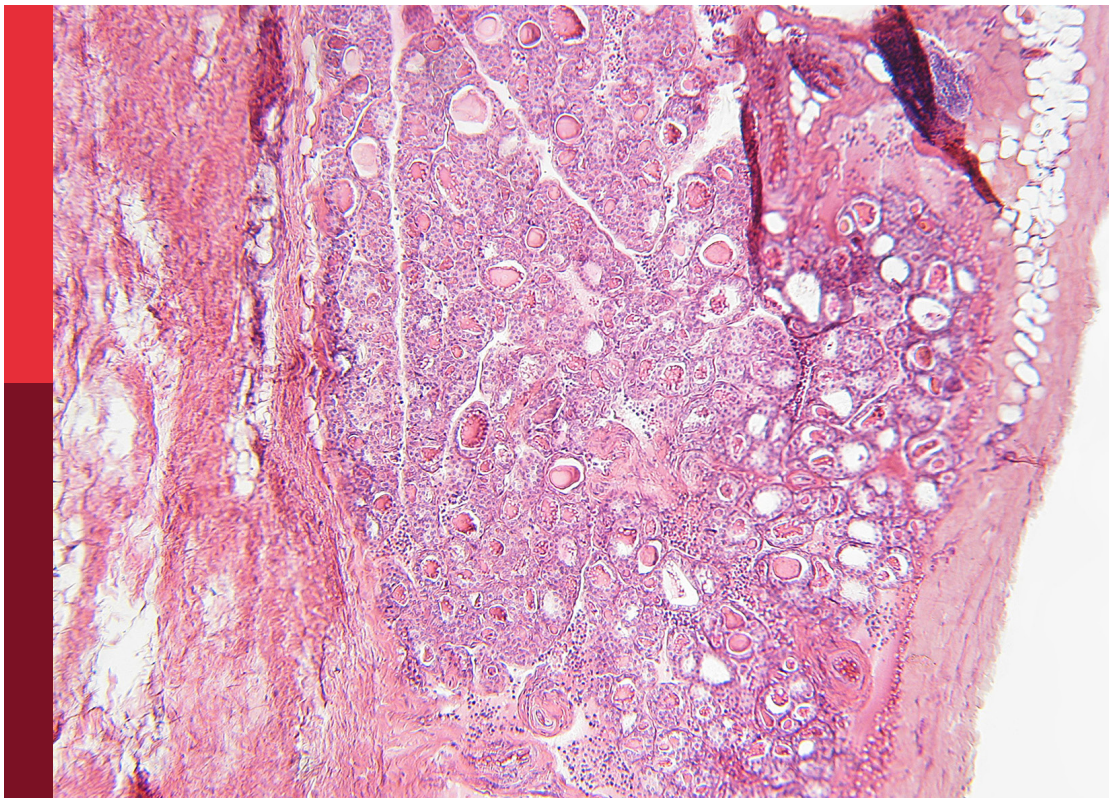
frontiers | Research Topics

Exploring the optimal endometrial preparation protocol for frozen-thawed embryo transfer

Edited by
Fan Qu, Zhen-Gao Sun, Yifei Liu and Emre Pabuccu

Coordinated by
Jing-Yan Song

Published in
Frontiers in Endocrinology



ISSN 1664-8714
ISBN 978-2-8325-4551-5
DOI 10.3389/978-2-8325-4551-5

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Exploring the optimal endometrial preparation protocol for frozen-thawed embryo transfer

Topic editors

Fan Qu — Zhejiang University, China

Zhen-Gao Sun — Affiliated Hospital of Shandong University of traditional Chinese Medicine, China

Yifei Liu — Yale New Haven Health System, United States

Emre Pabuccu — Ufuk University, Türkiye

Topic Coordinator

Jing-Yan Song — Shandong University of Traditional Chinese Medicine, China

Citation

Qu, F., Sun, Z.-G., Liu, Y., Pabuccu, E., Song, J.-Y., eds. (2024). *Exploring the optimal endometrial preparation protocol for frozen-thawed embryo transfer*.

Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-4551-5

Table of contents

frontiers | Frontiers in Endocrinology

TYPE Editorial
PUBLISHED 22 February 2024
DOI 10.3389/fendo.2024.1377488

OPEN ACCESS

EDITED AND REVIEWED BY
Richard Ivell,
University of Nottingham, United Kingdom

*CORRESPONDENCE
Jing-Yan Song
hanlingjuzei91@126.com
Zhen-Gao Sun
sunzhengao77@126.com

RECEIVED 27 January 2024
ACCEPTED 02 February 2024
PUBLISHED 22 February 2024

CITATION
Xu Y, Song J-Y and Sun Z-G (2024) Editorial: Exploring the optimal endometrial preparation protocol for frozen-thawed embryo transfer.
Front. Endocrinol. 15:1377488.
doi: 10.3389/fendo.2024.1377488

Editorial: Exploring the optimal endometrial preparation protocol for frozen-thawed embryo transfer

Ying Xu[1], Jing-Yan Song[1*] and Zhen-Gao Sun[2*]

[1]The First Clinical College, Shandong University of Traditional Chinese Medicine, Jinan, China, [2]Reproductive Center of Integrated Medicine, The Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, China

KEYWORDS

frozen embryo transfer, natural cycle, hormone replacement cycle, corpus luteum (CL), pregnancy outcomes

Editorial on the Research Topic
Exploring the optimal endometrial preparation protocol for frozen-thawed embryo transfer

A successful frozen embryo transfer (FET) rate has gradually increased due to the continuous improvement of cryopreservation technology. The convenience of storing frozen embryos for an extended period allows patients to have more choice and flexibility. In recent years, FET has proven to be a more viable option than fresh embryo transfer (1). Studies have indicated that FET has a higher live birth rate (LBR) than fresh embryo transfer (2). While FET cycles are becoming increasingly popular, it remains unclear what is the best protocol for preparing the endometrium prior to this procedure. More and more studies have recently discussed the optimal endometrial preparation protocol.

Obstetric and perinatal complications

The researchers who contributed to this Research Topic have published a total of 11 thematic articles, including 1 review, 2 meta-analyses, 7 original research articles, and 1 study protocol, which highlight optimal endometrial preparation protocols. Hormone replacement therapy (HRT) is currently the most widely used treatment due to its broad applicability and low cycle cancellation rate (3). Nevertheless, it is associated with an increased risk of complications during obstetrics and delivery (4). Three of the articles focused on obstetric and perinatal outcomes in different FET cycles, among which Epelboin et al. highlighted that prolonged doses of exogenous estrogen-progesterone may be harmful to gestational vascular pathologies, while the corpus luteum (CL) in ovulatory cycle (OC-FET) has a protective effect on the prevention of gestational vasculopathies. Also, Zhao et al. found that artificial cycles (AC-FET) have a negative impact on obstetric and perinatal complications, including gestational hypertension, preeclampsia, gestational diabetes mellitus, large for gestational age, macrosomia, placenta previa, small for gestational age, preterm labor, postpartum hemorrhage, placental abruption, and premature rupture of

membranes at a premature pregnancy. Additionally, Wang et al. found that AC-FET may lead to abnormal placental attachment, which may result in obstetric complications such as preeclampsia, postpartum hemorrhage, and a higher incidence of cesarean delivery. A lack of CL may be responsible for this phenomenon in AC-FET, and the absence of CL could impair maternal hemodynamics and cardiovascular fitness for pregnancy in the first trimester, which leads to adverse pregnancy outcomes (e.g., preeclampsia) (5), as well as the fact that the CL produces a variety of vasoactive molecules, like relaxin, prorenin and other unknown molecules, which contribute to the global changes that occur during pregnancy and help reduce hypertensive disorders later (6, 7). Ultimately, precaution should be exercised whenever AC-FET is used, and specific approaches can be explored to mitigate the increased risk of obstetric and perinatal complications.

Influencing factors of pregnancy outcomes

Several articles have explored which factors affect pregnancy outcomes. In a study conducted by Wang et al., it was found that atosiban can improve implantation rate, positive pregnancy rate, clinical pregnancy rate (CPR), and LBR in repeated implantation failure (RIF) patients, which may be attributed to atosiban's beneficial effects on uterine contractility and endometrial receptivity (8–10). According to Liu et al., progesterone supplementation duration did not significantly affect AC-FET outcomes, and single-blastocyst transfers were recommended, as blastocysts have improved gestational outcomes than cleavage embryos because the blastocyst reselection process crosses developmental barriers and culls embryos with poor developmental potential (11, 12). In their study, Rodríguez-Varela et al. found that the duration of estrogen exposure prior to exogenous progesterone administration in HRT cycles did not affect clinical outcomes, allowing patients, physicians, and clinics to adjust dates optimally to meet their needs. As determined by Demirel et al., nearly half of NC-FETs require luteal phase support (LPS) because of low progesterone, and the authors also recommend that 25 mc subcutaneous progesterone salvages LPS and produces a similar ongoing pregnancy rate, and that LPS should be initiated prior to embryo transfer in all NC-FET cases. Data from Guler et al. report that higher serum luteinizing hormone (LH) levels are associated with higher CPRs and LBRs prior to initiation of FET with progestogens. Given that early initiation of estrogen therapy may be associated with lower LH levels prior to progestogen administration, delaying estrogen initiation to day 4 of the AC-FET cycle may be a favorable strategy to improve pregnancy outcomes. According to Huang et al., RNA-seq-based ER testing (rsERT) and preimplantation genetic testing (PGT) were both effective for improving ART efficacy, and endocrine dysregulation caused by multiple endocrine neoplasia type 1 (MEN1), rather than mutations in the MEN1 gene, was responsible for endometrial receptivity abnormalities. In this case, we demonstrate how treatment guidelines for patients suffering from MEN1 infertility can be developed by integrating multiple approaches, such as rsERT, PGT, and multidisciplinary team management.

Final considerations

In summary, this Research Topic contributes to our understanding of optimal endometrial preparation regimens, and further studies are required to determine the optimal regimen and dosage of HRT supplementation for various estrogens and progestogens, as well as to identify specific strategies for preventing adverse outcomes in AC-FET cases that cannot be avoided.

Author contributions

YX: Writing – original draft. J-YS: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. Z-GS: Funding acquisition, Supervision, Writing – review & editing.

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

References

1. Wong KM, Mastenbroek S, Repping S. Cryopreservation of human embryos and its contribution to *in vitro* fertilization success rates. *Fertil Steril* (2014) 102:19–26. doi: 10.1016/j.fertnstert.2014.05.027

2. Evans J, Hannan NJ, Edgell TA, Vollenhoven BJ, Lutjen PJ, Osianlis T, et al. Fresh versus frozen embryo transfer: backing clinical decisions with scientific and clinical evidence. *Hum Reprod Update* (2014) 20:808–21. doi: 10.1093/humupd/dmu027

3. Yu J, Chen P, Luo Y, Lv M, Lou L, Xiao Q, et al. GnRH-agonist pretreatment in hormone replacement therapy improves pregnancy outcomes in women with male-factor infertility. *Front Endocrinol (Lausanne)* (2022) 13:1014558. doi: 10.3389/fendo.2022.1014558

4. Chen ZJ, Shi Y, Sun Y, Zhang B, Liang X, Cao Y, et al. Fresh versus Frozen Embryos for Infertility in the Polycystic Ovary Syndrome. *N Engl J Med* (2016) 375:523–33. doi: 10.1056/NEJMoa1513873

5. Post Uiterweer ED, Koster MPH, Jeyabalan A, Kuc S, Siljee JE, Stewart DR, et al. Circulating pregnancy hormone relaxin as a first trimester biomarker for preeclampsia. *Pregnancy Hypertens* (2020) 22:47–53. doi: 10.1016/j.preghy.2020.07.008

6. Verdonk K, Saleh L, Lankhorst S, Smilde JE, van Ingen MM, Garrelds IM, et al. Association studies suggest a key role for endothelin-1 in the pathogenesis of preeclampsia and the accompanying renin-angiotensin-aldosterone system suppression. *Hypertension* (2015) 65:1316–23. doi: 10.1161/HYPERTENSIONAHA.115.05267

7. Verdonk K, Visser W, Van Den Meiracker AH, Danser AH. The renin-angiotensin-aldosterone system in pre-eclampsia: The delicate balance between good and bad. *Clin Sci (Lond)* (2014) 126:537–44. doi: 10.1042/CS20130455

8. Kim SH, Riaposova L, Ahmed H, Pohl O, Chollet A, Gotteland JP, et al. Oxytocin receptor antagonists, atosiban and nolasiban, inhibit prostaglandin F(2alpha)-induced contractions and inflammatory responses in human myometrium. *Sci Rep* (2019) 9:5792. doi: 10.1038/s41598-019-42181-2

9. Penrod LV, Allen RE, Rhoads ML, Limesand SW, Arns MJ. Oxytocin stimulated release of PGF2alpha and its inhibition by a cyclooxygenase inhibitor and an oxytocin receptor antagonist from equine endometrial cultures. *Anim Reprod Sci* (2013) 139:69–75. doi: 10.1016/j.anireprosci.2013.04.010

10. Sztachelska M, Ponikwicka-Tyszko D, Sokolowska G, Anisimowicz S, Czerniecki J, Lebiedzinska W, et al. Oxytocin antagonism reverses the effects of high oestrogen levels and oxytocin on decidualization and cyclooxygenase activity in endometrial tissues. *Reprod BioMed Online* (2019) 39:737–44. doi: 10.1016/j.rbmo.2019.06.002

11. Magli MC, Jones GM, Gras L, Gianaroli L, Korman I, Trounson AO. Chromosome mosaicism in day 3 aneuploid embryos that develop to morphologically normal blastocysts *in vitro*. *Hum Reprod* (2000) 15:1781–6. doi: 10.1093/humrep/15.8.1781

12. Capalbo A, Hoffmann ER, Cimadomo D, Ubaldi FM, Rienzi L. Human female meiosis revised: New insights into the mechanisms of chromosome segregation and aneuploidies from advanced genomics and time-lapse imaging. *Hum Reprod Update* (2017) 23:706–22. doi: 10.1093/humupd/dmx026

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 23 March 2023
DOI 10.3389/fendo.2023.1161707

OPEN ACCESS

EDITED BY
Zhen-Gao Sun,
Affiliated Hospital of Shandong University of Traditional Chinese Medicine, China

REVIEWED BY
Lang Qin,
Sichuan University, China
Ying Ni,
Shanghai Jiao Tong University School of Medicine, China

*CORRESPONDENCE
Guicheng Xia
xiaguicheng2020@163.com
Yong Tan
yongtan2022@163.com

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

RECEIVED 08 February 2023
ACCEPTED 13 March 2023
PUBLISHED 23 March 2023

CITATION
Wang R, Huang H, Tan Y and Xia G (2023) Efficacy of atosiban for repeated embryo implantation failure: A systematic review and meta-analysis.
Front. Endocrinol. 14:1161707.
doi: 10.3389/fendo.2023.1161707

Efficacy of atosiban for repeated embryo implantation failure: A systematic review and meta-analysis

Ruxin Wang[1,2], Haixia Huang[1,2], Yong Tan[1,2]* and Guicheng Xia[2]*

[1]First Clinical Medical College, Nanjing University of Chinese Medicine, Nanjing, Jiangsu, China, [2]Department of Reproductive Medicine, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, Jiangsu, China

Background: Repeated embryo implantation failure (RIF) posed a significant challenge in assisted reproduction. Evidence of its therapeutic effectiveness including atosiban used around embryo transfer to improve pregnancy outcomes in RIF patients undergoing *in vitro* fertilization-embryo transfer (IVF-ET) remained inconsistent. This study aimed to explore the efficacy of atosiban on pregnancy outcomes of patients with RIF who received IVF-ET.

Methods: The research was designed using the PICOS format. A systematic search of four English databases, PubMed, EMBASE, Web of Science, Cochrane Library, and one Chinse database, China National Knowledge Infrastructure (CNKI) was conducted. The time range was from inception to December 10, 2022. Then trials comparing the efficacy of atosiban and control group on pregnancy outcomes in RIF patients who receive IVF-ET were included. Subgroup analysis and sensitivity analysis were performed to reduce the influence of heterogeneity between included studies. Risk ratio (RR) and 95% confidence interval (CI) were calculated. The main outcome measure was clinical pregnancy rate (CPR). For the analyses, StataMP 17.0 (Stata Corporation, USA) was used.

Results: Two prospective randomized controlled trials (RCTs), one prospective cohort study and four retrospective cohort studies were included. Our results showed that atosiban was associated with higher clinical pregnancy rate (RR=1.54, 95% CI: 1.365–1.735, $P < 0.001$, $I^2 = 0.0\%$). The results of subgroup analysis based on study types (prospective randomized controlled clinical trial, retrospective cohort study and prospective cohort study) showed that in all types of studies, CPR of atosiban group was significantly higher than controlled group. The results of subgroup analysis based upon the diagnostic criteria of number of previous embryo transfer failures showed that the intervention of atosiban improved the CPR whether in participants with 2 previous ET failures or in

participants with 3 previous ET failures. Nevertheless, the incidence of ectopic pregnancy, multiple pregnancy, and miscarriages were not significantly different between the case and control groups.

Conclusion: For women who are undergoing IVF-ET and have experienced repeated embryo implantation failure, atosiban may be an important factor in enhancing pregnancy outcomes. To confirm this conclusion, more thorough, prospective randomized controlled studies of sizable sample sizes with well design are required.

KEYWORDS

atosiban, repeated embryo implantation failure, *in vitro* fertilization-embryo transfer (IVF-ET), meta-analysis, clinical pregnancy rate (CPR)

1 Introduction

One of the most crucial stages in reproduction, embryo implantation is the process by which the embryo connects to the luminal surface of the endometrium, moves through the luminal epithelium, and infiltrates the deep layer to become fixed in the deeper layer (1). In assisted reproductive technology (ART), ultrasonographic evidence of an intrauterine gestational sac suggests that the progress of implantation is completed successfully which necessitates a competent blastocyst, a receptive endometrium and synchronous communication between the maternal and embryonic tissues (2). Embryo abnormalities, poor endometrial receptivity as well as insufficient interaction between embryo and maternal endometrium can lead to implantation failure.

Repeated embryo implantation failure (RIF) is an unsolved and challenging technical problem during *in vitro* fertilization-embryo transfer (IVF-ET). At present, no standard definition has been established for the total number of transferred embryos or the number of failed cycles. It is however accepted that RIF can be considered as the inability to successfully achieve a clinical pregnancy after receiving embryo transfers (ETs) of high-quality embryo three or more times or ≥10 embryos transferred at different times with the precise numbers of transfers to be chosen by each different reproductive medical centers (3). Accordingly, there are different definitions for RIF in different centers practicing IVF. It is also well-accepted that failure of pregnancy after two or more embryo transfer cycles for individuals constitute RIF (4, 5). Those failures may bring these infertile couples tremendous mental and economic pressure (6).

At present, more attention has been attracted regarding how to improve pregnancy outcomes of patients experienced RIF. Traditionally, the quality of embryo has been considered as the main cause for RIF. Indeed, impaired uterine receptivity was thought to be one of the main causes of treatment failure when high-quality embryos were transplanted (7). Generally, structural uterus abnormal including uterine congenital abnormalities and acquired diseases (8), thickness of endometrium (9, 10), chronic endometritis (11), endometrial perfusion (12) and uterine peristalsis (13) may impact on endometrial receptivity and thus embryo implantation. Previous studies proved that in fresh and frozen-thawed embryo transfer cycles, uterine peristalsis had a significant impact in embryo mobility and implantation (14) and was even associated with the clinical pregnancy outcome (15, 16). Such as, with an increase in uterine peristalsis, the rates of implantation, clinical pregnancy, and continued pregnancy gradually reduced (17). Excessive uterine peristalsis could move the implanted embryo out of the uterus. Thus, uterine peristalsis was considered as a potential triggers on decreasing implantation rates in ART cycles. In contrast, uterine peristalsis has been neglected in diagnostic measures, and it has not been demonstrated that treatments around ET like beta agonists or non-steroid anti-inflammatory medications (NSAIDs) are beneficial in decreasing uterine peristalsis (18).

Atosiban, a vasopressin V1a and oxytocin receptor antagonist, was selected as the treatment for preterm labour by reducing uterine peristalsis (19). The application of atosiban in IVF that may decrease uterine peristalsis to improve uterine receptivity during ET was first reported by Pierzynski et al. in 2007 (20). In recent years, many clinical studies evaluating more outcome measures, such as clinical pregnancy, live birth, miscarriage, multiple pregnancy, implantation and ectopic pregnancy rates has been conducted on this issue (21–26). The effectiveness of atosiban intervention in IVF-ET still remained controversial and ambiguous based on the published evidence.

Taking into account the difficulties in treating RIF, atosiban is being applied to reduce uterine peristalsis as an adjuvant to IVF in RIF-affected women. It is necessary to provide objective evidence on the application of atosiban on RIF patients who undergo IVF-ET. This systematic review and meta-analysis were designed to investigate effects of atosiban on IVF-ET-assisted pregnancy outcomes in women with RIF.

2 Materials and methods

The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) statement was followed when conducting this study (27).

2.1 Protocol registration

This review protocol has been registered in the PROSPERO International Prospective Register of Systematic Reviews (Registration Number: CRD42022382312).

2.2 PICOS

This study was designed as "PICOS" principle (population, intervention, comparison, outcome and study design). Population: Participants undergoing IVF-ET who had experienced RIF were included. Intervention and comparison: Trials comparing the use of atosiban around ET versus either no treatment or a placebo were eligible for inclusion. Outcome: Trials with the following outcomes were included: positive pregnancy test rate, clinical pregnancy rate, live birth rate, implantation rate, miscarriage rate, multiple pregnancy rate and ectopic pregnancy rate. Study design: Published clinical research (observational studies/clinical trials) were eligible for inclusion.

2.3 Literature search

Electronic databases were systematically searched to find all pertinent studies, including PubMed, EMBASE, Web of Science, Cochrane Library, and China National Knowledge Infrastructure (CNKI) by two authors (RXW and HXH) from inception to December 10, 2022. The databases were searched using the following search terms:(*in vitro* fertilization-embryo transfer [Title/Abstract]) OR (IVF-ET [Title/Abstract]) OR (repeated embryo implantation failure [Title/Abstract])) OR (recurrent embryo implantation failure [Title/Abstract]) OR (RIF [Title/Abstract]) OR (intracytoplasmic sperm injection [Title/Abstract]) OR (ICSI [Title/Abstract]) OR (assisted reproductive techniques [Title/Abstract]) OR (ART [Title/Abstract]) OR (*in vitro* fertilization [Title/Abstract]) OR (IVF [Title/Abstract]) OR (embryo transfer [Title/Abstract]) OR (ET [Title/Abstract]) AND (atosiban [Title/Abstract]).

2.4 Eligibility criteria

All clinical research (observational studies/clinical trials) examining impacts of atosiban on patients with RIF undergoing IVF-ET were included in this review.

Inclusion criteria (1): The studies involved patients undergoing IVF-ET who had experienced RIF. RIF was defined as ≥2 failed ET cycles (2). The case group was composed of patients treated with atosiban around ET. (3) Patients in the control group underwent either no treatment or a placebo. (4) Confirmed pregnancy outcomes were reported, including at least the following three outcome indicators: implantation rate, clinical pregnancy rate, miscarriage rate. (5) The raw data were available in the articles.

Exclusion criteria: (1) Animal experiments. (2) No usable data was provided. (3) Studies that did not have a control group or a full text available. (4) Reviews and case reports

2.5 Study selection and data extraction

Two writers (RXW and HXH) independently selected the studies and extracted the data. All articles from the electronic searches, including abstracts, were evaluated. Citations that met the criteria for inclusion were obtained. A PRISMA flow diagram was created to display the search results as well as the number of trials that were included and excluded. For all included studies, characteristics were summarized in tables, including authors' names, title, year of publication, number of patients, year of patients, type of study, RIF diagnostic criteria, type of interventions, controlled ovarian stimulation (COS) protocol, ET protocol and outcomes.

2.6 Evaluation of bias risk and methodological quality in included studies

The bias risks of included RCTs were evaluated by the criteria of the Cochrane' risk of bias assessment tool (28). Two evaluators evaluated the reports in terms of the following items independently, assigning scores of "high" "low" and "unclear": (1) Random sequence generation. (2) Allocation concealment. (3) Blinding of participants and personnel. (4) Blinding of outcome assessment. (5) Incomplete outcome data. (6) Selective reporting. (7) Other sources of bias.

The Newcastle-Ottawa Scale (NOS) was used to evaluate the quality of the cohort studies that were included (29). The NOS checklist involves 3 quality parameters: (1) Selected population. (2) Comparability of groups. (3) Assessment of either the exposure or outcome of interest for case-control or cohort studies. Each study received a grade ranging from 0 to 9. High quality studies were those whose scores were greater than or equal to 7 (30–32).

2.7 Synthesis and analysis of information

Using both fixed and random effects models, the pooled risk ratio (RR) with 95% confidence interval (CI) were derived from individual research (33). The results of the meta-analyses were graphically displayed using the forest plot. Statistics were deemed significant at $P < 0.05$. Cochrans Q and the I^2 statistic were employed to calculate the degree of statistical heterogeneity. A value of 0% indicated no heterogeneity, while values greater than

50% indicated significant heterogeneity (34). When the heterogeneity was less than 50%, a fixed-effect model was chosen; otherwise, a random effects model was chosen. The subgroup analyses were conducted based on study types and the diagnostic criteria of number of previous embryo transfer failures to explore whether the type of the study and the diagnostic criteria influenced the results of meta-analysis. Moreover, to assess the stability of the results, sensitivity analyses were performed. For the analyses, StataMP 17.0 (Stata Corporation, USA) was used. The potential publication bias was graphically evaluated using the Egger's test ($P > 0.05$).

2.8 Definition of outcomes

The clinical pregnancy rate (CPR) was the main outcome indicator of this study; secondary outcome indicators included positive pregnancy test rate (PPTR), live birth rate (LBR), Implantation rate (IR), miscarriage rate (MR), multiple pregnancy rate (MPR), ectopic pregnancy rate (EPR). Clinical pregnancy was verified when the heartbeat of the fetal sac in the uterus was confirmed by ultrasonography. A successful delivery of live-born baby (after 20 weeks of gestation) was defined as a live birth. The implantation rate was identified as the percentage of transferred embryos that successfully underwent implantation, that was the total number of pregnancy sacs per total number of embryos transferred. Miscarriage was commonly defined as a pregnancy loss prior to viability. A pregnancy with more than one fetus was considered as multiple pregnancy. Ectopic pregnancy meant that a fertilized egg implanted outside the main cavity of the uterus.

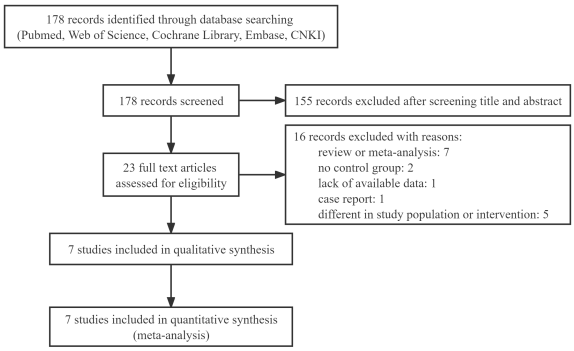


FIGURE 1
PRISMA flow diagram of study selection process.

3 Results

3.1 Description of studies

This review retrieved 178 relevant records. An assessment of the titles and abstracts revealed 23 records that would be acceptable for inclusion. Among them, due to obvious ineligibility, 16 records were excluded, including meta-analyses, reviews, and case reports, no control group, a lack of available data and different in study population and intervention. Finally, the meta-analysis included 7 studies. A PRISMA flow diagram depicted the selection process in detail (Figure 1).

Table 1 showed the characteristics of the selected studies in detail. Two of the studies that were included were prospective, randomized,

TABLE 1 Characteristics of included studies in the review.

Study	Design	Number of patients	Age (years)	RIF diagnostic criteria	Intervention	COS protocol	ET protocol	Outcome
Tang et al. (2022) (35)	Prospective, randomized, double-blind controlled clinical trial	194 Atosiban group: 97 Placebo group:97	less than 40	At least 3 fresh or frozen-thawed transfer cycles failure of four good-quality embryos	Atosiban group: a bolus intravenous dose of 6.75 mg atosiban 30 min before ET; Placebo group: normal saline infusion for the same duration	GnRH agonist protocol; GnRH antagonist protocol	Fresh embryo transfer	PPTR;CPR; OPR; LBR; MR; MPR; IR; EPR; CAR
Li et al. (2021) (36)	Retrospective cohort study	388 Atosiban group: 193 Control group: 195	20 – 39	At least 3 previous embryo implantation failure (including fresh cycle and frozen-thawed cycle)	Atosiban group:a bolus intravenous dose of 6.75 mg atosiban 30 min before ET; Control group: no treatment	N/A	Artificial FET cycles	PPTR; CPR; IR; MR; EPR
Liu et al. (2017) (37)	Retrospective cohort study	262 Atosiban group: 97 Control group: 168	less than 40	At least 3 ET failures (including fresh and frozen cycles); or at least one good-quality embryos in a minimum of 10 embryos	Atosiban group:a bolus intravenous dose of 6.75 mg atosiban 30 min before ET; Control group: no treatment	N/A	Natural FET cycles; Artificial FET cycles; Ovulation induction FET cycles	CPR; IR; MR;MPR; EPR; LBR

(Continued)

10.3389/fendo.2023.1161707

TABLE 1 Continued

Study	Design	Number of patients	Age (years)	RIF diagnostic criteria	Intervention	COS protocol	ET protocol	Outcome
He et al. (2016) (38)	Prospective cohort study	536 1st ET: 178 2nd ETs: 151 3rd ETs: 119 >3 ETs: 88	20 – 45	With 3 or more transfer cycles	Treatment group: a bolus intravenous dose of 6.75 mg atosiban 30 min before ET; Control group: No treatment	N/A	Natural FET cycles; Artificial FET cycles	PPTR; IR; CPR; MR
Zhang et al. (2014) (39)	Retrospective cohort study	240 Atosiban group: 120 Control group:120	22 – 39	≥2 embryo transfers; the total number of transplanted embryos ≥6; at least one high-quality embryo in each transfer cycle	Atosiban group: intravenous administration of atosiban about 1 hour before the transfer with a bolus dose of 37.5 mg during one hour; Control group: no treatment	N/A	Natural FET cycles; Artificial FET cycles;	IR; CPR; MR; MPR; EPR; LBR
Jiang et al. (2014) (40)	Prospective, randomized controlled clinical trial	188 Atosiban group: 84 Comtrol group: 104	20 – 40	>2 transfer cycles failure of good-quality embryos	Atosiban group: a bolus intravenous dose of 6.75 mg atosiban 30 min before ET; Control group: no treatment	Ultra Long GnRH agonist protocol; Long GnRH agonist protocol; Short GnRH agonist protocol; GnRH antagonist protocol ; Mild stimulation protocol	Natural FET cycles; Artificial FET cycles;	CPR; IR; MPR; MR
Chou et al. (2011) (41)	Retrospective cohort study	150 Group 1: 80 Group 2: 40 Group 3: 30	Group 1: 34.8 ±3.76 Group 2: 34.63 ±4.21 Group 3: 34.63 ±4.21	2 or more previous IVF failures after the transfer of good-quality embryos	Group 1: no treatment; Group 2: a single bolus dose (6.75 mg, 0.9 mL/vial) of atosiban intravenously before ET; Group 3: a bolus dose of 6.75 mg atosiban at 18 mg/hr for 3 hours	Long luteal-phase GnRH agonist protocol; Short GnRH agonist protocol; GnRH antagonist protocol	Fresh embryo transfer	IR, CPR, MR,LBR, MPR

PPTR, Positive pregnancy test rate; CPR, Clinical pregnancy rate; OPR, Ongoing pregnancy rate; IR, Implantation rate; LBR, Live birth rate; MR, Miscarriage rate; MPR, Multiple pregnancy rate; EPR, Ectopic pregnancy rate; CAR, Congenital abnormality rate; N/A, Not applicable.

double-blind clinical trials (35, 40), one was prospective cohort study (38) and four were retrospective cohort studies (36, 37, 39, 41). 1958 women, 903 participants in case groups, and 1055 participants in control groups were all part of single-center investigations. All patients received IVF or ICSI treatment, with fresh ET or frozen-thawed ET. Three research (39–41) examined the impact of atosiban on patients who underwent two or more ET cycles, while three studies (35–37) involved patients who underwent three or more cycles. One study (38) divided patients into four subgroups based on the number of previous ETs (patients undergoing ET for the first/second/third or more time). Our study included patients who undergoing the third and more than the third ET. The prospective cohort study by He et al. (38) also measured uterine contractions and serum oxytocin (OT), Prostaglandin F2α (PGF2α) level. The number of transfer cycles and serum OT levels were found to positively correlate with uterine contractions, and patients who had higher uterine contractions (43.1 wave/min) were more likely to be the RIFs and benefited more from atosiban treatment.In all studies, atosiban was given intravenously. In six studies, the dose of atosiban was 6.75mg, while the dose was 37.5mg in the study by Zhang Yue et al. (39). The study by Chou et al. (41) investigated if the different methods of atosiban use had an effect on efficacy. Patients who received atosiban were divided into two groups by the usage, a single bolus dose (6.75 mg, 0.9 mL/vial) before ET or a bolus dose of 6.75 mg atosiban followed by a 3-hour infusion at 18 mg/hr after ET. Results indicated that the clinical pregnancy rate and the implantation rate were significantly higher in the group who received a single bolus dose of atosiban before ET. That may suggest a better usage of atosiban.

3.2 Risk of bias assessment and quality evaluation

Based on various quality domains of the Cochrane Collaboration tool, the risks of bias of the included RCTs were showed in Table 2. One of the RCTs (35) was at low risk of bias for

TABLE 2 Bias risks of the included RCT.

Study, year	Domain 1	Domain 2	Domain 3	Domain 4	Domain 5	Domain 6	Domain 7
Tang et al. (2022) (35)	Low	Low	Low	Low	Low	Low	Low
Jiang et al. (2014) (40)	High	High	High	Low	Low	Low	Low

Bias risk was determined using the Cochrane risk of bias tool: 1: Random sequence generation (selection bias); 2: Allocation concealment (selection bias); 3: Blinding of participants and personnel (performance bias); 4: Blinding of outcome assessment (detection bias); 5: Incomplete outcome data (attrition bias); 6: Selective reporting (reporting bias); 7: Other sources of bias (other bias).

method of randomization, allocation concealment, performance bias, detection bias, attrition bias and reporting bias. In the study (40), methods for random sequence generation and random allocation concealment were at high risk of selection bias. Patients in that study were randomized according to the ET day and informed consent and there was no blinding in this study. Since the outcomes of implantation, clinical pregnancy, multiple pregnancies, and miscarriage are all evaluated objectively by serum human chorionic gonadotrophin (HCG) detection and ultrasound scan, it was improbable that the assessment of pregnancy outcome would be subjective. Thus we believed that detection bias of all studies were at a low risk.

The Newcastle-Ottawa Scale (NOS) was used to assess the quality of the included cohort studies in Table 3. The NOS scores of included cohort studies ≥ 7 points were considered to be of high quality. None of the included studies mentioned non-response rate.

Considering the various study designs that were used, we performed a sensitivity analysis based on the main outcome indicator (clinical pregnancy rate) which showed a stable and reliable outcome. The pooled analysis was not significantly impacted by any of the studies. Regardless of any study excluded, the results remained statistically significant (RR=1.54, 95% CI: 1.37–1.73) (Figure 2). The Egger regression asymmetry test revealed no statistically significant publication bias (Egger's test; t = 0.76, $P = 0.482$).

3.3 Outcome measures

3.3.1 Positive pregnancy test rate

The rates of PPTR were examined in three studies. A total of 603 participants were included. The combined PPTR was 55.7% in the atosiban group and 42.0% in the control group. Treatment with atosiban strongly improved positive pregnancy test rate by the fixed-effects model and the RR was 1.32(95% CI: 1.12 – 1.56, P=0.001, I^2 = 36.4%) (Table 4; Supplemental Figure 1).

3.3.2 Clinical pregnancy rate

Results showed that atosiban significantly improved CPRs in all included studies in women with RIF, according to the fixed effect forest plot (RR=1.54, 95% CI: 1.37–1.74, P<0.001, I^2 = 0.0%) (Table 4; Supplemental Figure 2).

In view of the different types of the included researches, subgroup analysis was performed based on study types (prospective randomized controlled clinical trial, retrospective cohort study and prospective cohort study). The results showed that in all types of studies, whether RCTs or cohort studies, CPR of atosiban group was significantly higher than controlled group (Figure 3). However, there was a subgroup of RCTs with high level of heterogeneity (65%). The heterogeneity was complicated and may due to many factors. First, age range, sample size, body mass index, sex hormone level and other base line information were different or unknown. Second, low quality of study, such as blindness, methods for random sequence generation and random allocation concealment, may affect the credibility of the results. Third, the control measures were different in two studies. One is no treatment while the other one is placebo.

The diagnostic criterias of RIF were somewhat different among the included studies. In four studies, women with 2 or more transfer cycle failures of good-quality embryos were selected as participants. In other three studies, women with 3 or more transfer cycle failures were selected. Hence, subgroup analysis was undertaken based upon the diagnostic criteria of number of previous embryo transfer failures. The results showed that the intervention of atosiban improved the CPR whether in participants with 2 previous ET failures or in participants with 3 previous ET failures (Figure 4).

3.3.3 Live birth rate

LBR was selected as one of two crucial outcomes for IVF/ICSI by ESHRE in their 2019 guideline on ovarian stimulation (42). Four studies examined the effects of atosiban on the LBRs in women with RIF, including 846 participants. Results of the meta-analysis indicated that the administration of atosiban was associated with a higher LBR in RIF patients who receive IVF-ET (RR=1.58, 95% CI: 1.18 – 2.11, P=0.002, I^2 = 49.8%) (Table 4; Supplemental Figure 3).

3.3.4 Implantation rate

All included studies assessed the implantation rate. We pooled the data and discovered that application of atosiban significantly increased the implantation rates (RR=1.54, 95% CI: 1.37–1.74, P<0.001, I^2 = 15.9%) (Table 4; Supplemental Figure 4).

3.3.5 Miscarriage rate

The comparison of MRs for 726 patients was conducted in seven related studies. Miscarriage occurred in 46 of 392 (11.7%) patients in the atosiban group and in 40 of 334 (12.0%) patients in the control group. Regarding the rates of miscarriage, no significant difference was found between the two groups; the RR was 0.94 (95%

TABLE 3 Bias risks of the included cohort studies.

