely to receive education about appropriate weight gain throughout pregnancy during prenatal care, in order to reduce the occurrence of known pregnancy complications, such as preeclampsia and fetal macrosomia. Therefore, these women might pay more attention to their dietary habits, have more physical activity, and ultimately have less GWG than women without GDM (31).

The overall chance of a successful TOLAC was 71.1%. Previous findings found that women who had delivered vaginally had a higher chance of vaginal birth (30). Our study also confirmed most prior reports that abnormal pre-pregnancy weight is linked to an increased rate of failed TOLAC (32, 33). Shi and his colleagues performed a retrospective cohort study that encompassed all TOLAC cases in the US from 2012 to 2020 to determine if overweight women were significantly less likely to undergo TOLAC (32). Herman and his colleagues found a link between maternal BMI and TOLAC utilization in a trial involving 536 pregnant women (33). In addition, consistent with previous studies (34, 35), we found that induction of labor and gestational hypertension were associated with failed TOLAC.

There is limited research on GWG and TOLAC outcomes. The likelihood of successful VBAC was reported to be 40% lower in patients who gained over 40 pounds by Juhasz and his colleagues (20), and Shi Hanxu and his team also found that weight gain exceeding 20 pounds during pregnancy was linked to a lower VBAC rate (32). In contrast, Jenny and her colleagues did not observe any effect of excessive GWG on TOLAC success rates (19). However, these studies did not performed a stratified analysis by pre-pregnancy BMI and gestational stage, which are potential modifiers of the relationship

TABLE 5 Multivariable logistic regression of GWG and VBAC.

Variables	Vaginal birth (%) $n = 364$	Cesarean (%) $n = 148$	*P*-value	Adjust† OR	95% confidence internal
GWG Before 28 weeks (IOM category)			0.272		
Not excess	245 (67.3)	94 (63.5)		ref	-
Excessive	119 (32.7)	54 (36.5)		0.81	0.52–1.23
GWG After 28 weeks (IOM category)			<0.001		
Not excess	232 (63.7)	47 (31.8)		ref	-
Excessive	132 (36.3)	101 (68.2)		0.31	0.20–0.48

GWG, gestational week gain; IOM, Institute of Medicine. †Adjusted for pre-pregnancy body mass index, induction of labor, hypertension in pregnancy, previous Vaginal birth. All of which were significant at the $p < 0.05$ level with VBAC in univariable analysis.

TABLE 6 Associations of GWG after 28 weeks with VBAC stratified by GWG before 28 weeks.

GWG before28weeks (IOM category)	GWG after 28 weeks (IOM category)	Vaginal birth *n* (%)	Cesarean *n* (%)	*p*	Crude OR (95% CI)	Adjust† OR (95% CI)
Not excess	Not excess	33 (35.1)	165 (67.3)	<0.001	ref	ref
	Excessive	61 (64.9)	80 (32.7)		0.26 (0.16–0.43)	0.32 (0.19–0.55)
Excessive	Not excess	14 (25.9)	67 (56.3)	<0.001	ref	ref
	Excessive	40 (74.1)	52 (43.7)		0.27 (0.13–0.55)	0.27 (0.12–0.60)

GWG, gestational week gain; IOM, Institute of Medicine; OR, odds ratio; 95%CI, 95% confidence internal. †Adjusted for pre-pregnancy body mass index, induction of labor, hypertension in pregnancy, previous Vaginal birth.

between GWG and TOLAC outcomes. Our results revealed that excessive GWG after 28 weeks was association with failed TOLAC. Further analysis found that increased risk of failed TOLAC was linked to excessive GWG after 28 weeks among women with or without excessive GWG before 28 weeks. Inconsistent with our study findings, IS et al. (24) suggested that trimester-specific GWG was not significantly associated with cesarean delivery in general outside the framework of TOLAC.

Two population-based studies analyzed the association between GWG and cesarean section stratified by pre-pregnancy BMI in pregnant women generally and arrived at different conclusions. Xu and his colleagues conducted a population-based cohort study with 174,953 singleton pregnancies and found that high GWG was associated with a higher overall risk of cesarean delivery for all BMI groups (36). On the other hand, a population-based cohort study which included 245,526 singleton term pregnancies, suggested that high weight gain during pregnancy increased the risk of cesarean delivery for obese women (37). In our study, we found that excessive GWG after 28 weeks was associated with a higher chance of cesarean delivery for all BMI groups (underweight, normal weight, and overweight), whereas the vaginal delivery was not influenced by excessive weight gain before 28 weeks.

The associations between excessive GWG after 28 weeks and the increased risks of failed TOLAC in our study might owing to several mechanisms. Excessive GWG after 28 weeks is positively related to pre-pregnancy overweight, induction of labor, and hypertension in pregnancy, which in turn could contribute to the increased risks of failing TOLAC (20, 23, 29). Moreover, excessive GWG may impair uterine contraction by increasing the amount of fat and cholesterol in the uterine muscle, resulting in reduced uterine contractility, longer labor duration, and more cesarean deliveries (38). However, it is unknown if the distribution of excessive fat differs before and after 28 weeks of gestation. Since fat distribution is an important factor in uterine contraction, whether excessive weight gain in the third trimester is more likely to lead to a relative increase in the distribution of fat in the uterus requires further research.

Some limitations of our study should be acknowledged. First, our study was conducted in a single center and excluded obese women, which may affect the applicability of our findings to other populations. Second, this was a retrospective study, which may lead to selection bias. It is possible that women with a high pre-pregnancy BMI were counseled toward choosing elective repeat cesarean delivery, and this may be one of the reasons why there were so few obese people in this study. Third, due to data limitations, we were unable to perform further analyses based on metabolic-related laboratory indicators (blood glucose, hemoglobin, etc.) and physical examination indicators (abdominal circumference, subcutaneous fat, etc.). Fourth, although our analysis stratified by different pre-conception BMI groups, there may be other unknown confounding variables which may influenced VBAC we did not account for in our analysis. Moreover, we did not further analyze women with gestational diabetes, such as whether exercise would affect their weight. Therefore, more studies are needed to explore this issue in depth in the future.

5. Conclusion

To sum up, our findings showed that regardless of pre-pregnancy weight status and weight gain before 28 weeks, excessive weight gain after 28 weeks of pregnancy was associated with decreased rates of VBAC. This finding suggests the importance of weight management in the third trimester to improve the chance of VBAC, especially in

overweight women, who were more likely to gain excessive weight after 28 weeks. Our results suggest that women can increase their likelihood of achieving VBAC by managing their weight during pregnancy. Based on our results, we recommend that TOLAC women should be counseled to avoid excessive GWG after 28 weeks, no matter their pre-pregnancy BMI, to increase their possibility of VBAC success.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The study was approved by the Ethics Committee of the Fourth affiliated Hospital of Hebei Medical University (2022KS010). The patients/participants provided their written informed consent to participate in this study.

Author contributions

GL and HS conceived and designed the study and drafted the manuscript. GL, JZ, and HZ collected the clinical data. GL and CZ analyzed the data. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Potential molecular targets for intervention in pelvic organ prolapse

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Pelvic organ prolapse (POP) is a concerning gynecological benign illness in middle-aged and senior women. Its etiology is complex, the incidence rate is high, symptoms are clinically subjective, and its influence tends to be polarized. At present, for those who need medical treatment, whether surgical or non-surgical, complications cannot be ignored, and treatment effect needs to be optimized. However, there is a lack of accurate molecular biological interventions for the prevention, diagnosis, progression delay, and treatment of POP. Here, we reviewed the current state of understanding of the molecular mechanisms and factors associated with POP etiology. These factors include cyclins, matrix metal peptidases/tissue inhibitors of metalloproteinases, microRNAs, homeobox A11, transforming growth factor β1, insulin-like growth factor 1, fibulin 5, lysyl oxidase-like 1, oxidative stress, inflammatory response, estrogen, and other potential biomarkers associated with POP. In addition, relevant molecular targets that may be used to intervene in POP are summarized. The aim of this review was to provide more information to identify accurate potential biomarkers and/or molecular targets for the prevention, diagnosis, progression delay, and treatment of POP, with the goal of improving medical treatment for patients at-risk for POP or having POP. Continued research is needed to identify additional details of currently accepted molecular mechanisms and to identify additional mechanisms that contribute to POP.

KEYWORDS

potential biomarkers, intervention, pelvic organ prolapse, micro-mechanism, molecular targets

1. Introduction

The current understanding of the pelvic organ prolapse is mostly based on the integral theory of the pelvic floor proposed by Petros and Ulmsten, the theory of "Levels of Support" proposed by Delancey, and the theory of "Boat in dry dock" proposed by Norton (1–3) (Figures 1–3), Pelvic organ prolapse (POP) is considered to be a disease of pelvic floor defects caused by vulnerability of the support structure owing to diverse factors, and then leads to the decline and displacement of pelvic organs, resulting in anomalous anatomical location and dysfunction (4). The etiology of POP is complex and diverse, and it is generally divided into microfactors, such as cyclin, matrix metal peptidases/tissue inhibitors of metalloproteinase (MMPs/TIMPs), and microRNAs (miRNAs); and macrofactors, such as age, vaginal delivery, obesity, and chronic respiratory diseases (4, 5). Despite extensive research on the etiology of POP, it has not been fully clarified. In addition, the clinical symptoms and effects of POP are diverse and tend to be generally polarized. Many women show asymptomatic POP, which may

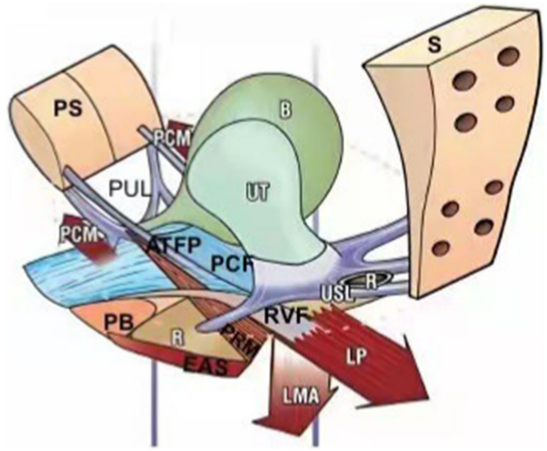

FIGURE 1
In 1990, Petros and Ulmsten first proposed the integral theory of the pelvic floor. It points out that different levels of vaginal support axis in different compartments together constitute an anatomical and functional organic whole, and weakening any structure will lead to the imbalance of the whole function, resulting in pelvic floor dysfunction disease. PS, pubic symphysis; PUL, pubourethral ligament; PCM, pubococcygeus muscle; ATFP, arcus tendineus fascia pelvis; PB, perineal body; PCF, pubocervocal fascia; EAS, external anal sphincter; PRM, pubic rectum muscle; RVF, rectovaginal fascia; LMA, longitudinal muscle of anus; LP, levator plate; USL, uterosacral ligament; S, sacrum; R: rectum; B, bladder; UT, uterus.

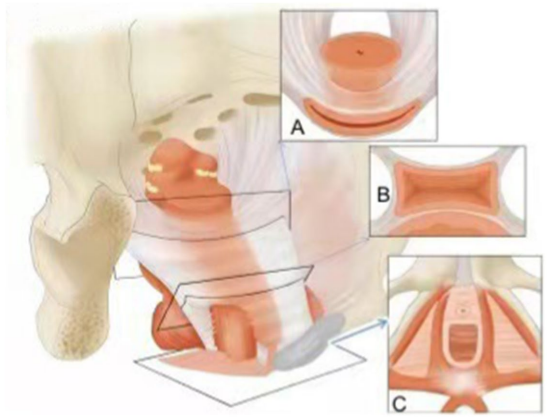

FIGURE 2
In 1994, theory of "Levels of Support" was proposed by Delancey, level 1 **(A)** is the upper supporting structure (main ligament-uterine low ligament complex); level 2 **(B)** is the lateral supporting structure (levator ani muscle group and bladder, rectovaginal fascia), level 3 **(C)** is the distal supporting structure (perineum and sphincter).

only be detected *via* a routine gynecological physical examination; such cases are not considered pathological and life and work are unaffected (5). However, symptomatic patients with POP may experience abnormal pelvic pressure, vaginal prolapse, dysfunctional urination and defecation, and sexual dysfunction (4, 5). Some patients have other complications, such as tissue ulcers, bleeding, and infection, which seriously affect quality of life and mental health (4, 5). It is reported that about 40% of women will suffer from pelvic organ prolapse (4), and this is predicted to climb as the population ages. By 2050, the number of women suffering from POP in the United States is projected to increase by approximately 50% (5). The total number of women undergoing POP surgery is anticipated to increase from 166,000 in 2010 to 245,970 in 2050 in United States (6). Besides, complications of POP cannot be ignored. At present, clinical treatment of POP can be classified as nonsurgical or surgical. Nonsurgical treatment may have complications such as tissue ischemia, necrosis or fistula formation, incarceration, bleeding, and infection (5), whereas complications from surgical treatment include mesh erosion, lower urinary tract symptoms, sexual dysfunction, and recurrent prolapse (5, 7). These phenomena cannot be ignored, and the therapeutic effect needs to be optimized. There remains a lack of accurate molecular biological intervention for POP. In this study, we reviewed and summarized the current knowledge of the molecular mechanisms of POP etiology associated with cyclins, MMPs/TIMPs, miRNAs, homeobox A11 (HOXA11), transforming growth factor β1 (TGF-β1), insulin-like growth factor 1 (IGF-1), fibulin 5 (FBLN5), lysyl oxidase-like 1 (LOXL1), oxidative stress, inflammatory response, estrogen, and biomarkers (Figure 4). This review aimed to clarify POP pathogenesis and provide more accurate potential biomarkers and intervention targets for the prevention, diagnosis, progression delay, and treatment of POP. However, as other, yet unknown, mechanisms may also lead to POP, further research is required in this field (Figures 1, 2).