Study, year	Quality evaluation	Selection					Comparability	Exposure		
		Case definition	Representa-tiveness	Selection of controls	Definition of controls		Comparability	Ascertainment of exposure	Same method	Non-Response rate
Li et al. (2021) (36)	8	1	1	1	1		2	1	1	0
Liu et al. (2017) (37)	7	1	1	1	1		1	1	1	0
He et al. (2016) (38)	8	1	1	1	1		2	1	1	0
Zhang et al. (2014) (39)	7	1	1	1	1		1	1	1	0
Chou et al. (2011) (41)	8	1	1	1	1		2	1	1	0

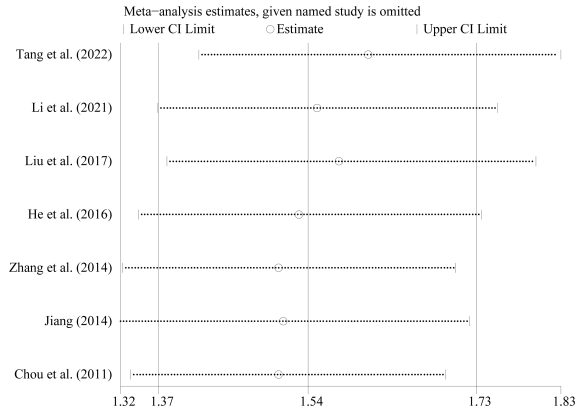


FIGURE 2
Sensitivity analysis of the included studies The abscissa refer to the RR and the ordinate represented each study and year.

CI: 0.63–1.39, P=0.747, I^2 = 0.0%) in the fixed-effects model (Table 4; Supplemental Figure 5).

3.3.6 Multiple pregnancy rate

For the MPR, we merged the outcomes of five studies with 448 participants. The combined MPR was 26.3% in the atosiban group and 19.9% in the control group. Results of analysis indicated that there was no significant difference between the atosiban group and the control group in MPR (RR=1.26, 95% CI: 0.88–1.79, P=0.212, I^2 = 0.0%) ((Table 4; Supplemental Figure 6).

3.3.7 Ectopic pregnancy rate

Four studies with 393 participants focused on the EPR. In the atosiban group, the total EPR was 2.8%, whereas in the control group, it was 4.5%. Results of the meta-analysis showed that differences in the EPR for intervention and control groups were not statistically significant (RR=0.64, 95% CI: 0.23–1.83, P=0.409, I^2 = 0.0%) (Table 4; Supplemental Figure 7).

4 Discussion

This study aimed to learn more about the effects of atosiban medication in patients with RIF undergoing IVF-ET by comparing larger samples of atosiban intervention patients and control patients. In this paper, results indicated that atosiban was associated with a higher positive pregnancy test rate, a higher clinical pregnancy rate, a higher live birth rate and a higher implantation rate. The outcomes demonstrated that there was no discernible difference in the rates of miscarriage, multiple pregnancy, or ectopic pregnancy between the atosiban intervention and control groups.

There were some previous systematic reviews and meta-analyses (43–45) published about the use of atosiban in IVF treatment, but not for patients with RIF. They concluded that in the majority of women who experienced IVF, atosiban might only have a little impact on pregnancy outcomes. However, based on the

TABLE 4 Meta-analysis of all studies comparing pregnancy outcomes between case and control groups in patients with RIF.

Pregnancy outcomes	Number of studies	Number of participants	Positive/total in case group	Positive/total in control group	Risk Ratio	95% Confidence Interval	I^2	*P*	Analysis model
Positive pregnancy test rate	3	1118	55.7% (165/296)	42.0% (129/307)	1.32	1.12 - 1.56	36.4%	0.001	fixed-effects
Clinical pregnancy rate	7	1958	52.4% (348/664)	34.7% (270/779)	1.54	1.37 - 1.74	0.0%	<0.001	fixed-effects
Live birth rate	4	846	40.4% (154/381)	26.7% (124/465)	1.58	1.18 – 2.11	49.8%	0.002	random-effects
Implantation rate	7	1958	34.2% (460/1345)	22.8% (341/1496)	1.54	1.37 – 1.74	15.9%	<0.001	fixed-effects
Miscarriage rate	7	1958	11.7% (46/392)	12.0% (40/334)	0.94	0.63 – 1.39	0.0%	0.747	fixed-effects
Multiple pregnancy rate	5	1034	26.3% (65/247)	19.9% (40/201)	1.26	0.88 – 1.79	0.0%	0.212	fixed-effects
Ectopic pregnancy rate	4	1084	2.8% (6/214)	4.5% (8/179)	0.64	0.23 – 1.83	0.0%	0.409	fixed-effects

results of this study, atosiban has significant therapeutic effects on patients with RIF.

Maternal age played a crucial role in the success of IVF and it was one of risk factors for RIF. It was reported that oocyte yield, blastocyst formation and endometrial thickness all decreased in patients over 35 years of age (46). Body mass index (BMI) (47), psychological stress (48), alcohol abuse and smoking (49) were also risk factors for RIF. Embryo and endometrial synchrony was under influence of many factors, such as embryonic and parental genetics, anatomical factors, maternal immune system, endocrine milieu, hematologic factors and reproductive tract microbiome (50). Besides, one of the essential elements of uterine receptivity, uterine contractions, played an important role in embryo implantation (51). Uterine contractions were caused by the synthesis of oxytocin, which was strongly influenced by estradiol (E_2) level (52). By enhancing the oxytocin receptor gene expression in the uterus, a high amount of E_2 strengthened the effects of oxytocin, leading to uterine contractions even without pregnancy (53). Also, a high level of E_2 may induce indirectly the synthesis or release of prostaglandin F2a (PGF2a), which may produce the strong and frequent uterine contractions and inhibit maternal recognition of pregnancy (54). During fresh embryo transfer cycle after controlled ovarian hyperstimulation or in artificial preparation cycles for frozen embryo transfer, women undergoing IVF-ET were likely to be exposed to supraphysiologic levels of estradiol, which could affect uterine contractions and negatively affect implantation. It was reported that RIF patients may experience more uterine contractions (55). Patients with RIF experienced more hormone stimulations and more instrumental operations, such as ovarian stimulation, constantly transvaginal ultrasound supervision, transvaginal oocyte retrieval, embryo transfer or even hysteroscopy which may lead to a hyperactivated autocrine/paracrine OT/OTR system in the endometrial epithelium that can result in the high level of serum OT and PGF2α and thereby to high uterine contractions (38). This provided some level of support for the application of atosiban in patients with RIF. Correspondingly, there may be a reduced pregnancy rate among women who experience frequent uterine contractions. Therefore, drugs or treatments to decrease uterine contractions around embryo transfer are becoming more appealing options for improving pregnancy outcomes of RIF patients. Atosiban, as a combined oxytocin/vasopressin V1A antagonist, could be a choice to reduce uterine contractions. Apart from the reduction in uterine contractions, atosiban has been found to prevent early luteal regression and embryonic loss, and inhibit contractions and inflammation, by inhibiting the endometrial production of PGF2a (56, 57). Another significant effect of atosiban may be that it reversed the consequences of high estradiol and oxytocin on endometrial receptivity parameters (58). Its safety and few side effects have been evidenced in trustworthy documents in related studies (59). The phenomenon of improved pregnancy rates in patients with RIF who received atosiban could be attributed to its effects on uterine contractility and beneficial effects on endometrial receptivity.

This study provided documented evidence for the use of atosiban in cases of RIF and the potential indication for ET by comparing the pregnancy outcomes of RIF patients treated with atosiban and control. It also showed that the application of atosiban around embryo transfer could improve the pregnancy outcomes of patients with RIF.

This was the most up-to-date review, which included a large sample of patients with RIF on this subject. Both observational and randomized controlled trials confirmed the increased risk of pregnancy caused by atosiban. However, there were some potential limitations in this meta-analysis. First, the therapeutic schedules, including ovulation induction protocol, embryo transfer protocol and luteal support regimen differed among patients undergoing IVF-ET. In addition, confounding factors included the different class and number of transferred embryos. Secondly, the included studies contained various types of study design, such as

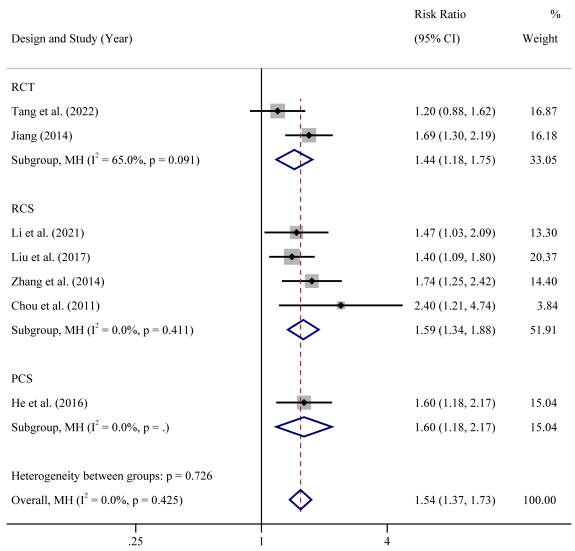


FIGURE 3
Subgroup analysis based on study design of clinical pregnancy rate RCT: In view of the different designs of the included studies, RCS: In view of the different designs of the included studies, PCS: In view of the different designs of the included studies.

randomized controlled trial and cohort study. Also, in some of the included studies, blinding was not applied. As a result, biases in implementation and measurement were unavoidable. Thirdly, the patients enrolled in the included studies represented a wide range in age, from 20 to 40. The lack of sufficient data on age in included studies meant that age specific analyses could not be performed. It has previously been indicated that the addition of atosiban to FET cycles did not decrease uterine peristalsis, but may be beneficial to the group of advanced age (60). It will be more accurate and objective if the clinical trial can be carried out by age groups.

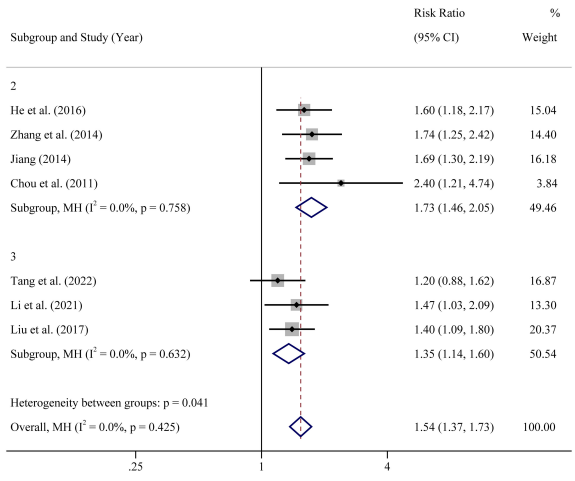


FIGURE 4
Subgroup analysis based on RIF diagnostic criteria of clinical pregnancy rate.

5 Conclusion

In conclusion, the application of atosiban around the time of ET could increase the implantation rates, positive pregnancy test rates, clinical pregnancy rates, and live birth rates for RIF patients undergoing IVF-ET and had no effect on the rates of miscarriage, multiple pregnancy and ectopic pregnancy when compared to control groups. To investigate the efficacy of atosiban during ET in ART for RIF in more depth, further large, well-designed, prospective randomized placebo-controlled trials with large numbers of patients grouped by age and reporting on live births and adverse clinical outcomes should be conducted.

Author contributions

RW: Research scheme design, data collection and analysis, manuscript writing. HH: Database searching, research extracting and evaluating. GX and YT: Research design and guidance. All authors contributed to the article and approved the submitted version.

Funding

This systematic review was supported by National Natural Science Foundation of China (Grant NO. 8217151456, Grant NO. 81873333) and Jiangsu Provincial Health Planning Commission Science, Education, and Health Strengthening Project: Jiangsu Provincial Clinical Medical Center of Gynecology of Traditional Chinese Medicine (YXZXB2016006).

Acknowledgments

We are grateful to GX and YT for their guidance and assistance with our studies.

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/fendo.2023.1161707/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Forest plots of positive pregnancy test rate.

SUPPLEMENTARY FIGURE 2
Forest plots of clinical pregnancy rate.

SUPPLEMENTARY FIGURE 3
Forest plots of live birth rate.

SUPPLEMENTARY FIGURE 4
Forest plots of implantation rate.

SUPPLEMENTARY FIGURE 5
Forest plots of miscarriage rate.

SUPPLEMENTARY FIGURE 6
Forest plots of multiple pregnancy rate.

SUPPLEMENTARY FIGURE 7
Forest plots of ectopic pregnancy rate.

References

1. Coughlan C, Ledger W, Wang Q, Liu F, Demirol A, Gurgan T, et al. Recurrent implantation failure: definition and management. *Reprod BioMed Online.* (2014) 28 (1):14–38. doi: 10.1016/j.rbmo.2013.08.011

2. Galan A, Herrer R, Remohi J, Pellicer A, Simon C. Embryonic regulation of endometrial epithelial apoptosis during human implantation. *Hum Reprod* (2000) 15 Suppl 6:74–80.

3. Thornhill AR, deDie-Smulders CE, Geraedts JP, Harper JC, Harton GL, Lavery SA, et al. ESHRE PGD consortium 'Best practice guidelines for clinical preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS)'. *Hum Reprod* (2005) 20(1):35–48. doi: 10.1093/humrep/deh579

4. Tan BK, Vandekerckhove P, Kennedy R, Keay SD. Investigation and current management of recurrent IVF treatment failure in the UK. *BJOG.* (2005) 112(6):773–80. doi: 10.1111/j.1471-0528.2005.00523.x

5. Polanski LT, Baumgarten MN, Quenby S, Brosens J, Campbell BK, Raine-Fenning NJ. What exactly do we mean by 'recurrent implantation failure'? a systematic review and opinion. *Reprod BioMed Online.* (2014) 28(4):409–23. doi: 10.1016/j.rbmo.2013.12.006

6. Stanhiser J, Steiner AZ. Psychosocial aspects of fertility and assisted reproductive technology. *Obstet Gynecol Clin North Am* (2018) 45(3):563–74. doi: 10.1016/j.ogc.2018.04.006

7. Miller PB, Parnell BA, Bushnell G, Tallman N, Forstein DA, Higdon HL3rd, et al. Endometrial receptivity defects during IVF cycles with and without letrozole. *Hum Reprod* (2012) 27(3):881–8. doi: 10.1093/humrep/der452

8. Taylor E, Gomel V. The uterus and fertility. *Fertil Steril.* (2008) 89(1):1–16. doi: 10.1016/j.fertnstert.2007.09.069

9. Richter KS, Bugge KR, Bromer JG, Levy MJ. Relationship between endometrial thickness and embryo implantation, based on 1,294 cycles of *in vitro* fertilization with transfer of two blastocyst-stage embryos. *Fertil Steril.* (2007) 87(1):53–9. doi: 10.1016/j.fertnstert.2006.05.064

10. Alfer J, Happel L, Dittrich R, Beckmann MW, Hartmann A, Gaumann A, et al. Insufficient angiogenesis: Cause of abnormally thin endometrium in subfertile patients? *Geburtshilfe Frauenheilkd* (2017) 77(7):756–64. doi: 10.1055/s-0043-111899

11. Johnston-MacAnanny EB, Hartnett J, Engmann LL, Nulsen JC, Sanders MM, Benadiva CA. Chronic endometritis is a frequent finding in women with recurrent implantation failure after *in vitro* fertilization. *Fertil Steril.* (2010) 93(2):437–41. doi: 10.1016/j.fertnstert.2008.12.131

12. Silva Martins R, Helio Oliani A, Vaz Oliani D, Martinez de Oliveira J. Subendometrial resistence and pulsatility index assessment of endometrial receptivity in assisted reproductive technology cycles. *Reprod Biol Endocrinol* (2019) 17(1):62. doi: 10.1186/s12958-019-0507-6

13. Zhang Y, Qian J, Zaltzhendler O, Bshara M, Jaffa AJ, Grisaru D, et al. Analysis of *in vivo* uterine peristalsis in the non-pregnant female mouse. *Interface Focus.* (2019) 9 (4):20180082. doi: 10.1098/rsfs.2018.0082

14. Orisaka M, Kurokawa T, Shukunami K, Orisaka S, Fukuda MT, Shinagawa A, et al. A comparison of uterine peristalsis in women with normal uteri and uterine leiomyoma by cine magnetic resonance imaging. *Eur J Obstet Gynecol Reprod Biol* (2007) 135(1):111–5. doi: 10.1016/j.ejogrb.2006.07.040

15. Zhu L, Che HS, Xiao L, Li YP. Uterine peristalsis before embryo transfer affects the chance of clinical pregnancy in fresh and frozen-thawed embryo transfer cycles. *Hum Reprod* (2014) 29(6):1238–43. doi: 10.1093/humrep/deu058

16. Long Y, Liang R, Zhang J, Fang F, Cheng C, Lu Q, et al. Identification and characterization of uterine micro-peristalsis in women undergoing *in vitro* fertilization and embryo transfer *via* dynamic ultrasound features. *Arch Gynecol Obstet* (2019) 300 (6):1729–39. doi: 10.1007/s00404-019-05327-1

17. Fanchin R, Righini C, Olivennes F, Taylor S, de Ziegler D, Frydman R. Uterine contractions at the time of embryo transfer alter pregnancy rates after in-vitro fertilization. *Hum Reprod* (1998) 13(7):1968–74. doi: 10.1093/humrep/13.7.1968

18. Mesen TB, Kacemi-Bourhim L, Marshburn PB, Usadi RS, Matthews M, Norton HJ, et al. The effect of ketorolac on pregnancy rates when used immediately after oocyte retrieval. *Fertil Steril.* (2013) 100(3):725–8. doi: 10.1016/j.fertnstert.2013.04.048

19. Tsatsaris V, Carbonne B, Cabrol D. Atosiban for preterm labour. *Drugs.* (2004) 64(4):375–82. doi: 10.2165/00003495-200464040-00003

20. Pierzynski P, Reinheimer TM, Kuczynski W. Oxytocin antagonists may improve infertility treatment. *Fertil Steril* (2007) 88(1):213.e19–22. doi: 10.1016/j.fertnstert.2006.09.017

21. Lan VT, Khang VN, Nhu GH, Tuong HM. Atosiban improves implantation and pregnancy rates in patients with repeated implantation failure. *Reprod BioMed Online.* (2012) 25(3):254–60. doi: 10.1016/j.rbmo.2012.05.014

22. Mishra V, Agarwal H, Goel S, Roy P, Choudhary S, Lamba S. A prospective case-control trial to evaluate and compare the efficacy and safety of atosiban versus placebo in *In vitro* fertilization-embryo transfer program. *J Hum Reprod Sci* (2018) 11(2):155–60. doi: 10.4103/jhrs.JHRS_7_17

23. Moraloglu O, Tonguc E, Var T, Zeyrek T, Batioglu S. Treatment with oxytocin antagonists before embryo transfer may increase implantation rates after IVF. *Reprod BioMed Online.* (2010) 21(3):338–43. doi: 10.1016/j.rbmo.2010.04.009

24. Ng EH, Li RH, Chen L, Lan VT, Tuong HM, Quan S. A randomized double blind comparison of atosiban in patients undergoing IVF treatment. *Hum Reprod* (2014) 29(12):2687–94. doi: 10.1093/humrep/deu263

25. Wu MH, Lin CW, Su PF, Lai EC, Sie FC, Mau YL, et al. Atosiban and pregnancy outcomes following *In vitro* fertilization treatment for infertile women requiring one, two, or more embryo transfer cycles: A longitudinal cohort study. *Reprod Sci* (2020) 27 (3):853–9. doi: 10.1007/s43032-019-00088-3

26. Yuan C, Song H, Fan L, Su S, Dong B. The effect of atosiban on patients with difficult embryo transfers undergoing *In vitro* fertilization-embryo transfer. *Reprod Sci* (2019) 26(12):1613–7. doi: 10.1177/1933719119831791

27. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-p) 2015 statement. *Syst Rev* (2015) 4(1):1. doi: 10.1186/2046-4053-4-1

28. Cumpston M, Li T, Page MJ, Chandler J, Welch VA, Higgins JP, et al. Updated guidance for trusted systematic reviews: a new edition of the cochrane handbook for systematic reviews of interventions. *Cochrane Database Syst Rev* (2019) 10:ED000142. doi: 10.1002/14651858.ED000142

29. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa scale (NOS) for assessing the quality if nonrandomized studies in meta-analyses. (2009).

30. Zhai T, Li SZ, Fan XT, Tian Z, Lu XQ, Dong J. Circulating nesfatin-1 levels and type 2 diabetes: A systematic review and meta-analysis. *J Diabetes Res* (2017) 2017:7687098. doi: 10.1155/2017/7687098

31. Liu Q, Li X, Li W, Du X, He JQ, Tao C, et al. Influence of carbapenem resistance on mortality of patients with pseudomonas aeruginosa infection: a meta-analysis. *Sci Rep* (2015) 5:11715. doi: 10.1038/srep11715

32. Feng Y, Wang S, Chen R, Tong X, Wu Z, Mo X. Maternal folic acid supplementation and the risk of congenital heart defects in offspring: A meta-analysis of epidemiological observational studies. *Sci Rep* (2015) 5:8506. doi: 10.1038/srep08506

33. Blettner M, Sauerbrei W, Schlehofer B, Scheuchenpflug T, Friedenreich C. Traditional reviews, meta-analyses and pooled analyses in epidemiology. *Int J Epidemiol.* (1999) 28(1):1–9. doi: 10.1093/ije/28.1.1

34. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ.* (2003) 327(7414):557–60. doi: 10.1136/bmj.327.7414.557

35. Tang CL, Li QY, Chen FL, Cai CT, Dong YY, Wu YY, et al. A randomized double blind comparison of atosiban in patients with recurrent implantation failure undergoing IVF treatment. *Reprod Biol Endocrinol* (2022) 20(1):124. doi: 10.1186/s12958-022-00999-y

36. Li FJ, Pei L, Huang GN, Ye H. Analysis of the influence of atosiban on the pregnancy outcome of freezethaw embryo transfer in patients with different ages. *J Pract Obstetrics Gynecology.* (2021) 37(2):147–51.

37. Liu Jie ZJ, Yalan L, Wen X, Ming X, Hong S. Influence of atoxiban on pregnancy outcome in patients with repeated implantion failure in blastcyst thawed embryo transfer. *J Pract Med* (2017) 33(6):979–82.

38. He Y, Wu H, He X, Xing Q, Zhou P, Cao Y, et al. Application of atosiban in frozen-thawed cycle patients with different times of embryo transfers. *Gynecol Endocrinol* (2016) 32(10):811–5. doi: 10.1080/09513590.2016.1180680

39. Zhang Yue CY, Haining L, Yunshan Z, Fengxia X. Influence of atosiban on pregnancy outcome in patients with repeated implantation failure in freezing embryo transfer cycle. *Chin J Family Planning.* (2014) 22(5):325–8.

40. Jiang W. The use of oxytocin antagonist in repeated implantation failure. Wuhan, China: Huazhong University of Science and Technology (2014).

41. Chou PY, Wu MH, Pan HA, Hung KH, Chang FM. Use of an oxytocin antagonist in *in vitro* fertilization-embryo transfer for women with repeated implantation failure: a retrospective study. *Taiwan J Obstet Gynecol* (2011) 50 (2):136–40. doi: 10.1016/j.tjog.2011.04.003

42. Ovarian Stimulation T, Bosch E, Broer S, Griesinger G, Grynberg M, Humaidan P, et al. ESHRE guideline: ovarian stimulation for IVF/ICSI(dagger). *Hum Reprod Open* (2020) 2020(2):hoaa009. doi: 10.1093/hropen/hoaa009

43. Huang QY, Rong MH, Lan AH, Lin XM, Lin XG, He RQ, et al. The impact of atosiban on pregnancy outcomes in women undergoing *in vitro* fertilization-embryo transfer: A meta-analysis. *PLoS One* (2017) 12(4):e0175501. doi: 10.1371/journal.pone.0175501

44. Schwarze JE, Crosby J, Mackenna A. Atosiban improves the outcome of embryo transfer. a systematic review and meta-analysis of randomized and non-randomized trials. *JBRA Assist Reprod* (2020) 24(4):421–7. doi: 10.5935/1518-0557.20200016

45. Li J, Chen Y, Wang A, Zhang H. A meta-analysis of atosiban supplementation among patients undergoing assisted reproduction. *Arch Gynecol Obstet.* (2017) 296 (4):623–34. doi: 10.1007/s00404-017-4455-0

46. Shapiro BS, Daneshmand ST, Desai J, Garner FC, Aguirre M, Hudson C. The risk of embryo-endometrium asynchrony increases with maternal age after ovarian stimulation and IVF. *Reprod BioMed Online.* (2016) 33(1):50–5. doi: 10.1016/j.rbmo.2016.04.008

47. Orvieto R, Meltcer S, Nahum R, Rabinson J, Anteby EY, Ashkenazi J. The influence of body mass index on *in vitro* fertilization outcome. *Int J Gynaecol Obstet.* (2009) 104(1):53–5. doi: 10.1016/j.ijgo.2008.08.012

48. Pasch LA, Gregorich SE, Katz PK, Millstein SG, Nachtigall RD, Bleil ME, et al. Psychological distress and *in vitro* fertilization outcome. *Fertil Steril.* (2012) 98(2):459–64. doi: 10.1016/j.fertnstert.2012.05.023

49. Waylen AL, Metwally M, Jones GL, Wilkinson AJ, Ledger WL. Effects of cigarette smoking upon clinical outcomes of assisted reproduction: A meta-analysis. *Hum Reprod Update.* (2009) 15(1):31–44. doi: 10.1093/humupd

50. Franasiak JM, Alecsandru D, Forman EJ, Gemmell LC, Goldberg JM, Llarena N, et al. A review of the pathophysiology of recurrent implantation failure. *Fertil Steril.* (2021) 116(6):1436–48. doi: 10.1016/j.fertnstert.2021.09.014

51. Fanchin R, Ayoubi JM. Uterine dynamics: Impact on the human reproduction process. *Reprod BioMed Online.* (2009) 18 Suppl 2:57–62. doi: 10.1016/1472-6483(10) 60450-6

52. Chibbar R, Wong S, Miller FD, Mitchell BF. Estrogen stimulates oxytocin gene expression in human chorio-decidua. *J Clin Endocrinol Metab* (1995) 80(2):567–72. doi: 10.1210/jcem.80.2.7852522

53. Richter ON, Kubler K, Schmolling J, Kupka M, Reinsberg J, Ulrich U, et al. Oxytocin receptor gene expression of estrogen-stimulated human myometrium in extracorporeally perfused non-pregnant uteri. *Mol Hum Reprod* (2004) 10(5):339–46. doi: 10.1093/molehr/gah039

54. Liedman R, Hansson SR, Howe D, Igidbashian S, McLeod A, Russell RJ, et al. Reproductive hormones in plasma over the menstrual cycle in primary dysmenorrhea compared with healthy subjects. *Gynecol Endocrinol* (2008) 24(9):508–13. doi: 10.1080/09513590802306218

55. Liang YL, Kuo TC, Hung KH, Chen TH, Wu MH. Oxytocin antagonist for repeated implantation failure and delay of delivery. *Taiwan J Obstet Gynecol.* (2009) 48 (3):314–6. doi: 10.1016/S1028-4559(09)60314-4

56. Penrod LV, Allen RE, Rhoads ML, Limesand SW, Arns MJ. Oxytocin stimulated release of PGF2alpha and its inhibition by a cyclooxygenase inhibitor and an oxytocin receptor antagonist from equine endometrial cultures. *Anim Reprod Sci* (2013) 139(1-4):69–75. doi: 10.1016/j.anireprosci.2013.04.010

57. Kim SH, Riaposova L, Ahmed H, Pohl O, Chollet A, Gotteland JP, et al. Oxytocin receptor antagonists, atosiban and nolasiban, inhibit prostaglandin F (2alpha)-induced contractions and inflammatory responses in human myometrium. *Sci Rep* (2019) 9(1):5792. doi: 10.1038/s41598-019-42181-2

58. Sztachelska M, Ponikwicka-Tyszko D, Sokolowska G, Anisimowicz S, Czerniecki J, Lebiedzinska W, et al. Oxytocin antagonism reverses the effects of high oestrogen levels and oxytocin on decidualization and cyclooxygenase activity in endometrial tissues. *Reprod BioMed Online* (2019) 39(5):737–44. doi: 10.1016/j.rbmo.2019.06.002

59. Worldwide Atosiban versus Beta-agonists Study G. *Effectiveness and safety of the oxytocin antagonist atosiban versus beta-adrenergic agonists in the treatment of preterm labour* Vol. 108. The Worldwide Atosiban versus Beta-agonists Study Group. BJOG (2001) p. 133–42.

60. Buddhabunyakan N, Sothornwit J, Seejorn K, Buppasiri P, Salang L. Effects of atosiban on uterine peristalsis following frozen embryo transfer: A randomized controlled trial. *Eur J Obstet Gynecol Reprod Biol* (2021) 265:96–101. doi: 10.1016/j.ejogrb.2021.08.017

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 22 May 2023
DOI 10.3389/fendo.2023.1182148

OPEN ACCESS

EDITED BY
Zhen-Gao Sun,
Affiliated Hospital of Shandong University of Traditional Chinese Medicine, China

REVIEWED BY
Xiufeng Ling,
Nanjing Medical University, China
Beihong Zheng,
Fujian Women and Children Hospital, China

*CORRESPONDENCE
Sylvie Epelboin
sylvie.epelboin@aphp.fr

†These authors have contributed equally to this work and share first authorship

RECEIVED 08 March 2023
ACCEPTED 04 May 2023
PUBLISHED 22 May 2023

CITATION
Epelboin S, Labrosse J, De Mouzon J, Devaux A, Gervoise-Boyer M-J, Hesters L, Jonveaux P, Levy R, Sermondade N, Fauque P and Pessione F (2023) Higher risk of pre-eclampsia and other vascular disorders with artificial cycle for frozen-thawed embryo transfer compared to ovulatory cycle or to fresh embryo transfer following *in vitro* fertilization. *Front. Endocrinol.* 14:1182148. doi: 10.3389/fendo.2023.1182148

Higher risk of pre-eclampsia and other vascular disorders with artificial cycle for frozen-thawed embryo transfer compared to ovulatory cycle or to fresh embryo transfer following *in vitro* fertilization

Sylvie Epelboin[1*†], Julie Labrosse[2†], Jacques De Mouzon[3], Aviva Devaux[4], Marie-José Gervoise-Boyer[5], Laetitia Hesters[6], Philippe Jonveaux[7], Rachel Levy[8], Nathalie Sermondade[8], Patricia Fauque[9] and Fabienne Pessione[7]

[1]Department of Reproductive Medicine, Gynecology and Obstetrics, Hôpital Bichat-Claude Bernard, Assistance Publique-Hôpitaux de Paris Nord, Université de Paris, Paris, France, [2]Department of Reproductive Medicine, Gynecology and Obstetrics, American Hospital of Paris, Neuilly-sur-Seine, France, [3]Department of Reproductive Biology, Unilabs, Direction médicale, Clichy La Garenne, France, [4]Department of Reproductive Biology, Centre Hospitalier Universitaire, Amiens, France, [5]Department of Reproductive Medicine, Hôpital Saint Joseph, Marseille, France, [6]Department of Reproductive Biology, Hôpital Antoine Béclère, Hôpitaux Universitaires Paris-Sud, Assistance Publique - Hôpitaux de Paris, Clamart, France, [7]Department of Procreation, Agence de la biomédecine, La Plaine Saint Denis, France, [8]Department of Reproductive Biology, Hôpital Tenon, Hôpitaux Universitaires Est Parisien, Assistance Publique – Hôpitaux de Paris, Paris, France, [9]Department of Reproductive Biology, Université Bourgogne Franche-Comté - INSERM UMR1231, Dijon, France

Background: Risks of maternal morbidity are known to be reduced in pregnancies resulting from frozen embryo transfer (FET) compared to fresh-embryo transfer (*fresh*-ET), except for the risk of pre-eclampsia, reported to be higher in FET pregnancies compared to *fresh*-ET or natural conception. Few studies have compared the risk of maternal vascular morbidities according to endometrial preparation for FET, either with ovulatory cycle (OC-FET) or artificial cycle (AC-FET). Furthermore, maternal pre-eclampsia could be associated with subsequent vascular disorders in the offspring.

Methods: A 2013-2018 French nationwide cohort study comparing maternal vascular morbidities in 3 groups of single pregnancies was conducted: FET with either OC or AC preparation, and *fresh*-ET. Data were extracted from the French National Health System database. Results were adjusted for maternal characteristics and infertility (age, parity, smoking, obesity, history of diabetes or hypertension, endometriosis, polycystic ovary syndrome and premature ovarian insufficiency).

Results: A total of 68025 single deliveries were included: *fresh*-ET (n=48152), OC-FET (n=9500), AC-FET (n=10373). The risk of pre-eclampsia was higher in

AC-FET compared to OC-FET and *fresh*-ET groups in univariate analysis (5.3% *vs.* 2.3% and 2.4%, respectively, *P*<0.0001). In multivariate analysis the risk was significantly higher in AC-FET compared to *fresh*-ET: aOR=2.43 [2.18-2.70], *P*<0.0001). Similar results were observed for the risk of other vascular disorders in univariate analysis (4.7% *vs*. 3.4% and 3.3%, respectively, *P*=0.0002) and in multivariate analysis (AC-FET compared to *fresh*-ET: aOR=1.50 [1.36-1.67], *P*<0.0001). In multivariate analysis, the risk of pre-eclampsia and other vascular disorders were comparable in OC-FET and *fresh*-ET: aOR=1.01 [0.87-1.17, *P*= 0.91 and aOR=1.00 [0.89-1.13], *P*=0.97, respectively).Within the group of FET, the risks of pre-eclampsia and other vascular disorders in multivariate analysis were higher in AC-FET compared to OC-FET (aOR=2.43 [2.18-2.70], *P*<0.0001 and aOR=1.5 [1.36-1.67], *P*<0.0001, respectively).

Conclusion: This nationwide register-based cohort study highlights the possibly deleterious role of prolonged doses of exogenous estrogen-progesterone supplementation on gestational vascular pathologies and the protective role of the *corpus luteum* present in OC-FET for their prevention. Since OC-FET has been demonstrated not to strain the chances of pregnancy, OC preparation should be advocated as first-line preparation in FET as often as possible in ovulatory women.

KEYWORDS

pre-eclampsia, artificial cycle, ovulatory cycle, frozen embryo transfer, fresh embryo transfer, endometrial preparation

1 Introduction

The practice of frozen-thawed embryo transfer (FET) has increased over the past decades in connection with technological improvements resulting in higher cumulative live birth rates (1–5). FET enables single embryo transfer, reduces the risk of ovarian hyperstimulation syndrome, optimizes endometrial receptivity and facilitates fertility preservation (2–8). According to the 2020 annual report of the French Biomedicine Agency, FET was performed in up to 33 350 couples, representing 41.6% of *in vitro* Fertilization (IVF) transfers, of which 65% were transfers of frozen-thawed blastocysts. Although there is no transnational report on the type of protocol most frequently used for FET, it seems that the artificial cycle (AC) is the most performed worldwide since it facilitates the organization of ART centers compared with endometrial preparation by ovulatory cycles (OC), whether natural/modified natural or stimulated.

Assisted Reproductive Technologies (ART) have been associated to various maternal morbidities for which controlled ovarian stimulation regimens (9), embryo culture media (10, 11), and/or subfertility in itself might play a role (12–14). Risks of maternal and perinatal morbidity (placenta previa, placental abruption, premature birth, small for gestational age, and perinatal mortality) are known to be reduced in pregnancies resulting from FET compared to fresh embryo transfer (*fresh*-ET), except for the risk of pre-eclampsia and severe pre-eclampsia that were reported to be significantly higher in pregnancies resulting from FET compared to *fresh*-ET or natural conception (9, 15–21). Gestational hypertension is a disease of pregnancy that combines an increase in blood pressure > 140 mmHg and/or 90 mmHg occurring after the 20th week of amenorrhea, measured twice, with or without proteinuria > 0.3g/24h and/or oedemas and clinical other symptoms (22). The severe form of pre-eclampsia is defined by systolic pressure is ≥ 160 mmHg and/or diastolic pressure ≥ 110 mmHg or hypertension not controlled by treatment, more or less associated with impaired renal function with proteinuria ≥ 3g per 24 hours, oliguria, high blood levels of creatinine and/or liver enzymes, and low levels of blood platelets (22). Preeclampsia can lead to eclampsia, a serious complication that results in seizures.

Pre-eclampsia is considered as a multi-systemic disorder occurring in about 2-5% of pregnancies and as one of the leading causes of maternal and perinatal mortality and morbidity, particularly in developing countries (23). In France, although maternal mortality secondary to hypertensive pathologies in pregnancy has decreased by 50% in 10 years according to the latest report of the confidential national survey on maternal deaths, pre-eclampsia and severe pre-eclampsia remains one of the main causes of mortality (24). Moreover, beyond maternal mortality, pre-eclampsia can be the cause of significant severe maternal morbidity: in 10% of cases, pre-eclampsia progresses to a severe form, which can lead to organic dysfunctions, sometimes persistent in the medium and long term. Severe pre-eclampsia is a risk factor for postpartum thromboembolic complications. Pre-eclampsia is also responsible for a third of premature births in France (25).

Therefore, it is important to prevent it by refining the knowledge of risk factors. An increased risk of pre-eclampsia might be explained by altered trophoblastic invasion leading to an inadequate remodelling of spiral arteries, insufficiently dilated vessels, an imbalance between angiogenic and anti-angiogenic factors or by endothelial dysfunction occurring after the release of placental factors into maternal circulation (26). Some causes of female infertility, such as diminished ovarian reserve or endometriosis may be a risk factor for pre-eclampsia and placental malperfusion lesions (27, 28).

Concerning the impact of the different types of endometrial preparation protocols for FET on the risk of pregnancy-induced vascular disorders, some studies have suggested that the presence of a *corpus luteum* (CL) in OC might be a protective factor, whereas the non-physiological and prolonged doses of the estrogen-progesterone combination in AC may have a deleterious impact (20, 29).Thus, as most recent studies demonstrate an equal live birth rate with endometrial preparation either by OC or AC, others have compared the risk of gestational vascular morbidities between the two types of protocols (20, 29). A systematic review of 2021 on hypertensive disorders in pregnancy following a FET cycle observed that hypertensive disorders were significantly increased after AC-FET when compared with natural cycle or mild OC-FET cycle (30). In a 2022 meta-analysis of 12 studies, Busneli et al. (31) observed a higher risk of pre-eclampsia, hypertensive disorders of pregnancy (HDP) and pregnancy-induced hypertension in AC-FET pregnancies compared to NC.

Many studies have also highlighted other manifestations of gestational vascular disorders, not only on pregnancy, but on the child's growth, in case of assisted procreation techniques or treatments (30–35). Nevertheless, we have chosen to focus this study on the association between endometrial preparation protocols for ET and the incidence of pre-eclampsia and other maternal vascular disorders during pregnancy. The study of fetal growth disorders following medically assisted reproduction according to maternal context or techniques was specifically studied from the same national cohort by the same research group from the French Biomedecine Agency.

The objective of this extensive 5-year nationwide register-based cohort study was to evaluate, in the French population, the risk of pre-eclampsia and other gestational vascular disorders between FET, according to endometrial preparation by either ovulatory cycle (OC-FET) or artificial cycle (AC-FET) and *fresh*-ET. The aim was to report broad real-life data considering the numerous confounding factors accessible in the database, including underlying female infertility.

2 Materials and methods

This study is a nationwide register-based cohort study. Data were extracted from the French National Health System database (*Système National des Données de Santé* – *SND*S) that includes > 99% of national deliveries, in which all outpatients and hospitalizations from 2008 to 2018 (in any public hospital and private clinic) were registered. The database contains information on patient characteristics, diagnoses and treatments registered in outpatient consultations. Maternal records were merged anonymously and with previous hospitalisations through a specific software making it impossible to retrieve patient identity but allowing to cross information through anonymized codes. The access to this database was legally approved.

We conducted a comparative analysis of the cohort of singleton births (deliveries ≥22 weeks of gestation (WG) and/or > 500g of birthweight) occurring in France and resulting from *fresh*-ET or FET from IVF and intracytoplasmic sperm injection (ICSI) cycles performed over a 5-year period (2013-2017). All women with a history of delivery from IVF with fresh-ET, intrauterine insemination or FET within the previous 5 years were excluded from the analysis As it was specified above, the database analysis made it possible to identify health events of our patients since the year 2008. Data available in the hospitalization database were parity, multiple pregnancy, maternal age, active smoking during pregnancy, obesity, maternal history of diabetes (type 1 or 2) or hypertension, diagnosis of endometriosis, polycystic ovary syndrome (PCOS) or premature ovarian insufficiency (POI), mode of conception (*fresh*-ET or FET) and term. Patients with twin deliveries or history of hypertensive disorders (pregnancy-induced or not) were excluded.

2.1 Comparison groups

Three comparison groups of singletons were analyzed: 1/pregnancies resulting from OC-FET (natural, modified natural or stimulated cycle); 2/pregnancies resulting from AC-FET; 3/pregnancies resulting from *fresh*-ET.

2.2 Endometrial preparation protocols

Endometrial preparation with OC included natural cycles, modified natural cycles (natural cycles with ovulation triggering by hCG and/or luteal phase support) and stimulated cycles (mild ovarian stimulation by gonadotropins). Luteal phase support with vaginal micronized progesterone (VMPg, 200 to 400 mg/day) was administered for 6 to 10 weeks of gestation (WG) in case of pregnancy.

Endometrial preparation by AC consisted in the sequential administration of exogenous estrogens and progesterone. According to ART centers, supplementation by estrogens was usually started on Day 1 (orally at 4-8 mg/day and/or transdermally at 200 µg/3days). In case of AC protocols with previous down regulation by GnRH-agonist, estrogen supplementation was started 10 to 15 days after GnRH-agonist introduction. Once adequate endometrial thickness was obtained, VMPg (600 to 1200 mg daily) was started and continued until the 12th WG in case of pregnancy.

Embryos were obtained from conventional IVF or ICSI cycles. In FET groups, embryos were frozen at cleavage stage or blastocyst stage (according to the evolution of laboratory policies).

2.3 Vascular disorders

Vascular disorders were classified into 2 groups according to the International Classification of Disease (ICD-10) codes: (i) hospitalization for pre-eclampsia and/or eclampsia (O14 and O15, grouped under the term pre-eclampsia), and (ii) hospitalization for other vascular disorders during pregnancy (gestational hypertension with or without proteinuria (O13, O16) or isolated proteinuria with oedema (O12). Although the terms used in literature are most often hypertensive disorders of pregnancy, (grouping pre-eclampsia and pregnancy induced hypertension with or without proteinuria), we made the choice to scrupulously follow the diagnoses according to the ICD10 codes internationally validated. We grouped under the term "other vascular disorders" the cases of hospitalization for isolated proteinuria with oedema with those for gestational hypertension with or without proteinuria, and distinguished them from pre-eclampsia/eclampsia, because of the difference in the severity of the maternal-fetal consequences. For isolated proteinuria with oedema, we make it clear that these are cases that have generated hospitalization.

2.4 Statistical analysis

Univariate and multivariate analyses were performed using logistic regression models to compare the risk of pregnancy-induced vascular disorders between *fresh*-ET and FET, and between the two types of endometrial preparation in FET cycles (OC-FET *versus* AC-FET). Two risk estimations were performed in multivariate analysis: the risk of pre-eclampsia and the risk of other pregnancy-induced vascular disorders, compared to pregnancies without any vascular disorder. Adjusted Odds Ratios (aOR) and their 95% confidence intervals (CI) were estimated.

3 Results

The study included 68 025 single deliveries following embryo transfer occurring nationwide from 2013 to 2017. A total of 48 152 were cases of *fresh*-ET and 19 873 were FET cycles, among which 9 500 were AC-FET and 10 373 were OC-FET.

3.1 Maternal characteristics

Comparison of maternal characteristics according to the type of treatment is presented in Table 1. Patients with *fresh*-ET were younger and more often primiparous ($P < 0.0001$ in the overall comparison). Within FET groups, in univariate analysis, women with AC were more often primiparous (56.1% *vs.* 54.5%, $P = 0.03$) and obese (4.3% *vs.* 3.6%, $P = 0.01$) and were more often diagnosed with endometriosis (13.3% *vs.* 10.8%, $P < 0.0001$), PCOS (3.5% *vs.* 2.0%, $P < 0.0001$) and POI (1.4% *vs.* 0.6%, $P < 0.0001$).

TABLE 1 Maternal characteristics according type of embryo transfer and endometrial preparation protocol.

	Fresh-ET N=48152		FET without AC N=9500		FET with AC N=10373		*P**
	N	%	N	%	N	%	
Age (years)	33.2	4.4	33.4	4.2	33.5	4.3	
30-29	10101	21.0	1755	18.5	1948	18.8	< 0.0001
30-39	34080	70.8	6980	73.5	7486	72.2	
≥ 40	3971	8.3	765	8.1	939	9.1	
Primiparous	31221	64.8	5181	54.5	5816	56.1	< 0.0001
Smoking	1048	2.2	163	1.7	211	2.0	0.02
Obesity BMI > 30	1818	3.8	340	3.6	445	4.3	0.02
Diabetes	365	0.8	63	0.7	83	0.8	0.51
Endometriosis	6080	12.6	1025	10.8	1374	13.3	< 0.0001
PCOS	1077	2.2	185	2.0	362	3.5	< 0.0001
POI	676	1.4	60	0.6	141	1.4	< 0.0001
Pregnancy-induced vascular disorders							
Pre-eclampsia	1162	2.4	214	2.3	546	5.3	< 0.0001
Other vascular disorders	1621	3.4	309	3.3	486	4.7	< 0.0001

Fresh-ET, fresh embryo transfer; FET, frozen-thawed embryo transfer; AC, artificial cycle; BMI, body mass index; PCOS, polycystic ovarian syndrome; POI, premature ovarian insufficiency.
*P-values in the overall comparison.

10.3389/fendo.2023.1182148

3.2 Risk of pre-eclampsia and other vascular disorders

The frequency of hospitalizations for pre-eclampsia was 2.8% (n = 1 922) and 3.6% (n = 2 416) for other vascular disorders. A total of 63 687 (93.6%) women were not hospitalized for any vascular disorder.

Risk factors of pre-eclampsia and other vascular disorders compared to women without any vascular disorder in multivariate analysis are presented in Tables 2, 3, respectively. The risk of pre-eclampsia increased with age, primiparity, obesity, history of diabetes and POI (Table 2). There was no increased risk of pre-eclampsia in case of endometriosis or PCOS. The risk of vascular disorders other than pre-eclampsia increased with age,

TABLE 2 Risk of pre-eclampsia compared to no vascular disease in multivariate analysis (N=65609).

	Risk of pre-eclampsia*					
	N	%	Adjusted OR	CI 95%		*P*
All	1922	2.9				
Maternal age						
20-29	360	2.6	1			
30-39	1332	2.7	1.19	1.05	1.34	0.005
≥ 40	230	4.1	1.76	1.49	2.09	< 0.0001
Smoking						
No	1879	2.8	1			
Yes	43	3.0	1.02	0.45	1.40	0.88
Primiparous						
No	467	1.8	1			
Yes	1455	3.5	2.13	1.91	2.37	< 0.0001
Obesity						
No	1742	2.7	1			
Yes	180	6.9	2.72	2.31	3.20	< 0.0001
Diabetes						
No	1881	2.8	1			
Yes	41	8.0	2.97	2.13	4.14	< 0.0001
Endometriosis						
No	1684	2.8	1			
Yes	238	2.8	0.97	0.45	1.12	0.71
PCOS						
No	1870	2.8				
Yes	52	3.2	0.99	0.45	1.32	0.97
POI						
No	1876	2.8	1			
Yes	46	5.3	1.77	1.31	2.40	0.0002
Treatment						
Fresh-ET (N=46531)	1162	2.5	1			
FET without AC (N=9191)	214	2.3	1.01	0.87	1.17	0.91
FET with AC (N=9887)	546	5.5	2.43	2.18	2.70	< 0.0001

Pre-eclampsia/eclampsia is defined according to the International Classification of Disease (ICD-10) codes: (O14 and O15).
Patients with other vascular diseases are excluded: N =65609: 1922 with preeclampsia vs. 63687 without any vascular disease.
Fresh-ET, fresh embryo transfer; FET, frozen-thawed embryo transfer; AC, artificial cycle; confidence intervals; aOR, adjusted odds ratio; PCOS, polycystic ovarian syndrome; POI, premature ovarian insufficiency.

10.3389/fendo.2023.1182148

TABLE 3 Risk of other pregnancy-induced vascular diseases compared to no vascular disease in multivariate analysis (N =66103).

	Risk of other vascular disorders					
	N	%	Adjusted OR	CI95%		*P*
All	2416	3.7				
Maternal age						
20-29	477	3.6	1			
30-39	1691	3.6	1.07	0.96	1.19	0.20
≥ 40	248	4.6	1.37	1.17	1.61	< 0.0001
Smoking						
No	2348	3.6	1			
Yes	68	4.9	1.36	1.06	1.74	0.02
Primiparous						
No	774	3.1	1			
Yes	1642	4.0	1.3	1.26	1.51	< 0.0001
Obesity						
No	2276	3.6	1			
Yes	140	5.8	1.60	1.34	1.91	< 0.0001
Diabetes						
No	2370	3.6	1			
Yes	46	9.8	2.72	1.99	3.71	< 0.0001
Endometriosis						
No	2107	3.6	1			
Yes	309	3.8	1.02	0.90	1.15	0.73
PCOS						
No	2349	3.6	1			
Yes	67	4.3	1.11	0.87	1.43	0.40
POI						
No	2384	3.7	1			
Yes	32	3.9	1.02	0.71	1.45	0.93
Treatment						
Fresh-ET (N=46990)	1621	3.4	1			
FET without AC (N=9286)	309	3.3	1.00	0.89	1.13	0.98
FET with AC (N=9827)	486	5.0	1.50	1.36	1.67	< 0.0001

Other vascular disorders are defined according to the International Classification of Disease codes: hospitalization for (gestational hypertension with or without proteinuria (O13, O16) or isolated proteinuria with oedema (O12).

Patients with preeclampsia are excluded: N=66103: 2416 other vascular disease and 63687 without any vascular disease.

Fresh-ET, fresh embryo transfer; FET, frozen-thawed embryo transfer; AC, artificial cycle; CI, confidence intervals; aOR, adjusted odds ratio; PCOS, polycystic ovarian syndrome; POI: premature ovarian insufficiency.

active smoking, primiparity, obesity and history of diabetes (Table 3). There was no increased risk based on any maternal infertility.

The risk of pre-eclampsia was significantly higher in the AC-FET group compared to OC-FET and *fresh*-ET groups in univariate analysis (5.3% *vs.* 2.3% and 2.4%, respectively, $P < 0.0001$). Similar results were observed for the risk of other vascular disorders in AC-FET compared to OC-FET and *fresh*-ET in univariate analysis (4.7% *vs.* 3.4% and 3.3%, $P = 0.0002$) (Table 1).

In multivariate analysis, the risk of pre-eclampsia compared to no vascular disease was significantly higher in AC-FET compared to *fresh*-ET (aOR = 2.43 [2.18-2.70], $P < 0.0001$) (Table 2). The risk

10.3389/fendo.2023.1182148

was similar between OC-FET and *fresh*-ET: (aOR = 1.01 [0.87-1.17], $P = 0.91$) (Table 2).

In multivariate analysis, the risk of other vascular disease compared to no vascular disease was significantly higher in AC-FET compared to *fresh*-ET (aOR = 1.50 [1.36-1.67], $P < 0.0001$) (Table 3). The risk was similar between OC-FET and *fresh*-ET (aOR = 1.00 [0.89-1.13], $P = 0.97$, respectively) (Table 3).

To confirm the difference between the two endometrial preparation protocols for FET, the same multivariate analyses were performed excluding patients with *fresh*-ET. The risk of pre-eclampsia and other vascular disorders (compared to no vascular disease) was significantly higher after AC-FET compared to OC-FET: aOR=2.42 [2.06-2.85], $P < 0.0001$ and aOR=1.50 [1.29-1.74], $P < 0.0001$, respectively) (Figure 1).

4 Discussion

In all, the findings from this large 5-year nationwide observational study demonstrated that endometrial preparation by AC was associated with an increased risk of pre-eclampsia and other vascular disorders compared to *fresh*-ET and OC-FET. These results remained significant after multivariate analysis adjusted on maternal characteristics. The risk was similar between OC-FET and *fresh*-ET.

Several recent publications are in line with our findings. A 2022 retrospective cohort study analysing the incidence of pre-eclampsia in 536 pregnant patients (from either autologous cycles or egg donation) after OC-FET (n = 325) or AC-FET (n= 211) showed that pre-eclampsia was significantly higher in AC cycles (11.8% *vs.* 3.7%, respectively, $P < 0.001$). Results remained significant after multivariate logistic regression analysis (AC-FET *vs.* OC-FET: OR: 2.9, 95% CI 1.4–6.0, $P = 0.005$) (36). Similarly, a 2022 meta-analysis including 9 studies (n= 8 327 patients with PCOS, pregnant after AC-FET or OC-FET) showed that preterm birth and pre-eclampsia rates were significantly higher with AC-FET compared to OC-FET (37). In addition to the obstetrical aspect, long-term consequences of pre-eclampsia are becoming a concern. A 2021 population-based cohort study using Danish national health registers including 2 491 340 individuals born in Denmark from 1977 to 2018 suggest that the existence of maternal hypertensive disorders during pregnancy is associated with a 23% increased risk of early-onset cardiovascular disease in children (32). Notably, results show an increased risk of specific cardiovascular diseases such as hypertension (HR = 2.11 [1.96-2.27]; $P < 0.001$), myocardial infarction (HR = 1.49 [1.12-1.98]; $P = 0.007$), pulmonary embolism (HR = 1.33 [1.11-1.58]; $P = 0.002$) and heart failure (HR = 1.30 [1.02-1.66]; $P = 0.037$). Moreover, a 2022 multinational population-based cohort study collecting data from Danish, Finnish and Swedish national registries (including 8 475 819 births, of which 188 670 (2.2%) were exposed to maternal pre-eclampsia) showed that children had an increased risk of ischemic heart disease (aOR = 1.33 [1.12-1.58]) and stroke (aOR = 1.34 [1.17-1.52]) in case of maternal pre-eclampsia (38). These associations were independent from preterm or small for gestational age, but dependent on the severity of pre-eclampsia.

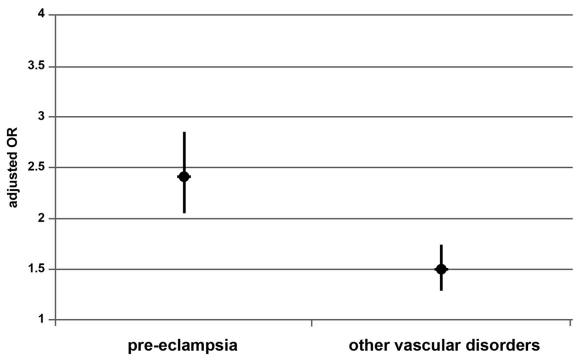


FIGURE 1
Risk of pre-eclampsia and other vascular disorders after AC-FET compared to OC-FET. AC-FET, artificial cycle for frozen-embryo transfer; OC-FET (line 1), ovulatory cycle for frozen-embryo transfer.

In line with our findings, recent studies describe increased obstetrical risks in the absence of CL (18). The pathophysiology of vascular disorders found increased in the absence of CL seems to involve multiple factors. Indeed, the CL is a major source of estradiol, progesterone and their metabolites, as well as relaxin and vasoactive and angiogenic substances that might optimize implantation and placentation. Therefore, the presence of a CL in endometrial preparation by OC possibly leads to more physiological protein secretion profiles compared to AC (39, 40). A hypothesis for the increased risk of pre-eclampsia in the absence of CL could be the imbalance of steroid hormones and their metabolites influencing early physiological processes such as decidualisation, implantation, angiogenesis and maternal haemodynamic (41). Moreover, serum relaxin levels are almost undetectable in pregnant women without CL. This absence of circulating relaxin may also be at risk of abnormal placentation or compromised maternal cardiovascular adaptation. In 2019, Von Versen-Höynck et al. prospectively assessed rates of gestational vascular pathologies in relation to carotid-femoral pulse wave velocity and transit time before, during and after pregnancy, according to number of CL: 0 (n = 26), 1 (n = 23) or >1 (n = 22) (42). AC-FET cycles (0-CL) were associated with higher rates of pre-eclampsia (12.8% *vs.* 3.9%, $P = 0.02$) and severe pre-eclampsia (9.6% *vs.* 0.8%, $P = 0.002$) compared to modified natural FET cycles (1 CL). Authors suggested that altered vascular health in early pregnancy in women with 0 CL (AC cycles) might lead to insufficient cardiovascular adaptation contributing to an increased risk of pre-eclampsia (43). Moreover, within the same time, Boutet et al. published a case-control study on maternal and fetal concentrations of haemopexin, a glycoprotein protective of the vascular endothelium, in pre-eclamptic IVF-pregnancies according to presence or not of CL at embryo transfer (44). After adjustment, maternal haemopexin was higher in IVF with CL compared to natural conception in normotensive women ($P = 0.04$) and in case of pre-eclampsia ($P = 0.01$), and lower in case of pre-eclampsia in IVF pregnancies without CL compared to IVF pregnancies with CL ($P = 0.002$). In cord blood, in case of pre-eclampsia, hemopexin was higher in IVF with CL when compared to spontaneous pregnancies ($P = 0.04$). These

physiological differences support the hypothesis that CL activity may influence perinatal outcomes.

The strength of our extensive 5-year nationwide register-based cohort study covering 68025 single deliveries including 19873 resulting from FET, almost equally between preparation by artificial (AC-FET: 10373) or ovulatory cycle (OC-FET: 9500), and 48152 fresh-ET controls, relies in the number and exhaustiveness of subjects analyzed. Moreover, our national database allows us to report broad real-life data considering the numerous confounding factors accessible in the database, including underlying detailed female infertility.

The limitations are linked to the register-based nature of the cohort data, which, although collected prospectively for the National Health Data System, were analyzed retrospectively according to a non-predetermined reading protocol. Therefore, it did not enable to refine the risk according to details of techniques (embryo stage, culture media, slow freezing or vitrification) and treatments (such as use or not of antiplatelet agents) in each group. Our national database provides some information on certain maternal characteristics (age, obesity, POI, PCOS, endometriosis, etc…) but does not enable to establish a possible link with the indication of FET (avoid fresh transfer to prevent the risk of OHSS, deferred transfer of supernumerary embryo after failure of fresh transfer or after a previous pregnancy). Nevertheless, except for POI, maternal underlying infertility (including PCOS) did not impact the incidence of gestational vascular disorders when comparing fresh-ET and both FET populations (Table 2). Therewith, we did not investigate the respective number of deliveries for the same mother over the period studied, which could result either from two successive IVFs with fresh-transfer if there are no supernumerary embryos after the first delivery, or FET for another child after successful IVF and fresh-transfer delivery, or FET for a child followed by IVF with fresh-transfer for the next one, or again2 deliveries after FET. The hypothesis of an increased risk of gestational vascular pathologies in the event of a prior history of gestational hypertensive disorder could be relevant. Nevertheless, the interpretation would be complex since a vascular gestational history induces a preventive therapeutic framework for the subsequent pregnancy, and because of the multiple possible successions of protocols. In this study, except for ET protocols, the risk of pre-eclampsia and other vascular disorders also increased with age, primiparity, obesity, history of diabetes and POI. Given the multicentric and retrospective nature of this national cohort, it was not possible for us to test the hypothesis of any association between those factors and the protocol choose for ET

In the growing trend of ART centers to practice more and more embryonic freeze-all, the choice of endometrial preparation in full knowledge of its side effects is of primary importance. The two lessons learned from these broad national data are as much warning information regarding the risk factor for preeclampsia and other vascular disorders represented by the artificial cycle preparation, than a reassuring message stemming from the data concerning the ovulatory cycle.

The choice of treatment for FET is possible in women whose ovarian function is present. Growing evidence highlights the possibly fundamental nature of the contribution of the CL in the prevention of adverse obstetrical and perinatal outcomes during pregnancy (45). Pereira et al. (41) stated that a better understanding of the critical roles of the secretory products of the CL during early pregnancy held the promise of improving the efficacy and safety of ART based on programmed FET cycles.

Until recently, the decision on the endometrial preparation protocol most often depended on center procedures, based on medical arguments, expected success rates, pregnancy loss rates, feasibility, organization and regulation of the center's activity and women's comfort. Medical arguments are essentially based on the possibility of ovulation of the woman. Obviously, dysovulations or anovulations determine a preparation with AC. If AC cycle with or without GnRH-agonist pre-treatment has long been the first choice for PCOS patients, current trends follow the principles of individualization, securitization and optimization in endometrial preparation, as mean endometrial thickness, implantation rates, clinical pregnancy rates, ongoing pregnancy rates and live birth rates are similar in artificial cycle and stimulated cycle for endometrial preparation prior to FET in PCOS (46, 47). Guo et al's recent retrospective study on 1413 cases suggested that natural cycle, hormone replacement cycle, or hormone replacement treatment with GnRHa pretreatment showed no superiority or inferiority in pregnancy and perinatal outcomes in patients with endometriosis (48). Furthermore, the organizational argument has long prevailed in favor of AC-FET, which makes it possible to regulate transfers along the week. However, the use of antagonists in modified natural cycles allows an almost comparable flexibility. The argument of women's comfort between a few days of subcutaneous injections in the stimulated protocols then about 6 weeks of progesterone (the vaginal route being the most used in France) in OC, and 3 months of oral estrogen intake and vaginal progesterone in AC protocol, is a very subjective consideration. The cost-effectiveness argument between the two protocols, including the cost of pregnancy concerns (such as hospitalization for pregnancy loss or pre-eclampsia and its complications), has poorly been studied and varies according to the country and the financial support. A recent retrospective study considering overweight/obese women with PCOS, suggested that midly stimulated preparation for FET demonstrated a higher LBR and a lower pregnancy loss rate than that in the AC-FET, and may be considered. as the most cost-effective treatment with the least adverse effects on patients (49). The argument for success has long been in favor of AC-FET, until considering the increased number of miscarriages and therefore the lower live birth rate (LBR) with AC when compared with OC."

Zhang et al. (37) suggested that endometrial preparation by OC might be superior to AC, with significantly higher live birth rates and lower risks of miscarriage, preterm birth and pre-eclampsia, even for women with PCOS (37). Von Versen-Höynck et al. also concluded that pregnancy in the absence of CL could lead to adverse maternal and foetal risks and suggested that the existing evidence was already sufficient to discourage the use of AC-FET in women who ovulate, as they generate a deviation from physiology, exposing the patient and fetus to an avoidable health risk with no apparent benefit (42).

Hence, wider implications of this nationwide register-based cohort study are that it highlights two important information for

physicians: i) the possible deleterious role of non-physiological and prolonged doses of exogenous estrogen-progesterone supplementation on gestational vascular pathologies ii) the protective role of the CL present in stimulated or spontaneous OC for their prevention. Our conclusions could help to change habits, especially since the possible addition of antagonists in OC allows a satisfactory programming of embryo transfer, which is the principal advantage of using AC in ART centers. Since results obtained by OC do not strain the chances of pregnancy, OC preparation could be advocated as first-line endometrial preparation in FET as often as the choice is possible in ovulatory women. Nevertheless, one must consider what specific management could be proposed for cases in which AC is unavoidable, as egg donation for pre-menopausal or menopausal women, or irreducible cases of anovulation or dysovulation generating a long and painful stimulation in women. Developing strategies to reduce the risk of pre-eclampsia are required. The preventive efficacy of antiplatelet agents in AC remains to be established. The present study should also be extended by a long-term follow-up of the cohort in order to evaluate the possible association of maternal pre-eclampsia with an increased risk of subsequent vascular pathologies in the offspring.

Data availability statement

The datasets presented in this article are not readily available because we used the French National hospitalization database (PMSI), included in the large French National Health System database (Système National des Données de Santé (SNDS), in which all hospitalizations (in any public hospital or private clinic) are registered, containing information on patient characteristics, diagnoses and treatments. Data were anonymized at data entry through a specific software making it impossible to retrieve patient identity but enabling to follow all hospitalizations through anonymized codes. Access to PMSI data and SNDS was legally approved in accordance with French Public Health Law (decree N° 2016-1871). Access to PMSI and SNDS data for organizations that do not have permanent access or matching with other databases already available goes through an authorization procedure that involves several organizations: the National Data Institute health (INDS, which in 2019 became the health data platform, the Expertise Committee for research, studies and evaluations in the field of health (CESREES). Consequently, data are available after obtaining legal authorization (at https://www.indsante.fr/) and from the CNIL (Commission Nationale Informatique et Liberte; CNIL, https://www.cnil.fr/). According to the French Public Health Law, non-interventional studies on humans do not require approval from an Institutional Review Board or written consent from participants. The study was conducted according to institutional and ethical rules concerning research on tissue specimens and patients. Requests to access the datasets should be directed to sylvie.epelboin@aphp.fr.

Ethics statement

Ethical review and approval were not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

SE, FP: study conception and design. SE, JL, PF, FP: methodology and investigation. FP: formal analysis. JM, M-JG-B, LH, NS, RL, PJ: resources. SE, JL, PF, FP: writing—original draft preparation. SE, JL, PF, FP: writing—review and 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.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Groenewoud ER, Cohlen BJ, Macklon NS. Programming the endometrium for deferred transfer of cryopreserved embryos: hormone replacement versus modified natural cycles. *Fertil Steril* (2018) 109:768–74. doi: 10.1016/j.fertnstert.2018.02.135

2. Healy MW, Patounakis G, Connell MT, Devine K, DeCherney AH, Levy MJ, et al. Does a frozen embryo transfer ameliorate the effect of elevated progesterone seen in fresh transfer cycles? *Fertil Steril* (2016) 105:93–99.e1. doi: 10.1016/j.fertnstert.2015.09.015

3. Rienzi L, Gracia C, Maggiulli R, LaBarbera AR, Kaser DJ, Ubaldi FM, et al. Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. *Hum Reprod Update* (2017) 23:139–55. doi: 10.1093/humupd/dmw038

4. Roque M, Valle M, Guimarães F, Sampaio M, Geber S. Freeze-all policy: fresh vs. frozen-thawed embryo transfer. *Fertil Steril* (2015) 103:1190–3. doi: 10.1016/j.fertnstert.2015.01.045

5. Roque M, Haahr T, Geber S, Esteves SC, Humaidan P. Fresh versus elective frozen embryo transfer in IVF/ICSI cycles: a systematic review and meta-analysis of reproductive outcomes. *Hum Reprod Update* (2019) 25:2–14. doi: 10.1093/humupd/dmy033

6. Devroey P, Polyzos NP, Blockeel C. An OHSS-free clinic by segmentation of IVF treatment. *Hum Reprod* (2011) 26:2593–7. doi: 10.1093/humrep/der251

7. Groenewoud ER, Cantineau AEP, Kollen BJ, Macklon NS, Cohlen BJ. What is the optimal means of preparing the endometrium in frozen-thawed embryo transfer cycles? a systematic review and meta-analysis. *Hum Reprod Update* (2017) 23:255–61. doi: 10.1093/humupd/dmw046

8. Racca A, Vanni VS, Somigliana E, Reschini M, Viganò P, Santos-Ribeiro S, et al. Is a freeze-all policy the optimal solution to circumvent the effect of late follicular elevated progesterone? a multicentric matched-control retrospective study analysing cumulative live birth rate in 942 non-elective freeze-all cycles. *Hum Reprod* (2021) 36:2463–72. doi: 10.1093/humrep/deab160

9. Vidal M, Vellvé K, González-Comadran M, Robles A, Prat M, Torné M, et al. Perinatal outcomes in children born after fresh or frozen embryo transfer: a Catalan cohort study based on 14,262 newborns. *Fertil Steril* (2017) 107:940–7. doi: 10.1016/j.fertnstert.2017.01.021

10. Maheshwari A, Kalampokas T, Davidson J, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of blastocyst-stage versus cleavage-stage embryos generated through *in vitro* fertilization treatment: a systematic review and meta-analysis. *Fertil Steril* (2013) 100:1615–1621.e1–10. doi: 10.1016/j.fertnstert.2013.08.044

11. Wale PL, Gardner DK. The effects of chemical and physical factors on mammalian embryo culture and their importance for the practice of assisted human reproduction. *Hum Reprod Update* (2016) 22:2–22. doi: 10.1093/humupd/dmv034

12. Pinborg A, Wennerholm UB, Romundstad LB, Loft A, Aittomaki K, Söderström-Anttila V, et al. Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? systematic review and meta-analysis. *Hum Reprod Update* (2013) 19:87–104. doi: 10.1093/humupd/dms044

13. Luke B. Pregnancy and birth outcomes in couples with infertility with and without assisted reproductive technology: with an emphasis on US population-based studies. *Am J Obstet Gynecol* (2017) 217:270–81. doi: 10.1016/j.ajog.2017.03.012

14. Epelboin S, Labrosse J, Fauque P, Levy R, Gervoise-Boyer M-J, Devaux A, et al. Endometriosis and assisted reproductive techniques independently related to mother-child morbidities: a French longitudinal national study. *Reprod BioMed Online* (2021) 42:627–33. doi: 10.1016/j.rbmo.2020.11.017

15. Toner JP, Coddington CC, Doody K, Van Voorhis B, Seifer DB, Ball GD, et al. Society for assisted reproductive technology and assisted reproductive technology in the united states: a 2016 update. *Fertil Steril* (2016) 106:541–6. doi: 10.1016/j.fertnstert.2016.05.026

16. Maheshwari A, Pandey S, Amalraj Raja E, Shetty A, Hamilton M, Bhattacharya S. Is frozen embryo transfer better for mothers and babies? can cumulative meta-analysis provide a definitive answer? *Hum Reprod Update* (2018) 24:35–58. doi: 10.1093/humupd/dmx031

17. Qin J, Liu X, Sheng X, Wang H, Gao S. Assisted reproductive technology and the risk of pregnancy-related complications and adverse pregnancy outcomes in singleton pregnancies: a meta-analysis of cohort studies. *Fertil Steril* (2016) 105:73–85.e1–6. doi: 10.1016/j.fertnstert.2015.09.007

18. Saito K, Kuwahara A, Ishikawa T, Morisaki N, Miyado M, Miyado K, et al. Endometrial preparation methods for frozen-thawed embryo transfer are associated with altered risks of hypertensive disorders of pregnancy, placenta accreta, and gestational diabetes mellitus. *Hum Reprod* (2019) 34:1567–75. doi: 10.1093/humrep/dez079

19. Wei D, Liu J-Y, Sun Y, Shi Y, Zhang B, Liu J-Q, et al. Frozen versus fresh single blastocyst transfer in ovulatory women: a multicentre, randomised controlled trial. *Lancet* (2019) 393:1310–8. doi: 10.1016/S0140-6736(18)32843-5

20. Ginström Ernstad E, Wennerholm U-B, Khatibi A, Petzold M, Bergh C. Neonatal and maternal outcome after frozen embryo transfer: increased risks in programmed cycles. *Am J Obstet Gynecol* (2019) 221:126.e1–126.e18. doi: 10.1016/j.ajog.2019.03.010

21. Pessione F, De Mouzon J, Deveaux A, Epelboin S, Gervoise-Boyer M-J, Jimenez C, et al. Risques de morbidité maternelle et périnatale en fécondation *in vitro* : une étude nationale de cohorte française. *Gynécol Obstétrique Fertilité Sénol* (2020) 48:351–8. doi: 10.1016/j.gofs.2020.02.002

22. Bonnet M-P, Garnier M, Keita H, Compere V, Arthuis C, Raia-Barjat T, et al. Reprint of: severe pre-eclampsia: guidelines for clinical practice from the French society of anesthesiology and intensive care (SFAR) and the French college of gynaecologists and obstetricians (CNGOF). *Gynecol Obstet Fertil Senol* (2022) 50:2–25. doi: 10.1016/j.gofs.2021.11.003

23. Poon LC, Shennan A, Hyett JA, Kapur A, Hadar E, Divakar H, et al. The international federation of gynecology and obstetrics (FIGO) initiative on pre-eclampsia: a pragmatic guide for first-trimester screening and prevention. *Int J Gynaecol Obstet* (2019) 145 Suppl 1:1–33. doi: 10.1002/ijgo.12802

24. SPF. *Les Morts maternelles en France: mieux comprendre pour mieux prévenir. 5e rapport de l'Enquête nationale confidentielle sur les morts maternelles (ENCMM), 2010-2012*. Available at: https://www.santepubliquefrance.fr/maladies-et-traumatismes/maladies-cardiovasculaires-et-accident-vasculaire-cerebral/maladie-vasculaire-de-la-grossesse/les-morts-maternelles-en-france-mieux-comprendre-pour-mieux-prevenir.-5e-rapport-de-l-enquete-nationale-confidentielle-sur-les-morts (Accessed April 30, 2023).