2. Cyclin

Some cell cycle regulatory proteins are involved in the metabolism of collagen and other extracellular matrix (ECM) proteins, resulting in POP. For example, p53 is a tumor suppressor protein that monitors whether cells should continue their cell cycle, and p21, an inhibitor of G1 cyclin-dependent kinase, which regulates the initiation and progression of the cell cycle. By regulating p21, p53 inhibits the abnormal high-level proliferation and growth of cells in the ECM (8, 9). In one study, the expression of p53 and p21 decreased in prolapsed fibroblasts, and thus cells could not enter the inactive period, but they could enter the S phase from the late G1 stage (9). This led to decreased elastin synthesis and deposition, resulting in weakness or even loss of the supporting function of the pelvic connective tissue. Similarly, another study (10) on cell aging showed that the protein level of p53 was significantly lower in main ligament fibroblasts in patients with uterine prolapse compared with that of the control group,. The decrease in p53 protein expression in prolapsed fibroblasts may lead to higher proliferation activity and decreased synthesis and deposition of ECM components; the functional changes in supporting ligament fibroblasts were related to the mechanism of uterine prolapse. Further research (11) showed that p53 and p21 were lower in the uterosacral ligament (USL) of patients with POP than in non-POP patients, and that the levels of the two protein were positively correlated. This low expression is believed to lead to abnormal fibroblast proliferation in the pelvic support system, reduce the synthesis of elastin and other ECM components that should be secreted during the inactive period, and decrease connective tissue in support structures, such as the USL, which is considered to be linked to the appearance of POP. Furthermore, low expression of p53 in the USL increased the risk of uterine prolapse (POP-Q stage III-IV) 20.25 times, suggesting an effect on the metabolic balance of the ECM associated with the USL,

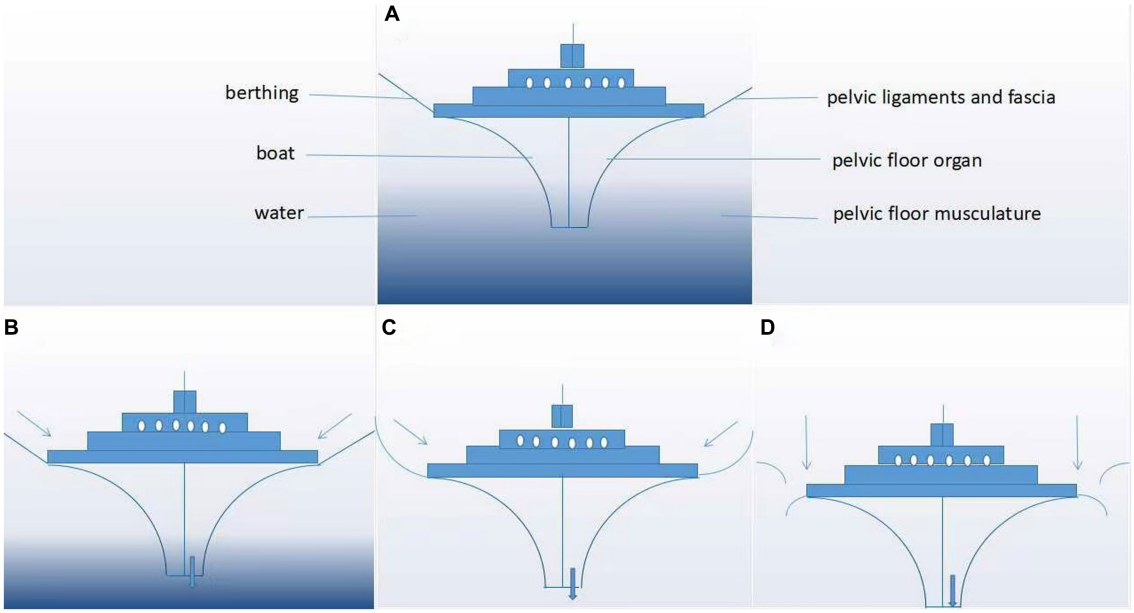

FIGURE 3
"Boat in dry dock" conception of pelvic floor disorders. As shown in panels **(A–D)**, the boat represents the pelvic floor organ, its berthing represents the pelvic ligaments and fascia, and the water represents the pelvic floor musculature. **(A)** Normal pelvic floor tissues and **(B–D)** evolution of pelvic organs after progressive damage to the pelvic floor support tissue.

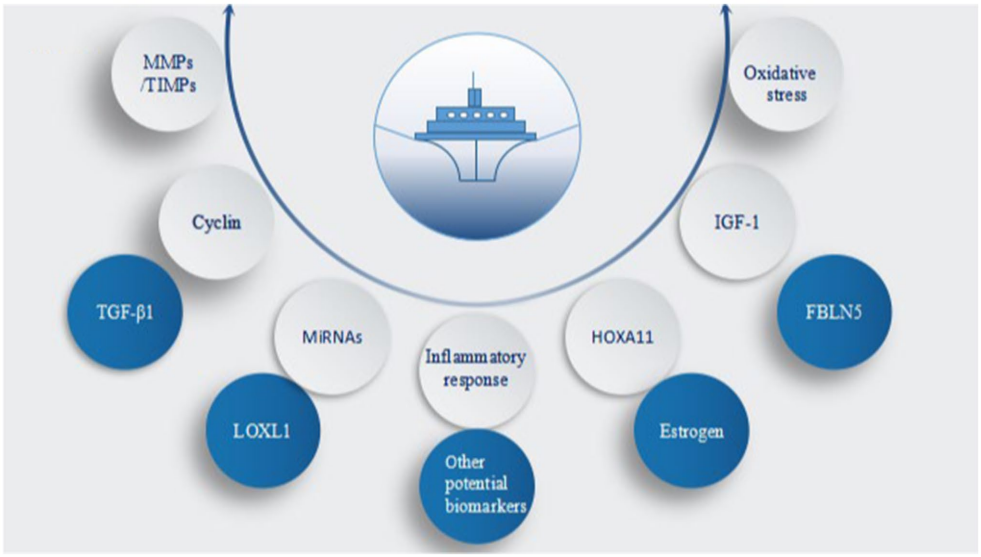

FIGURE 4
Core factors involved in pelvic organ prolapse (POP). MMPs/TIMPs, matrix metal peptidases/tissue inhibitors of metalloproteinases; TGF-β1, transforming growth factor β1; miRNAs, microRNAs; LOXL1, lysyl oxidase-like 1; HOXA11, homeobox A11; IGF-1, insulin-like growth factor 1; FBLN5, fibulin-5; oxidative stress, inflammatory response, estrogen, and other potential biomarkers.

resulting in insufficient elasticity of the ligament to support the pelvic organs (12). Therefore, through proper regulation of the expression of p53 and p21, the synthesis of ECM components such as elastin may be increased, and the macroscopic supporting structure of the pelvic floor may be strengthened, which would be beneficial for alleviating POP (Figure 3).

3. MMPs/TIMPs

MMPs are a family of zinc-dependent endopeptidases that participate in degrading a variety of ECM proteins, including collagen and elastin. TIMPs bind to MMPs in a 1:1 stoichiometric ratio, inhibiting MMP activity, and the MMP/TIMP ratio usually

decides the degree of decomposition of ECM proteins and tissue reshaping (13). According to one study (14), type I collagen expression decreased and MMP-1 expression increased in the USL of patients with POP compared with that of the control group, suggesting an association with POP regulation. Another study (15) showed that MMP-2 and MMP-9 was more likely to be elevated in patients with POP with low amounts of collagen, indicating that an increase in these proteolytic enzymes is related to human POP disease. However, some different results were observed in other studies. For example, Gabriel et al. (16) discovered that when compared with the control, MMP-2 in the USL of patients with POP was enhanced, while MMP-1 was not. In contrast, another study (17) revealed that the expression of MMP-1 increased in patients with POP, but no difference in MMP-2 expression was found. Despite these differences in results, it is generally believed that an increase in proteolytic enzymes is closely related to prolapse. Further research (18) showed that women with POP had higher MMP-2 and lower TIMP-2 mRNA and protein expression in the USL than that of women without POP. Thus, an increase in MMP-2 expression and a decrease in TIMP-2 expression results in an increase in the degradation of ECM, which may lead to pelvic floor tissue defects and ultimately contribute to POP. A subsequent study (19) showed that in patients with POP, the expression level of MMP-2 gelatinase activity and ADAMTS-2 (procollagen I N-proteinase; a disintegrin and metalloproteinase with thrombospondin motifs) was increased in pelvic floor tissue. In addition, MMP-12 protein expression was upregulated in patients with POP in the proliferative phase of the menstrual cycle, while expression of TIMP-1–4 genes and TIMP-1 protein, which antagonizes MMPs, was decreased. These results suggest that an imbalance in the MMPs/TIMPs system may lead to connective tissue defects, which may result in POP. Therefore, it seems feasible that blocking ECM degradation by downregulating MMPs/TIMPs could be used to interfere in the occurrence and progression of POP (Figure 4).

4. miRNAs

miRNAs are noncoding small RNAs belonging to the family of gene regulatory factors that affect many biological functions, including cell proliferation, differentiation, apoptosis, organ development, and aging, by regulating the translation of mRNAs (20, 21). Recently, miRNAs have been the focus of considerable research, including in association with POP. For example, one study (22) confirmed that, compared to patients without POP, miR-19-3p expression increased, whereas expression of type I collagen and IGF-1 decreased in patients with POP. miR-19-3p contributed to vaginal fibroblast autophagy and apoptosis, and inhibited the production of type I collagen in POP *via* the protein kinase B (Akt) /mTOR/p70S6K pathway by targeting IGF-1. Another study (23) demonstrated that, compared with non-POP groups, the expression level of miR-4,429 in human USL fibroblasts from patients with POP decreased, and that miR-4,429 overexpression reduced the increase in phosphatase and tensin homolog (PTEN) expression and fibroblast apoptosis. In addition, compared with the control group (pelvic organ prolapse quantitation POP-Q < stage II), the expression of miR-30d and miR-181a in the USL of the POP group (POP-Q ≥ stage II) significantly increased (24), which shows that these miRNAs are related to POP. Both of these miRNAs are important post-transcriptional regulators of HOXA11, and abnormal expression of these miRNAs may also contribute to the pathogenesis of POP through pathways other than HOXA11 dysregulation. It can be seen that the decrease in miR-19-3p expression and the overexpression of miR-4,429 may lower the occurrence of POP by regulating the proliferation, differentiation, and apoptosis of fibroblasts. However, the decrease in miR-30d and miR-181a will negatively affect POP. These miRNAs may be used as biomarkers or potential molecular targets for clinical monitoring and intervention of POP.

5. HOXA11

HOXA11 is a transcription regulator that influences the development of urogenital embryos and the USL of mice and humans. POP is related to a decrease in HOXA11 and type III collagen expression, and an increase in MMP-2 expression in humans. In a mouse model with *HOXA11* deficiency, *in vitro* studies verified that The directional deletion of *Hoxa11* in mice led to an absent development of the USL. In addition, expression of HOXA11 decreased MMP-2 and increased type III collagen in the mouse fibroblasts, which benefitted collagen synthesis over degradation (25). A signal conduction defect in HOXA11 may lead to functional development or repair defects in the USL, which will alter the biomechanical strength of the USL and lead to the development of POP. Furthermore, the cytoarchitecture and smooth muscle content of the USL in women with prolapse were both reduced compared with that of women with normal pelvic support (26, 27). In addition, the number of cells in the USL of women with POP was reduced, as was the expression of HOXA11. HOXA11 can promote the proliferation of mouse fibroblasts and primary human USL cells *in vitro*, indicating that the decrease in HOXA11 in the USL of women with POP leads to a decrease in cell proliferation. Moreover, the expression of HOXA11 not only increased cell proliferation, but also decreased p53 expression, indicating that HOXA11 is involved in the regulation of the p53 inhibiting signal transduction pathway, promoting cell proliferation, and possibly reducing apoptosis (28). Using a Hoxa11-knockout (KO) model, the expression levels of type I and type III collagen and TIMP1 in the USL were shown to be significantly decreased. Meanwhile, the levels of pro-MMP-2, pro-MMP-9, and activated MMP-2 increased (29). These results indicate that Hoxa11-KO enhanced ECM degradation by regulating the expression level of the MMP/TIMP system, which is the likely mechanism by which the female pelvic floor support weakens. In addition, HOXA11 and TGF-β1 have a synergistic effect on the expression levels of collagen and MMPs (30), which jointly promote the synthesis of collagen, inhibit its degradation, and help inhibit POP. The decrease in ECM caused by the reduction of HOXA11 and TGF-β1 is a key factor in POP. Therefore, we speculate that the expression of HOXA11 can not only promote fibroblast proliferation by regulating the cell cycle, but also increase the ECM by regulating MMP/TIMP, which hinders the occurrence and progression of POP. Furthermore, the synergistic effect of HOXA11 and TGF-β1 also helps prevent the occurrence of POP. HOXA11 may be a potential biomarker for POP intervention.

6. TGF-β1

TGF-β1 is a profibrotic cytokine that is widely involved in fibrosis and degenerative fibrosis disease. The cytokine can induce fibroblast differentiation in cells from different tissues, leading to ECM deposition and secretion of paracrine and autocrine growth factors; moreover, TGF-β1 is important for fibroblast proliferation and ECM metabolism (31). However, in previous studies, the role of TGF-β1 in POP differed. Qi et al. (32) reported that TGF-β1 in pubic cervical fascia was negatively correlated with POP. In contrast, Mijerink et al. (33) reported that TGF-β1 in the vaginal wall was positively correlated with POP, while Leegant et al. (34) did not find any difference in TGF-β1 expression between USL samples (POP-Q ≥ II) and non-POP controls. However, in recent years, TGF-β1 has been shown to be negatively correlated with POP or its stages (30, 35–38). In one study (35), excessive mechanical stress and H2O2 treatment of USL fibroblasts reduced the level of TGF-β1, which was proven to reduce cell proliferation and ECM components, while increasing the ratio of MMP-2/TIMP2 mRNA. This indicated that the TGF-β1 signaling pathway could affect ECM remodeling by disrupting the MMP/TIMP balance. Another study (36) showed that TGF-β1 pretreatment could stimulate TIMP-2 synthesis and inhibit MMP-2/9 activity through the TGF-β1/Smad3 signaling pathway, reducing the loss of ECM in POP USL fibroblasts subjected to excessive mechanical stress. A recent study (37) showed that the expression of TGF-β1 was similar between symptomatic patients with POP and the controls, who did not show any signs of prolapse. Compared with moderate/mild cases, TGF-β1 was more commonly expressed in severe prolapse, suggesting its association with the progression of POP (i.e., repair after injury). Another recent study (38) showed that the expression of phospho-p44/42 and TGF-β1 declined in patients with POP than without POP and it was positively related to collagen expression; the low-level of expression was deemed to be linked to the presence of POP. In addition, further study (39) proposed that the crosstalk between calpain and TGF-β1 activated the TGF-β1 Smad2/3 and non-Smad (Akt) pathways, enhancing type I collagen synthesis in human lung fibroblasts and pulmonary fibrosis. This may also be one of the mechanisms of POP. According to the above research findings, TGF-β1 may have a role in the occurrence and/or progression of POP by negatively regulating collagen synthesis and interfering with ECM metabolism. However, based on some differences in the findings of the above studies, further research is needed to clarify its molecular biological mechanism.