25. Ancel P-Y, Goffinet FEPIPAGE-2 Writing Group, , Kuhn P, Langer B, Matis J, et al. Survival and morbidity of preterm children born at 22 through 34 weeks' gestation in France in 2011: results of the EPIPAGE-2 cohort study. *JAMA Pediatr* (2015) 169:230–8. doi: 10.1001/jamapediatrics.2014.3351

26. Shanmugalingam R, Wang X, Motum P, Fulcher I, Lee G, Kumar R, et al. Clinical influence of nonadherence with prophylactic aspirin in preventing preeclampsia in high-risk pregnancies. *Hypertension* (2020) 75:1125–32. doi: 10.1161/HYPERTENSIONAHA.119.14107

27. Herman HG, Volodarsky-Perel A, Ton Nu TN, Machado-Gedeon A, Cui Y, Shaul J, et al. Diminished ovarian reserve is a risk factor for preeclampsia and placental malperfusion lesions. *Fertil Steril* (2023). doi: 10.1016/j.fertnstert.2023.01.029

28. Epelboin S, Labrosse J, Fauque P, Levy R, Gervoise-Boyer M-J, Devaux A, et al. Endometriosis and assisted reproductive techniques independently related to mother-child morbidities: a French longitudinal national study. *Reprod Biomed Online* (2021) 42:627–33. doi: 10.1016/j.rbmo.2020.11.017

29. Jing S, Li X, Zhang S, Gong F, Lu G, Lin G. Increased pregnancy complications following frozen-thawed embryo transfer during an artificial cycle. *J Assist Reprod Genet* (2019) 36:925–33. doi: 10.1007/s10815-019-01420-1

30. Mumusoglu S, Polat M, Ozbek IY, Bozdag G, Papanikolaou EG, Esteves SC, et al. Preparation of the endometrium for frozen embryo transfer: a systematic review. *Front Endocrinol (Lausanne)* (2021) 12:688237. doi: 10.3389/fendo.2021.688237

31. Busnelli A, Schirripa I, Fedele F, Bulfoni A, Levi-Setti PE. Obstetric and perinatal outcomes following programmed compared to natural frozen-thawed embryo transfer cycles: a systematic review and meta-analysis. *Hum Reprod* (2022) 37:1619–41. doi: 10.1093/humrep/deac073

32. Huang C, Li J, Qin G, Liew Z, Hu J, László KD, et al. Maternal hypertensive disorder of pregnancy and offspring early-onset cardiovascular disease in childhood, adolescence, and young adulthood: a national population-based cohort study. *PloS Med* (2021) 18:e1003805. doi: 10.1371/journal.pmed.1003805

33. Rosalik K, Carson S, Pilgrim J, Luizzi J, Levy G, Heitmann R, et al. Effects of different frozen embryo transfer regimens on abnormalities of fetal weight: a systematic review and meta-analysis. *Hum Reprod Update* (2021) 28:1–14. doi: 10.1093/humupd/dmab037

34. Asserhøj LL, Spangmose AL, Aaris Henningsen A-K, Clausen TD, Ziebe S, Jensen RB, et al. Adverse obstetric and perinatal outcomes in 1,136 singleton pregnancies conceived after programmed frozen embryo transfer (FET) compared with natural cycle FET. *Fertil Steril* (2021) 115:947–56. doi: 10.1016/j.fertnstert.2020.10.039

35. Makhijani R, Bartels C, Godiwala P, Bartolucci A, Nulsen J, Grow D, et al. Maternal and perinatal outcomes in programmed versus natural vitrified-warmed blastocyst transfer cycles. *Reprod BioMed Online* (2020) 41:300–8. doi: 10.1016/j.rbmo.2020.03.009

36. Roelens C, Blockeel C. Impact of different endometrial preparation protocols before frozen embryo transfer on pregnancy outcomes: a review. *Fertil Steril* (2022) 118:820–7. doi: 10.1016/j.fertnstert.2022.09.003

37. Zhang Y, Wu L, Li TC, Wang CC, Zhang T, Chung JPW. Systematic review update and meta-analysis of randomized and non-randomized controlled trials of ovarian stimulation versus artificial cycle for endometrial preparation prior to frozen embryo transfer in women with polycystic ovary syndrome. *Reprod Biol Endocrinol* (2022) 20:62. doi: 10.1186/s12958-022-00931-4

38. Yang F, Janszky I, Gissler M, Roos N, Wikström A-K, Yu Y, et al. Association of maternal preeclampsia with offspring risks of ischemic heart disease and stroke in Nordic countries. *JAMA Netw Open* (2022) 5:e2242064. doi: 10.1001/jamanetworkopen.2022.42064

39. Casper RF, Yanushpolsky EH. Optimal endometrial preparation for frozen embryo transfer cycles: window of implantation and progesterone support. *Fertil Steril* (2016) 105:867–72. doi: 10.1016/j.fertnstert.2016.01.006

40. Fauser BC, de Jong D, Olivennes F, Wramsby H, Tay C, Itskovitz-Eldor J, et al. Endocrine profiles after triggering of final oocyte maturation with GnRH agonist after cotreatment with the GnRH antagonist ganirelix during ovarian hyperstimulation for *in vitro* fertilization. *J Clin Endocrinol Metab* (2002) 87:709–15. doi: 10.1210/jcem.87.2.8197

41. Pereira MM, Mainigi M, Strauss JF. Secretory products of the corpus luteum and preeclampsia. *Hum Reprod Update* (2021) 27:651–72. doi: 10.1093/humupd/dmab003

42. von Versen-Höynck F, Narasimhan P, Selamet Tierney ES, Martinez N, Conrad KP, Baker VL, et al. Absent or excessive corpus luteum number is associated with altered maternal vascular health in early pregnancy. *Hypertension* (2019) 73:680–90. doi: 10.1161/HYPERTENSIONAHA.118.12046

43. Conrad KP, von Versen-Höynck F, Baker VL. Risk of preeclampsia in artificial frozen embryo transfer as a result of insufficient corpus luteum hormone levels: a response. *Am J Obstet Gynecol* (2022) 227:676–7. doi: 10.1016/j.ajog.2022.06.011

44. Boutet ML, Youssef L, Erlandsson L, Hansson E, Manau D, Crispi F, et al. Maternal and fetal haemopexin and α1-microglobulin concentrations in pre-eclamptic IVF pregnancies according to presence of corpus luteum at embryo transfer. *Reprod BioMed Online* (2022) 45:135–45. doi: 10.1016/j.rbmo.2022.01.005

45. Singh B, Reschke L, Segars J, Baker VL. Frozen-thawed embryo transfer: the potential importance of the corpus luteum in preventing obstetrical complications. *Fertil Steril* (2020) 113:252–7. doi: 10.1016/j.fertnstert.2019.12.007

46. Yu J, Ma Y, Wu Z, Li Y, Tang L, Li Y, et al. Endometrial preparation protocol of the frozen-thawed embryo transfer in patients with polycystic ovary syndrome. *Arch Gynecol Obstet* (2015) 291:201–11. doi: 10.1007/s00404-014-3396-0

47. Bosdou JK, Anagnostis P, Kolibianakis EM. Is pretreatment with GnRH agonist necessary for endometrial preparation for frozen embryo transfer cycles in women with polycystic ovary syndrome? *BJOG* (2021) 128:675. doi: 10.1111/1471-0528.16473

48. Guo Y, Fang Z, Yu L, Sun X, Li F, Jin L. Which endometrial preparation protocol provides better pregnancy and perinatal outcomes for endometriosis patients in frozen-thawed embryo transfer cycles? a retrospective study on 1413 patients. *J Ovarian Res* (2023) 16:7. doi: 10.1186/s13048-023-01095-4

49. Guan L, Wu H, Wei C, Pang C, Liu D, Yu X, et al. The effect of mildly stimulated cycle versus artificial cycle on pregnancy outcomes in overweight/obese women with PCOS prior to frozen embryo transfer: a retrospective cohort study. *BMC Pregnancy Childbirth* (2022) 22:394. doi: 10.1186/s12884-022-04728-6

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 06 June 2023
DOI 10.3389/fendo.2023.1202044

OPEN ACCESS

EDITED BY
Zhen-Gao Sun,
Affiliated Hospital of Shandong University of Traditional Chinese Medicine, China

REVIEWED BY
Keiichi Matsubara,
Ehime University, Japan
Jie Zhang,
Shanghai Jiao Tong University, China
Ali Khatibi,
Sahlgrenska University Hospital, Sweden
Heather Burks,
University of Oklahoma Health Sciences Center, United States

*CORRESPONDENCE
Hong Li
hongliszivf@163.com
Qingxia Meng
mqx593204@163.com

†These authors have contributed equally to this work

RECEIVED 07 April 2023
ACCEPTED 25 May 2023
PUBLISHED 06 June 2023

CITATION
Wang F, Wang Q, Song Y, Ding J, Li H and Meng Q (2023) Programmed frozen embryo transfer cycles are associated with a higher risk of abnormal placental development: a retrospective cohort study of singleton live births.
Front. Endocrinol. 14:1202044.
doi: 10.3389/fendo.2023.1202044

Programmed frozen embryo transfer cycles are associated with a higher risk of abnormal placental development: a retrospective cohort study of singleton live births

Fuxin Wang[1,2†], Qi Wang[1,2†], Ye Song[2,3], Jie Ding[1,2], Hong Li[1,2*] and Qingxia Meng[1,2*]

[1]Center of Human Reproduction and Genetics, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou, China, [2]Suzhou Municipal Hospital, Gusu School, Nanjing Medical University, Suzhou, China, [3]Department of Obstetrics, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou, China

Introduction: Abnormal placental development can lead to adverse outcomes for both mother and fetus. The effect of different types of endometrium preparation regimens of frozen-thawed cycles on the placental development features associated with the perinatal outcomes remains unclear. Hence, we conducted a retrospective cohort study to assess the impact of specific endometrial preparation regimens on placenta-mediated pregnancy complications in singleton live births.

Methods: A retrospective cohort study was conducted evaluating data of all singleton live births both conceived naturally or by *in vitro* fertilization (IVF) therapy from 2018 to 2020 at our hospital. Two exposed groups of frozen-thawed embryo transfer (FET) were created by the endometrium preparation regimen as the modified natural cycles (mNC) and the programmed cycles. The nonexposed group was the singleton pregnancies conceived naturally. The obstetrical and perinatal outcomes were compared among the three groups using multivariate analysis to adjust the results for determinants potentially associated with the abnormal placental development.

Results: A total of 2186 pregnant women with singleton live births were included in our final analysis and were divided into three groups as naturally conceived group (n=1334), mNC-FETs group (n=217) and programmed-FETs group (n=635). After adjusting for maternal age and parity, no significant difference was observed on the risk of placental disorders between mNC-FET cycles and natural conceived pregnancies (aOR 1.16; 95%CI 1.31-7.01), while programmed-FET cycles were associated with a higher occurrence of placental disorders (aOR 5.36; 95%CI 3.63-8.05). Using the mNC-FET group as a reference and adjusting for confounders such as maternal age, parity, endometrial thickness, and number of embryos transferred, we found that the main manifestation of abnormal

placentation in programmed FET cycles was abnormal placental attachment, including placental adhesion and placenta increta (aOR 2.50, 95%CI 1.36-4.90). The dysfunction of placentation in programmed-FET cycles was independently associated with the type of infertility, the total dose of Femostone and thinner endometrium. Additionally, placental disorders in the programmed-FET group were associated with higher rate of preeclampsia, postpartum hemorrhage and Cesarean section.

Conclusion: Our retrospective study revealed that the programmed-FET has a substantial impact on placental development, resulting in a higher incidence of preeclampsia, postpartum hemorrhage and Cesarean section. These findings have significant implications on clinical decision-making.

KEYWORDS

frozen-embryo transfer, endometrial preparation regimen, hormone replacement treatment, natural cycle treatment, placental disorder

Introduction

With the improvements in cryo-techniques, the cycles of frozen embryo transfers (FETs) have increased dramatically since 1983. FETs are beneficial not only for reducing the rate of ovarian hyperstimulation syndrome (OHSS), but also for allowing time for preimplantation genetics testing and facilitating fertility preservation (1).

Although the pregnancy rates after cryothawing and fresh embryo transfer are similar, the effect of embryo cryopreservation on obstetric and neonatal outcomes is still under debate. It has been reported that compared to fresh embryo transfer, FET has been shown to result in lower rates of low birth weight and premature birth, but higher rates of hypertensive disorders during pregnancy, induced labor, cesarean section, and lower Apgar scores of the newborns (2–4).

Despite the cyro-technique itself, the different endometrial preparation protocols used for FETs in daily practice are crucial to the outcome of newborns. There are three commonly used endometrial preparation regimens for FETs, natural cycles, modified natural cycles (mNC) and programmed cycles. The different endometrial preparation programs will result in different endometrial environments and changes in implantation and placental formation (5). Placental data add to the understanding of intrauterine processes and a comprehensive assessment of placental histopathological patterns may shed light on the potential causes of different pregnancy outcomes in different endometrial preparation regimens of FET pregnancies. Recently, it has been reported that embryo vitrification has a significant effect on the placental histopathology pattern and is associated with a higher prevalence of dysfunctional labor (6). Meanwhile, there is a higher rate of anatomic and vascular placental pathology of pregnancies arising from frozen embryo transfers than those from fresh transfers (7). However, only a few studies have evaluated the impact of different endometrial preparation protocols of FET on placental development and perinatal outcomes (6, 8, 9).

The aim of our study is to assess the effects of different endometrial preparation regimens of FETs on the incidence of placental disorders and the perinatal outcomes of single birth.

Materials and methods

Study design and patients

This was a retrospective study that evaluated data of all singleton live births, both conceived naturally or through *in vitro* fertilization (IVF) therapy, at Suzhou Municipal Hospital and the Center for Reproduction and Genetics at Suzhou Municipal Hospital, Jiangsu Province, from January 2018 to December 2020. The study protocol was approved by the institutional review board of the hospital. Data were collected from medical records, including baseline characteristics, treatment-related information and reproductive outcomes reported up to live birth. Multiple pregnancy and fresh cycles of IVF were excluded.

Endometrium preparation before embryo transfer

In our center, there are mainly two endometrial preparation programs based on patient preference or the discretion of physician, the mNC-FET and programmed-FET.

mNC-FET refers to a modified natural cycle, as a dose of human chorionic gonadotropin (c) trigger is given based on ultrasonic measurements of the dominant follicle and luteal support was also administered. The day of ovulation was confirmed by transvaginal ultrasound. Luteal phase support started on the day after ovulation

with oral dydrogesterone (Duphaston; Abbott, OLST, Netherlands) at a dose of 30mg three times daily. Cleavage-stage embryo or blastocyst-stage embryo were thawed and transferred on day 3 or 5 after ovulation.

For patients in programmed-FET group, oral estradiol valerate (Progynova; Bayer Schering Pharma AG, Germany; at a dose of 2-3mg twice a day) was given on the second or third day of menstrual cycle, after confirming that patients were in the early proliferative phase for menstrual cycle. When the endometrial thickness reached at least 7 mm, vaginal progesterone gel at a dose of 90 mg once daily or micronized progesterone at a dose of 400 mg twice daily combined with oral dydrogesterone at a dose of 30 mg three times daily was added. If the thickness of the endometrium could not meet 7 mm, the dose of oral estradiol valerate was increased to 8 mg or by adding of one or two vaginal 17-βestradiol tablets (Femoston; Duphaston; Abbott, OLST, Netherlands, brick red tablets containing 2mg estradiol). FET was scheduled for 4 days for cleavage-stage embryos and 6 days for blastocyst-stage embryos from the starting of progesterone.

Placental examination

Macroscopic placental examinations were performed for all deliveries in accordance with the institutional protocol employed during the study period. The evaluation of placental morphology and structure included assessment of placental size and weight, umbilical cord, fetal and maternal surfaces, and placental parenchyma. Any abnormalities detected were documented. The main placental disorders found in our study are shown in Figure 1.

Study outcomes

We obtained information on obstetric complications from the medical records and defined diagnoses according to the International Classification of Diseases and Related Health Problems (ICD), 10th revision (ICD-10). Hypertensive disorders in pregnancy were defined as the O13–15 code (pregnancy-induced hypertension, preeclampsia, and eclampsia); preeclampsia, O14; PPROM, O420; placenta previa, O44; placental abruption, O45; induction of labor, O61; postpartum hemorrhage (PPH), O72 with bleeding > 500 mL; and CS, O82. Preeclampsia was defined as gestational hypertension combined with proteinuria and/or organ dysfunction. Intrahepatic cholestasis of pregnancy was defined as O26. Gestational diabetes was defined as O24.9. All diagnoses were allocated by medical doctors. Perinatal outcomes evaluated were child's sex, gestational age (post-term birth [> 42 weeks], preterm birth [PTB; <37 weeks]), birth weight (low birthweight <2500g, high birthweight>4000g)), small gestational age (SGA), and large gestational age (LGA), which were defined as <–2 standard deviation and >+2 standard deviation difference from the expected sex-specific birth weight for the given gestational age, respectively. In all groups gestational age was calculated based on a first-trimester ultrasonography scan.

Statistical analysis

Our main comparison was programmed-FET versus mNC-FET. In addition, we also compared the programmed-FET group, mNC-FET group to the natural conceiving group. All statistical

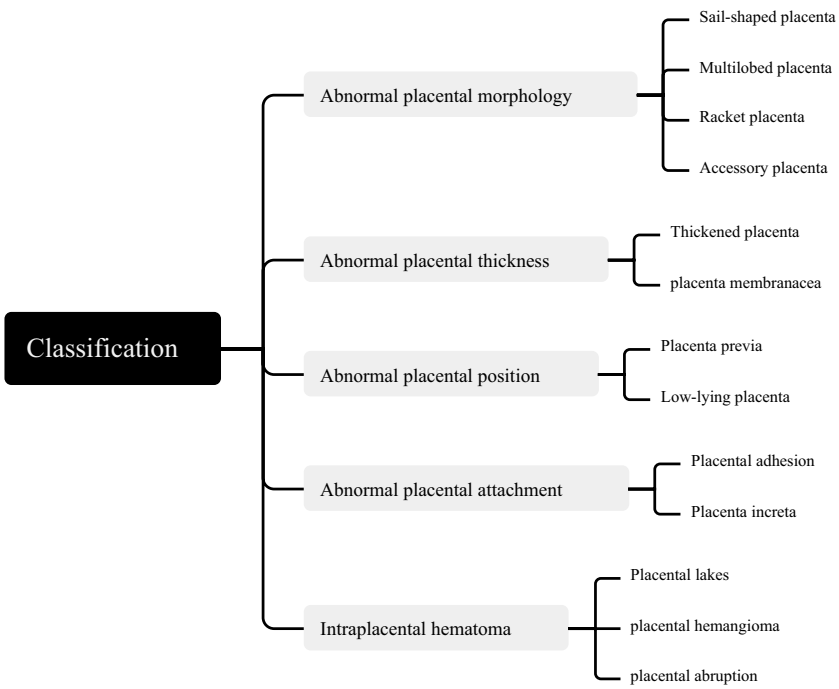


FIGURE 1
Classification of placental developmental abnormalities.

analyses were performed with IBM SPSS Statistics (version 25.0) or the free software computing environment R (version 4.1.0). Data distributions were evaluated with the Shapiro-Wilk test. Normally distributed data were expressed as mean ± standard deviation (SD) and non-normally distributed data were expressed as median (interquartile range). A nonparametric test (Kruskal-Wallis test) was used to compare the rank means between multiple groups of skewed distributions. After the Kruskal-Wallis test, if the differences were statistically significant, the rank sum test for multiple comparisons was continued to be completed. Categorical information was expressed as the number of cases (as a percentage of the total) and assessed by Pearson's chi-square test or Fisher's exact probability method. Univariate logistic regression analysis was used to determine the various factors affecting placental development and multivariate logistic regression analysis was used to adjust for confounding factors to investigate the effect of relevant factors on abnormal placental development during hormone replacement cycles. P-value < 0.05 was considered statistically significant.

Results

Baseline characteristics

A total of 2,186 patients who met the inclusion and exclusion criteria were included in this analysis. Of these, 1334 were conceived naturally, 217 were conceived through mNC-FET cycles and 635 were through programmed-FET cycles, respectively (Figure 2). The baseline characteristics were shown in Table 1. The maternal and paternal ages were differed among the 3 groups, with the youngest in the naturally conceived group and oldest in the modified natural cycle group.

Maternal and neonatal outcomes

The maternal and neonatal outcomes were categorized by the type of conception and the type of endometrial preparation protocols (Table 2). There were no statistically significant differences in the rates of intrahepatic cholestasis of pregnancy, fetal intrauterine growth restriction, and the Apgar score among the three groups. Compared to naturally conceiving group, the rates of gestational diabetes, preeclampsia, Cesarean section, premature birth and postpartum hemorrhage were significantly higher in the FET groups. Furthermore, the incidences of preeclampsia and postpartum hemorrhage were highest in the programmed FET group with statistically significance. The rates of low birth weight and high birth weight were both increased in the programmed-FET group.

Placental disorders in natural conceived group and in the FET groups with different endometrium preparation regimens

The prevalence of abnormal placental thickness and intraplacental hematoma (such as placental lakes, placental hemangioma and placental abruption) was similar among the three groups. After adjusting for maternal age and parity, the prevalence of abnormal placental morphology, including sail-shaped placenta, multilobed placenta, racket placenta and accessory placenta, the incidence of abnormal placental position, including placenta previa and low-lying placenta, the incidence of abnormal placental attachment including placental adhesion and placenta increta, were similar between mNC-FET cycles and naturally conceived group. However, programmed-FET group was associated with a higher occurrence of abnormal placental

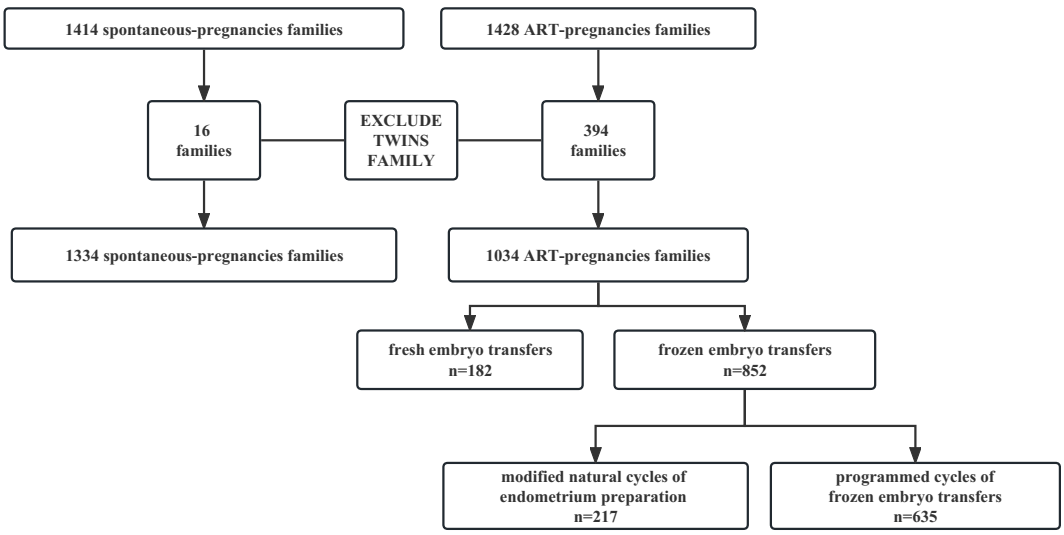


FIGURE 2
Flowchart of patients' selection process.

10.3389/fendo.2023.1202044

TABLE 1 Demographic, clinical and IVF treatment characteristics.

Variables	Naturally conceived (n=1334)	Frozen embryo transfers		*P-value*
		Modified natural cycle (n=217)	Programmed cycle (n=635)	
Maternal age (years)	28.9 (4.8)	31.1 (5.6)	30.2 (5.1)	<0.001***
Paternal age (years)	30.2 (5.4)	32.2 (6.9) *	31.4 (6.0) *	<0.001
Maternal BMI (kg/m^2)	20.8 (3.1)	21.5 (3.9) *	21.8 (4.1) *	<0.001
Parity (n (%))				<0.001***
• first	983 (73.7)	181 (83.4)	586 (92.3)	
• high order	351 (26.3)	36 (16.6)	49 (7.7)	
Duration of infertility (m)		33.0 (27.0)	34.0 (27.0)	<0.001
Type of infertility				0.144
• primary infertility		112 (51.6)	364 (57.3)	
• secondary infertility		105 (48.4)	271 (42.7)	
Cause of infertility				0.103
• female		105	320	
• male		28	83	
• mixes		74	209	
• unexplained		9	66	
b-AMH (ng/mL)		3.6 (3.2)	4.9 (5.3)	0.859
Total antral follicle count		14 (8.75)	15 (8)	0.071
EMT (mm)		9.7 (2.6)	9.0 (2.0)	0.002
Embryo stage at transfer				0.863
• Cleavage stage		68 (31.3)	195 (30.7)	
• blastocyst		149 (68.7)	440 (69.3)	
No. of embryos transferred				<0.001
• single		135 (62.2)	242 (38.1)	
• double		79 (36.4)	383 (60.3)	
• triple		3 (1.4)	10 (1.6)	

BMI, body mass index.
AMH, anti-Müllerian hormone.
E_2, serum estradiol.
EMT, endometrial thickness.
Skewed data were presented as median (with interquartile range).
Categorical data was expressed as the number of cases (as a percentage of the total).
P-value <0.05 was considered statistically significant.
*Significantly different from the naturally conceived group.
**Significantly different from the naturally conceived group and natural cycle group.
***Significant differences were found between any two groups.

morphology (aOR 5.27; 95%CI 2.00-16.44), abnormal placental position (aOR 3.84; 95%CI 1.82-5.82) and abnormal placental attachment (aOR 8.67; 95%CI 5.24-14.98) compared to natural conceived group. Overall, no significant difference was observed in the risk of placental disorders between mNC-FET cycles and natural conceived pregnancies (aOR 1.16; 95%CI 1.31-7.01), while programmed-FET cycles were associated with a higher occurrence of placental disorders (aOR 5.36; 95%CI 3.63-8.05) (Table 3).

We then focused on the dysfunction of placentation in programmed cycles (Table 4). Using the mNC-FET group as a reference and adjusting for confounders such as maternal age, parity, endometrial thickness, and number of embryos transferred, we found that abnormal placentation in programmed cycles mainly manifested as abnormal placental attachment, including placental adhesion and placenta increta (aOR 2.50, 95% CI 1.36-4.90). Additionally, this dysfunction of placentation in

TABLE 2 Obstetric and perinatal outcomes.

Variables	Naturally conceived (n=1334)	Frozen embryo transfers		*P-value*
		Modified natural cycle (n=217)	Programmedcycle (n=635)	
Maternal complications during pregnancy (n (%))				
• Gestational diabetes	307 (23.0)	69 (31.7)*	180 (28.3)*	0.003
• Preeclampsia	23 (1.7)	5 (2.3)*	39 (6.1)**	<0.001
• Intrahepatic cholestasis of pregnancy	9 (0.7)	4 (1.8)	9 (1.4)	0.146
• Fetal intrauterine growth restriction	9 (0.7)	0 (0.0)	5 (0.8)	0.222
Gestational week of delivery (weeks)	39 (1.3)	39 (2.0)*	39 (2.0)*	<0.001
Delivery mode (n (%))				<0.001***
• Vaginal delivery	842 (63.1)	92 (42.4)	186 (29.3)	
• Cesarean section	492 (36.9)	125 (57.6)	449 (70.7)	
Premature birth rate (n (%))	43 (3.2)	13 (6.0)*	47 (7.4)*	<0.001
Postpartum hemorrhage (n (%))	89 (6.7)	10 (4.6)	95 (15.0)	<0.001***
Neonatal outcomes				
• Apgar1' (mean score)	10 (0)	10 (0)	10 (0)	0.968
• Apgar5' (mean score)	10 (0)	10 (0)	10 (0)	0.978
• Birth weight (g)	3360 (520)	3350 (600)	3450 (585)*	<0.001
• Low birth weight (n (%))	28 (2.1)	8 (3.7)	25 (3.9)*	0.048
• high birth weight (n (%))	81 (6.1)	14 (6.4)	63 (9.9)*	0.008

Skewed data were presented as median (with interquartile range).
Categorical data was expressed as the number of cases (as a percentage of the total).
P-value <0.05 was considered statistically significant.
*Significantly different from the naturally conceived group.
**Significantly different from the naturally conceived group and natural cycle group.
***Significant differences were found between any two groups.

TABLE 3 Unadjusted and adjusted odd ratios of placental developmental abnormalities in different cycles of frozen embryo transfer.

Variables	Naturally conceived Reference group (n=1334)	Frozen embryo transfers							
		Modified natural cycle (n=217)				Programmed cycle (n=635)			
		unadjusted		adjusted		unadjusted		adjusted	
		OR (95%CI)	*P*-value	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
Abnormal placental morphology		14.39 (4.39-47.14)	<0.001	4.35 (4.14-6.84)	0.148	8.60 (2.86-25.81)	<0.001	5.27 (2.00-16.44)	0.002
Abnormal placental thickness		/	0.140	/	0.986	/	/	/	/
Abnormal placental position		3.39 (1.34-8.59)	0.016	3.83 (3.03-7.21)	0.143	3.48 (1.73-6.99)	<0.001	3.84 (1.82-8.52)	<0.001
Abnormal placental attachment		2.84 (1.28-6.33)	0.016	2.53 (2.08-4.73)	0.318	8.80 (5.32-14.55)	<0.001	8.67 (5.24-14.98)	<0.001
Intraplacental hematoma		1.0 (0.99-1.00)	1.000	/	0.973	1.26 (0.30-5.30)	1.000	0.51 (0.07-2.44)	0.427
Abnormal placental development		4.19 (2.51-6.99)	<0.001	1.16 (1.31-7.01)	0.879	6.80 (4.71-9.83)	<0.001	5.36 (3.63-8.05)	<0.001

Adjusted for: maternal age, parity.

TABLE 4 Unadjusted and adjusted odd ratios of significant placental pathology results.

	Programmed versus modified natural cycle of endometrium preparation			
	unadjusted		adjusted	
Variables	OR (95%CI)	*P-value*	OR (95%CI)	*P-value*
Abnormal placental morphology	0.60 (0.26-1.37)	0.220	0.54 (0.22-1.34)	0.165
Abnormal placental thickness	1.00 (0.99-1.00)	0.225	NA	0.985
Abnormal placental position	1.01 (0.42-2.41)	0.983	0.65 (0.29-1.51)	0.294
Abnormal placental attachment	3.10 (1.52-6.29)	0.001	2.50 (1.36-4.90)	0.005
Intraplacental hematoma	1.00 (1.00-1.01)	0.575	NA	0.987
Abnormal placental development	1.63 (1.03-2.57)	0.036	1.37 (0.86-2.22)	0.192

Adjusted for: maternal age, parity, EMT (endometrial thickness), number of embryos transferred.

programmed cycles was independently associated with the type of infertility, the total dose of Femostone, and endometrial thickness.

Factors associated with abnormal placental phenotypes in programmed cycles

Since the incidence of placental disorders was higher in the programmed-FET group, we further analyzed the related factors (Table 5). Using univariate logistic regression analysis, we found that secondary infertility, older age, the addition of Femostone, the total dose of Femostone, and the thinner endometrium were correlated with the abnormal placental development. After analyzing these factors by multivariate logistic regression, the secondary infertility, the total dose of Femostone and thinner endometrium were still related to the prevalence of placental disorders.

Association of different placental changes with the perinatal outcomes in programmed-FET cycles

In order to study the abnormal placental development on the incidence of maternal and neonatal complications, we divided the programmed FET group based on whether placental disorders had occurred (Table 6). Data revealed that, placental disorders in the programmed-FET group were associated with higher rate of preeclampsia, postpartum hemorrhage and Cesarean section, but were not related to the occurrence of gestational diabetes, premature birth, low birth weight or high birth weight.

Discussion

Our retrospective cohort study aimed to investigate the effect of different endometrial preparation protocols of FET on the placental development pattern in singleton live births. The placenta develops from the outer trophoblastic layer following the differentiation of the embryo and is more susceptible to epigenetic regulatory changes caused by environmental interventions and influences during assisted reproductive technology. Placenta not only regulates the development of the fetus, but also dysplasia of placenta will lead to poor maternal and perinatal outcomes as well as long-term health risks later in life, including neurodevelopmental disorders, tumors, and adult metabolic syndrome (10, 11). There is sufficient evidence that ART may be related to changes in placental morphology and structure, growth dynamics, imprinted and non imprinted genes, and other aspects that regulate placental formation (12, 13). Several studies have shown that the incidence of placenta previa in ART is significantly higher than in natural pregnancy (14). In addition, the placental weight of ART pregnancies is significantly larger, and the ratio of placental weight to birth weight is also higher (15). Some observations indicate that increased placental thickness during pregnancy obtained through ART leads to a higher incidence of hematoma (9). But few studies focus the different FET protocols on placentation. As a result, it is very meaningful to investigate the association between different intrauterous environment created by different endometrial preparation protocols on the placental changes and intrauterine fetal development.

When maternal age and parity did not take into consideration, the odds ratios of abnormal placental morphology, abnormal placental position and abnormal placental attachment were considerably higher from mNC-FET and programmed-FET groups compared to naturally conceived ones. However, after adjustment of maternal age and parity, the difference of abnormal placental disorder rate was more obvious only in the programmed-FET group compared to the spontaneously conceived pregnancies. Therefore, we speculated that part of the defective placental formation may be related to advanced maternal age.

Then we focus on the dysfunction of placentation in programmed cycles. Using mNC-FET group as reference and adjusting the cofounders like maternal age, parity, endometrium thickness, number of embryo transferred, we found that the abnormal placentation of programmed cycles mainly lied in abnormal placental attachment including placental adhesion and placental increta (aOR 2.50, 95%CI 1.36-4.90). Additionally, this dysfunction of placentation of programmed-FET cycles was independently associated with the infertility type, the total dose of Femostone and the endometrium thickness.

TABLE 5 Logistic regression analysis of factors associated with abnormal placental development in hormone replacement cycles.

Univariate logistic regression analysis			
Variables	OR	95%CI	*P*
Primary infertility	0.43	0.28-0.67	<.001*
Age(years)	1.08	1.02-1.14	.004*
BMI (kg/m^2)	0.97	0.91-1.04	0.473
AMH (ng/mL)	0.95	0.90-1.00	0.086
Progynova treatment	0.73	0.42-1.32	0.282
Total dose of Progynova(mg)	1	0.99-1.00	0.318
Addition of Femoston treatment	2.42	1.56-3.77	<.001*
Total dose of Femoston(mg)	1.01	1.01-1.02	<.001*
EMT (mm)	0.79	0.69-0.91	<.001*
Previous cesarean section	0.49	0.20-1.02	0.08

BMI, body mass index.
AMH, anti-Müllerian hormone.
EMT, endometrial thickness.

Multivariate logistic regression			
Variables	adj.OR	95%CI	adj.*P*
Primary infertility	0.53	0.33-0.85	.008*
Age(years)	1.05	0.99-1.11	0.102
Total dose of Femoston(mg)	1.01	1.00-1.02	.014*
EMT (mm)	0.86	0.75-0.99	.038*

EMT, endometrial thickness.
*P-value <0.05 was considered statistically significant.

		abnormal placental development	without abnormal placental development	*P*
Additon of Femoston treatment	n=139	50	89	<0.001
No addition of Femoston treatment	n=418	46	372	
EMT<8 mm	n=101	26	75	0.001
EMT≥8 mm	n=534	70	464	

EMT, endometrial thickness.
*P-value <0.05 was considered statistically significant.

Infertility is often multifactorial, especially for primary infertility, with both male and female factors and a combination of genetic causes, environmental impacts and underlying disruption of hormonal and endocrine homeostasis. The etiology of primary infertility is different from secondary infertility, with a higher rate of unexplained infertility, ovulatory dysfunction, male factor and least common etiologies of tubal factor (16). Therefore, we speculate that the complicated causes behind primary infertility may be the reason related to placental development disorders. Secondly, in our study, we found that addition of Fenmotone was associated with an increased incidence of placental abnormalities. In our center, when the endometrium cannot meet 7mm using routine dosage of Progynova, we will add Femoston, which in turn resulted a higher cumulative estrogen dose and duration. This has been attributed to reduced endometrial thickness (17), but high-dose estrogen alone may also affect obstetric outcomes and placental findings. Recently published data also showed a similar phenomenon that the higher estrogen dose administered resulted in a higher rate of bilobated placentas, accessory lobes, accelerated villous maturation (18). Exposure to high estradiol concentrations may change the gene expression involved in endometrial remodeling (19). *In vivo* studies have shown that the placental junction region undergoes excessive growth and the ratio of fetus to placenta is low during superovulation, which may be related to decreased oxygenation and consequent placental dysfunction (20). However, research addressing this correlation in programmed cycles is still rare.

TABLE 6 Association of abnormal placental development with the occurrence of perinatal complications in programmed cycles.

	abnormal placental development	without abnormal placental development	χ^2	*P*
Variables	n=96	n=539		
Gestational diabetes	24(25.0)	156(28.9)	0.624	0.430
Preeclampsia	11(11.5)	28(5.2)	5.546	0.019
Premature birth	11(11.5)	36(6.7)	2.716	0.099
Cesarean section	83(86.5)	366(67.9)	13.545	<0.001
Postpartum hemorrhage	34(35.4)	61(11.3)	37.199	<0.001
Low birth weight	6(6.3)	19(3.5)	1.600	0.206
High birth weight	7(7.3)	56(10.4)	0.875	0.350

Categorical data was expressed as the number of cases (as a percentage of the total).
P-value <0.05 was considered statistically significant.

Abnormal placentation in the programmed-FET group is a cause of increased risk of preeclampsia, cesarean section, and postpartum hemorrhage. The link between programmed cycles and hypertensive disorders has gained a lot of attention during recent years (21). Programmed cycles lack corpus luteum, which produces several vasoactive molecules like relaxin, prorenin and other unknown molecules that contribute to the global changes that occur in early pregnancy and serve to reduce the risk of hypertensive disorders later in pregnancy (22, 23). At the same time, the prematurely elevated level of estradiol in programmed cycles may suppress the trophoblastic invasion of spiral arteries which may attribute to the increased risk of hypertensive disorders (24). Abnormal placentation, especially the increased rate of placental attachment, may be the result of a combination of the above factors, and is the origin of the complications of the obstetrical complications of postpartum hemorrhage and higher rate of cesarean section.

Strengths and limitations

The study's strengths lie in its novel approach to assess the placental morphology and structure to explore the effectiveness of different endometrial preparation protocol of FET cycles. The consistency of our study can also be assured as it was conducted in a single center, and placental analyses were carried out by the same pathologist blinded to patient background data. Additionally, multiple pregnancy was excluded, because multiple pregnancy only was reported as a risk factor for maternal and neonatal complications.

One limitation of our study is that it is a retrospective study, which may introduce biases and confounding factors. Although we adjusted for potential confounders, such as maternal age, parity, endometrium thickness, and number of embryos transferred, there may still be residual confounding factors that we did not measure or adjust for. In addition, the sample size of our study is relatively small, which may limit the generalizability of our findings. Further studies with larger sample sizes and prospective designs are needed to confirm our results. Moreover, according to the "developmental origins of health and disease" (DOHaD) hypothesis and the Barker hypothesis, placental dysplasia leads to poor perinatal outcomes as well as long-term health risks later in life (25, 26). It is necessary to perform a more rigorous assessment of placental function and also a follow-up on ART offspring after childbirth.

Conclusion

In conclusion, our study suggests that programmed cycles of FET are associated with an increased risk of abnormal placental attachment, which may lead to obstetric complications such as preeclampsia, postpartum hemorrhage, and a higher rate of cesarean section. The high total dose of estrogen may also contribute to the abnormal placental development pattern in programmed cycles. Clinicians should be aware of these risks when counseling patients about their options for endometrial preparation for FET cycles. Further studies are needed to explore the underlying mechanisms and potential interventions to reduce the risk of abnormal placentation in programmed cycles.

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 approved by Reproductive Medicine Ethics Committee of Suzhou Municipal Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

HL and QM supervised the entire study, including the procedures, design interpretation of the study data, and revisions

of the article. FW: collected the data, drafted the manuscript and reviewed the manuscript. QW: collected the data, data analysis and drafted the article. YS: assessed the placenta. JD: collected the data. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by The National Key Research and Development Program of China(2022YFC2702901), Suzhou key clinical diseases funding (LCZX202109), Gusu School, Nanjing Medical University (GSKY20212226 and GSKY20220401), and Project of Maternal and Child Health of Jiangsu Province (F201913).

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

References

1. Roque M, Haahr T, Geber S, Esteves SC, Humaidan P. Fresh versus elective frozen embryo transfer in IVF/ICSI cycles: a systematic review and meta-analysis of reproductive outcomes. *Hum Reprod Update* (2018) 25(1):2–14. doi: 10.1093/humupd/dmy033

2. Fan L, Li N, Liu X, Li X, Cai H, Pan D, et al. Hormone replacement treatment regimen is associated with a higher risk of hypertensive disorders of pregnancy in women undergoing frozen-thawed embryo transfer. *Front Endocrinol* (2023) 14:1133978. doi: 10.3389/fendo.2023.1133978

3. Zhou R, Zhang X, Huang L, Wang S, Li L, Dong M, et al. The impact of different cycle regimens on birthweight of singletons in frozen-thawed embryo transfer cycles of ovulatory women. *Fertil Steril* (2022) 117(3):573–82. doi: 10.1016/j.fertnstert.2021.09.033

4. Takeshima K, Ezoe K, Onogi S, Kawasaki N, Hayashi H, Kuroda T, et al. Endometrial preparation and maternal and obstetrical outcomes after frozen blastocyst transfer. *AJOG Global Rep* (2022) 2(4):100081. doi: 10.1016/j.xagr.2022.100081

5. Wang Y, Chen Q, Wang Y. Effects of different endometrial preparation regimens during IVF on incidence of ischemic placental disease for FET cycles. *J Clin Med* (2022) 11(21):6506. doi: 10.3390/jcm11216506

6. Volodarsky-Perel A, Ton Nu TN, Orvieto R, Mashiach R, Machado-Gedeon A, Cui Y, et al. The impact of embryo vitrification on placental histopathology features and perinatal outcome in singleton live births. *Hum Reprod* (2022) 37(10):2482–91. doi: 10.1093/humrep/deac167

7. Sacha CR, Harris AL, James K, Basnet K, Freret TS, Yeh J, et al. Placental pathology in live births conceived with in vitro fertilization after fresh and frozen embryo transfer. *Am J Obstet Gynecol* (2020) 222(4):360.e1–.e16. doi: 10.1016/j.ajog.2019.09.047

8. Pereira N, Petrini AC, Lekovich JP, Schattman GL, Rosenwaks Z. Comparison of perinatal outcomes following fresh and frozen-thawed blastocyst transfer. *Int J Gynecol Obstet* (2016) 135(1):96–100. doi: 10.1016/j.ijgo.2016.04.007

9. Joy J, Gannon C, McClure N, Cooke I. Is assisted reproduction associated with abnormal placentation? *Pediatr Dev Pathol Off J Soc Pediatr Pathol Paediatric Pathol Soc* (2012) 15(4):306–14. doi: 10.2350/11-11-1115-OA.1

10. Lesseur C, Chen J. Adverse maternal metabolic intrauterine environment and placental epigenetics: implications for fetal metabolic programming. *Curr Environ Health Rep* (2018) 5(4):531–43. doi: 10.1007/s40572-018-0217-9

11. Fall CHD, Kumaran K. Metabolic programming in early life in humans. philosophical transactions of the royal society of London series b. *Biol Sci* (2019) 374 (1770):20180123. doi: 10.1098/rstb.2018.0123

12. Manna C, Lacconi V, Rizzo G, De Lorenzo A, Massimiani M. Placental dysfunction in assisted reproductive pregnancies: perinatal, neonatal and adult life outcomes. *Int J Mol Sci* (2022) 23(2). doi: 10.3390/ijms23020659

13. Ganer Herman H, Mizrachi Y, Farhadian Y, Shevach Alon A, Gluck O, Bar J, et al. Placental disorders of pregnancy in subsequent IVF pregnancies - a sibling cohort. *Reprod BioMed Online* (2021) 42(3):620–6. doi: 10.1016/j.rbmo.2020.11.018

14. Tao Y, Kuang Y, Wang N. Risks of placenta previa and hypertensive disorders of pregnancy are associated with endometrial preparation methods in frozen-thawed embryo transfers. *Front Med* (2021) 8:646220. doi: 10.3389/fmed.2021.646220

15. Haavaldsen C, Tanbo T, Eskild A. Placental weight in singleton pregnancies with and without assisted reproductive technology: a population study of 536,567 pregnancies. *Hum Reprod* (2012) 27(2):576–82. doi: 10.1093/humrep/der428

16. Sadecki E, Weaver A, Zhao Y, Stewart EA, Ainsworth AJ. Fertility trends and comparisons in a historical cohort of US women with primary infertility. *Reprod Health* (2022) 19(1):13. doi: 10.1186/s12978-021-01313-6

17. Ganer Herman H, Farhadian Y, Shevach Alon A, Mizrachi Y, Ariel D, Raziel A, et al. Complications of the third stage of labor in in vitro fertilization pregnancies: an additional expression of abnormal placentation? *Fertil Steril* (2021) 115(4):1007–13. doi: 10.1016/j.fertnstert.2020.10.004

18. Ganer Herman H, Volodarsky-Perel A, Nu TNT, Machado-Gedeon A, Cui Y, Shaul J, et al. The effect of oestrogen dose and duration in programmed frozen cycles on obstetric outcomes and placental findings. *Reprod BioMed Online* (2023) 46(4):760-6. doi: 10.1016/j.rbmo.2023.01.003

19. Senapati S, Wang F, Ord T, Coutifaris C, Feng R, Mainigi M. Superovulation alters the expression of endometrial genes critical to tissue remodeling and placentation. *J Assisted Reprod Genet* (2018) 35(10):1799–808. doi: 10.1007/s10815-018-1244-z

20. Pereira N, Reichman DE, Goldschlag DE, Lekovich JP, Rosenwaks Z. Impact of elevated peak serum estradiol levels during controlled ovarian hyperstimulation on the birth weight of term singletons from fresh IVF-ET cycles. *J Assisted Reprod Genet* (2015) 32(4):527–32. doi: 10.1007/s10815-015-0434-1

21. Bortoletto P, Prabhu M, Baker VL. Association between programmed frozen embryo transfer and hypertensive disorders of pregnancy. *Fertil Steril* (2022) 118 (5):839–48. doi: 10.1016/j.fertnstert.2022.07.025

22. Verdonk K, Visser W, Van Den Meiracker AH, Danser AH. The renin-angiotensin-aldosterone system in pre-eclampsia: the delicate balance between good and bad. *Clin Sci (London Engl 1979)* (2014) 126(8):537–44. doi: 10.1042/CS20130455

23. Verdonk K, Saleh L, Lankhorst S, Smilde JE, van Ingen MM, Garrelds IM, et al. Association studies suggest a key role for endothelin-1 in the pathogenesis of preeclampsia and the accompanying renin-angiotensin-aldosterone system suppression. *Hypertension (Dallas Tex 1979)* (2015) 65(6):1316–23. doi: 10.1161/HYPERTENSIONAHA.115.05267

24. Bonagura TW, Babischkin JS, Aberdeen GW, Pepe GJ, Albrecht ED. Prematurely elevating estradiol in early baboon pregnancy suppresses uterine artery remodeling and expression of extravillous placental vascular endothelial growth factor and $\alpha1\beta1$ and $\alpha5\beta1$ integrins. *Endocrinology* (2012) 153(6):2897–906. doi: 10.1210/en.2012-1141

25. Barker DJ. The developmental origins of chronic adult disease. *Acta Paediatrica (Oslo Norway 1992) Suppl* (2004) 93(446):26–33. doi: 10.1111/j.1651-2227.2004.tb00236.x

26. Bhattacharya A, Freedman AN, Avula V, Harris R, Liu W, Pan C, et al. Placental genomics mediates genetic associations with complex health traits and disease. *Nat Commun* (2022) 13(1):706. doi: 10.1038/s41467-022-28365-x

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 27 July 2023
DOI 10.3389/fendo.2023.1193826

OPEN ACCESS

EDITED BY
Emre Pabuccu,
Ufuk University, Türkiye

REVIEWED BY
Hongzhan Zhang,
Shenzhen Zhongshan Urological Hospital, China
Guanhua Qian,
Chongqing Medical University, China
Xiushan Feng,
Fujian Medical University, China

*CORRESPONDENCE
Ling Liu
eye99@163.com

†These authors have contributed equally to this work

RECEIVED 25 March 2023
ACCEPTED 22 June 2023
PUBLISHED 27 July 2023

CITATION
Liu L, Zhou H, Hu J, Sun X, Liu D and Huang G (2023) Association between duration of progesterone supplementation and clinical outcomes in artificial frozen-thawed embryo transfer cycles. *Front. Endocrinol.* 14:1193826. doi: 10.3389/fendo.2023.1193826

Association between duration of progesterone supplementation and clinical outcomes in artificial frozen-thawed embryo transfer cycles

Ling Liu[1*†], Hongyan Zhou[1†], Jie Hu[1], Xingyu Sun[2], Doudou Liu[1] and Guiying Huang[1]

[1]Reproductive Medicine Center, The Affiliated Hospital of Southwest Medical University, Luzhou, China, [2]Department of Gynecology, The Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University, Luzhou, Sichuan, China

Objective: The administration of progesterone before transfer in hormone replacement treatment (HRT) is crucial for the clinical outcomes of frozen-thawed embryo transfer (FET), but the optimal duration of progesterone remains controversial. This study aimed to investigate the effect of the duration of progesterone administration on the clinical outcomes of FET cycles.

Methods: This prospective cohort study included 353 artificial FET cycles conducted at a reproductive medicine center between April and October 2021. The FET cycles were stratified into four groups based on the duration of progesterone supplementation before the procedure and the embryonic development stage: group P3 (73 patients) received intramuscular progesterone for 3 days and group P4 (87 patients) for 4 days before Day 3 frozen embryo transfer, group P5 (70 patients) for 5 days and group P6 (123 patients) for 6 days before frozen blastocyst transfer. This trial was performed using one or two vitrified embryo(s) when the endometrial thickness reached 7 mm after estrogen supplementation in an artificial cycle. The primary outcome was clinical pregnancy, and secondary outcomes included biochemical pregnancy, implantation, early pregnancy loss, and live births.

Results: There were no significant differences in the demographic and clinical characteristics between the groups. No significant difference was observed in the clinical pregnancy rates between groups: 23/73 (31.5%) in group P3 vs 28/87 (32.2%) in group P4 ($P = 0.927$). Compared to group P5 (41/70, 58.6%), the clinical pregnancy rate was not significantly different in group P6 (77/123, 62.6%, $P = 0.753$). There was no significant difference in the implantation rates between groups: 33/136 (24.3%) in group P3 vs 34/166 (20.5%) in group P4 ($P = 0.431$), and 62/133 (46.6%) in group P5 vs 107/231 (46.3%) in group P6 ($P = 0.956$). The duration of progesterone supplementation (mean: 3.5 ± 0.5 days; range:3–4 days) before Day 3 frozen embryo transfer did not impact clinical pregnancy (odds ratio [OR] 1.048; 95% confidence interval [CI], 0.518–2.119). The duration of progesterone administration (mean: 5.6 ± 0.5 days; range:5–6 days) before

frozen blastocyst transfer may not affect clinical pregnancy (OR 1.339; 95% CI, 0.717–2.497).

Conclusion: There may be no significant correlation between the duration of progesterone supplementation and pregnancy outcomes in artificial FET cycles, although the clinical pregnancy rate was higher when progesterone supplementation was extended for one day before FET.

KEYWORDS

artificial endometrial preparation, endometrial transformation time, frozen-thawed embryo transfer (FET), implantation rate, pregnancy outcome, window of implantation (WOI)

1 Introduction

Due to recent developments in clinical practice and laboratory technology, embryo cryopreservation has become a central component of assisted reproductive technology (ART). Improved laboratory technology has contributed to an increased number of embryos available for *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycles. The vitrification method, improved embryo survival rates after thawing (1, 2), and implementation of a single embryo transfer policy to reduce multiple pregnancies without reducing the cumulative delivery rate (3), have boosted the number of FET cycles performed worldwide. A freeze-all strategy is now adopted for a growing number of indications, including the prevention of ovarian hyperstimulation syndrome, preimplantation genetic testing (PGT), progesterone elevation in the late follicular phase, endometrial abnormality, embryo–endometrium asynchrony, egg freezing, and fertility preservation, and so on (4–11). As a result, the proportion of autologous frozen embryo transfers has increased dramatically worldwide over the last decade, which is of great significance in improving the clinical outcomes of FET (12).

Despite the emerging importance of the FET cycle, optimal endometrial preparation before it remains unclear. Although several randomized trials have examined the effects of different cycle regimens on FET, there is still no evidence for a single optimal endometrial priming protocol for FET cycles (13–16). Hormone replacement therapy (HRT) is currently the most widely administered therapy due to its wide range of applications, low cycle cancellation rate, and no requirement for frequent follow-ups (17). Patients can participate in the HRT program irrespective of their ovarian functional status and menstrual irregularities (18). Several studies have suggested that HRT cycles are comparable to natural cycles in terms of pregnancy, miscarriage, and live birth rates (14–16). Most reproductive medicine centers primarily use HRT in endometrial preparation for various reasons. In artificial FET cycles, estrogen and progesterone are sequentially implemented to synchronize the embryo transfer with the window of implantation (WOI) (19). Supplementation of estrogen before FET promotes endometrial thickening, and estrogen is continued as a daily supplementation of progesterone, which is initiated a few days before scheduled embryo transfer. There are two significant factors for successful implantation and pregnancy, namely euploid embryos with developmental potential and a synchronous endometrium (20).

In contrast to estrogen, progesterone appears to be a significant determinant of the WOI because the endometrial WOI is confined to the stenosis interval in the luteal phase (19, 21). Endometrial WOI was first characterized by studies applying hormone preparations in recipients with donor oocytes, indicating that endometrial receptivity is significantly reduced when the frozen embryo is administered prior to or after this critical period (22, 23). Therefore, determining the optimal duration of progesterone exposure before FET is crucial for maximizing the success of ART (24). No clear and definite conclusion regarding the superiority of one protocol over another can be drawn thus far. Most researchers maintain that a stable clinical pregnancy rate can be obtained by transferring the 3rd day (Day 3) embryos with progesterone supplementation for 3 days and blastocysts with progesterone administration for 5 days in the FET cycle (25, 26). However, there are always some unavoidable factors that lead to the incomplete synchronous development of the embryos and endometrium (27, 28). Although some previous studies have indicated that appropriately delaying the transfer time may improve the outcomes of FET (29), another data did not show any difference in clinical pregnancy rates between protocols with or without prolonged progesterone supplementation before FET (30).

As for the lack of evidence regarding to the optimal duration of progesterone administration, there is still much more to be learned about endometrial preparation and synchronization to maximize endometrial function and receptivity, and select the best time for embryo transfer (31). This study assessed whether the duration of progesterone administration before FET influenced the clinical outcomes of HRT of during the FET cycle.

2 Materials and methods

2.1 Study design and participants

This prospective study was conducted at the Reproductive Medical Center of the Affiliated Hospital of Southwest Medical

University. This study included patients who underwent FET in an artificial cycle from April 2021 to October 2021 and were allocated to one of four groups as soon as the endometrial thickness reached 7 mm after estrogen supplementation. One or two embryo(s) were transferred according to Health Commission legislation. The inclusion criteria were patients aged 22–45 years and on hormone replacement cycles. The exclusion criteria included known allergic reactions to progesterone products, uptake of an experimental medicine within 30 days before study initiation, and cancellation of FET for various reasons. The cycles were stratified into four groups based on the duration of progesterone supplementation before embryo transfer and the embryonic development stage. The cycles were assigned to groups P3 or group P5 from April 2021 to June 2021. The cycles were assigned to Groups P4 and P6 from July 2021 to October 2021. Patients in groups P3 and P4 were administered intramuscular progesterone for 3 and 4 days, respectively, before frozen-thawed Day 3 cleavage-stage embryo transfer. Patients in groups P5 and P6 were separately supplemented with intramuscular progesterone for 5 and 6 days, respectively, before the frozen-thawed blastocyst transfer. This trial was performed using one or two vitrified-warmed embryo(s) when the endometrial thickness reached 7 mm after estrogen supplementation in an artificial cycle. This study was approved by the Institutional Review Board of the hospital. Written informed consent was obtained from all participants.

2.2 Endometrial preparation and embryo transfer

All cycles were performed using the same artificial protocol. Oral estradiol valerate (Progynova, BayerSchering Pharma AG, Germany) was administered on the second or third day of the menstrual cycle after verifying that the patients were in the early proliferative phase of the menstrual cycle. Typically, 3 mg of estradiol valerate was prescribed twice daily. If the endometrial thickness was <7 mm 14 days later, patients were required to comply with a step-up protocol that involved the addition of vaginal estrogen supplementation (Femoston, Abbott Healthcare Products; 1 mg once daily), to the oral administration of estradiol valerate, based on the physician's preference and experience. Serum estradiol and progesterone levels were determined on the day before the initiation of progesterone treatment.

Intramuscular progesterone (60 mg once a day) was initiated, supplemented with oral dydrogesterone (10 mg thrice a day) when the endometrial thickness was 7 mm. Embryo transfer was performed days 4, 5, 6 and 7 of progesterone exposure in groups P3, P4, P5 and P6 respectively. No more than two embryos were transferred in any FET cycles. All the embryos were thawed on the morning of the transfer. Embryos were evaluated according to the conventional classification system currently used in our IVF laboratory: number of blastomeres, degree of cytoplasmic fragmentation and the equality of blastomeres (32). Embryos with seven to nine cells on Day 3 and with no multinucleation, cytoplasmic fragmentation, or less than 10% fragmentation were considered good-quality embryos. The blastocyst morphology was evaluated according to the Gardner and Schoolcraft grading system (33). A morphological quality score was assigned to the blastocysts at the moment of transfer; blastocysts with inner cell mass/trophectoderm (ICM/TE) score types AA, AB, or BA were considered good-quality embryos (30). The daily estrogen and progesterone protocol was continued if there was a negative pregnancy test result after FET. If pregnancy was achieved, hormone administration was continued until approximately 11–12 weeks of gestation.

2.3 Outcome measures

The independent variable of interest was the duration of progesterone exposure, defined as the number of days from the initiation of progesterone treatment to the completion of embryo transfer. To evaluate whether the duration of progesterone administration before FET affected the clinical outcomes, the primary outcome of the study was clinical pregnancy, which was defined as the presence of one or more gestational sac(s) with an embryonic pole indicating the fetal heartbeat on transvaginal ultrasound at 7 weeks of gestation. Secondary outcomes included biochemical pregnancy, rate of implantation, live births, early pregnancy loss, and miscarriage.

Fourteen days after embryo transfer, having a serumβ-hCG level of >5 IU/L was considered a biochemical pregnancy. The implantation rate was determined by dividing the number of intrauterine sacs inspected using transvaginal ultrasound divided by the number of embryos transferred. Live birth was defined as the delivery of viable infant(s) at ≥24 gestational weeks. Early pregnancy loss was considered when no gestational sac was observed even after a serum β-hCG ≥ 5 mIU/mL or loss occurring after the presence of an intrauterine gestational sac was confirmed.

2.4 Statistical analysis

Normally distributed measurement data are presented as the "mean ± standard"; data that is not normally distributed are presented as "median (range)." The Shapiro- Wilk (SW) test was used to determine whether the continuous variables were normally distributed. Statistical comparisons between the two groups were performed using the Mann-Whitney U test. Categorical variables were described as frequencies or percentages, and comparisons between groups were performed using Pearson's chi-squared test or Fisher's exact test. All statistical analyses were performed using IBM SPSS Statistics for Windows (version 19.0; IBM, Corp., Armonk, NY, USA. Statistical significance was set at $P < 0.05$.

3 Results

Overall, 353 FET cycles were performed with HRT were analyzed in this study, including FET of cleavage embryos or

blastocysts. All FET cycles were stratified into four groups (P3, P4, P5, and P6) according to the embryonic development stage and the duration of progesterone administration before FET. At the time of analysis, groups P3, P4, P5 and P6 were compared.

3.1 Baseline characteristics

Comparison of baseline demographic and cycle characteristics based on the duration of progesterone administration before frozen-thawed Day 3 cleavage-stage embryo transfer. The baseline characteristics are presented in Table 1. There were no significant differences in the baseline characteristics between groups P3 and P4. Comparison of baseline demographic and cycle characteristics according to the duration of progesterone exposure before frozen blastocyst transfer was performed. There were no significant differences in demographic and clinical characteristics between groups P5 and P6 (Table 2).

3.2 Pregnancy outcomes

Pregnancy outcomes stratified into two groups according to the duration of progesterone administration prior to frozen-thawed Day 3 cleavage-stage embryo transfer are listed in Table 3. No significant differences were observed between groups in the rates of clinical pregnancy, biochemical pregnancy, implantation, live birth, early pregnancy loss, and miscarriage. The pregnancy outcomes stratified into two groups according to the duration of progesterone exposure before frozen blastocyst transfer are listed in Table 4. There were no significant differences between groups in the rates of clinical pregnancy, biochemical pregnancy, implantation, live birth, early pregnancy loss rate, or miscarriage.

Baseline demographics and cycle characteristics were compared between patients who achieved clinical pregnancy after frozen-thawed Day 3 cleavage-stage embryo transfer (Table 5). Patients who achieved pregnancy after the transfer had a mean duration of 3.5 ± 0.5 days (range: 3–4 days) of progesterone administration

TABLE 1 Baseline and cycle characteristics for frozen-thawed day 3 embryo transfer cycles.

Parameters	Group P3 (n=73)	Group P4 (n=87)	*P*
Female age (year)	32.4 ± 5.8	33.3 ± 5.4	0.314[a]
Male age (year)	34.2 ± 6.3	35.2 ± 5.9	0.291[a]
Previous conception (%)	33(45.2%)	30(34.5%)	0.167[b]
Years of infertility	5.9 ± 4.0	5.5 ± 4.6	0.530[a]
BMI (kg/m2)	22.9 ± 3.5	23.2 ± 3.1	0.648[a]
Basic FSH(mIU/ml)	9.2 ± 4.5	10.0 ± 5.0	0.279[a]
Basic LH(mIU/ml)	4.1 ± 2.8	4.4 ± 2.8	0.502[a]
Basic E2(pg/ml)	35.3 ± 15.3	32.9 ± 16.9	0.340[a]
Basic PRL	13.4 ± 8.2	14.4 ± 8.9	0.447[a]
Basic P	0.7 ± 0.5	0.6 ± 0.5	0.445[a]
AMH(ng/ml)	3.3 ± 3.2	2.9 ± 3.3	0.352[a]
AFC	16.8 ± 9.3	16.3 ± 9.2	0.757[a]
Endometrial thickness (mm)	9.7 ± 1.9	9.8 ± 2.2	0.822[a]
No. of embryos transferred	1.9 ± 0.4	1.9 ± 0.3	0.425[a]
No. of good-quality embryos transferred	1.4 ± 0.7	1.4 ± 0.7	0.554[a]
E2 level on the day of planning FET	543.6 ± 515.4	490.3 ± 468.4	0.494[a]
P level on the day of planning FET	53.6 ± 12.1	52.7 ± 12.9	0.678[a]
Concomitant infertility factors			
Tubal factor (%)	49(67.1%)	59(67.8%)	0.926[b]
Ovulation disorder (%)	11(15.1%)	12(13.8%)	0.819[b]
Decreased ovarian reserve (%)	4(5.1%)	7(8.0%)	0.523[b]
Male factor (%)	9(12.3%)	9(10.3%)	0.692[b]
Length of estradiol supplement	17.8 ± 4.3	18.4 ± 4.7	0.436[a]

[a]Mann–Whitney U test, [b]Pearson's Chi squared test.
BMI, body mass index; FSH, Follicle stimulating hormone; LH, Luteinizing hormone; E2, estrogen; PRL, prolactin; P, progesterone; AMH, anti-Müllerian hormone; AFC, antral follicle count.

10.3389/fendo.2023.1193826

TABLE 2 Baseline and cycle characteristics for frozen-thawed blastocysts transfer cycles.

Parameters	Group P5 (n=70)	Group P6 (n=123)	*P*
Female age (year)	30.5 ± 4.1	31.4 ± 4.5	0.187[a]
Male age (year)	32.0 ± 4.3	33.4 ± 6.0	0.058[a]
Previous conception (%)	37(52.9%)	49(39.8%)	0.080[b]
Years of infertility	5.2 ± 3.8	5.1 ± 3.5	0.918[a]
BMI (kg/m^2)	22.8 ± 3.2	22.6 ± 3.5	0.721[a]
Basic FSH(mIU/ml)	7.9 ± 1.7	8.3 ± 2.3	0.247[a]
Basic LH(mIU/ml)	4.7 ± 3.0	5.3 ± 5.1	0.431[a]
Basic E2(pg/ml)	43.0 ± 35.2	35.3 ± 27.3	0.090[a]
Basic PRL	12.9 ± 6.6	14.1 ± 7.8	0.286[a]
Basic P	0.6 ± 0.6	0.6 ± 0.4	0.634[a]
AMH(ng/ml)	5.1 ± 4.1	4.9 ± 4.1	0.661[a]
AFC	23.3 ± 11.1	22.6 ± 10.1	0.633[a]
Endometrial thickness (mm)	10.4 ± 2.4	10.1 ± 2.4	0.473[a]
No. of embryos transferred	1.9 ± 0.3	1.9 ± 0.3	0.647[a]
No. of good-quality embryos transferred	1.5 ± 0.8	1.3 ± 0.8	0.184[a]
E2 level on the day of planning FET	503.5 ± 476.3	556.0 ± 569.0	0.515[a]
P level on the day of planning FET	54.1 ± 12.3	55.9 ± 10.4	0.285[a]
Concomitant infertility factors			
Tubal factor (%)	40(57.1%)	87(70.7%)	0.056[b]
Ovulation disorder (%)	11(15.7%)	13(10.6%)	0.298[b]
Decreased ovarian reserve (%)	2(2.9%)	1(0.8%)	0.270[b]
Male factor (%)	17(24.3%)	12(9.8%)	0.014[b]
Length of estradiol supplement	18.0 ± 4.6	18.5 ± 4.3	0.487[a]
Day 5 blastocysts(%)	56(80.0%)	104(84.6%)	0.419[b]

[a]Mann–Whitney U test, [b]Pearson's Chi squared test.
BMI, body mass index; FSH, Follicle stimulating hormone; LH, Luteinizing hormone; E2, estrogen; PRL, prolactin; P, progesterone; AMH, anti-Müllerian hormone; AFC, antral follicle count.

TABLE 3 Outcomes of frozen-thawed day 3 cleavage stage embryo transfer cycles.

Outcomes	Group P3 (n=73)	Group P4 (n=87)	*P*
Primary outcome			
Clinical pregnancy (%)	23(31.5%)	28(32.2%)	0.927[b]
Secondary outcomes			
Biochemical pregnancy (%)	29(39.7%)	37(42.5%)	0.720[b]
Implantation (%)	33/136(24.3%)	34/166(20.5%)	0.431[b]
Live birth (%)	22/73(30.1%)	25/87(28.7%)	0.846[b]
Early pregnancy loss(%)	6/73(8.2%)	9/87(10.3%)	0.646[b]
Miscarriage (%)	1/23(4.3%)	1/26(3.6%)	0.440[b]

[a]Mann–Whitney U test, [b]Pearson's Chi squared test.

TABLE 4 Outcomes of frozen-thawed blastocysts transfer cycles.

Outcomes	Group P5 (n=70)	Group P6 (n=123)	*P*
Primary outcome			
Clinical pregnancy (%)	41(58.6%)	77(62.6%)	0.581[b]
Secondary outcomes			
Biochemical pregnancy (%)	45(68.6%)	81(65.9%)	0.826[b]
Implantation (%)	62/133(46.6%)	107/231(46.3%)	0.956[b]
Live birth (%)	35/70(50.0%)	66/123(53.7%)	0.625[b]
Early pregnancy loss(%)	6/70(8.6%)	10/123(8.1%)	0.915[b]
Miscarriage (%)	5/40(12.5%)	10/77(13.0%)	0.940[b]

[a]Mann–Whitney U test, [b]Pearson's Chi squared test.

before FET. After controlling for age, body mass index, endometrial thickness at transfer, whether the embryo had a morphology grade of ≥730, and the number of days of progesterone administration, the odds of achieving a clinical pregnancy were not modified (odds ratio [OR] 1.048; 95% confidence interval [CI], 0.518–2.119; P = 0.897), Table 5).

The baseline demographics and cycle characteristics were compared between patients who achieved clinical pregnancy after frozen blastocyst transfer (Table 6). Patients who achieved pregnancy after the transfer had a mean duration of 5.6 ± 0.5 days (range: 5–6 days) of progesterone administration before FET. After controlling for age, body mass index, endometrial thickness at transfer, embryonic day of development at freezing, whether the embryo had a morphological grade of 3BB or better, and the number of days of progesterone administration, the odds of achieving clinical pregnancy were not modified (OR 1.339; 95% CI, 0.717–2.497; P = 0.36), Table 6).

4 Discussion

HRT for FET cycles includes the sequential administration of exogenous estrogen and progesterone to imitate the physiological hormonal exposure of the endometrium in a normal menstrual cycle and to accurately time the transfer of the thawed embryo to the receptive endometrium (19, 34). In HRT cycles, progesterone is initiated to promote the final phase of endometrial preparation prior to embryo transfer (28, 31). Empirically, progesterone supplementation is usually initiated 3 days before the embryo transfer with excellent pregnancy rates in artificially prepared cycles (35). Most reproductive medicine centers believe that a stable clinical pregnancy rate can be obtained by administering the protocol of transferring the Day 3 embryos with progesterone administration for 3 days and transferring blastocysts with progesterone administration for 5 days in HRT cycles (25, 26, 35). Some reproductive medicine centers still consider that a similar or even higher clinical pregnancy rates can be achieved by prolonging the program by one day (29, 30). These scholars maintained that prolonging the time of endometrial transformation using progesterone can diminish uterine contractions, improve endometrial receptivity, and foreshorten the period between development and implantation after embryo transplantation in the FET cycle (29).

However, the hypothesis that prolonging the duration of progesterone administration could increase the pregnancy rates has not yet been confirmed (29, 30, 36, 37). Better implantation outcomes can be obtained by prolonging the exposure of progesterone to the endometrium during HRT cycles, which prolongs the endometrial receptivity period by increasing the interaction between the embryo and endometrium (38). However, this assumption was not fully confirmed in the present study. Although the clinical pregnancy rates of FET Day 3 embryos on the fifth day of progesterone administration were higher than those on the fourth day of

TABLE 5 A comparison of baseline demographic and characteristics according to whether patients achieved a clinical pregnancy after frozen day 3 cleavage embryo transfer.

Demographics and cycle characteristics	Clinical pregnancy (n=51)	Non clinical pregnancy (n=109)	Adjusted OR (95%CI)	*P*
Female age (year)	31.6 ± 5.1	33.6 ± 5.7	0.935 (0.874-1.000)	0.049
No. of good-quality embryos transferred	1.6 ± 0.6	1.3 ± 0.7	1.771 (1.016-3.085)	0.044
Endometrial thickness (mm)	10.2 ± 1.7	9.5 ± 2.2	1.206 (1.019-1.427)	0.029
BMI (kg/m^2)	23.3 ± 2.7	23.0 ± 3.5	1.05 (0.946-1.165)	0.358
Duration of progesterone supplementation	3.5 ± 0.5	3.5 ± 0.5	1.048 (0.518-2.119)	0.897

CI, confidence interval; OR, odds ratio.

TABLE 6 A comparison of baseline demographic and characteristics according to whether patients achieved a clinical pregnancy after frozen blastocyst transfer.

Demographics and cycle characteristics	Clinical pregnancy (n=118)	Non clinical pregnancy (n=75)	Adjusted OR (95%CI)	*P*
Female age (year)	30.6 ± 4.5	31.7 ± 4.2	0.949 (0.885-1.018)	0.142
No. of good-quality embryos transferred	1.4 ± 0.8	1.3 ± 0.8	1.142 (0.769-1.696)	0.509
Endometrial thickness (mm)	10.6 ± 2.5	9.6 ± 2.1	1.21 (1.053-1.390)	0.007
BMI (kg/m^2)	22.8 ± 3.1	22.6 ± 3.3	1.019 (0.925-1.123)	0.701
Duration of progesterone supplementation	5.6 ± 0.5	5.6 ± 0.5	1.339 (0.717-2.497)	0.36

CI, confidence interval; OR, odds ratio.

progesterone administration, there was no statistically significant difference between the two groups. This research showed that transferring vitrified-warmed Day 3 cleavage stage embryos on the fifth day of progesterone administration may not increase pregnancy rates compared to transferring on the fourth day of progesterone administration. In addition, this study demonstrated that transferring vitrified-thawed blastocysts on the seventh day of progesterone supplementation may not improve the pregnancy rates compared to transferring blastocysts on the sixth day of progesterone supplementation. To our knowledge, this is the first prospective trial comparing the effects of different duration of progesterone administration before FET. Although various studies have indicated a trend toward better pregnancy outcomes with shorter progesterone supplementation, no definite conclusion has been reached (39–43). Furthermore, it has been proposed that WOI switches approximately 48 h after the initiation of progesterone and is sustained for at least 4 days (44). This study showed that similar pregnancy outcomes were achieved with or without extending progesterone by one day, which may indicate that WOI may be longer than estimated (40, 42). Previous studies have shown that delayed endometrial development during the luteal phase occurs in approximately 25% of the general population. Thus, delaying the day of embryo transfer is feasible (36, 37). Perhaps, the extended day is also theoretically covered by the implantation window (29). This viewpoint seems to have been recognized in the present study.

The hypothesis that prolonging the duration of progesterone administration could diminish the early pregnancy loss rate has not yet been confirmed (42). This hypothesis was not verified in the present study. A higher risk of early pregnancy loss may occur when the duration of progesterone administration before transplantation is shorter than embryonic age (42). Thus, extended progesterone administration before transfer is recommended (45). Implantation after the normal endometrial receptivity period is closely associated with early pregnancy loss (46). A prolonged window of endometrial receptivity may account for the delayed implantation of severely damaged embryos and the subsequent early pregnancy loss (47). A higher incidence of early pregnancy loss was not detected in between group P3, which was administered progesterone for 3 days, and P4, which was administered progesterone for 4 days before transferring vitrified-warmed embryos at the third day cleavage stage, This was not observed in either group P5, which was administered progesterone for 5 days, or P6, which was administered progesterone for 6 days before transferring vitrified-thawed blastocysts, which may be because the duration of progesterone administration was no shorter than the age of the embryo (42). According to these data, there may also be no difference in the rate of early pregnancy loss between the two groups with or without prolonged progesterone administration, which may be due to adaptation to the normal period of endometrial receptivity (46). In contrast, Day 3 embryos of group P4 were transferred on the fifth day of progesterone supplementation, and blastocysts of group P6 were transferred on the seventh day of progesterone supplementation, which might have been too long, although no evidence exists that longer progesterone supplementation had a negative impact on success rates (43).

Based on these results, it may be concluded that enhanced flexibility would be possible when programming vitrified-warmed embryos transfer after progesterone administration, because no differences were observed between the duration of progesterone administration ≥1 day of embryonic age as far as clinical pregnancy, implantation, live birth, and early pregnancy loss rates are concerned (29, 30, 36, 37). This study demonstrated that there may be no correlation between the developmental stage of the blastocyst and clinical pregnancy in the FET cycle in day 5 embryos or day 6 blastocysts when an equal duration of progesterone supplementation was employed, considering that the duration of progesterone supplementation for day 5 and day 6 blastocysts was equal before FET (48, 49). In the light of this result, it could be concluded that clinical pregnancy may not be related to the developmental stage of blastocysts, but rather to the quality of the embryo, as morphologically high-quality day 5 and 6 blastocysts resulted in similar pregnancy outcomes, possibly due to the lack of difference between high-quality embryos on days 5 and 6 regarding aneuploidy (48, 49). However, this assumption may be challenged because other data from some scholars indicated that a blastocyst on day 6 has diverse developmental potential and a specific synchronization between the embryo and endometrium with a different WOI compared with a blastocyst on day 5 (50). On the basis of these results, programming the transfer of a vitrified-warmed blastocyst on day 6 requires further investigation, since these “delayed” blastocysts may encounter different and possibly narrower WOI compared with day 5 embryos. Consequently, the optimal duration of progesterone administration remains controversial.

In addition, this study discovered that blastocysts might have higher pregnancy outcomes in FET cycles compared with that of cleavage-stage embryos (51), which might be related to differences in intimal thickness between them. Additionally, differences in age and the number of good-quality embryos transferred may be related

to these pregnancy outcomes as well. An explanation for these findings may be that blastocysts undergo a re-selection procedure that spans the 8-cell-stage developmental block and eliminates embryos with poor developmental potential compared to cleavage embryos (52–54). Moreover, this study found that a stable clinical pregnancy may have no correlation with the number of good-quality embryos, indicating the feasibility of single blastocyst transfer, effectively reducing the incidence of ovarian hyper stimulation syndrome, multiple birth rates, and fetal and maternal risks while ensuring relatively higher pregnancy and live birth rates (55–57). In addition, the current trend in assisted reproduction may become a more feasible approach in extending the duration of *in vitro* embryo culture to obtain more developed embryos, determining the implantation window for these mature embryos, and further improving the success rate of assisted reproduction (29). Single embryo transfer (SET) may be safer than double embryo transfer and as effective as single blastocyst transfer if patients are thoroughly informed of the reasons for the proposition (55, 56). The question may not be whether to apply SET, but how to apply it in terms of patient selection, patient-centered counselling, and coverage of treatment (3).

The data also displayed that the pregnancy outcome of embryos at the cleavage stage might be related to the woman's age, while the blastocyst pregnancy outcome might be uncorrelated with it (53). It is possible that the aneuploidy rate of oocytes increases with age and then undergoes an embryo developmental block or poor developmental potential (58). However, blastocysts could have better quality and higher euploidy because of a re-selection procedure that spans developmental blocks and eliminates embryos with poor developmental potential compared to that of cleavage embryos (52–54). These results indicate that the difference in pregnancy outcomes between the transfer of blastocysts and day 3 cleavage embryos may largely depend on the quality of the embryos transferred (59).

This study had similar or different results from those of other studies, possibly due to epidemiological variables, limitations of its design, a high level of heterogeneity in the study populations, and insufficient sample size. Although this study adjusted for multiple potential confounding factors, caution should be taken when considering the results as a basis for definitive policy due to the prospective design of a single-center study with a limited sample size, as well as possible differences in research protocol and clinical performance. Consequently, confirmation of these findings in a multi-center study with a large sample size and a more rigorous research design is warranted.

5 Conclusion

In summary, although the results of this study need to be confirmed in multicenter randomized trials, they suggest that obtaining as many high-quality blastocysts as possible and programming single blastocyst transplantation deserved consideration. Although the optimal duration of progesterone supplementation remains to be clarified, it is recommended to transfer embryos on days 3 and 5 with a degree of flexibility. However, there may be different and possibly narrower WOI for the blastocyst on the sixth day of vitrification. Further research is required to optimize FET protocols and distinguish other contributing factors that may affect pregnancy outcomes after transferring frozen-thawed embryos.

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 Affiliated Hospital of Southwest Medical University Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

LL and HZ: conceptualization and writing-original draft prepatation. JH and XS: data curation DL and GH: revising the manuscript critically for important intellectual content. All authors contributed to the article and approved the submitted version.

Funding

The study was supported by a project of the Science Foundation of Affiliated Hospital of Southwest Medical University (no. 20014).

Acknowledgments

We thank the survey participants and all members involved in this study for their painstaking efforts in conducting the data collection.