7. IGF-1

IGF-1 belongs to the insulin-like growth factor family, which includes insulin-like polypeptides mainly synthesized by the liver. IGF-1 can regulate ECM metabolism and various biological processes, such as cell proliferation, differentiation, and apoptosis (40, 41). One study (40) have shown that IGF-1 levels in vaginal wall tissues were lower in patients with POP than in non-POP controls, and it induced the proliferation of vaginal wall fibroblasts, activated mitogen-activated protein kinase (MAPK) and nuclear factor-κ-gene binding (NF-κB) pathways, promoted the metabolism of type I and III collagen another study (42) reported that IGF-1 can be used as an inhibitor of apoptosis and it may also stimulate fibroblasts to release ECM molecules, such as polysaccharides and proteins. Further study (22) proposed that, the expression of IGF-1 decreased in the vaginal wall of patients with POP, and it inhibited autophagy and apoptosis and promoted expression of type I collagen, affecting the metabolism of the ECM by activating the Akt/mTOR/p70S6K pathway in vaginal fibroblasts. In all, IGF-1 may stimulate the proliferation of fibroblasts; activate Akt/mTOR/p70S6K, MAPK, and NF-κB pathways; promote collagen synthesis; and block the occurrence and progression of POP.

8. FBLN5 and LOXL1

FBLN5 is a calcium-dependent elastic fiber-related protein belonging to the short fibrin family (43). LOXL1 belongs to the family of lysyl oxidases, and it activates tropoelastin through specific localization and binding with the fibrin-5 domain. Tropoelastin is converted to mature elastin through covalent crosslinking, which is crucial for the synthesis and assembly of elastic fibers (44). However, as discussed here, studies on FBLN5 and LOXL1 in humans have produced conflicting results. Kluetke et al. (45) measured the elastin protein content in the USL using western blot analysis and LOXL1 and FBLN5 mRNA levels using real-time quantitative polymerase chain reaction (RT-qPCR). They found that compared with women with normal pelvic support, the level of LOXL1 in the USL biopsy of the POP group (POP-Q ≥ III) was reduced, while that of FBLN5 was increased, and the elastin content decreased significantly. In a similar study using the same techniques, Jung et al. (46) found that the mRNA and protein expression levels of FBLN5 significantly decreased in the advanced POP (POP-Q III-IV) group, while that of LOXL1 increased compared with that of the non-prolapsed group. Although both authors used similar tissue samples and detection techniques, they observed opposite results. Kluetke et al. (45) surmised that the increased expression of FBLN5 might be a secondary effect of tissue injury in patients with POP; it has been shown that the level of fibrin-5 mRNA increases when lung tissue is injured by elastase (47). Jung et al. (46) interpreted the increase in LOXL1 expression as a compensatory mechanism secondary to abnormal crosslinking, although the structural disorder of elastin has not been studied. Recently, Garcia et al. (48) used western blotting and an enzyme-linked immunosorbent assay (ELISA) to quantify LOXL1 and FBLN5 protein expression in vaginal secretions of women with and without POP; LOXL1 protein expression was higher in patients with POP, while the expression of FBLN5 did not significantly differ between the two groups. The increase in LOXL1 expression was believed to reflect a compensatory mechanism in women with POP. This also seems to be in agreement with the findings in most studies that FBLN5 is reduced in POP, although the statistical significance has not been demonstrated. In another study (49), immunohistochemical staining revealed a decrease in expression of FBLN5 and LOXL1 in abdominal hysterectomy ligament samples of the POP group (POP-Q ≥ II) compared with the control group, suggesting that this low expression may be important in weakening the supporting structure of the pelvic floor. Similarly, Takacs et al. (50) showed that FBLN5 mRNA and protein levels were significantly lower in women with anterior vaginal wall prolapse than in women without anterior vaginal wall prolapse, and this low expression was considered to be involved in POP. Alarab et al. (51) reported that LOXL1 mRNA and protein expression in the vaginal tissue of POP group was lower compared with that in

asymptomatic control group, which may have led to assembly defects in the pelvic tissue. In addition, a large number of animal model experiments have also been carried out to help elucidate the possible role and related mechanism of FBLN5 and LOXL1 in POP, as discussed below.

8.1. FBLN5

A previous study (52) showed that compared with wild-type (WT) mice, *Fbln5*-deficient mice exhibited the same phenotype as that of women with POP, such as descending and extending vagina and cervix, bulging vaginal wall, increased genital hiatus, and bulging bladder in some cases. As young as 3 months old, virgin *Fbln5*-KO mice developed a bulging urogenital system. By 6 months, 92% (33/36 cases) of the female *Fbln5*-KO mice had POP. Severe prolapse occurred in mice aged ≥6 months. In this case, a defect in *Fbln5* was thought to be related to POP. In addition, in *Fbln5*-KO mice, pelvic floor suspension connective tissues, such as the USL, were either missing or stunted. Moreover, in the older (6 months old) *Fbln5*-KO mice, the USL in females was either missing or weakened, indicating that FBLN5 is related to defective congenital development, weakness, and acquired repair of the pelvic floor support structure. Another study (53) showed that compared with WT mice, approximately 90% of *Fbln5*-KO mice prolapsed with age. Compared with non-pregnant mice, *Fbln5*-KO prolapsed mice showed biomechanical changes, such as decreased hardness, decreased maximum load, and increased expansibility, indicating that impaired elastin function and pelvic floor biomechanical changes led to prolapse. In addition, compared with those in WT mice, MMP-9 and MMP-2 levels were enhanced in the vaginal tissues of mature *Fbln5*-KO mice, the pelvic organ support deteriorated progressively, and 90% of *Fbln5*-KO mice prolapsed at the age of 6 months (54). Notably, the lack of *Fbln5* expression in the vaginal wall not only contributed to genetic defects in the synthesis and assembly of elastic fibers, but it also led to increased protease activity and elastin decomposition, which inhibited the repair or synthesis of new elastic fibers. This was also believed to be the cause of the failure of matrix regeneration in connective tissues supporting the pelvic floor and the occurrence of POP. By negatively regulating the interaction of β1 integrin and fibronectin in the vagina of mice, FBLN5 inhibited the pro-MMP-9 and active MMP-9, increased the density of elastic fibers, and improved collagen fibers, which was not conducive to POP (15). In addition to the upregulation of MMP-2 and MMP-9, serine protease inhibitors (serpina1a [a1-antitrypsin] and elafin) were reduced in vaginal tissues of POP and were dysregulated in the epithelium of *Fbln5*-deficient mice. MMP-9 and a trypsin-like serine protease were upregulated in the *Fbln5*-KO mice. PRSS3, a major extra-pancreatic trypsinogen, is expressed in human vaginal tissues (55). It is suggested that other proteases and protease inhibitors are also involved in POP, and the balance between proteases and their inhibitors may provide insight into POP in humans and mice. The histological changes were further verified, for example, in *Fbln5*-deficient mice (55), no change was seen in the size of perineal eminence over the course of the gravidity. However, elastic fiber breakage and inflammatory infiltration appeared in the vaginal wall at the beginning of the postpartum period (2–24 h). The seriousness of POP increased nearly 1 week after delivery, which further showed that the deletion of *Fbln5* contributed to failed repair of the birth-related injury. A study of vaginal dilation using a balloon to simulate labor (56) showed that the activities of MMP-2 and MMP-9 increased in the vaginal wall of non-gravid and gravid animals, with noticeable fragmented and broken elastic fibers in the vaginal wall. Compared with WT mice, vaginal dilation led to accelerated POP in non-pregnant *Fbln5*-KO mice, which never recovered. Similar to the results of previous studies, it can be seen that FBLN5 is also important for the protection and recovery of labor- and elastase-induced prolapse. In addition, another study of vaginal dilatation-simulated childbirth (57) showed that the levels of markers(p53 and γ-H2Ax) of cell senescence decreased in WT mice 1 week after distention, but not in *Fbln5*-KO mice. This suggests that WT mice can carry out cell proliferation and repair 1 week after injury; however, in *Fbln5*-KO mice, aging markers cannot be downregulated to repair tissues, leading to the accumulation of cell senescence and destruction of the ECM and connective tissue, which may be a potential injury mechanism of POP. Furthermore, PTK7 and β-catenin, which are involved in elastin production after vaginal mechanical expansion, were upregulated in WT mice, but not detected in *Fbln5*-KO mice, indicating that these proteins may also participate in POP in *Fbln5*-KO mice (58). In summary, FBLN5 deficiency seems to affect the pathology of POP by not only promoting an increase in markers of cell senescence and proteases/protease inhibitors, but also by reducing β1 integrin and fibronectin.