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

References

1. Nagy ZP, Shapiro D, Chang CC. Vitrification of the human embryo: a more efficient and safer *in vitro* fertilization treatment. *Fertil Steril* (2020) 113(2):241–7. doi: 10.1016/j.fertnstert.2019.12.009

2. Chang CC, Shapiro DB, Nagy ZP. The effects of vitrification on oocyte quality. *Biol Reprod* (2022) 106(2):316–27. doi: 10.1093/biolre/ioab239

3. Mejia RB, Capper EA, Summers KM, Ten Eyck P, Van Voorhis BJ. Elective transfer of one embryo is associated with a higher cumulative live birth rate and improved perinatal outcomes compared to the transfer of two embryos with *in vitro* fertilization. *F S Rep* (2020) 2(1):50–7. doi: 10.1016/j.xfre.2020.10.011

4. Singh B, Reschke L, Segars J, Baker VL. Frozen-thawed embryo transfer: the potential importance of the corpus luteum in preventing obstetrical complications. *Fertil Steril* (2020) 113(2):252–7. doi: 10.1016/j.fertnstert.2019.12.007

5. Mizrachi Y, Horowitz E, Farhi J, Raziel A, Weissman A. Ovarian stimulation for freeze-all IVF cycles: a systematic review. *Hum Reprod Update* (2020) 26(1):118–35. doi: 10.1093/humupd/dmz037

6. Abe M, Yamamoto Y, Noguchi H, Tamura K, Aoki H, Takeda A. Is a freeze-all strategy necessary for all embryo transfers : fresh embryo transfer without progesterone elevation results in an equivalent pregnancy rate to cryopreserved embryo transfer. *J Med Invest* (2022) 69(3.4):224–9. doi: 10.2152/jmi.69.224

7. Munné S, Kaplan B, Frattarelli JL, Child T, Nakhuda G, Shamma FN, et al. Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen-thawed embryo transfer in good-prognosis patients: a multicenter randomized clinical trial. *Fertil Steril* (2019) 112(6):1071–1079.e7. doi: 10.1016/j.fertnstert.2019.07.1346

8. Yang Y, Zhu D, Wang Q, Ma C, Li D, Wang J, et al. Frozen embryo transfer in the menstrual cycle after moderate-severe ovarian hyperstimulation syndrome: a retrospective analysis. *BMC Pregnancy Childbirth* (2022) 22(1):907. doi: 10.1186/s12884-022-05239-0

9. Racca A, Santos-Ribeiro S, De Munck N, Mackens S, Drakopoulos P, Camus M, et al. Impact of late-follicular phase elevated serum progesterone on cumulative live birth rates: is there a deleterious effect on embryo quality? *Hum Reprod* (2018) 33(5):860–8. doi: 10.1093/humrep/dey031

10. Groenewoud E, Cohlen BJ, Maclon N. Programming the endometrium for deferred transfer of cryopreserved embryos: hormone replacement versus modified natural cycles. *Fertil Steril* (2018) 109:768–74. doi: 10.1016/j.fertnstert.2018.02.135

11. Maheshwari A, Bell JL, Bhide P, Brison D, Child T, Chong HY, et al. Elective freezing of embryos versus fresh embryo transfer in IVF: a multicentre randomized controlled trial in the UK (E-freeze). *Hum Reprod* (2022) 37(3):476–87. doi: 10.1093/humrep/deab279

12. Sciorio R, Esteves SC. Clinical utility of freeze-all approach in ART treatment: a mini-review. *JCryobiology* (2020) 92:9–14. doi: 10.1016/j.cryobiol.2019.11.041

13. Chada A, Hipp HS. 'Let'rozole it grow: endometrial preparation methods for frozen-thawed embryo transfer. *Fertil Steril* (2022) 118(4):699–700. doi: 10.1016/j.fertnstert.2022.08.003

14. Ghobara T, Gelbaya TA, Ayeleke RO. Cycle regimens for frozen-thawed embryo transfer. *Cochrane Database Syst Rev* (2017) 7:Cd003414. doi: 10.1002/14651858.CD003414.pub3

15. Wang B, Zhu Q, Wang Y. Pregnancy outcomes after different cycle regimens for frozen-thawed embryo transfer: a retrospective study using propensity score matching. *Front Med (Lausanne)* (2020) 7:327. doi: 10.3389/fmed.2020.00327

16. Wang B, Zhang J, Zhu Q, Yang X, Wang Y. Effects of different cycle regimens for frozen embryo transfer on perinatal outcomes of singletons. *Hum Reprod* (2020) 35(7):1612–22. doi: 10.1093/humrep/deaa093

17. Yu J, Chen P, Luo Y, Lv M, Lou L, Xiao Q, et al. GnRH-agonist pretreatment in hormone replacement therapy improves pregnancy outcomes in women with male-factor infertility. *Front Endocrinol (Lausanne)* (2022) 13:1014558. doi: 10.3389/fendo.2022.1014558

18. Li SJ, Zhang YJ, Chai XS, Nie MF, Zhou YY, Chen JL, et al. Letrozole ovulation induction: an effective option in endometrial preparation for frozen–thawed embryo transfer. *Arch Gynecol Obstet* (2014) 289(3):687–93. doi: 10.1007/s00404-013-3044-0

19. Sekhon L, Feuerstein J, Pan S, Overbey J, Lee JA, Briton-Jones C, et al. Endometrial preparation before the transfer of single, vitrified-warmed, euploid blastocysts: does the duration of estradiol treatment influence clinical outcome? *Fertil Steril* (2019) 111(6):1177–1185.e3. doi: 10.1016/j.fertnstert.2019.02.024

20. Álvarez M, Gaggiotti-Marre S, Martínez F, Coll L, García S, González-Foruria I, et al. Individualised luteal phase support in artificially prepared frozen embryo transfer cycles based on serum progesterone levels: a prospective cohort study. *Hum Reprod* (2021) 36(6):1552–60. doi: 10.1093/humrep/deab031

21. Ruiz-Alonso M, Valbuena D, Gomez C, Cuzzi J, Simon C. Endometrial receptivity analysis (ERA): data versus opinions. *Hum Reprod Open* (2021) 2021(2):hoab011. doi: 10.1093/hropen/hoab011

22. Lessey BA, Young SL. What exactly is endometrial receptivity? *Fertil Steril* (2019) 111(4):611–7. doi: 10.1016/j.fertnstert.2019.02.009

23. Navot D, Bergh PA, Williams M, Garrisi GJ, Guzman I, Sandler B, et al. An insight into early reproductive processes through the *in vivo* model of ovum donation. *J Clin Endocrinol Metab* (1991) 72(2):408–14. doi: 10.1210/jcem-72-2-408

24. Franasiak JM, Ruiz-Alonso M, Scott RT, Simon C. Both slowly developing embryos and a variable pace of luteal endometrial progression may conspire to prevent normal birth in spite of a capable embryo. *Fertil Steril* (2016) 105(4):861–6. doi: 10.1016/j.fertnstert.2016.02.030

25. Mumusoglu S, Polat M, Ozbek IY, Bozdag G, Papanikolaou EG, Esteves SC, et al. Preparation of the endometrium for frozen embryo transfer: a systematic review. *Front Endocrinol (Lausanne)* (2021) 12:688237. doi: 10.3389/fendo.2021.688237

26. Yang X, Bu Z, Hu L. Live birth rate of frozen-thawed single blastocyst transfer after 6 or 7 days of progesterone administration in hormone replacement therapy cycles: a propensity score-matched cohort study. *Front Endocrinol (Lausanne)* (2021) 12:706427. doi: 10.3389/fendo.2021.706427

27. Glujovsky D, Pesce R, Fiszbajn G, Sueldo C, Hart RJ, Ciapponi A. Endometrial preparation for women undergoing embryo transfer with frozen embryos or embryos derived from donor oocytes. *Cochrane Database Syst Rev* (2010) 20(1):CD006359. doi: 10.1002/14651858.CD006359.pub2

28. Mackens S, Santos-Ribeiro S, van de Vijver A, Racca A, Van Landuyt L, Tournaye H, et al. Frozen embryo transfer: a review on the optimal endometrial preparation and timing. *Hum Reprod* (2017) 32(11):2234–42. doi: 10.1093/humrep/dex285

29. Xu M, Yan Y, Shen X, Sun H, Yan G, Kong N, et al. Prolonging the time of progesterone supplementation to improve the pregnancy outcomes of single day 6 blastocyst transfer in frozen-thawed cycles: study protocol for a randomized controlled trial. *Trials* (2022) 23(1):1024. doi: 10.1186/s13063-022-07013-1

30. Roelens C, Santos-Ribeiro S, Becu L, Mackens S, Van Landuyt L, Racca A, et al. Frozen-warmed blastocyst transfer after 6 or 7 days of progesterone administration: impact on live birth rate in hormone replacement therapy cycles. *Fertil Steril* (2020) 114(1):125–32. doi: 10.1016/j.fertnstert.2020.03.017

31. Bulletti C, Bulletti FM, Sciorio R, Guido M. Progesterone: the key factor of the beginning of life. *Int J Mol Sci* (2022) 23(22):14138. doi: 10.3390/ijms232214138

32. Herbemont C, Sarandi S, Boujenah J, Cedrin-Durnerin I, Sermondade N, Vivot A, et al. Should we consider day-2 and day-3 embryo morphology before day-5 transfer when blastocysts reach a similar good quality? *Reprod BioMed Online* (2017) 35(5):521–8. doi: 10.1016/j.rbmo.2017.07.014

33. Gardner DK, Schoolcraft WB. Culture and transfer of human blastocysts. *Curr Opin Obstet Gynecol* (1999) 11(3):307–11. doi: 10.1097/00001703-199906000-0013

34. Boomsma CM, Kavelaars A, Eijkemans MJ, Amarouchi K, Teklenburg G, Gutknecht D, et al. Cytokine profiling in endometrial secretions: a non-invasive window on endometrial receptivity. *Reprod BioMed Online* (2009) 18(1):85–94. doi: 10.1016/S1472-6483(10)60429-4

35. Mizrachi Y, Weissman A, Rozen G, Rogers PAW, Stern C, Polyakov A. Timing of progesterone luteal support in natural cryopreserved embryo transfer cycles: back to basics. *Reprod BioMed Online* (2022) 45(1):63–8. doi: 10.1016/j.rbmo.2022.03.021

36. Murray MJ, Meyer WR, Zaino RJ, Lessey BA, Novotny DB, Ireland K, et al. A critical analysis of the accuracy, reproducibility, and clinical utility of histologic endometrial dating in fertile women. *Fertil Steril* (2004) 81(5):1333–43. doi: 10.1016/j.fertnstert.2003.11.030

37. Diaz-Gimeno P, Ruiz-Alonso M, Blesa D, Bosch N, Martinez-Conejero JA, Alama P, et al. The accuracy and reproducibility of the endometrial receptivity array is superior to histology as a diagnostic method for endometrial receptivity. *Fertil Steril* (2013) 99(2):508–17. doi: 10.1016/j.fertnstert.2012.09.046

38. Saupstad M, Freiesleben NC, Skouby SO, Andersen LF, Knudsen UB, Petersen KB, et al. Preparation of the endometrium and timing of blastocyst transfer in modified natural cycle frozen-thawed embryo transfers (mNC-FET): a study protocol for a randomised controlled multicentre trial. *BMJ Open* (2019) 9(12):e031811. doi: 10.1136/bmjopen-2019-031811

39. van de Vijver A, Drakopoulos P, Polyzos NP, Van Landuyt L, Mackens S, Santos-Ribeiro S, et al. Vitrified-warmed blastocyst transfer on the 5th or 7th day of progesterone supplementation in an artificial cycle: a randomized controlled trial. *Gynecol Endocrinol* (2017) 33(10):783–6. doi: 10.1080/09513590.2017.1318376

40. An BGL, Chapman M, Tilia L, Venetis C. Is there an optimal window of time for transferring single frozen-thawed euploid blastocysts? a cohort study of 1170 embryo transfers. *Hum Reprod* (2022) 37(12):2797–807. doi: 10.1093/humrep/deac227

41. Fonttis AA, Napolitano R, Borda C. Successful pregnancy and delivery after delaying the initiation of progesterone supplementation in a postmenopausal donor oocyte recipient. *Reprod BioMed Online* (2004) 9(6):611–3. doi: 10.1016/s1472-6483(10)61769-5

42. van de Vijver A, Polyzos NP, Van Landuyt L, Mackens S, Stoop D, Camus M, et al. What is the optimal duration of progesterone administration before transferring a vitrified-warmed cleavage stage embryo? a randomized controlled trial. *Hum Reprod* (2016) 31(5):1097–104. doi: 10.1093/humrep/dew045

43. Escriba MJ, Bellver J, Bosch E, Sanchez M, Pellicer A, Remohi J. Delaying the initiation of progesterone supplementation until the day of fertilization does not compromise cycle outcome in patients receiving donated oocytes: a randomized study. *Fertil Steril* (2006) 86(1):92–7. doi: 10.1016/j.fertnstert.2005.12.048

44. Bartels CB, Ditrio L, Grow DR, O'Sullivan DM, Benadiva CA, Engmann L, et al. The window is wide: flexible timing for vitrified-warmed embryo transfer in natural cycles. *Reprod BioMed Online* (2019) 39(2):241–8. doi: 10.1016/j.rbmo.2019.04.003

45. Nawroth F, Ludwig M. What is the "ideal" duration of progesterone supplementation before the transfer of cryopreserved-thawed embryos in estrogen/progesterone replacement protocols? *Hum Reprod* (2005) 20(5):1127–34. doi: 10.1093/humrep/deh762

46. Wilcox AJ, Baird DD, Weinberg CR. Time of implantation of the conceptus and loss of pregnancy. *N Engl J Med* (1999) 340(23):1796–9. doi: 10.1056/NEJM199906103402304

47. Salker M, Teklenburg G, Molokhia M, Lavery S, Trew G, Aojanepong T, et al. Natural selection of human embryos: impaired decidualization of endometrium disables embryo-maternal interactions and causes recurrent pregnancy loss. *PLoS* (2010) 5(4):e10287. doi: 10.1371/journal.pone.0010287

48. Shi W, Zhou H, Chen L, Xue X, Shi J. Live birth rate following frozen-thawed blastocyst transfer is higher in high-grade day 6 blastocysts than in low-grade day 5 blastocysts. *Front Endocrinol (Lausanne)* (2023) 13:1066757. doi: 10.3389/fendo.2022.1066757

49. Niu X, Wang CT, Li R, Haddad G, Wang W. Is day 7 culture necessary for *in vitro* fertilization of cryopreserved/warmed human oocytes? *Reprod Biol Endocrinol* (2020) 18(1):4. doi: 10.1186/s12958-020-0565-9

50. Bourdon M, Pocate-Cheriet K, Finet de Bantel A, Grzegorczyk-Martin V, Amar Hoffet A, Arbo E, et al. Day 5 versus day 6 blastocyst transfers: a systematic review and meta-analysis of clinical outcomes. *Hum Reprod* (2019) 34(10):948–1964. doi: 10.1093/humrep/dez163

51. Wang SS, Sun HX. Blastocyst transfer ameliorates live birth rate compared with cleavage-stage embryos transfer in Fresh*In Vitro*Fertilization or intracytoplasmic sperm injection cycles: reviews and meta-analysis. *Yonsei Med J* (2014) 55(3):815–25. doi: 10.3349/ymj.2014.55.3.815

52. Magli MC, Jones GM, Gras L, Gianaroli L, Korman I, Trounson AO. Chromosome mosaicism in day 3 aneuploid embryos that develop to morphologically normal blastocysts *in vitro*. *Hum Reprod* (2000) 15(8):1781–6. doi: 10.1093/humrep/15.8.1781

53. Fernández-Shaw S, Cercas R, Braña C, Villas C, Pons I. Ongoing and cumulative pregnancy rate after cleavage-stage versus blastocyst-stage embryo transfer using vitrification for cryopreservation: impact of age on the results. *J Assist Reprod Genet* (2015) 32(2):177–84. doi: 10.1007/s10815-014-0387-9

54. Capalbo A, Hoffmann E, Cimadomo D, Maria Ubaldi F, Rienzi L. Human female meiosis revised: new insights into the mechanisms of chromosome segregation and aneuploidies from advanced genomics and time-lapse imaging. *Hum Reprod Update* (2017) 23(6):706–22. doi: 10.1093/humupd/dmx026

55. Sullivan EA, Wang YA, Hayward I, Chambers GM, Illingworth P, McBain J, et al. Single embryo transfer reduces the risk of perinatal mortality, a population study. *Hum Reprod* (2012) 27(12):3609–15. doi: 10.1093/humrep/des315

56. Su W, Xu J, Arhin SK, Liu C, Zhao J, Lu X. The feasibility of all-Blastocyst-Culture and single blastocyst transfer strategy in elderly women: a retrospective analysis. *BioMed Res Int* (2020) 2020:5634147. doi: 10.1155/2020/5634147

57. Mancuso A, Kapfhamer J. With a good quality blastocyst, single embryo transfer remains the best choice. *Fertil Steril* (2018) 110(4):631. doi: 10.1016/j.fertnstert.2018.06.024

58. Wasielak-Politowska M, Kordowitzki P. Chromosome segregation in the oocyte: what goes wrong during aging. *Int J Mol Sci* (2022) 23(5):2880. doi: 10.3390/ijms23052880

59. Dai X, Gao T, Xia X, Cao F, Yu C, Li T, et al. Analysis of biochemical and clinical pregnancy loss between frozen-thawed embryo transfer of blastocysts and day 3 cleavage embryos in young women: a comprehensive comparison. *Front Endocrinol (Lausanne* (2021) 12:785658. doi: 10.3389/fendo.2021.785658

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 01 August 2023
DOI 10.3389/fendo.2023.1233685

OPEN ACCESS

EDITED BY
Emre Pabuccu,
Ufuk University, Türkiye

REVIEWED BY
Mehtap Polat,
Anatolia IVF Center, Türkiye
Hiroshi Kishi,
The Jikei University School of Medicine, Japan

*CORRESPONDENCE
Cristina Rodríguez-Varela
cristina.rodriguez@ivirma.com

RECEIVED 02 June 2023
ACCEPTED 07 July 2023
PUBLISHED 01 August 2023

CITATION
Rodríguez-Varela C, Salvaleda-Mateu M and Labarta E (2023) Duration of oestrogen exposure does not affect reproductive outcome in artificial cycles: a retrospective analysis of more than 7000 hormonal replacement therapy cycles for an embryo transfer.
Front. Endocrinol. 14:1233685.
doi: 10.3389/fendo.2023.1233685

Duration of oestrogen exposure does not affect reproductive outcome in artificial cycles: a retrospective analysis of more than 7000 hormonal replacement therapy cycles for an embryo transfer

Cristina Rodríguez-Varela[1]*, Maria Salvaleda-Mateu[1] and Elena Labarta[1,2]

[1]Valencian Infertility Institute (IVI) Foundation – Health Research Institute (IIS) La Fe, Valencia, Spain, [2]Human Reproduction Department, Valencian Infertility Institute (IVI) Reproductive Medicine Associates (RMA) Valencia, Valencia, Spain

Introduction: Optimal duration of oestrogen exposure before an embryo transfer in artificial cycles has not been defined yet, as its correlation with reproductive outcome remains controversial. The length of oestrogen treatment before starting luteal phase support varies significantly among patients.

Materials and methods: In this study, we conducted a retrospective analysis of a huge database of our own clinical results in artificial cycles in the past five years. The aim of this study was to assess the effect of the length of estrogen exposure on reproductive outcome and to evaluate if there is any optimal duration of estrogen exposure in order to maximize success rates.

Results: Differences in pregnancy rates according to oestrogen length, if present, were not clinically relevant.

Discussion: Our results suggest that the length of oestrogen exposure (in days) before exogenous progesterone administration do not affect clinical outcomes.

KEYWORDS

oestrogen, artificial cycle, pregnancy, embryo transfer, *in vitro* fertilization

1 Introduction

Artificial endometrial preparation with hormonal replacement therapy (HRT) is frequently used for frozen embryo transfer (FET) and egg donation cycles (1). This protocol involves the administration of exogenous estrogen and progesterone trying to mimic the hormonal changes happening physiologically in a natural cycle.

Whereas the number of days of progesterone administration until the embryo transfer (ET) is clearly defined according to the embryo development stage, the part of the protocol that involves the length of the oestrogen exposure is highly variable among patients. The latter usually extends between six and twenty-five days before progesterone administration onset, although it may vary relying on the ability of each patient to reach the minimum endometrial thickness, as well as special conditions such as personal decisions or waiting for the donor to be ready in oocyte donation cycles. However, the optimal duration of oestrogen exposure in HRT cycles for an ET hasn't been defined yet, and its correlation with reproductive outcome remains controversial.

Several studies have claimed that the duration of oestrogen exposure before ET does not affect the final pregnancy outcome in cycles with own oocytes, both with (2) or without preimplantation genetic testing for aneuploidies (PGT-A) (3). These two above mentioned studies covered a range of the oestrogen exposure therapy between 10 and 39 days, approximately. In contrast, Bourdon et al. claimed that a duration of oestrogen exposure until ET longer than 29 days was correlated to significantly lower live birth rates (LBR), and a length longer than 36 days was correlated to significantly higher probability of miscarriage (4).

While these studies evaluated the impact of the length of oestrogen exposure until the day of ET (including the luteal phase prior to transfer), the vast majority of studies addressing this issue have analyzed their results by comparing the length of oestrogen exposure until the day of onset of progesterone administration. In this regard, Jiang et al. in (5) did not find any significant effect on pregnancy rates between 7 or 14 days of oestrogen exposure (5). Indeed, higher clinical pregnancy rates were observed in a group of patients without pituitary suppression and a length of oestrogen exposure shorter than 20 days, in comparison to a length of 20 days or more (6).

In addition, in oocyte donation cycles, the length of estrogen exposure can be also extended in order to facilitate the synchronization between patient and donor. In this type of cycles, the optimal length of oestrogen exposure seems to be between 12 and 19 days, approximately (7, 8).

Nevertheless, the length of oestrogen exposure may exert an effect in other parameters, such as gestational age at delivery. In Sekhon et al. (2), in spite of having denied the correlation between the length of oestrogen exposure and pregnancy rates, each additional day of estrogen therapy was significantly associated with a reduction in the gestational age at delivery (in weeks) (β = -0.07 ± 0.03, p = 0.01) (2).

Finally, the onset of oestrogen therapy (day 2-5 of cycle vs. day 6 onwards), and not its length, has been proven to have a significant impact on endometrial thickness, as well as on biochemical and clinical pregnancy, being higher in the late onset group. In contrast, the same relationship has not been proven for ongoing pregnancy rate (9).

The aim of the current study is to retrospectively analyze the impact of the duration of oestrogen exposure (in days) until the onset of progesterone administration on pregnancy outcome, in the context of an artificial endometrial preparation cycle for an ET. This issue will be addressed using a large database of 7390 cycles performed in the past 5 years in IVI RMA Valencia (Spain), including both own and oocyte donation cycles. Results from this study could help to elucidate which is the optimal length of oestrogen exposure in order to maximize success rates in this type of ET cycles.

2 Materials and methods

2.1 Design and setting

Retrospective study conducted at IVI RMA Valencia (Spain) between November 2018 and July 2022. This study was approved by the Institutional Review Board of IVI RMA Valencia (Code: 2212-VLC-181-EL).

2.1.1 Study population

The study enrolled 7390 infertile patients scheduled for an ET in the context of an artificial endometrial preparation cycle with HRT. Participating women were ≤ 50 years old with adequate endometrial pattern (triple layer) and thickness (6.5 mm) after estrogen treatment in the proliferative phase. Luteal phase support (LPS) was performed with micronized vaginal progesterone (MVP; 400 mg twice daily for 5 days) before ET. One or two blastocysts were transferred. Patients with uterine or adnexal anomalies were excluded from the study.

2.1.2 Endpoints

The primary endpoint was the ongoing pregnancy rate (OPR) based on the length of oestrogen exposure before the onset of exogenous progesterone administration, in the overall population and according to the type of cycle (oocyte donation, own oocytes and own oocytes with PGT-A). OPR was defined as the presence of at least one viable fetus beyond Week 12.

Secondary endpoints included: the comparison of the mean length of oestrogen exposure according to pregnancy outcome variables (biochemical pregnancy, clinical pregnancy, biochemical miscarriage and clinical miscarriage), in the overall population and according to the type of cycle (oocyte donation, own oocytes without PGT-A, own oocytes with PGT-A); the impact of the length of oestrogen exposure on biochemical, clinical and ongoing pregnancy rates taking into account confounding variables (such as embryo quality, serum progesterone (P4) levels on the ET day, age, BMI, route of oestrogen administration, etc.); the assessment of pregnancy outcomes according to the length of oestrogen exposure as a categorical variable (divided into 4 different groups taking into account quartiles), in the overall population and according to the type of cycle.

Pregnancy outcome was determined by a positive β-hCG test (serum levels of β-hCG > 10 IU/ml 11 days after ET); clinical pregnancy was defined as the presence of at least one gestational sac on ultrasound; implantation was defined as the presence of a gestational sac per embryo transferred; miscarriage rate was defined as any pregnancy loss before Week 12, including biochemical miscarriage with a positive β-hCG test without evidence of a gestational sac and clinical miscarriage after confirmation of an intrauterine gestational sac; ectopic pregnancy

was defined as a gestational sac located outside the uterine cavity; and LBR was defined as the number of deliveries that resulted in at least one live born neonate.

2.2 Study protocol

2.2.1 Endometrial preparation

After transvaginal ultrasound to confirm ovarian quiescence, oestrogen treatment commenced on days 2–3 of menstruation. Oestrogens were administered orally at either 6 mg/day of estradiol valerate (Progynova®, Bayer Hispania, Barcelona, Spain; Meriestra®, Novartis, Barcelona, Spain) or transdermally with two patches of 75 mg estradiol hemihydrate (Evopad®, Janssen Cilag, Madrid, Spain) every 48 h. Patients who underwent egg donation cycles using fresh embryos were given a GnRH agonist (Decapepty® 3.75 mg, single dose, Ipsen Pharma, Barcelona, Spain) administered in the mid-luteal phase of the previous menstrual cycle, or a gonadotropin-releasing hormone (GnRH) antagonist (0.25 mg/day) for 5 days from the first day of menstruation (Orgalutran® 0.25 mg/0.5 ml, single dose, Merck Sharp & Dohme, Madrid, Spain) (10). After 10–14 days on estrogens, a vaginal two-dimensional (2D) ultrasound was performed to measure endometrial thickness (EMT) and to confirm a triple-layer pattern, and a blood sample was drawn for estradiol (E2) and P4 determinations to ensure that no spontaneous ovulation had occurred. If EMT was >6.5 mm, the endometrial pattern was trilaminar, and serum P < 1.0 ng/ml, ET was scheduled.

LPS began 5 days before ET with MVP at a dose of 400 mg twice daily (Utrogestan®, SEID, Barcelona, Spain; Progeffik®, Effik, Madrid, Spain; or Cyclogest®, Gedeon Richter, Barcelona, Spain). If pregnancy occurred, hormonal treatment was maintained until pregnancy week 12 in accordance with routine practice.

2.2.2 IVF laboratory

Intracytoplasmic sperm injection (ICSI) was used in all cases, as it is the main method of fertilization used in our center. Either fresh or vitrified oocytes were used in oocyte donation cycles since there are no differences in pregnancy rates between them (11, 12). Likewise, ET was performed with fresh or thawed blastocysts.

Embryo quality was classified according to the Spanish ASEBIR (*Asociación para el estudio de la biología de la reproducción*) classification (13). This classification allocates each blastocyst to a category from A to D based on the trophectoderm and the inner cell mass morphology, being A the best and D the worst quality. Only embryos graded A to C were transferred.

Embryo transfers were performed in lithotomy position by senior gynecologists under transabdominal ultrasound guidance with full bladder.

2.3 Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics v25 software (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation (SD), whereas categorical variables were expressed as percentages.

Categorical variables were compared with a Chi-squared test, and a ANOVA test was used to compare the continuous variables between the two or more groups. P-value < 0.05 was considered statistically significant.

To analyze the correlation between the length of oestrogen therapy and pregnancy outcome, a binary logistic regression analysis was performed. Variables that were correlated to pregnancy outcome in a univariable analysis were included in the model (age, BMI, embryo quality (A, B or C), type of cycle (oocyte donation, own oocytes and own oocytes with PGT-A), route of oestrogen administration (patches or pills), and serum P4 levels on ET day).

The length of oestrogen therapy was also compared as a categorical variable grouped as: i) 9 categories according to percentiles (<10; 10<11; 11<12; 12<13; 13<14; 14<15; 15<17; 17 ≤ 19; >19 days), and ii) 2 categories according to the cut-off point of 14 days.

3 Results

3.1 Descriptive analysis

Of the 7390 HRT-ET cycles included in the study, 5044 (68.3%) involved oocyte donation cycles, 1049 (14.2%) were cycles with own oocytes and 1297 (17.6%) were cycles with own oocytes with PGT-A.

Cycles with own oocytes, with or without PGT-A, were always transferred in the context of a frozen embryo transfer (n=2346), while cycles with donated oocytes could be transferred in the context of a fresh embryo transfer (n=2066) or a frozen embryo transfer (n=2978).

Mean characteristics of the study population are shown in Table 1.

The range of oestrogen exposure prior to luteal phase supplementation ranged from 6 to 36 days in the overall population. In oocyte donation cycles it ranged from 6 to 36 days, from 6 to 28 days in cycles of own oocytes without PGT-A, and from 6 to 31 days in cycles with own oocytes with PGT-A.

Oestrogens were administered orally in the form of pills in 79.2% of cases, in the form of transdermal patches in 15.3% and using a combination of both in the remaining 5.5%.

Embryos transferred were classified as A in 18.9% of cases, as B in 65.0% of cases and as C in the remaining 16.1% of cases. The majority of cycles were single embryo transfers (92.2%), while 7.8% of them were double embryo transfers.

3.2 Clinical outcome according to the length of oestrogen exposure before progesterone onset

Mean ongoing pregnancy rate was 45.9%. Mean length of oestrogen exposure before progesterone administration onset was 14.3 ± 3.7 days in ongoing pregnancies vs. 14.1 ± 3.6 in non-ongoing pregnancies (p=0.044). This difference continued to be

TABLE 1 Cycle and patients' characteristics of the cycles analyzed.

	Overall N=7390	Oocyte donation N=5044	Own oocytes N=1049	Own oocytes with PGT-A N=1297	P value
Age (y.o.)	40.5 ± 4.8	42.1 ± 4.3	35.2 ± 3.8	38.3 ± 3.5	**<0.001**
BMI (kg/m^2)	23.6 ± 4.2	23.7 ± 4.2	23.1 ± 4.2	23.3 ± 3.8	**<0.001**
Last serum E2 before progesterone onset (pg/mL)	312.7 ± 376.6	322.3 ± 387.0	290.0 ± 360.2	291.0 ± 344.2	**0.017**
Last serum P4 before progesterone onset (ng/mL)	0.21 ± 0.29	0.23 ± 0.27	0.18 ± 0.27	0.18 ± 0.37	**<0.001**
Last EMT before progesterone onset (mm)	8.9 ± 1.6	8.9 ± 1.7	9.0 ± 1.6	8.8 ± 1.5	**0.029**
Days oestrogen exposure until progesterone onset	14.2 ± 3.7	14.7 ± 3.8	12.8 ± 3.0	13.1 ± 3.1	**<0.001**
Serum P4 levels on day of ET (ng/mL)	13.2 ± 6.1	13.2 ± 6.1	12.8 ± 5.4	13.3 ± 6.6	0.070

E2, estradiol; P4, progesterone; EMT, endometrial thickness; ET, embryo transfer; PGT-A, preimplantational genetic testing for aneuploidies.
Data are shown as mean ± standard deviation. ANOVA test. P-value < 0.05 is considered statistically significant.
Bold values, statistically significant differences.
Coloured line, is the variable in which we have focused in this article.

significant only in the subgroup of patients in oocyte donation cycles (14.9 ± 3.8 in ongoing pregnancies vs. 14.6 ± 3.7 in non-ongoing pregnancies; p=0.013) (Table 2) after the stratification of patients according to the type of cycle (oocyte donation, own oocytes and own oocytes with PGT-A).

Also in oocyte donation cycles, the mean length of oestrogen exposure was significantly shorter in cycles that ended in a clinical miscarriage. In contrast, cycles with own oocytes with or without PGT-A didn't show any significant differences in the length of oestrogen exposure according to pregnancy outcome (Table 2).

OPR was statistically different according to the percentiles of oestrogen duration (p=0.022), although any trend was observed (Figure 1).

When classifying the population into 2 groups according to be above or below the cut-off point of 14 days of oestrogen exposure, a higher miscarriage rate was observed in patients with a shorter

TABLE 2 Mean length of oestrogen exposure (days) according to clinical outcome.

		Overall N=7390	Oocyte donation N=5044	Own oocytes N=1049	Own oocytes with PGT-A N=1297
Biochemical pregnancy (%)	Yes	14.19 ± 3.7	14.78 ± 3.8	12.79 ± 2.9	13.04 ± 3.0
	No	14.10 ± 3.6	14.60 ± 3.8	12.71 ± 3.0	13.29 ± 3.1
	P-value	0.274	0.102	0.645	0.148
Clinical pregnancy (%)	Yes	14.20 ± 3.7	14.80 ± 3.8	12.75 ± 2.9	13.02 ± 3.0
	No	14.11 ± 3.6	14.61 ± 3.7	12.77 ± 3.1	13.26 ± 3.2
	P-value	0.254	0.071	0.902	0.155
Ongoing pregnancy (%)	Yes	14.25 ± 3.6	14.86 ± 3.8	12.76 ± 3.0	13.05 ± 3.0
	No	14.08 ± 3.6	14.59 ± 3.7	12.76 ± 3.0	13.21 ± 3.1
	P-value	**0.044**	**0.013**	0.976	0.360
Biochemical miscarriage (%)	Yes	14.17 ± 3.6	14.65 ± 3.7	13.00 ± 3.0	13.25 ± 3.5
	No	14.16 ± 3.7	14.72 ± 3.8	12.74 ± 3.0	13.12 ± 3.0
	P-value	0.954	0.753	0.429	0.705
Clinical miscarriage (%)	Yes	13.90 ± 3.4	14.43 ± 3.6	12.72 ± 2.5	12.81 ± 2.8
	No	14.18 ± 3.7	14.74 ± 3.8	12.76 ± 3.0	13.16 ± 3.1
	P-value	0.051	0.086	0.880	0.247

Data are shown as mean ± standard deviation. Results are shown in the overall population and each type of cycle. ANOVA test. P-value < 0.05 is considered statistically significant.
Bold values, statistically significant p-value.

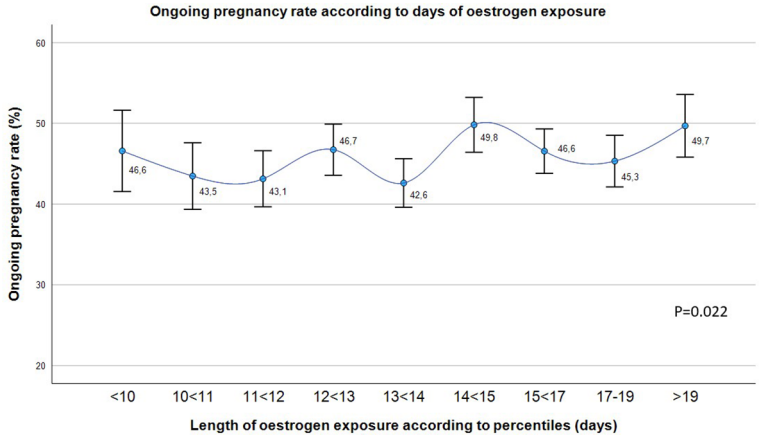


FIGURE 1
Ongoing pregnancy rate (%) in the overall population according to the length of oestrogen exposure in days divided into 9 groups. Chi-squared test. P-value < 0.05 is considered statistically significant.

duration of oestrogens (18.3 vs 15.8%, p=0.040). Table 3 shows the clinical outcomes according to the length of oestrogen exposure (≤ or > 14 days), in the overall population and in the three types of cycle.

Logistic regression model showed that the length of oestrogen exposure was not an independent factor for increasing the rate of biochemical pregnancy (OR: 1.00 IC95% (0.99-1.01), p=0.99), clinical pregnancy (OR: 1.00 IC95% (0.99-1.01), p=0.91) and ongoing pregnancy (OR: 1.01 IC95% (0.99-1.02), p=0.38), after adjusting for all confounding variables mentioned above.

4 Discussion

This retrospective analysis of more than 7000 HRT cycles for embryo transfer suggests that the length of oestrogen exposure (in days) before exogenous progesterone administration do not affect clinical outcomes.

Despite ongoing pregnancies had a significantly longer duration of oestrogen exposure, the difference with respect to non-ongoing pregnancies is less than one day (14.3 ± 3.7 vs. 14.1 ± 3.6; p=0.044), thus not clinically relevant. This finding was observed in the overall population and particularly in oocyte donation cycles (Table 2).

However, the subsequent division of the length of oestrogen exposure into 9 groups according to percentiles shows how this statistically significant variation in OPR is not clinically relevant (the maximum difference between 2 groups is of 7 points) (Figure 1). Hence, statistically significant differences may be due to the large sample size analyzed.

In particular, the maximum difference in OPR happens around day 14 of oestrogen exposure (Figure 1), so that this was the cut-off point chosen to study the behavior of pregnancy rates. In addition, the mean number of days of oestrogen exposure is significantly different between the different types of cycles (oocyte donation, own oocytes and own oocytes with PGT-A) (Table 1), probably due to the necessity of synchronization between the donor and the recipient in oocyte donation cycles. For this reason, and also in order to avoid any potential bias related to embryo quality, its impact on pregnancy outcome has been also addressed separately.

Despite a slight tendency to higher pregnancy rates in cycles with longer duration of oestrogen therapy (lower clinical miscarriage rates and higher ongoing pregnancy rates in oocyte

TABLE 3 Clinical outcome according to the length of oestrogen exposure as a categorical variable, divided into 2 groups according to the cut-off of 14 days.

	Overall N=7390			Oocyte donation N=5044			Own oocytes N=1049			Own oocytes with PGT-A N=1297		
	≤ 14	> 14	p-value	≤ 14	> 14	p-value	≤ 14	> 14	p-value	≤ 14	> 14	p-value
Biochemical pregnancy (%)	61.7	62.2	0.654	61.3	62.8	0.278	61.8	62.1	0.921	62.6	58.1	0.142
Clinical pregnancy (%)	53.8	54.4	0.598	53.4	55.3	0.190	53.1	52.7	0.907	55.5	50.1	0.087
Ongoing pregnancy (%)	45.3	46.9	0.199	44.9	47.7	**0.046**	43.5	44.0	0.894	48.0	43.1	0.117
Biochemical miscarriage (%)	11.9	12.0	0.947	12.2	11.5	0.571	13.3	13.9	0.836	10.0	13.7	0.148
Clinical miscarriage (%)	18.3	15.8	**0.040**	18.8	15.4	**0.021**	20.1	18.8	0.738	15.4	15.9	0.883

Results are shown in the overall population and each type of cycle. Chi-squared test. P-value < 0.05 is considered statistically significant.
Bold values, statistically significant p-value.

donation cycles, although only lower clinical miscarriage rates in the overall population when >14 days), these differences were also not clinically relevant (Table 3).

This slight tendency to better results with longer oestrogen exposure contrasts with previous studies found in the literature, which have claimed that shorter periods of oestrogen exposure are more beneficial to pregnancy outcome [less than 24 days until progesterone onset in Bourdon et al. (4) and less than 20 days in Sunkara et al. (6)]. In addition, Borini and Younis proposed the optimal length of oestrogen exposure in oocyte donation cycles to be between 12 and 19 days (7, 8). In contrast, we didn't see any significant drop in pregnancy rates beyond 19 days (Table 3), also in line with previously evidences proving that periods of oestrogen exposure longer than 35 days in oocyte donation cycles do not hamper clinical outcomes (14).

Regarding cycles with own oocytes, our results don't show any difference in pregnancy outcomes regarding the length of oestrogen exposure, both with or without PGT-A (Table 3), as previously evidenced (2, 3). The number of days covered by these two studies ranges from 5 to 34, very similar to the range of the present study (6 to 36 days).

Hence, in this study we were not able to propose a potential optimal window for the length of oestrogen exposure in HRT-ET cycles, as the statistically significant differences observed are not clinically relevant. Indeed, binary regression models demonstrated that the length of oestrogen exposure was not an independent factor for increasing the rate of biochemical pregnancy, clinical pregnancy and ongoing pregnancy, after adjusting for all confounding variables (including the type of cycle).

The main advantage of HRT cycles for an ET is the flexibility they offer, allowing the best adjustment of dates taking into account the patient, the doctor and the clinic needs. This flexibility is possible by modifying the duration of oestrogen exposure until the onset of exogenous progesterone administration. Results from this study suggest that the variation in the length of oestrogen therapy do not exert any impact on pregnancy outcome, offering the possibility to continue with this clinical practice and favoring the logistics of this type of cycles to suit individual needs. Nevertheless, despite the large sample size analyzed, the main limitation of this study is its retrospective design and conclusions should be taken with caution.

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 approved by Research Ethics Committee of IVI Valencia. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

EL and CR-V significantly contributed to the study conception and design. MS-M significantly contributed to the preparation of the database used for the subsequent analysis included in this study, along with CR-V. CR-V performed statistical analyses and data interpretation, and drafted the article. MS-M also contributed to the elaboration of the background and context of the drafted article. EL carefully revised the drafted article. All authors contributed to the article and approved the submitted version.

Acknowledgments

The authors want to appreciate the invaluable support of Rubén Ferrando Llobell and the rest of the IT department of IVIRMA, involved in the generation of the database export.

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

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Groenewoud ER, Cohlen BJ, Macklon NS. Programming the endometrium for deferred transfer of cryopreserved embryos: hormone replacement versus modified natural cycles. *Fertil Steril* (2018) 109(5):768–74. doi: 10.1016/j.fertnstert.2018.02.135

2. Sekhon L, Feuerstein J, Pan S, Overbey J, Lee JA, Briton-Jones C, et al. Endometrial preparation before the transfer of single, vitrified-warmed, euploid blastocysts: does the duration of estradiol treatment influence clinical outcome? *Fertil Steril* (2019) 111(6):1177–1185.e3. doi: 10.1016/j.fertnstert.2019.02.024

3. Eid J, Pirtea P, Benammar A, Poulain M, De Ziegler D, Ayoubi JM. Optimal duration of estrogen priming during frozen embryo transfer (FET) cycles: Does it really matter? 77th Annual meeting of the American Society for Reproductive Medicine

(ASRM), P-289, 2021 Oct 16-20; Baltimore (Maryland), United States of America. *Fertil Steril* (2021) 116(3):e230. doi: 10.1016/j.fertnstert.2021.07.624

4. Bourdon M, Santulli P, Kefelian F, Vienet-Legue L, Maignien C, Pocate-Cheriet K, et al. Prolonged estrogen (E2) treatment prior to frozen-blastocyst transfer decreases the live birth rate. *Hum Reprod* (2018) 33(5):905–13. doi: 10.1093/humrep/dey041

5. Jiang WJ, Song JY, Sun ZG. Short (seven days) versus standard (fourteen days) oestrogen administration in a programmed frozen embryo transfer cycle: a retrospective cohort study. *J Ovarian Res* (2022) 15(1):36. doi: 10.1186/s13048-022-00967-5

6. Sunkara SK, Seshadri S, El-Toukhy T. The impact of the duration of estrogen supplementation on the outcome of medicated frozen-thawed embryo transfer (FET) cycles. 67th Annual meeting of the American Society for Reproductive Medicine (ASRM), O-142, 2011 Oct 15-19; Orlando (Florida), United States of America. *Fertil Steril* (2011) 96(3):s43. doi: 10.1016/j.fertnstert.2011.07.163

7. Borini A, Dal Prato L, Bianchi L, Violini F, Cattoli M, Flamigni C. Effect of duration of estradiol replacement on the outcome of oocyte donation. *J Assist Reprod Genet* (2001) 18(4):185–90. doi: 10.1023/a:1009472416305

8. Younis JS, Mordel N, Lewin A, Simon A, Schenker JG, Laufer N, et al. Artificial endometrial preparation for oocyte donation: The effect of estrogen stimulation on clinical outcome. *J Assist Reprod Genet* (1992) 9:222–7. doi: 10.1007/BF01203817

9. Ying Y, Wu Y, Liu S, Huang Q, Liu H. The timing for initiating estrogen stimulation in artificial cycle for frozen-thawed embryo transfer can be flexible. *Reprod Health* (2021) 18:181. doi: 10.1186/s12978-021-01229-1

10. Labarta E, Mariani G, Paolelli S, Rodriguez-Varela C, Vidal C, Giles J, et al. Impact of low serum progesterone levels on the day of embryo transfer on pregnancy outcome: a prospective cohort study in artificial cycles with vaginal progesterone. *Hum Reprod* (2021) 36(3):683–92. doi: 10.1093/humrep/deaa322

11. Cobo A, Kuwayama M, Pérez S, Ruiz A, Pellicer A, Remohí J. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. *Fertil Steril* (2008) 89(6):1657–64. doi: 10.1016/j.fertnstert.2007.05.050

12. Rienzi L, Romano S, Albricci L, Maggiulli R, Capalbo A, Baroni E, et al. Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. *Hum Reprod* (2010) 25(1):66–73. doi: 10.1093/humrep/dep346

13. Pons MC. Evluación morfológica del estadio de mórula al de blastocisto. D+4, D +5 y D+6. In: *Criterios ASEBIR de Valoración Morfológica de Ovocitos, Embriones Tempranos y Blastocistos Humanos*, *3rd edn*, vol. p. Madrid: Góbalo. Agencia Creativa Digital (2015). p. 58–68.

14. Remohí J, Gutiérrez A, Cano F, Ruiz A, Simón C, Pellicer A. Long oestradiol replacement in an oocyte donation programme. *Hum Reprod* (1995) 10(6):1387–91. doi: 10.1093/humrep/10.6.1387

frontiers | Frontiers in Endocrinology

TYPE Systematic Review
PUBLISHED 16 August 2023
DOI 10.3389/fendo.2023.1186068

OPEN ACCESS

EDITED BY
Zhen-Gao Sun,
Affiliated Hospital of Shandong University of Traditional Chinese Medicine, China

REVIEWED BY
Aleksander Makatsariya,
I.M. Sechenov First Moscow State Medical University, Russia
Fahiel Casillas,
Metropolitan Autonomous University, Mexico
Hilma Putri Lubis,
University of North Sumatra, Indonesia

*CORRESPONDENCE
Liangzhi Xu
xuliangzhiscu@163.com

†These authors have contributed equally to this work and share first authorship

RECEIVED 14 March 2023
ACCEPTED 14 July 2023
PUBLISHED 16 August 2023

CITATION
Zhao Z, Chen Y, Deng H, Huang L, Lu D, Shen X and Xu L (2023) The influence of embryo stage on obstetric complications and perinatal outcomes following programmed compared to natural frozen-thawed embryo transfer cycles: a systematic review and meta-analysis. *Front. Endocrinol.* 14:1186068. doi: 10.3389/fendo.2023.1186068

The influence of embryo stage on obstetric complications and perinatal outcomes following programmed compared to natural frozen-thawed embryo transfer cycles: a systematic review and meta-analysis

Zhonghua Zhao[1,2,3,4†], Yan Chen[1,2,3,4†], Hongxia Deng[1,2,3,4], Lu Huang[1,2,3,4], Danhua Lu[1,2,3,4], Xiaoyang Shen[1,2,3,4] and Liangzhi Xu[1,2,3,4*]

[1]Department of Obstetrics and Gynecology, West China Second University Hospital, Sichuan University, Chengdu, China, [2]Reproductive Endocrinology and Regulation Laboratory, West China Second University Hospital, Sichuan University, Chengdu, China, [3]Key Laboratory of Birth Defects and Related Diseases of Women and Children, Ministry of Education, Sichuan University, Chengdu, China, [4]The Joint Laboratory for Reproductive Medicine of Sichuan University, The Chinese University of Hong Kong, Chengdu, China

Objective: To investigate the effect of embryo stage at the time of transfer on obstetric and perinatal outcomes in programmed frozen-thawed embryo transfer (FET) versus natural FET cycles.

Design: Systematic review and meta-analysis.

Setting: Not applicable.

Patient(s): Women with programmed frozen-thawed embryo transfer (FET) and natural FET.

Intervention(s): The PubMed, MEDLINE, and EMBASE databases and the Cochrane Central Register of Controlled Trials (CCRT) were searched from 1983 to October 2022. Twenty-three observational studies were included.

Primary outcome measure: The primary outcomes were hypertensive disorders of pregnancy (HDPs), gestational hypertension and preeclampsia (PE). The secondary outcomes were gestational diabetes mellitus (GDM), placenta previa, postpartum haemorrhage (PPH), placental abruption, preterm premature rupture of membranes (PPROM), large for gestational age (LGA), small for gestational age (SGA), macrosomia, and preterm delivery (PTD).

Result(s): The risk of HDP (14 studies, odds ratio (OR) 2.17; 95% confidence interval (CI) 1.95-2.41; P<0.00001; I^2 = 43%), gestational hypertension (11 studies,

10.3389/fendo.2023.1186068

OR 1.38; 95% CI 1.15-1.66; P=0.0006; I^2 = 19%), PE (12 studies, OR 2.09; 95% CI 1.88-2.32; P<0.00001; I^2 = 0%), GDM (20 studies, OR 1.09; 95% CI 1.02-1.17; P=0.02; I^2 = 8%), LGA (18 studies, OR 1.11; 95% CI 1.07-1.15; P<0.00001; I^2 = 46%), macrosomia (12 studies, OR 1.15; 95% CI 1.07-1.24; P=0.0002; I^2 = 31%), PTD (22 studies, OR 1.21; 95% CI 1.15-1.27; P<0.00001; I^2 = 49%), placenta previa (17 studies, OR 1.2; 95% CI 1.02-1.41; P=0.03; I^2 = 11%), PPROM (9 studies, OR 1.19; 95% CI 1.02-1.39; P=0.02; I^2 = 40%), and PPH (12 studies, OR 2.27; 95% CI 2.02-2.55; P <0.00001; I^2 = 55%) were increased in programmed FET cycles versus natural FET cycles with overall embryo transfer. Blastocyst transfer had a higher risk of HDP (6 studies, OR 2.48; 95% CI 2.12-2.91; P<0.00001; I^2 = 39%), gestational hypertension (5 studies, OR 1.87; 95% CI 1.27-2.75; P=0.002; I^2 = 25%), PE (6 studies, OR 2.23; 95% CI 1.93-2.56; P<0.00001; I^2 = 0%), GDM (10 studies, OR 1.13; 95% CI 1.04-1.23; P=0.005; I^2 = 39%), LGA (6 studies, OR 1.14; 95% CI 1.07-1.21; P<0.0001; I^2 = 9%), macrosomia (4 studies, OR 1.15; 95% CI 1.05-1.26; P<0.002; I^2 = 68%), PTD (9 studies, OR 1.43; 95% CI 1.31-1.57; P<0.00001; I^2 = 22%), PPH (6 studies, OR 1.92; 95% CI 1.46-2.51; P<0.00001; I^2 = 55%), and PPROM (4 studies, OR 1.45; 95% CI 1.14-1.83; P=0.002; I^2 = 46%) in programmed FET cycles than in natural FET cycles. Cleavage-stage embryo transfers revealed no difference in HDPs (1 study, OR 0.81; 95% CI 0.32-2.02; P=0.65; I^2 not applicable), gestational hypertension (2 studies, OR 0.85; 95% CI 0.48-1.51; P=0.59; I^2 = 0%), PE (1 study, OR 1.19; 95% CI 0.58-2.42; P=0.64; I^2not applicable), GDM (3 study, OR 0.79; 95% CI 0.52-1.20; P=0.27; I^2 = 21%), LGA (1 study, OR 1.15; 95% CI 0.62-2.11; P=0.66; I^2not applicable), macrosomia (1 study, OR 1.22; 95% CI 0.54-2.77; P=0.64; I^2 not applicable), PTD (2 studies, OR 1.05; 95% CI 0.74-1.49; P=0.79; I^2 = 0%), PPH (1 study, OR 1.49; 95% CI 0.85-2.62; P=0.17; I^2not applicable), or PPROM (2 studies, OR 0.74; 95% CI 0.46-1.21; P=0.23; I^2 = 0%) between programmed FET cycles and natural FET cycles.

Conclusion(s): The risks of HDPs, gestational hypertension, PE, GDM, LGA, macrosomia, SGA, PTD, placenta previa, PPROM, and PPH were increased in programmed FET cycles versus natural FET cycles with overall embryo transfer and blastocyst transfer, but the risks were not clear for cleavage-stage embryo transfer.

KEYWORDS

frozen–thawed embryo transfer, programmed cycle, natural cycle, embryo, embryo at time of transfer

Introduction

Frozen-thawed embryo transfer (FET) has increased dramatically since the first successful human pregnancy in 1983. This strategy enables the use of preimplantation genetic diagnosis/screening, facilitates fertility preservation, and reduces ovarian hyperstimulation syndrome in clinical practice (1). Compelling data have shown that FET results in a higher live birth rate than fresh embryo transfer. However, it was coupled with an increased risk for obstetric and perinatal complications (2). A meta-analysis performed by Roque et al. (2019) with 11 studies reported an increased risk of preeclampsia (PE) in pregnant women following FET compared with fresh ET (3). A pivotal multicentre RCT revealed a 3.13-fold increased risk of PE and a 1.6-fold increased risk of large for gestational age (LGA) in FET compared with fresh ET (4). To date, the reason FET leads to elevated obstetric and perinatal complications is unknown and possibly multifactorial. Recently, researchers proposed that the absence of the corpus luteum (CL) in the programmed endometrial preparation protocol, which is commonly used for FET, may be one potential contributor. Indeed, von Versen-Höynck et al. showed for the first time that the programmed FET cycle (0 CL) was associated with higher rates of preeclampsia and preeclampsia with severe features than the natural FET cycle (1 CL) (5). A meta-analysis including 9 studies reported a significant increase in hypertensive disorders of pregnancy (HDPs), PE, postpartum haemorrhage (PPH), placenta previa and preterm premature rupture of membranes (PPROM) following programmed FET cycles versus natural FET cycles (6). However, the abovementioned studies did not specify the embryo stage at the time of transfer, which may influence the pregnancy outcomes. Indeed, increased risks of preterm birth, LGA

and perinatal mortality (7), as well as placenta-related diseases, including placenta previa, placental abruption and pregnancy-induced hypertension (8, 9), have been observed following blastocyst transfers versus cleavage-stage embryo transfers. However, another study reported that the embryo stage at transfer has a neutral effect on obstetric and perinatal outcomes (10). Therefore, we conducted a review and meta-analysis to compare the obstetric and perinatal outcomes of programmed FET cycles and natural FETs according to the type of embryo stage at the time of transfer (cleavage stage or blastocyst stage).

Materials and methods

Data sources and search strategy

An electronic search of literature was performed from 1983 to November 2022 in the database of PubMed, MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials (CCRT), with the following search terms: ('frozen embryo transfer' OR 'frozen-thawed embryo transfer' OR 'FET' OR 'vitrified-warmed embryo transfer' OR 'frozen blastocyst transfer' OR 'frozen cleavage-stage transfer' OR 'D5-6 frozen embryo transfer' OR 'D2-3 frozen embryo transfer' OR 'programmed frozen embryo transfer' OR 'natural frozen embryo transfer cycle' OR 'endometrial preparation protocols' OR 'hormone replacement therapy' OR 'artificial frozen-thawed embryo' OR 'corpus luteum') AND ('obstetric complication' OR 'pregnancy complication' OR 'perinatal complication' OR 'neonatal complication' OR 'preterm birth' OR 'gestational hypertension' OR 'preeclampsia' OR 'hypertensive disorders of pregnancy' OR 'Pregnancy induced hypertension' OR 'post-partum hemorrhage' OR 'placenta previa' OR 'placental abruption' OR 'post-term birth' OR 'gestational diabetes mellitus' OR 'premature rupture of membranes' OR 'macrosomia' OR 'large for gestational age' OR 'small for gestational age'). Articles in the reference lists that met the inclusion criteria were also searched manually.Twenty-three observational studies that comparing obstetric and/or perinatal outcomes between programmed FET cycles and natural FET cycles were included (Flow chart of studies).

Criteria for inclusion and exclusion

Studies were included if they met the following criteria: (i) the study had at least two cohorts including programmed cycle FET versus natural FET cycle, and (ii) the study reported the obstetric and/or perinatal outcomes following programmed cycle FET versus natural FET cycle.

Data extraction and quality assessment

Three authors independently screened each the title and abstract of each. Full-text articles were read if the study met the inclusion criteria. Then, they extracted data with a standard extraction form. Details for the data extraction are shown in Table 1. Three o other authors independently assessed the risk of bias of the included studies using the Newcastle-Ottawa Scale (NOS) in three domains: selection of study groups, comparability of groups and ascertainment of exposure. Detailed scores are shown in Table 2. A discussion was conducted with a third author if there was any disagreement.

Outcome measures

The primary outcomes were hypertensive disorders of pregnancy (HDPs), gestational hypertension and preeclampsia. The secondary outcomes were as follows: gestational diabetes mellitus (GDM), placenta previa, postpartum haemorrhage (PPH), placental abruption, preterm premature rupture of membranes (PPROM), large for gestational age (LGA), small for gestational age (SGA), macrosomia, and preterm delivery.

Statistical analyses

Statistical analyses were performed using Review Manager 5.3 (Nordic Cochrane Centre, Cochrane Collaboration). Dichotomous outcomes are presented as odds ratios (ORs) with 95% confidence intervals (CIs). We used the Mantel-Haenszel method and fixed-effects model to estimate the pooled effect of variables. Heterogeneity was assessed by the I-squared statistic (I^2). When $I^2 > 50\%$, sensitivity analysis was applied to identify the sources of heterogeneity by excluding studies one by one. Differences were considered significant at $P < 0.05$.

Results

Study characteristics

The initial literature search identified a total of 3788 potentially relevant publications. After the titles and abstracts were thoroughly screened by two investigators independently, the full-text articles of 126 potential studies were selected for further review. Finally, 23 retrospective studies that met the inclusion criteria were included in this meta-analysis. The flow diagram of the selection procedure is presented in the flow chart of studies. The study characteristics are detailed in Table 1. The overall outcomes in the current study are presented in Table 3.

Hypertensive disorders of pregnancy

Fourteen studies reported HDPs, including 21769 natural cycles and 13666 programmed cycles. The risk of HDP was significantly higher in programmed FET cycles (OR=2.17, 95% confidence interval (CI): 1.95-2.41, $P<0.00001$, I^2 =43%). A subgroup analysis was performed to evaluate the effect of embryo stage at the time of transfer. With the use of blastocysts, there was an increased risk of HDP (OR 2.48, 95% CI 2.12-2.91, P <0.00001, I^2 =39%) in programmed FET cycles compared with natural FET cycles. Only

10.3389/fendo.2023.1186068

TABLE 1 Description of included studies.

Study	Country	Design	Endometrial preparation	Embryo stage	FET protocol	Freezing technique	PGT	Age	Study quality
Asserhøj et al., 2021 (11)	Denmark	Register-based cohort study	NC-FET	Cleavage-stage Blastocyst	HCG trigger: no P4 support: no	Unclear	Exclude	34.5 (4.3)	7
			PC-FET		GnRH agonist use: +/ -Estradiol: Yes P4 support: yes			33.5 (4.4)	
Dallagiovanna et al., 2021 (12)	Italy	Retrospective cohort study	NC-FET	Blastocyst	HCG trigger: no P4 support: no	Unclear	A part of	36(33-38)	8
			PC-FET		GnRH agonist use: no Estradiol: Yes P4 support: yes			34 (31–37)	
Fu et al., 2022 (13)	China	Retrospective study	NC-FET	Blastocyst	HCG trigger: no P4 support: yes	Vitrification	All	31.83 ±4.28	8
			PC-FET		GnRH agonist use: no estradiol: Yes P4 support: yes			31.76 ±4.21	
Ginström Ernstad et al., 2019 (14)	Sweden	Retrospective cohort study	NC-FET	Cleavage-stage Blastocyst	HCG trigger: none P4 support: none	Vitrification and slow freezing	Unclear	34.9 ±4.1	7
			PC-FET		GnRH agonist use: +/- Route of estradiol: unclear P4 support: yes			34.3 ±4.3	
Guan et al., 2016 (15)	China	Retrospective cohort study	NC-FET	Cleavage-stage	HCG trigger: yes P4 support: none	Vitrification	Unclear	31.2 ± 5.0	6
			PC-FET		GnRH agonist use: no Route of estradiol: yes P4 support: yes			31.3 ± 5.2	
Gu et al., 2023 (16)	China	Multicenter retrospective cohort study	NC-FET	Blastocyst	HCG trigger: no P4 support: yes	Unclear	Exclude	32 (29, 35)	8
			PC-FET		GnRH agonist use: +/- Estradiol: yes P4 support: yes			32 (29,35)	
Hu et al., 2021 (17)	China	Retrospective cohort study	NC-FET	Blastocyst	HCG trigger: +/- P4 support: yes	Vitrification	Exclude	32.3 (4.1)	8
			PC-FET		GnRH agonist use: no Estradiol: yes P4 support: yes			31.5 (4.0)	
Jing et al., 2019 (18)	China	Retrospective cohort study	NC-FET	Cleavage-stage Blastocyst	HCG trigger: no P4 support: yes	Vitrification	Unclear	31 (28, 35)	8
			PC-FET		GnRH agonist use: no Estradiol: yes P4 support: yes			31 (28, 35)	

(Continued)

10.3389/fendo.2023.1186068

TABLE 1 Continued

Study	Country	Design	Endometrial preparation	Embryo stage	FET protocol	Freezing technique	PGT	Age	Study quality
Li et al., 2021 (19)	China	Retrospective cohort study	NC-FET	Cleavage-stage Blastocyst	HCG trigger: no P4 support: no	Vitrification	Exclude	31.43 ± 3.74	6
			PC-FET		GnRH agonist use: no Estradiol: yes P4 support: yes			30.96 ± 3.70	
Li et al., 2022 (20)	China	Retrospective cohort study	NC-FET	Cleavage-stage Blastocyst	HCG trigger: yes P4 support: no	Vitrification	Exclude	31.0 (29.0–34.0)	7
			PC-FET		GnRH agonist use: no Estradiol: yes P4 support: ye			31.0 (29.0–34.0)	
Lin et al., 2020 (21)	China	Retrospective cohort study	NC-FET	Blastocyst	HCG trigger: +/- P4 support: yes	Vitrification	Unclear	28.74 ± 2.89	8
			PC-FET		GnRH agonist use: no Estradiol: yes P4 support: ye			29.08 ± 3.01	
Makhijani et al., 2020 (22)	USA	Retrospective cohort study	NC-FET	Blastocyst	HCG trigger: no P4 support: yes	Vitrification	Adjusted	35.0 ± 3.7	7
			PC-FET		GnRH agonist use: yes Estradiol: yes P4 support: ye			33.9 ± 3.9	
Man et al., 2021 (23)	China	Retrospective study	NC-FET	Blastocyst	HCG trigger: +/- P4 support: yes	Unclear	Unclear	29 (27-31)	8
			PC-FET		GnRH agonist use: no Estradiol: yes P4 support: ye			28 (26-31)	
Pan et al., 2020 (24)	China	Retrospective cohort study	NC-FET	Cleavage-stage	HCG trigger: no P4 support: yes	Vitrification	Unclear	28.49 ± 2.98	7
			PC-FET		GnRH agonist use: no Estradiol: yes P4 support: ye			28.18 ± 3.07	
Saito et al., 2017 (25)	Japan	Retrospective cohort study	NC-FET	Cleavage-stage Blastocyst	HCG trigger: unclear P4 support: unclear	Vitrified and slow-frozen embryo	Unclear	36.5 ± 3.7	6
			PC-FET		Unclear			35.3 ± 4.0	
Tatsumi et al., 2017 (26)	Japan	Retrospective cohort study	NC-FET	Cleavage-stage Blastocyst	Unclear	Not list	Unclear	37.9 (4.1)	8
			PC-FET		Unclear			36.6 (4.4)	
von Versen-Höynck et al., 2019 (5)	USA	Retrospective cohort study	NC-FET	Cleavage-stage Blastocyst	HCG trigger: yes P4 support: yes	Not list	Unclear	36.5 ±4.0	9
			PC-FET		GnRH agonist use: yes Estradiol:			35.4 ±4.2	

(Continued)

10.3389/fendo.2023.1186068

TABLE 1 Continued

Study	Country	Design	Endometrial preparation	Embryo stage	FET protocol	Freezing technique	PGT	Age	Study quality
					yes P4 support: yes				
Wang et al., 2020a (27)	China	Retrospective cohort study	NC-FET	Blastocyst	HCG trigger: yes P4 support: yes	Vitrification	Not clear	30.93 ± 4.16	8
			PC-FET		GnRH agonist use: no Estradiol: yes P4 support: yes			30.63 ± 4.14	
Wang et al., 2020b (28)	China	Retrospective cohort study	NC-FET	Cleavage-stage Blastocyst	HCG trigger: yes P4 support: yes	Vitrification	Exclude	32.51 ±4.26	8
			PC-FET		GnRH agonist use: no Estradiol: yes P4 support: yes			32.54 ±4.25	
Xu et al., 2022 (29)	China	Retrospective cohort study	NC-FET	Cleavage-stage Blastocyst	HCG trigger: no P4 support: yes	Vitrification	Exclude	32.68 ± 3.84	8
			PC-FET		GnRH agonist use: no Estradiol: yes P4 support: yes			31.66 ± 3.90	
Zaat et al., 2021 (30)	Netherlands	Retrospective analysis of a RCT	NC-FET	Cleavage-stage Blastocyst	HCG trigger: yes P4 support: yes	Not list	Not clear	33.3 ± 4.0	7
			PC-FET		GnRH agonist use: no Estradiol: yes P4 support: yes			33.8 ± 4.0	
Zhou et al., 2022 (31)	China	Retrospective cohort study	NC-FET	Cleavage-stage Blastocyst	HCG trigger: yes P4 support: yes	Vitrification	A part of	32.40 ±4.11	8
			PC-FET		GnRH agonist use: no Estradiol: yes P4 support: yes			32.43 ±4.49	
Zong et al., 2020 (32)	China	Retrospective cohort study	NC-FET	Blastocyst	HCG trigger: yes P4 support: yes	Vitrification	Exclude	30.5 ± 4.1	8
			PC-FET		GnRH agonist use: no Estradiol: yes P4 support: yes			30.8 ± 4.0	

Study quality was evaluated by the Newcastle-Ottawa Scale (NOS).
+/-, used in some patient but not all patients.

one study evaluated HDP for cleavage-stage embryo transfer and reported a non-significant increase in HDP in programmed FET cycles compared with natural FET cycles (OR=1.81, 95% CI: 0.32-2.02, P =0.65, I^2 not applicable) (Figure 1; Supplementary Figure S1).

Gestational hypertension

Nine studies including 9783 natural FET cycles and 7241 programmed FET cycles provided information on gestational hypertension. Compared with natural FET cycles, programmed FET cycles had a higher risk of gestational hypertension (OR 1.40, 95% CI 1.15-1.70, P =0.0006, I^2 =23%). The subgroup analysis for blastocyst transfer revealed a higher risk of gestational hypertension in programmed cycles versus natural cycles (OR 1.87, 95% CI 1.27-2.75, P =0.002, I^2 =25%). The subgroup analysis for cleavage-stage embryo transfer revealed no difference in gestational hypertension between programmed cycles and natural cycles (OR 0.85, 95% CI 0.48-1.51, P =0.59, I^2 =0%) (Figure 2; Supplementary Figure S2).

10.3389/fendo.2023.1186068

TABLE 2 Risk of bias and quality assessment.

	Selection				Comparability	Outcomes			
Study	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Outcome of interest was not present at the start of the study	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Enough follow up	Adequacy of follow-up of cohorts	Total
Asserhøj et al., 2021 (11)	1	1	0	1	1	1	1	1	7
Dallagiovanna et al., 2021 (12)	1	1	1	1	1	1	1	1	8
Fu et al., 2022 (13)	1	1	1	1	1	1	1	1	8
Ginström Ernstad et al., 2019 (14)	1	1	0	1	1	1	1	1	7
Guan et al., 2016 (15)	1	1	1	1	0	0	1	1	6
Gu et al., 2023 (16)	1	1	1	1	1	1	1	1	8
Hu et al., 2021 (17)	1	1	1	1	1	1	1	1	8
Jing et al., 2019 (18)	1	1	1	1	1	1	1	1	8
Li et al., 2021 (19)	1	0	1	1	1	0	1	1	6
Li et al., 2022 (20)	1	1	1	1	1	1	1	1	7
Lin et al., 2020 (21)	1	1	1	1	1	1	1	1	8
Makhijani et al., 2020 (22)	1	1	0	1	1	1	1	1	7
Man et al., 2021 (23)	1	1	1	1	1	1	1	1	8
Pan et al., 2020 (24)	1	1	1	1	1	0	1	1	7
Saito et al., 2017 (25)	1	1	0	1	1	0	1	1	6
Tatsumi et al., 2017 (26)	1	1	1	1	1	1	1	1	8
von Versen-Höynck et al., 2019 (5)	1	1	1	1	2	1	1	1	9
Wang et al., 2020a (27)	1	1	1	1	1	1	1	1	8
Wang et al., 2020b (28)	1	1	1	1	1	1	1	1	8
Xu et al., 2022 (29)	1	1	1	1	1	1	1	1	8
Zaat et al., 2021 (30)	1	1	1	1	1	0	1	1	7
Zong et al., 2020 (32)	1	1	1	1	1	1	1	1	8

The quality of stdies was assessed using the Newcastle-Ottawa Scale scoring system.

Preeclampsia

Eleven studies with a total of 20608 natural FET cycles and 10368 programmed FET cycles were pooled. We observed a higher risk of preeclampsia in programmed cycles than in natural cycles (OR 2.15, 95% CI 1.94-2.39, P <0.00001, I^2 =0%). The subgroup analysis revealed a higher risk of preeclampsia in programmed cycles than in natural cycles after blastocyst transfer (OR 2.23, 95% CI 1.93-2.56, P <0.00001, I^2 =0%). Only one study evaluated preeclampsia for cleavage-stage embryo transfer, showing no difference between programmed cycles and natural cycles (OR=1.19, 95% CI: 0.58-2.42, P =0.64, I^2 not applicable) (Figure 3; Supplementary Figure S3).

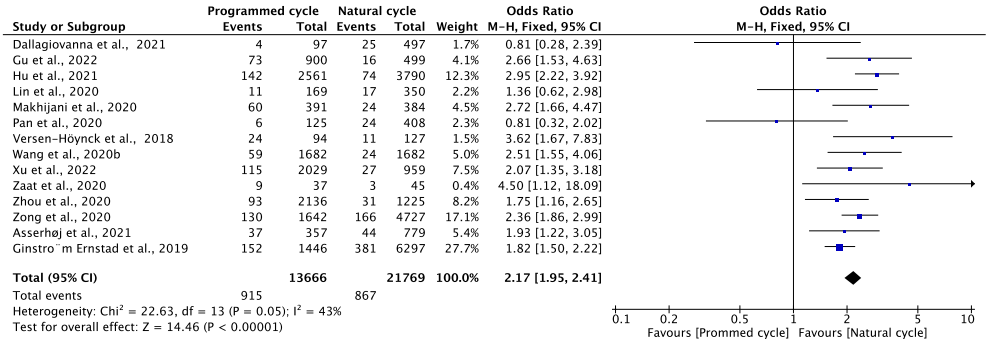


FIGURE 1
Hypertensive disorders of pregnancy.

Gestational diabetes mellitus

Eighteen studies reported GDM with 27349 natural FET cycles and 20768 programmed FET cycles. The incidence of GDM in programmed cycles was higher than that in natural cycles (OR 1.11, 95% CI 1.03-1.19, P =0.0006, I^2 =7%). The subgroup analysis in patients with blastocyst transfer also revealed a higher risk of GDM in programmed cycles than natural cycles (OR 1.13, 95% CI 1.04-1.23, P =0.005, I^2 =39%). However, the subgroup analysis for cleavage-stage embryo transfer revealed no difference in GDM between programmed cycles and natural cycles (OR 0.79, 95% CI 0.52-1.02, P =0.27, I^2 =21%) (Figure 4; Supplementary Figure S4).

Large for gestational age

Eighteen studies including 52676 natural cycles and 46099 programmed cycles provided information on LGA. A higher risk of LGA was observed in programmed cycles when compared with natural cycles (OR 1.11, 95% CI 1.07-1.15, P <0.00001, I^2 =46%). In the subgroup analysis for blastocyst transfer, there was an increased risk of LGA in programmed cycles versus natural cycles (OR 1.14, 95% CI 1.07-1.21, P <0.0001, I^2 =9%). 1.19). However, for cleavage-stage embryo transfer, no difference in LGA was found between programmed cycles and natural cycles in one study (OR 1.15, 95% CI 0.62-2.11, P =0.66, I^2 not applicable) (Figure 5; Supplementary Figure S5).

Macrosomia

Thirteen studies that included 29097 natural FET cycles and 25658 programmed FET cycles evaluated macrosomia. The risk of macrosomia was significantly higher in programmed cycles than in natural cycles (OR 1.14, 95% CI 1.06-1.23, P =0.0004, I^2 =30%). For blastocyst transfer, there was an increased risk of macrosomia in programmed cycles versus natural cycles (OR 1.15, 95% CI 1.05-1.26, P =0.002 I^2 =68%). Only one study evaluated macrosomia following cleavage-stage embryo transfer and reported no difference in macrosomia between programmed cycles and natural cycles (OR=1.22, 95% CI: 0.54-2.77, P =0.64, I^2 not applicable) (Figure 6; Supplementary Figure S6).

Secondary outcomes

Preterm delivery

Two studies, including 54314 natural FET cycles and 48870 programmed FET cycles, reported preterm delivery. The pooled result showed that the overall risk of PTD was significantly higher in programmed cycles than in natural cycles (OR 1.21, 95% CI 1.15-1.27, P <0.00001, I^2 =49%). The subgroup analysis for blastocyst transfer revealed an elevated risk of GDM in programmed cycles versus natural cycles (OR 1.43, 95% CI 1.31-1.57, P <0.00001, I^2 =22%). However, the subgroup analysis for cleavage-stage embryo transfer revealed no difference in GDM between

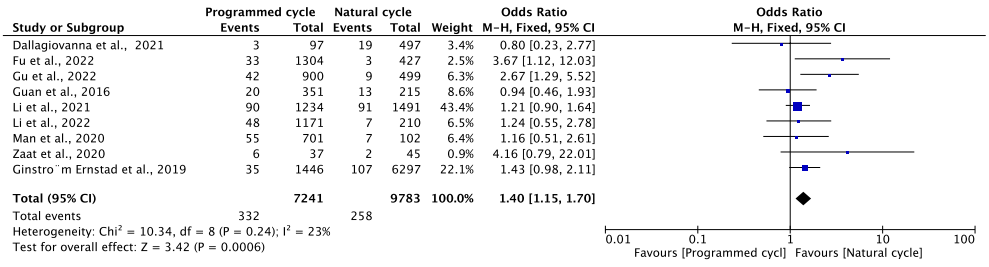


FIGURE 2
Gestational hypertention.

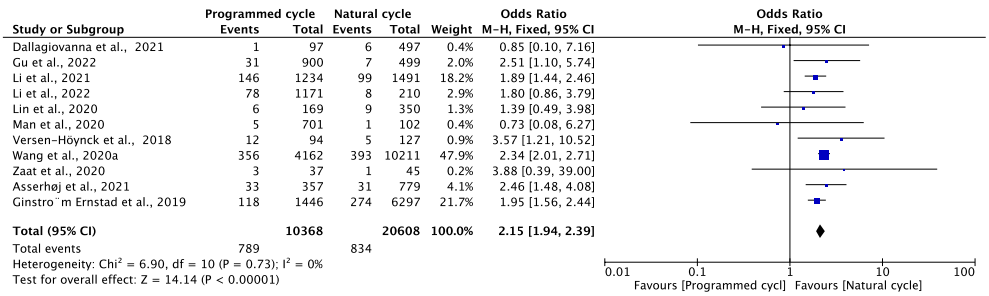


FIGURE 3
Preeclampsia.

programmed cycles and natural cycles (OR 1.05, 95% CI 0.74-1.49, P =0.96, I^2 =0%) (Figure 7; Supplementary Figure S7).

Small for gestational age

Eighteen studies, which included 52676 natural FET cycles and 46099 programmed FET cycles, were meta-analysed. There was no difference in the incidence of SGA between natural cycles and programmed cycles (OR 1.05, 95% CI 0.99-1.11, P =0.13, I^2 =36%). The results of the subgroup analyses revealed that the incidence of SGA in blastocyst transfer (OR 0.96, 95% CI 0.85-1.07, P =0.07, I^2 =36%) and cleavage-stage embryo transfer (OR 0.85, 95% CI 0.49-1.47, P =0.56, I^2not applicable) were consistent with the overall results (Figure 8; Supplementary Figure S8)

Placenta previa

Fifteen studies were included in the analysis (programmed cycles= 17288; natural cycles =30659). The overall risk of placenta previa was significantly higher in pregnancies resulting from programmed cycles (OR 1.30, 95% CI 1.10-1.55, P =0.003, I^2 =0%). The subgroup analyses revealed no difference in placenta previa between programmed cycles and natural cycles in either blastocyst transfer (OR 1.23, 95% CI 0.98-1.54, P =0.60, I^2 =0%) or cleavage-stage embryo transfer (OR 0.59, 95% CI 0.31-1.14, P =0.12, I^2 not applicable) (Figure 9; Supplementary Figure S9).

Placental abruption

Fifteen studies were included in the analysis (programmed cycles= 9205; natural cycles =18654). The overall risk of placental abruption was similar between programmed FET cycles and natural FET cycles (OR 1.23, 95% CI 0.81-1.89, P =0.33, I^2 =0%). The results of the subgroup analyses for blastocyst transfer (OR 1.28, 95% CI 0.72-2.28, P=0.4, I^2 =0%) and cleavage-stage embryo transfer (OR 0.86, 95% CI 0.10-7.42, P =0.89, I^2 not applicable) were consistent with the overall results (Figure 10; Supplementary Figure S10).

Preterm premature rupture of membranes

Seven studies reported PPROM, including 6880 natural cycles and 6803 programmed cycles. The overall risk of PPROM was higher among the pregnancies resulting from the programmed FET cycles (OR 1.22, 95% CI 1.02-1.46, P =0.03, I^2 =44%). The subgroup analysis for blastocyst transfer was consistent with the

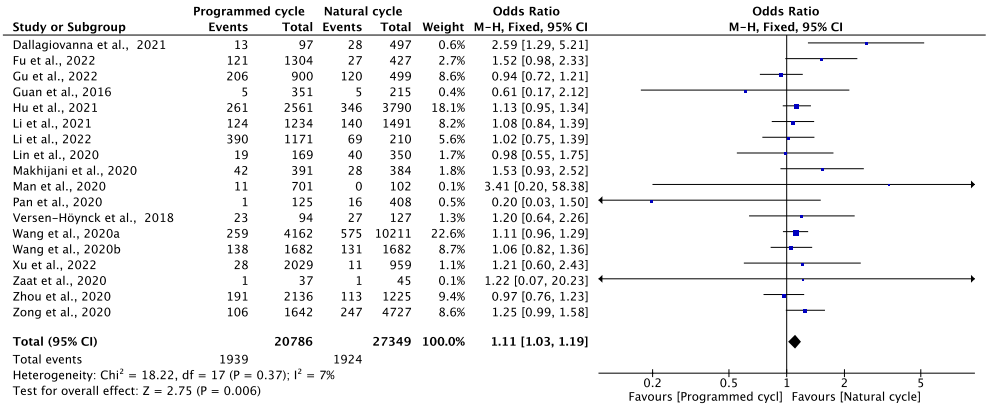


FIGURE 4
Gestational diabetes mellitus.