8.2. LOXL1

Liu et al. (59) showed that spontaneous pelvic floor disorder developed slowly in nulliparous Loxl1-KO mice. At the age of 1 year, approximately 50% of cases showed signs of pelvic organ decline, whereas WT animals did not show signs of POP or decline at 18 months. LOXL1 is associated with POP. Besides, POP occurs in mice with a *Loxl1* mutation after giving birth to either the first or second cub; however, no prolapse occurres in WT mice in the same age range (3–7 months). Parturition may be the most important trigger of POP in female *Loxl1*-KO mice. Lee et al. (60) also confirmed the above conclusion and reported that *Loxl1*-deficient mice could not rebuild standard elastic fibers during the reshaping of reproductive tract connective tissues after pregnancy and delivery, which contributed to POP. Liu et al. (61) showed that *Loxl1*-deficiency prevented deposition of normal elastic fibers in the uterus after delivery and demonstrated pathological manifestations of elastic fiber functional defects, such as POP, skin relaxation, and vascular abnormalities. Thus, LOXL1 plays an important part in the synthesis and assembly of elastic fibers in the process of pelvic floor injury repair. Alperin et al. (62) verified the change in mechanical properties in the vagina; compared with age-matched WT animals, *Loxl1*-deficient animals exhibited poor biomechanical properties of the vaginal supporting tissue complex. This characteristic was thought to be due to overall structural defects in the connective tissue rather than the loss of vaginal support itself. Morphometric analysis of elastic fibers in the cultivation of vaginal tissues demonstrated that compared with WT mice, the aspect ratio of elastic fibers in *Loxl1*-KO mice at 3 weeks of age was significantly smaller, proving that there was an increased number of shorter and broken elastic fibers (63). This showed that there was continuous elastic decomposition activity in the

culture tissue of *Loxl1*-KO cells, suggesting that both quantitative and qualitative facets of elastic fibers may be involved in the pathophysiology of POP. The results of a separate study (37) showed that compared with the levels in WT mice, the total and unit cell amounts of elastin and unit cell amount produced by non-epithelial vaginal cells in LOXL1-deficient mice were significantly reduced, while the ratio of MMP-9/TIMP-1 was relatively high. A recent study by Couri et al. (64) indicated that in contrast to WT mice, the mRNA levels of chemokine C-X-C motif ligand 12 and chemokine C-C motif ligand 7 (mediators of inflammatory response) were differentially upregulated in the tissues of virgin Loxl1-KO mice, and they were significantly upregulated in the vagina, urethra, bladder, and rectum of pregnant Loxl1-KO mice. However, in Loxl1-KO mice after vaginal childbirth, cytokines were expressed differently in terms of time, tissue, and concentration. Furthermore, the urethra and vagina may be especially susceptible to delivery injury. Based on the above studies, we concluded that LOXL1 deficiency may play a role in elastin synthesis and assembly by downregulating TGF-β1 activity and upregulating MMPs/TIMPs, chemokine C-X-C motif ligand 12, and chemokine C-C motif ligand 7 in POP. Moreover, LOXL1 deficiency seems to be particularly important in the repair process after an injury, such as a childbirth-related injury.

In summary, we can see that the results using animal models are consistent, suggesting that defects in Fbln5 and Loxl1 are involved in the pathological process of POP through different mechanisms. However, the different results reported for studies in humans may be related to the complexity of the structure and mechanisms at play in the human body, but differences in research design cannot be ruled out; thus, further research is needed.

9. Oxidative stress

Oxidative stress is caused by an imbalance in the oxidative and antioxidant defense system in cells, tissues, or organs, which leads to the accumulation of reactive oxygen species (ROSs), and oxidative damage of DNA, lipids, and proteins (65). The levels of oxidative stress biomarkers are higher in the pelvic floor of patients with POP than in control patients; these include isoprostanes (66), 8-hydroxy-2-deoxyguanosine (8-OHdG), 4-hydroxy-2-nonenal (4-HNE) (67), advanced glycation end-products (AGEs) (68–70), and mitofusin2 (Mfn2) (71–75). In contrast, the levels of antioxidant markers are lower in patients with POP than in control patients; these include glutathione peroxidase, superoxide dismutase (76), and nuclear factor erythroid-2-related factor 2 (Nrf2) (77). This may lead to damage of the pelvic floor tissue and contribute to the development of POP by regulating the MMP/TIMP balance (66) and inducing mitochondrial apoptosis (67) and other signal transduction pathways (68–79). One study (66) showed that the level of isoprostanes was higher in the fibroblasts of the main ligament and urine samples of women with uterine prolapse than in women without uterine prolapse, and MMP-2 mRNA expression in the main ligament of patients with uterine prolapse significantly increased. Oxidative stress is involved in POP, especially uterine prolapse, through direct regulation of the ECM or post-transcriptional regulation of MMP-2 by isoprostanes. Oxidative stress markers 8-OHdG and 4-HNE were markedly higher in the USLs of patients with POP (POP-Q III or IV stage) than in controls (67). In addition, a significant positive correlation was observed between oxidative stress markers and mitochondrial apoptosis markers in pelvic supporting connective tissue of patients with POP (67), indicating that oxidative stress weakens the pelvic-supporting tissue in patients with POP and suggesting a possible mechanism for mitochondrial apoptosis in the USL. Chen et al. (68) reported that the level of AGEs was higher in prolapsed tissues, while that of type I collagen was lower. Further experiments (69) showed that MMP-1 levels were higher in human vaginal fibroblasts of patients with POP than in controls. In addition, AGEs inhibited vaginal fibroblast proliferation in patients with POP and decreased the expression of type I collagen *via* receptor of advanced glycation end products (RAGE) and/or MAPK and nuclear factor-κB (NF-κB) pathways, which affects ECM metabolism and weakens the supporting structure of the pelvis. Vetusch et al. (70) showed that compared with the non-POP group, the anterior vaginal wall in the POP group exhibited a disordered normal myometrium structure and had upregulated AGE, extracellular signal-regulated kinase 1/2 (ERK1/2), Smad-2/3, MMP-3, and type III collagen in the myometrium. AGEs, ERK1/2, and Smads 2/3 may participate in the pathogenesis of POP. Mfn2 is a transcription product of oxidative stress and a crucial regulator of mitochondrial fusion and division, which is related to proliferation, apoptosis, and signal transduction (71, 72). The level of Mfn2 increased in USL fibroblasts obtained from patients with POP and decreased procollagen, and the increase in Mfn2 inhibited fibroblast proliferation and the cell cycle by regulating the Ras/Raf/ERK pathway (73–75). A decline in antioxidant defense ability is also involved in POP. Compared with the control group (POP-Q ≤ II stage), the expression of OHdG and 4-HNE in the main ligament in the POP group (POP-Q III-IV) were higher. However, the protein levels and enzyme activities of mitochondrial superoxide dismutase (MnSOD) and glutathione peroxidase 1 (GPx1) were lower. Compared with mild POP, the oxidative damage to pelvic-supporting ligaments in female patients with severe POP increased, while the antioxidant defense ability decreased. Thus, the accumulation of ROSs and a decrease in antioxidants may be involved in development of POP. In addition, Nrf2 and GPx are key transcription factors implicated in controlling the anti-oxidant defensive system (76). Lin et al. (77) analyzed discussed the expression of cyclooxygenase-2 (COX-2) and Nrf2/GPx3 in the lamina propria of the anterior vaginal tissue of women with or without POP. They showed that the arrangement of collagen fibers in the anterior vaginal wall was disordered and discontinuous in the POP group relative to the non-POP patients. The levels of Nrf2, GPx3, TIMP1, and type I and III collagen decreased significantly, while those of COX-2, prostaglandin E2, and MMP2 increased significantly in the POP group compared with those of the control group (77). These results demonstrated that oxidative stress and inflammation are closely related to POP. When exogenous H_2O_2 was used to treat primary cultured sacral ligament fibroblasts to establish the original oxidative stress cell model (78), it was concluded that oxidative stress might participate in the disorder of collagen metabolism by inhibiting the synthesis and metabolism of collagen or indirectly regulating MMPs, TIMPs, and TGF-β1. This had a negative effect on ECM production, destroying the pelvic floor support network, and likely participating in the pathophysiology of POP. Activation of the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt signaling pathway leads to the accumulation of ROSs, promotes the aging and apoptosis of fibroblasts in pelvic tissues and a decrease in type I collagen, and leads to the relaxation and dysfunction

of pelvic support (79). In addition, AGEs regulate the miR-4,429/PTEN/PI3K/Akt pathway, inducing apoptosis of human USL fibroblasts, which contributes to POP (23). Taken together, these studies indicate that oxidative stress leads to fibroblast apoptosis through complex molecular biological mechanisms and interferes with the metabolism of ECM, leading to the dysfunction of the pelvic floor support network. By monitoring oxidation and antioxidant markers in the pelvic floor support system, effective intervention programs may be formulated to block signaling pathways and ultimately prevent the pathophysiology of POP.

10. Microenvironment of the inflammatory response

In an examination of the inflammatory environment in the pelvic floor, patients with POP exhibited a higher level of inflammation in vaginal tissues than did controls with non-prolapsed tissues, which confirmed that the extensive changes in the inflammatory environment in the pelvic floor are part of the pathogenesis of POP (80). A further study (79) showed that COX-2, prostaglandin E2, and MMP-2 are more highly expressed in POP patients than in non-POP patients. The release and expression of inflammatory cytokines in the front vaginal wall in the POP group were greater than in the control group, which may affect collagen metabolization and lead to POP. In addition, the interaction of inflammation and oxidative stress further worsened the pelvic floor branch system. In an analysis (81) of severe anterior vaginal wall prolapse (AVP) tissue at the single-cell level, members of the FOS/JUN family, hyaluronan (HA) degradation genes, HA receptors, and collagen endocytic receptors (MRC1 and MRC2), which are thought to be related to the inflammatory response, were all upregulated in macrophages in POP samples. Moreover, IL18-CD48 pro-inflammatory cytokine interactions were discovered in fibroblasts and immune cells, and IL1B–IL1R1 inflammatory activators and their interactions were discovered in smooth muscle cells. These findings further support that inflammation is involved in POP and that the inflammatory microenvironment could be a key factor in POP intervention; however, further research is needed to enrich our understanding of the molecular biological mechanisms involved.

11. Estrogen

The role of estrogen in POP is controversial. It is generally believed that the high incidence of POP after menopause is due to the rapid decline in female estrogen secretion, the weak supporting structure of the pelvic floor, the downward displacement of pelvic organs, and the emergence of pelvic floor dysfunction (82, 83). However, a randomized, double-blind, placebo-controlled, multicenter study by Marschalek et al. (84) showed that there was no difference in subjective prolapse-related complaints over a 6 week period in patients using preoperative vaginal estrogen cream and placebo cream groups. This suggested that preoperative locally applied estrogen does not ameliorate prolapse-associated symptoms in postmenopausal women with symptomatic POP. However, the findings also indicated that longer observation time intervals may be needed. Jackson et al. (85) conducted a double-blind, placebo-controlled study for 6 months on postmenopausal women with stress urinary incontinence treated with estradiol valerate. The study showed that compared with the placebo control group, total collagen, mature crosslink histidinohydroxy lysino norleucine, AGEs, and non-fluorescent compound-1 (NFC-1) decreased in the periurethral biopsy tissues of the treated group. However, levels of pro-MMP-2 and immature crosslinked hydroxylysinoketo-norleucine increased significantly, while collagen type I/III ratios did not differ significantly between groups. These results suggested that estrogen therapy leads to increased protease activity, degradation of collagen and AGEs, and increased levels of immature protein. Furthermore, the results suggested that aged collagen degradation was only an initial reaction to estrogen stimulation.; a prolonged exposure interval may be needed to demonstrate the whole collagen content In an *in vitro* study (86), 17β-estradiol inhibited the proliferation of fibroblasts from the main ligament of patients with and without POP, but it was more evident in patients with POP. A decrease in fibroblast renewal may reduce the production of collagen, elastic fibers, and other ECM proteins, and weaken the supporting force of the main ligament, thereby contributing to POP. Erika et al. (87) reported that ongoing hormone treatment is highly associated with the descent of the rectal ampulla as well as Gh + Pb (genital hiatus + perineal body), as detected by ultrasound. Hormone therapy may increase rather than decrease the descent in pelvic organs. In contrast, Clark et al. (88) showed that after 5 months of estradiol treatment, collagen type I and III in pelvic floor connective tissue increased, and cystatin C, a proteinase inhibitor that prevents the degradation of collagen, also increased, suggesting that estrogen decreases the degradation of collagen by increasing cystatin C. Nunes et al. (89) showed that in a 30-day double-blind trial of estrogen and placebo in post-menopausal women, both with and without POP, the levels of hyaluronic acid and chondroitin sulfate at the top of the vagina were higher in women treated with estrogen than in those treated with placebo. Both hyaluronic acid and chondroitin sulfate are glycosaminoglycans, which are important components of the ECM. These results showed that estrogen increased the production of ECM. Other studies have shown that hyaluronic acid induces the vitality of fibroblasts and the production of collagen in the ECM (90, 91). In addition, estrogen applied topically for 6 weeks in postmenopausal women with POP, increased the thickness of epithelial and muscular layers of the vaginal wall at the macro-level and enhanced the synthesis of mature type I collagen at the micro-level (92). In contrast, type III collagen did not change significantly, resulting in an increase in the ratio of type I/III collagen and a decrease in the activity of the collagen-degrading enzyme MMP-9. Furthermore, 17β-estradiol inhibited expression of Mfn2 at the mRNA and protein level and increased fibroblast proliferation and procollagen 1A1/1A2/3A1 synthesis, while also increasing the expression of estrogen receptor and G protein-coupled receptor 30 in USL fibroblasts (93). A recent study (94) has shown that 17β-estradiol increased the protein and mRNA levels of anti-apoptosis poly-ADP-ribose polymerase (PARP1) and B-cell lymphoma-2 (Bcl-2). At the same time, the expression of estrogen receptor alpha, a target of poly-ADP-ribosylation of PARP1, was enhanced, and apoptosis and death of USL fibroblasts subjected to mechanical stress *in vitro* were reduced. In summary, there are significant differences in the efficacy of estrogen in pelvic floor support tissue, but further studies are needed to better understand the role of estrogen in the pathophysiology of POP.

12. Other potential biomarkers

Other potential biomarkers have been shown to be associated with POP. For example, Deng et al. (95) applied a non-targeted metabolomics approach using ultra-high performance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (UHPLC-Q-TOF-MS) to analyze and compare serum and urine of patients with POP with that of controls. And discovered that glycerophosphocholine, L-pyroglutamic acid, and inosine were increased while citrate was decreased in both serum and urine samples of patients with POP, which may be related to collagen synthesis or degradation, further lead to POP development This suggests that these six metabolites may be used as discriminatory POP biomarkers. Shama et al. (96) used capillary electrophoresis-tandem mass spectrometry (CE-MS) to study 17 amino acids in the pelvic connective tissue of patients with POP, and found that methionine, histidine, and glutamine levels were significantly higher in the POP group when comparing to their levels in none-POP group, and their increased levels could relate to the development of POP. A proteomic study by Sun et al. (97) using two-dimensional electrophoresis and matrix-assisted laser desorption/ionization TOF-MS (MALDI-TOF-MS) analysis of USL proteins of patients with POP and controls identified eight proteins (flavoprotein, apolipoprotein A-I, actin, transgelin, cofilin-1, cyclophilin A, myosin, and galectin-1) that were downregulated in the POP group. RT-qPCR was used to validate this conclusion at the mRNA level. These proteins may be involved in the pathophysiology of POP. Further proteomic analysis of the etiology and pathogenesis of POP using HPLC-MS/MS, iTRAQ, and ingenuity pathway analysis (IPA) techniques described by Li et al. (98), revealed five differentially expressed proteins (fibromodulin, collagen alpha-1 [XIV] chain, calponin-1, tenascin, and galectin-1) that appear to be involved in Metabolic mechanisms of the pelvic floor connective tissue. Wang et al. (99). further studied plasma samples using protein array analysis and ELISA in patients with POP and controls and found that the mean plasma levels of heat shock protein 10, zinc finger CCCH domain-containing protein 8, and unc-45 myosin chaperone A were lower than those in healthy controls, these proteins are diagnostic biomarkers for POP. In addition, a number of gene alterations affecting the genetic predisposition of POP have been studied. Certain candidate genes (*COL3A1, COL18A1, LAMC 1, MMP 1, MMP 3, MMP 9, MMP10, ZFAT*) (100–108) may be mediators of POP occurrence; the *COL1A1* rs1800012 polymorphism did not show a significant correlation with POP (109, 110). Contrary to the view outlined above, Cartwright et al. (111) reviewed some genetic correlation studies prior to May 1, 2014, and concluded that the rs1800012 polymorphism in the *COL1A1* gene was linked to anatomic prolapse. However, candidate genes *COL3A1, LAMC 1, MMP 1, MMP 3, and MMP 9* failed to show a significant predisposition to POP. A recent meta-analysis (112) of data related to the genetics of POP, collected between January 1, 2015 and November 1, 2020, yielded the same conclusions as those of Cartwright et al. (108). Furthermore, meta-analyses of the candidate genes *COL18A1* (collagen type 18), *ZFAT*, and *MMP10* did not yield significant predisposition to POP. Notably, some previous studies (113–116) found that *ESR* (estrogen receptor), *PGR* (progesterone receptor), and *Fbln5* are also involved in the pathophysiological mechanism of POP. Similarly, a meta-analysis report of Allen-Brady et al. (112) concluded that there is a significant correlation between *ESR1* RS2228480, *FBLN5* RS12589592, and *PGR* RS484389 and POP. Several other genetic biomarkers have also been explored; for example, Xie et al. (117) carried out an RNA-Seq study of USL specimens from patients with POP and controls and identified 81 POP signature genes. In addition, some ECM-related candidate genes, such as *COMP, NDP, and SNAI2*, were suggested to contribute to the pathological process of POP. Furthermore, components in neuroactive ligand-receptor interactions and the Wnt receptor signaling pathway were also indicated to be involved in the pathogenesis of POP. A single-cell transcriptome study (81) of severe AVP (POP-Q ≥ stage III) found abnormal gene expression in different cell types, including genes encoding ECM molecules (*FN1, LUM*, and *DCN*) or receptors for cellular uptake of HA and collagen (*LYVE1* and *MRC2*), which were widely upregulated in most cell types in the POP samples. In addition, two types of collagen endocytic receptors (*MRC1* and *MRC2*), HA degradation genes (*HYAL2, HYAL3*), and HA receptor (*LYVE1*), which are believed to regulate inflammation by converting signals from the ECM, were upregulated in macrophages in POP samples. Thus, fibroblasts and macrophages may play a significant role in the dysregulation of the ECM and immune disorders associated with POP. In a genome-wide association study using data from Iceland and the United Kingdom Biobank, Olafsdottir et al. (118) reported the discovery of eight sequence variants at seven loci associated with POP. These included *rs3820282–T* located in intron1 of *WNT4*, *rs12325192* located near *SALL1*, *rs9306894* located in the *3′-UTR of GDF7*, *rs1247943* close to *TBX5*, r*s7682992–T* close to the *FAT4* gene, r*s72624976* located in the *3′-UTR of IMPDH1*, and *rs3791675* and *rs1430191* partly located in and near *EFEMP1*. In addition, Natalia et al. (119) performed a genome-wide association meta-analysis and identified 26 loci significantly associated with POP, of which 7 loci are as described in the above studies, and the others are previously unidentified potential candidate genes, such as *VCL, CHRDL2, LOXL1-AS1, DUSP16, CRISPLD2, ADAMTS5, KLF13, MAFF in 2p24.1, 10q22.1, 11q13.4, 12p13.2, 16q24.1, 15q24.1, 15q13, 21q21.3*, and *22q13*, as well as *ACADVL, PLA2G6, and HOXD13*. In summary, a large number of potential POP biomarkers have been identified using metabolomics, proteomics, and genetic susceptibility, although some remain controversial. The described studies contribute to our global understanding and provide new insights into the molecular mechanisms of POP, further opening new avenues for future research.

13. Conclusion

POP is a concerning gynecological disease that occurs in middle-aged and senior women, and its molecular mechanism is complex. By exploring the mechanisms of various molecules associated with POP, we were able to summarize a large number of potential key molecular targets and/or signaling pathways involved in the evolution of POP. There is a current lack of accurate clinical molecular biological interventions to prevent, diagnose, progression delay, and improve the treatment of POP. We suggest that key molecular targets can be used to develop simple, rapid, and effective detection techniques for early screening in community health care centers to identify high-risk individuals. In addition, we propose reasonable preventive measures associated with risk factors to reduce the incidence of POP,

as well as identify asymptomatic POP patients. Finally, we provide scientific evidence for early diagnosis, intervention, and treatment of POP to delay progression of the disease and improve quality of life. The key molecular targets and signaling pathways associated with POP can be used to develop new biological meshes related to pelvic floor surgery, reduce complications such as postoperative recurrence, and develop molecular targeted drugs to accurately strengthen the pelvic floor structure, or even reverse prolapse. Finally, further research studies are needed, especially to address existing contradictions in the literature regarding the potential molecular mechanisms of POP.

Author contributions

XL conceptualized article. XW wrote first draft. TL plotted the figures. All authors contributed to the manuscript revision and approved the submitted version. All authors agreed to be accountable for all aspects of the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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