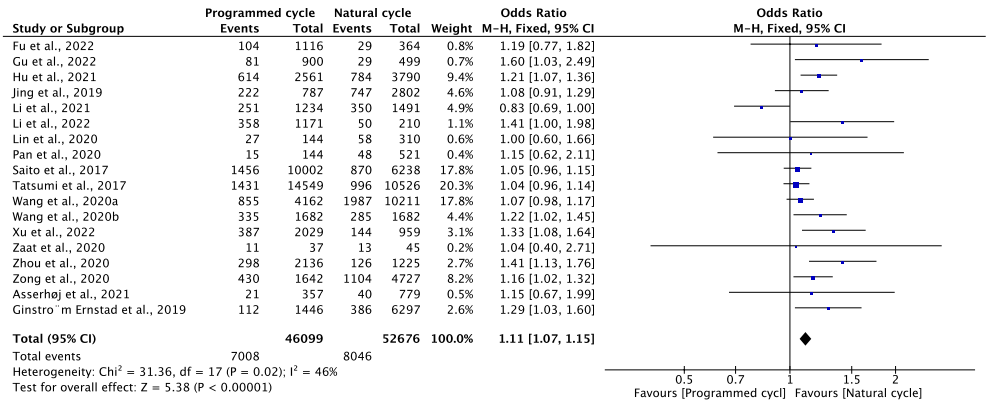


FIGURE 5
Large for gestational age.

overall results (OR 1.45, 95% CI 1.14-1.83, P =0.002, I^2 =46%). However, cleavage-stage embryo transfer showed no difference in PPROM between programmed cycles and natural cycles (OR=0.74, 95% CI: 0.46-1.21, P =0.23, I^2 =0%) (Figure 11; Supplementary Figure S11).

Postpartum haemorrhage

Ten studies reported PPH, including 11338 natural cycles and 19794 programmed cycles. The overall risk of PPH was higher in the pregnancies resulting from the programmed FET cycles than in those resulting from natural FET cycles (OR 2.40, 95% CI 2.12-2.72, P <0.00001, I^2 =53%). After a study suspected of being the source of heterogeneity was excluded (14), sensitivity analysis revealed that programmed cycles still had a significantly higher risk of PPH (OR 2.00, 95% CI 1.66-2.42, P <0.00001, I^2 =34%). The subgroup analysis for blastocyst transfer was consistent with the overall results (OR 1.92, 95% CI 1.46-2.51, P <0.00001, I^2 =55%). However, the subgroup analysis for cleavage-stage embryo transfer showed no difference in PPH between programmed cycles and natural cycles (OR 1.49, 95% CI: 0.85-2.62, P =0.17, I^2 not applicable) (Figure 12; Supplementary Figure S12).

Discussion

In our analysis of obstetric and perinatal outcomes between natural FET cycles and programmed FET cycles, we showed that programmed cycles had a higher risk of HDPs, GH, PE, GDM, LGA, macrosomia, PD, PP, PPROM and PPH than natural cycles, which is consistent with the meta-analysis (6). Similar results were also achieved in another meta-analysis (33). Via subgroup analysis, our present meta-analysis further evaluated the effect of embryo stage at the time of transfer on perinatal outcomes between programmed FET cycles and natural FET cycles and showed that the perinatal outcomes of blastocyst transfer were consistent with the overall results in programmed FET cycles; that is, the incidences of HDP, GH, PE, PPROM and PPH were elevated in programmed FET cycles compared with natural FET cycles following blastocyst transfer. However, the perinatal outcomes of cleavage-stage embryo transfers in programmed FET cycles are similar to those in natural FET cycles.

To date, studies evaluating the effect of cleavage-stage embryo and blastocyst transfers on pregnancy outcomes have shown conflicting results. Ginström Ernstad et al. reported that women with blastocyst transfer had a 2.08-fold increased risk of placenta previa and a 1.62-fold increased risk of placental abruption versus

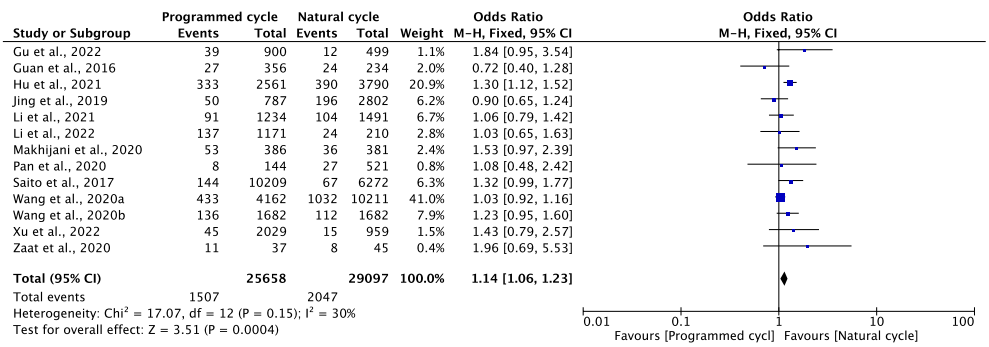


FIGURE 6
Macrosomia.

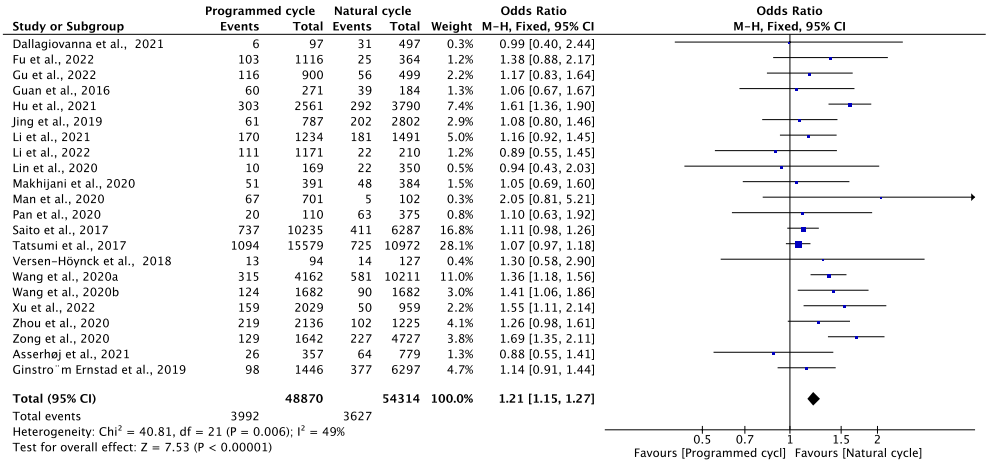


FIGURE 7
Preterm delivery.

cleavage-stage embryo transferss (34). In contrast, a meta-analysis performed by Rosalik et al. including 15 studies reported that both blastocyst transfers and cleavage-stage transfers could lead to a higher risk of LGA in programmed FET cycles versus natural FET cycles (35). Our present study finds a neutral effect for cleavage-stage transfers on obstetric and perinatal outcomes in programmed FET cycles versus natural FET cycles. Of note, the number of studies included here for cleavage-stage embryo transfer were too small to obtain reliable results, so these findings should be considered with great caution.

The reason for the higher risk of HDPs in the programmed FET cycles is unknown. The role of the corpus luteum has recently been a focus of attention for investigators. Indeed, a prospective cohort study of singleton pregnancy reported that women with 0 CL had elevated rates of PE (12.8% versus 3.9%; P=0.02) and PE with severe features (9.6% versus 0.8%; P=0.002) compared to those who conceived with 1 CL. After adjusting for confounders, 0 CL was shown to be a positive predictor of PE and sPE (5). Similar results were also achieved in another prospective study (35). Recently, a hypothesis was proposed that the absence of the CL in programmed cycles may lead to impaired maternal haemodynamic and cardiovascular adaptation to pregnancy in the first trimester, which is associated with adverse pregnancy outcomes such as preeclampsia (36). Compelling findings support the hypothesis that the expected decline in carotid-femoral pulse wave velocity (cfPWV) and the expected rise in transit time (cfPWTT) in the first trimester are attenuated in women with 0 CL (programmed FET cycle) relative to normal women, which suggests that arterial compliance is impaired in pregnant women with no CL (37). Moreover, pregnant women with 0 CL also have a blunted reactive hyperaemia index, an index that reflects endothelial function in early pregnancy, when compared to women with 1 CL (37). All these observations have prompted investigators to explore which factors are secreted into the circulation of women by the CL that may be important for maternal cardiovascular adaptation to pregnancy. Relaxin, a 6-kDa peptide, is probably a potential mediator (38). It is predominantly secreted by CL granulosa lutein cells in the late luteal phase (50-100 pg/ml) and

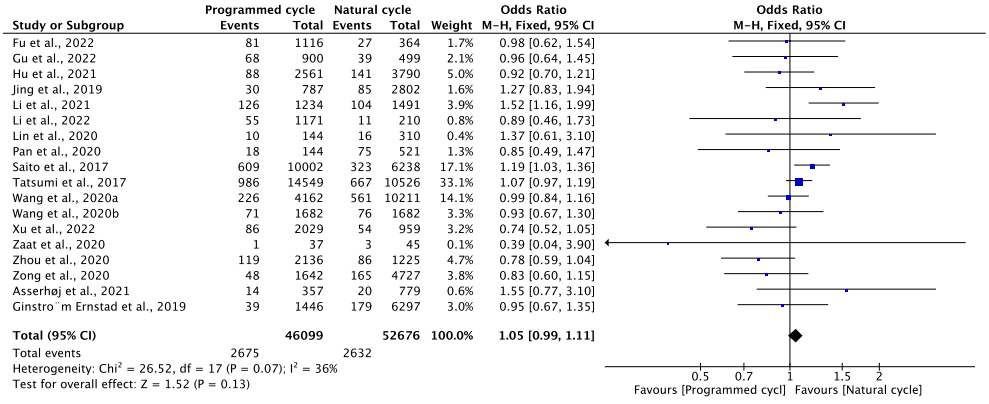


FIGURE 8
Small for gestational age.

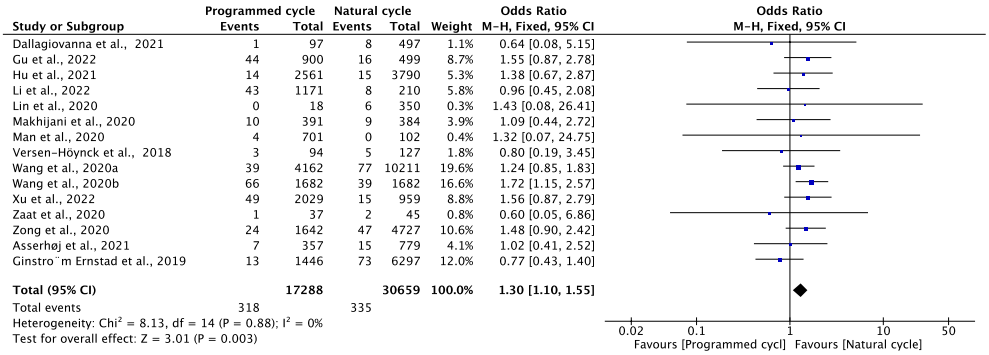


FIGURE 9
Placenta previa.

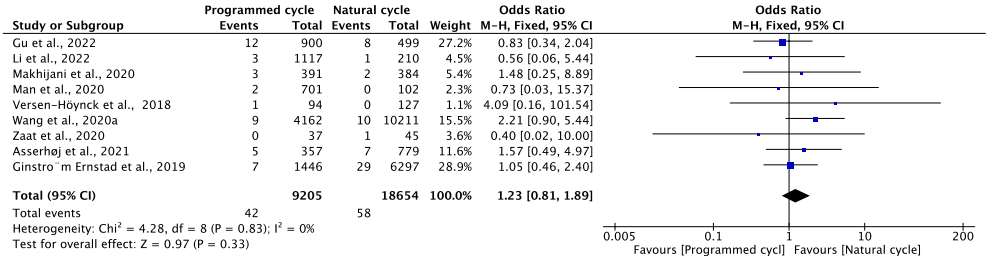


FIGURE 10
Placental abruption.

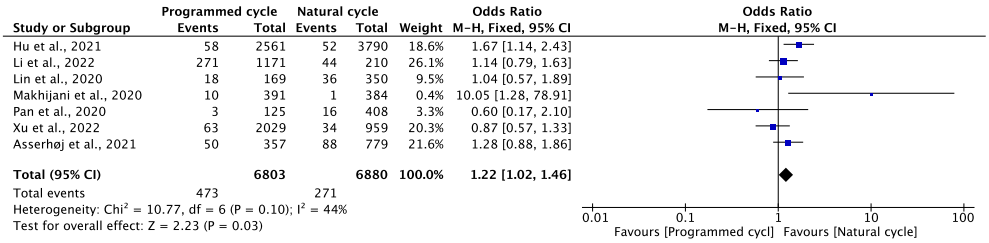


FIGURE 11
Preterm premature rupture of membranes.

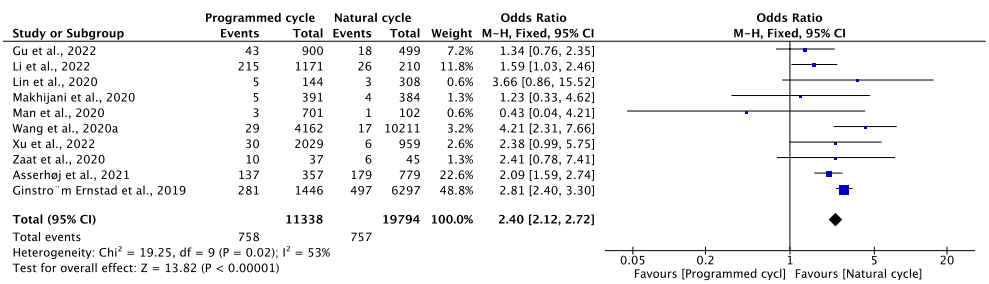


FIGURE 12
Post-partum hemorrhage.

10.3389/fendo.2023.1186068

TABLE 3 Results of the overall outcomes comparing natural and programmed cycle FET according to embryo stage at the time of transfer.

Outcome	Overall embryo transfer			Blastocyst transfer			Cleavage stage embryo transfers		
	No. of study	OR	GRADE	No. of study	OR	GRADE	No. of study	OR	GRADE
HDP	14	2.17 (1.95-2.41)	⊕⊕OO Low	6	2.48 (2.12-2.91)	⊕⊕OO Low	1	0.81 (0.32-2.02)	⊕OOO Very low
PE	11	2.15 (1.94, 2.39)	⊕⊕OO Low	6	2.23 (1.93-2.56)	⊕⊕OO Low	1	1.19 (0.58-2.42)	⊕OOO Very low
Gestational hypertension	9	1.40 (1.15-1.70)	⊕⊕OO Low	5	1.87 (1.27-2.75)	⊕⊕OO Low	2	0.85 (0.48-1.51)	⊕OOO Very low
GDM	18	1.11 (1.03-1.19)	⊕⊕OO Low	10	1.13 (1.04-1.23)	⊕⊕OO Low	3	0.79 (0.52-1.20)	⊕OOO Very low
LGA	18	1.11 (1.07-1.15)	⊕OOO Very low	6	1.14 (1.07-1.21)	⊕⊕OO Low	1	1.15 (0.62-2.11)	⊕OOO Very low
Macrosomia	13	1.14 (1.06-1.23)	⊕⊕OO Low	4	1.15 (1.05-1.26)	⊕⊕OO Low	1	1.22 (0.54-2.77)	⊕OOO Very low
PTD	22	1.21 (1.15-1.27)	⊕⊕OO Low	9	1.43 (1.31-1.57)	⊕⊕OO Low	2	1.05 (0.74, 1.49)	⊕OOO Very low
SGA	18	1.05 (0.99-1.11)	⊕OOO Very low	6	0.96(0.85-1.07)	⊕⊕OO Low	1	0.85 (0.49-1.47)	⊕OOO Very low
Placenta previa	15	1.30 (1.10-1.55)	⊕⊕OO Low	9	1.23 (0.98-1.54)	⊕⊕OO Low	1	0.59 (0.31-1.14)	⊕OOO; Very low
Placental abruption	9	1.23 (0.81-1.89)	⊕⊕OO Low	5	1.28 (0.72-2.28)	⊕OOO Very low	1	0.86 (0.10-7.42)	⊕OOO Very low
PPROM	7	1.22 (1.02-1.46)	⊕⊕OO Low	4	1.45 (1.14-1.83)	⊕⊕OO Low	2	0.74 (0.46-1.21)	⊕OOO Very low
PPH	10	2.00 (1.66-2.42)	⊕⊕OO Low	6	1.92(1.46-2.51)	⊕⊕OO Low	1	1.49 (0.85-2.62)	⊕OOO Very low

reaches a peak concentration of 1000-2500 pg/mL in the first trimester when pregnancy occurs (39). Circulating relaxin was undetectable in women lacking CL (40, 41). It binds to membrane-associated relaxin family peptide receptor 1 (RXFP1), which is a G protein-coupled receptor that is widely distributed in the uterus (myometrium and epithelial layer), ovary and placenta (42) and vascular smooth muscle (unpublished data). Relaxin is a potent vasodilator that mediates vasodilation through the activation of Gi/PI3K-induced cAMP and nitric oxide synthase (nNOS)-driven NO release (43). An experimental study showed that systemic and renal vasodilation and global arterial compliance in early pregnancy were decreased, which contributed to lower cardiac output and a lower glomerular filtration rate when a relaxin-neutralizing antibody was administered to gravid rats (44–46). In humans, the expected rise in 24-hour creatinine clearance was blunted in pregnant women with ovum donation (no circulating relaxin) compared with normal pregnant women, which suggests that the renal response is also impaired in human pregnancy (8, 41). Therefore, relaxin could play a similar role in the maternal circulatory changes that occur during the first trimester. Relaxin deficiency (0 CL) is a potential compromise of maternal cardiovascular adaptation to pregnancy, which is the basic circulatory pattern that occurs in preeclampsia. Indeed, Post Uiterweer et al. found that women with low relaxin concentrations (lowest centile: < p10) during the first trimester are at increased risk of developing late-onset preeclampsia (36). In addition, women with donor-fresh and donor-thawed treatment have significantly higher odds of HDPs relative to women undergoing autologous-fresh treatment. A common feature among donor oocyte cycles is the usage of programmed endometrial preparation, which has no functioning CL (47). Most strikingly, relaxin has been recommended as a potential therapeutic candidate for preeclampsia (38). On the other hand, relaxin is a potent stimulus of endometrial maturation (decidualization), which governs trophoblast invasion during pregnancy (48). Relaxin can enhance the effect of progestin on the induction of prolactin and insulin growth factor binding protein-1 (IGFBP-1) secretion as well as glycodelin expression from human endometrial stromal cells (hESCs), which are biomarkers of decidualization (42, 49). In an exogenous oestradiol- and progesterone-treated ovariectomized rhesus monkey model, giving relaxin can increase its resident endometrial lymphocyte number and promote endometrial

angiogenesis compared to controls (50, 51). Therefore, deficient CL-derived relaxin in early pregnancy probably contributes to aberrant decidualization, which is an important contributor to downregulated cytotrophoblast invasion, impaired placentation and consequently the genesis of placenta-related diseases, such as HDPs, placenta previa, PPROM and PPH (33, 52). The conventional theory is that preeclampsia causes impaired trophoblast invasion and uterine spiral artery remodelling, which can lead to impaired placentation, including reduced placental perfusion and placental ischaemia and reperfusion injury. In addition to CL, suboptimal steroid hormone administration in the programmed FET cycle may also affect trophoblast invasion through immunomodulation in the first trimester (48) and, as a result, influence placenta formation and function. For example, oestrogen has different effects on immune modulation depending on its concentration. Natural killer cells, which have been considered prime regulators of trophoblast invasion, are inhibited by pregnancy levels of oestradiol (E2) but are stimulated at dioestrus to proestrus levels of E2. IL-1 is stimulated by E2 at low concentrations but is inhibited by E2 at pregnancy levels. In addition, IL-6 is inhibited by E2 at periovulatory to pregnancy levels with no effects at early follicular or postmenopausal levels (53). Interestingly, these interleukins have been reported to play a role in trophoblast invasion during early pregnancy, which is involved in the genesis of HDPs. Indeed, Albrecht et al. found that elevated oestrogen levels in baboons lead to markedly decreased invasion of uterine spiral arteries by placental extravillous cytotrophoblasts, an important cell type involved in maintaining placental function (54). To date, we know little about the specific mechanism underlying the association between endometrial preparation for ET and adverse obstetric and neonatal complications.

Limitations

Our study has several limitations. The first is that the included studies are retrospective in design, and there are inherent biases across them that we cannot address. Another limitation is that we included preimplantation genetic testing cycles. In addition, we did not specify the true natural FET and modified natural cycles.

Conclusion

Our research showed that programmed FET cycles resulted in adverse obstetric and perinatal outcomes relative to natural FET cycles following mixed frozen embryo transfer (combined cleavage stage and blastocyst stage) or frozen blastocyst transfer, such as HDPs, gestational hypertension, PE, GDM,LGA, macrosomia, SGA, PTD, placenta previa, PPROM, and PPH. However, the obstetric and perinatal outcomes were similar after frozen cleavage-stage embryo transfers. Further investigations, including RCTs, should be conducted to elucidate the reason for obstetric and perinatal outcomes in programmed FET cycles.

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

Designer of the study: ZZ. Data acquisition and analysis: ZZ, YC, HD, XS, DL, LH. Draft of the manuscript and interpretation: ZZ. Revision of the manuscript: LX. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the National Natural Science Foundation of China (no. 81971354).

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/fendo.2023.1186068/full#supplementary-material

References

1. Singh B, Reschke L, Segars J, Baker VL. Frozen-thawed embryo transfer: the potential importance of the corpus luteum in preventing obstetrical complications. *Fertil Steril* (2020) 113:252–7. doi: 10.1016/j.fertnstert.2019.12.007

2. Chen ZJ, Shi Y, Sun Y, Zhang B, Liang X, Cao Y, et al. Fresh versus frozen embryos for infertility in the polycystic ovary syndrome. *N Engl J Med* (2016) 375:523–33. doi: 10.1056/NEJMoa1513873

3. Roque M, Haahr T, Geber S, Esteves SC, Humaidan P. Fresh versus elective frozen embryo transfer in IVF/ICSI cycles: a systematic review and meta-analysis of reproductive outcomes. *Hum Reprod Update* (2019) 25:2–14. doi: 10.1093/humupd/dmy033

4. Wei D, Liu JY, Sun Y, Shi Y, Zhang B, Liu JQ, et al. Frozen versus fresh single blastocyst transfer in ovulatory women: a multicentre, randomised controlled trial. *Lancet* (2019) 393:1310–8. doi: 10.1016/S0140-6736(18)32843-5

5. von Versen-Höynck F, Schaub AM, Chi YY, Chiu KH, Liu J, Lingis M, et al. Increased preeclampsia risk and reduced aortic compliance with *in vitro* fertilization cycles in the absence of a corpus luteum. *Hypertension* (2019) 73:640–9. doi: 10.1161/HYPERTENSIONAHA.118.12043

6. Busnelli A, Schirripa I, Fedele F, Bulfoni A, Levi-Setti PE. Obstetric and perinatal outcomes following programmed compared to natural frozen-thawed embryo transfer cycles: a systematic review and meta-analysis. *Hum Reprod* (2022) 37:1619–41. doi: 10.1093/humrep/deac073

7. Martins WP, Nastri CO, Rienzi L, van der Poel SZ, Gracia CR, Racowsky C. Obstetrical and perinatal outcomes following blastocyst transfer compared to cleavage transfer: a systematic review and meta-analysis. *Hum Reprod* (2016) 31:2561–9. doi: 10.1093/humrep/dew244

8. Smith MC, Murdoch AP, Danielson LA, Conrad KP, Davison JM. Relaxin has a role in establishing a renal response in pregnancy. *Fertil Steril* (2006) 86:253–5. doi: 10.1016/j.fertnstert.2005.11.070

9. Ishihara O, Araki R, Kuwahara A, Itakura A, Saito H, Adamson GD. Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: an analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan. *Fertil Steril* (2014) 101:128–33. doi: 10.1016/j.fertnstert.2013.09.025

10. Fernando D, Halliday JL, Breheny S, Healy DL. Outcomes of singleton births after blastocyst versus nonblastocyst transfer in assisted reproductive technology. *Fertil Steril* (2012) 97:579–84. doi: 10.1016/j.fertnstert.2011.12.032

11. Asserhøj LL, Spangmose AL, Aaris Henningsen AK, Clausen TD, Ziebe S, Jensen RB, et al. Adverse obstetric and perinatal outcomes in 1,136 singleton pregnancies conceived after programmed frozen embryo transfer (FET) compared with natural cycle FET. *Fertil Steril* (2021) 115:947–56. doi: 10.1016/j.fertnstert.2020.10.039

12. Dallagiovanna C, Cappellari M, D'Ambrosi F, Reschini M, Kordas K, Piani LL,, et al. Endometrial preparation does not affect the risk of hypertensive disorders of pregnancy in low-risk women undergoing frozen embryo transfer. *Gynecol Endocrinol* (2021) 38:238–42. doi: 10.1080/09513590.2021.1955342

13. Fu Y, Chen D, Cai B, Xu Y, Zhu S, Ding C, et al. Comparison of two mainstream endometrial preparation regimens in vitrified-warmed embryo transfers after PGT. *Reprod BioMed Online* (2022) 44:239–46. doi: 10.1016/j.rbmo.2021.09.009

14. Ginström Ernstad E, Wennerholm UB, Khatibi A, Petzold M, Bergh C. Neonatal and maternal outcome after frozen embryo transfer: Increased risks in programmed cycles. *Am J Obstet Gynecol* (2019) 221:126. doi: 10.1016/j.ajog.2019.03.010

15. Guan Y, Fan H, Styer AK, Xiao Z, Li Z, Zhang J, et al. A modified natural cycle results in higher live birth rate in vitrified-thawed embryo transfer for women with regular menstruation. *Syst Biol Reprod Med* (2016) 62:335–42. doi: 10.1080/19396368.2016.1199064

16. Gu F, Wu Y, Tan M, Hu R, Chen Y, Li X, et al. Programmed frozen embryo transfer cycle increased risk of hypertensive disorders of pregnancy: a multicenter cohort study in ovulatory women. *Am J Obstet Gynecol MFM* (2023) 5:100752. doi: 10.1016/j.ajogmf.2022.100752

17. Hu KL, Zhang D, Li R. Endometrium preparation and perinatal outcomes in women undergoing single-blastocyst transfer in frozen cycles. *Fertil Steril* (2021) 115:1487–94. doi: 10.1016/j.fertnstert.2020.12.016

18. Jing S, Li XF, Zhang S, Gong F, Lu G, Lin G. Increased pregnancy complications following frozen-thawed embryo transfer during an artificial cycle. *J Assist Reprod Genet* (2019) 36:925–33. doi: 10.1007/s10815-019-01420-1

19. Li C, He YC, Xu JJ, Wang Y, Liu H, Duan CC, et al. Perinatal outcomes of neonates born from different endometrial preparation protocols after frozen embryo transfer: a retrospective cohort study. *BMC Pregnancy Childbirth* (2021) 21:341. doi: 10.1186/s12884-021-03791-9

20. Li X, Xie Q, Luan T, Su Y, Zhang J, Zhang J, et al. Maternal and child-health outcomes in different endometrial preparation methods for frozen-thawed embryo transfer: a retrospective study. *Hum Fertil (Camb)* (2022), 1–12. doi: 10.1080/14647273.2022.2053593

21. Lin J, Zhao J, Hao G, Tan J, Pan Y, Wang Z, et al. Maternal and neonatal complications after natural vs. hormone replacement therapy cycle regimen for frozen single blastocyst transfer. *Front Med (Lausanne)* (2020) 7:338. doi: 10.3389/fmed.2020.00338

22. Makhijani R, Bartels C, Godiwala P, Bartolucci A, Nulsen J, Grow D, et al. Maternal and perinatal outcomes in programmed versus natural vitrified-warmed blastocyst transfer cycles. *Reprod BioMed Online* (2020) 41:300–8. doi: 10.1016/j.rbmo.2020.03.009

23. Man Y, Bian Y, Zhao S, Zhao R, Xu X, Wei D, et al. The effect of different endometrial preparations on women with polycystic ovary syndrome undergoing initial frozen embryo transfer: A historical cohort analysis. *Acta Obstet Gynecol Scand* (2021) 100:1116–23. doi: 10.1111/aogs.14058

24. Pan Y, Li B, Wang Z, Wang Y, Gong X, Zhou W, et al. Hormone replacement versus natural cycle protocols of endometrial preparation for frozen embryo transfer. *Front Endocrinol (Lausanne)* (2020) 11:546532. doi: 10.3389/fendo.2020.546532

25. Saito K, Miyado K, Yamatoya K, Kuwahara A, Inoue E, Miyado M, et al. Increased incidence of post-term delivery and cesarean section after frozen-thawed embryo transfer during a hormone replacement cycle. *J Assist Reprod Genet* (2017) 34:465–70. doi: 10.1007/s10815-017-0869-7

26. Tatsumi T, Jwa SC, Kuwahara A, Irahara M, Kubota T, Saito H. Pregnancy and neonatal outcomes following letrozole use in frozen-thawed single embryo transfer cycles. *Hum Reprod* (2017) 32:1244–8. doi: 10.1093/humrep/dex066

27. Wang Z, Liu H, Song H, Li X, Jiang J, Sheng Y, et al. Increased risk of pre-eclampsia after frozen-thawed embryo transfer in programming cycles. *Front Med (Lausanne)* (2020a) 7:104. doi: 10.3389/fmed.2020.00104

28. Wang B, Zhang J, Zhu Q, Yang X, Wang Y. Effects of different cycle regimens for frozen embryo transfer on perinatal outcomes of singletons. *Hum Reprod* (2020b) 35:1612–22. doi: 10.1093/humrep/deaa093

29. Xu J, Zhou H, Zhou T, Guo Y, Liang S, Jia Y, et al. The impact of different endometrial preparation protocols on obstetric and neonatal complications in frozen-thawed embryo transfer: a retrospective cohort study of 3,458 singleton deliveries. *Reprod Biol Endocrinol* (2022) 20:141. doi: 10.1186/s12958-022-01009-x

30. Zaat TR, Brink AJ, de Bruin JP, Goddijn M, Broekmans F, Cohlen BJ, et al. Increased obstetric and neonatal risks in artificial cycles for frozen embryo transfers. *Reprod BioMed Online* (2021) 42:919–29. doi: 10.1016/j.rbmo.2021.01.015

31. Zhou R, Zhang X, Huang L, Wang S, Li L, Dong M, et al. The impact of different cycle regimens on birthweight of singletons in frozen-thawed embryo transfer cycles of ovulatory women. *Fertil Steril* (2022) 117:573–82. doi: 10.1016/j.fertnstert.2021.09.033

32. Zong L, Liu P, Zhou L, Wei D, Ding L, Qin Y. Increased risk of maternal and neonatal complications in hormone replacement therapy cycles in frozen embryo transfer. *Reprod Biol Endocrinol* (2020) 18:36. doi: 10.1186/s12958-020-00601-3

33. Moreno-Sepulveda J, Espinós JJ, Checa MA. Lower risk of adverse perinatal outcomes in natural versus artificial frozen-thawed embryo transfer cycles: a systematic review and meta-analysis. *Reprod BioMed Online* (2021) 42:1131–45. doi: 10.1016/j.rbmo.2021.03.002

34. Ginström Ernstad E, Bergh C, Khatibi A, Källén KB, Westlander G, Nilsson S, et al. Neonatal and maternal outcome after blastocyst transfer: a population-based registry study. *Am J Obstet Gynecol* (2016) 214:378.e1–378.e10. doi: 10.1016/j.ajog.2015.12.040

35. Rosalik K, Carson S, Pilgrim J, Luizzi J, Levy G, Heitmann R, et al. Effects of different frozen embryo transfer regimens on abnormalities of fetal weight: a systematic review and meta-analysis. *Hum Reprod Update* (2021) 28:1–14. doi: 10.1093/humupd/dmab037

36. Post Uiterweer ED, Koster M, Jeyabalan A, Kuc S, Siljee JE, Stewart DR, et al. Circulating pregnancy hormone relaxin as a first trimester biomarker for preeclampsia. *Pregnancy Hypertens* (2020) 22:47–53. doi: 10.1016/j.preghy.2020.07.008

37. von Versen-Höynck F, Narasimhan P, Selamet Tierney ES, Martinez N, Conrad KP, Baker VL, et al. Absent or excessive corpus luteum number is associated with altered maternal vascular health in early pregnancy. *Hypertension* (2019) 73:680–90. doi: 10.1161/HYPERTENSIONAHA.118.12046

38. Pereira MM, Mainigi M, Strauss JF. Secretory products of the corpus luteum and preeclampsia. *Hum Reprod Update* (2021) 27:651–72. doi: 10.1093/humupd/dmab003

39. Aragón-Herrera A, Feijóo-Bandín S, Anido-Varela L, Moraña-Fernández S, Roselló-Lletí E, Portolés M, et al. Relaxin-2 as a potential biomarker in cardiovascular diseases. *J Pers Med* (2022) 12. doi: 10.3390/jpm12071021

40. Conrad KP, Graham GM, Chi YY, Zhai X, Li M, Williams RS, et al. Potential influence of the corpus luteum on circulating reproductive and volume regulatory hormones, angiogenic and immunoregulatory factors in pregnant women. *Am J Physiol Endocrinol Metab* (2019) 317:E677–85. doi: 10.1152/ajpendo.00225.2019

41. von Versen-Höynck F, Strauch NK, Liu J, Chi YY, Keller-Woods M, Conrad KP, et al. Effect of mode of conception on maternal serum relaxin, creatinine, and sodium concentrations in an infertile population. *Reprod Sci* (2019) 26:412–9. doi: 10.1177/1933719118776792

42. Hsu SY, Nakabayashi K, Nishi S, Kumagai J, Kudo M, Sherwood OD, et al. Activation of orphan receptors by the hormone relaxin. *Science* (2002) 295:671–4. doi: 10.1126/science.1065654

43. Devarakonda T, Salloum FN. Heart disease and relaxin: new actions for an old hormone. *Trends Endocrinol Metab* (2018) 29:338–48. doi: 10.1016/j.tem.2018.02.008

44. Novak J, Danielson LA, Kerchner LJ, Sherwood OD, Ramirez RJ, Moalli PA, et al. Relaxin is essential for renal vasodilation during pregnancy in conscious rats. *J Clin Invest* (2001) 107:1469–75. doi: 10.1172/JCI11975

45. Debrah DO, Novak J, Matthews JE, Ramirez RJ, Shroff SG, Conrad KP. Relaxin is essential for systemic vasodilation and increased global arterial compliance during early pregnancy in conscious rats. *Endocrinology* (2006) 147:5126–31. doi: 10.1210/en.2006-0567

46. Conrad KP. G-Protein-coupled receptors as potential drug candidates in preeclampsia: targeting the relaxin/insulin-like family peptide receptor 1 for treatment and prevention. *Hum Reprod Update* (2016) 22:647–64. doi: 10.1093/humupd/dmw021

47. Luke B, Brown MB, Eisenberg ML, Callan C, Botting BJ, Pacey A, et al. *In vitro* fertilization and risk for hypertensive disorders of pregnancy: associations with treatment parameters. *Am J Obstet Gynecol* (2020) 222:350.e1–350.e13. doi: 10.1016/j.ajog.2019.10.003

48. Sharma S, Godbole G, Modi D. Decidual control of trophoblast invasion. *Am J Reprod Immunol* (2016) 75:341–50. doi: 10.1111/aji.12466

49. Dimitriadis E, Stoikos C, Baca M, Fairlie WD, McCoubrie JE, Salamonsen LA. Relaxin and prostaglandin E(2) regulate interleukin 11 during human endometrial stromal cell decidualization. *J Clin Endocrinol Metab* (2005) 90:3458–65. doi: 10.1210/jc.2004-1014

50. Oron G, Sokal-Arnon T, Son WY, Demirtas E, Buckett W, Zeadna A, et al. Extended embryo culture is not associated with increased adverse obstetric or perinatal outcome. *Am J Obstet Gynecol* (2014) 211:165.e1–7. doi: 10.1016/j.ajog.2014.03.018

51. Garrido-Gomez T, Dominguez F, Quiñonero A, Diaz-Gimeno P, Kapidzic M, Gormley M, et al. Defective decidualization during and after severe preeclampsia reveals a possible maternal contribution to the etiology. *Proc Natl Acad Sci U.S.A.* (2017) 114:E8468–77. doi: 10.1073/pnas.1706546114

52. Conrad KP. Evidence for corpus luteal and endometrial origins of adverse pregnancy outcomes in women conceiving with or without assisted reproduction. *Obstet Gynecol Clin North Am* (2020) 47:163–81. doi: 10.1016/j.ogc.2019.10.011

53. Straub RH. The complex role of estrogens in inflammation. *Endocr Rev* (2007) 28:521–74. doi: 10.1210/er.2007-0001

54. Albrecht ED, Bonagura TW, Burleigh DW, Enders AC, Aberdeen GW, Pepe GJ. Suppression of extravillous trophoblast invasion of uterine spiral arteries by estrogen during early baboon pregnancy. *Placenta* (2006) 27:483–90. doi: 10.1016/j.placenta.2005.04.005

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 29 August 2023
DOI 10.3389/fendo.2023.1250847

OPEN ACCESS

EDITED BY
Zhen-Gao Sun,
Affiliated Hospital of Shandong University of Traditional Chinese Medicine, China

REVIEWED BY
Xian Ling Cao,
Fudan University, China
Meilan Mo,
Shenzhen Zhongshan Urological Hospital, China

*CORRESPONDENCE
Hsun-Ming Chang
changobs@hotmail.com

RECEIVED 30 June 2023
ACCEPTED 08 August 2023
PUBLISHED 29 August 2023

CITATION
Hsueh Y-W, Huang C-C, Hung S-W, Chang C-W, Hsu H-C, Yang T-C, Lin W-C, Su S-Y and Chang H-M (2023) Finding of the optimal preparation and timing of endometrium in frozen-thawed embryo transfer: a literature review of clinical evidence. *Front. Endocrinol.* 14:1250847. doi: 10.3389/fendo.2023.1250847

Finding of the optimal preparation and timing of endometrium in frozen-thawed embryo transfer: a literature review of clinical evidence

Ya-Wen Hsueh[1], Chien-Chu Huang[1], Shuo-Wen Hung[2], Chia-Wei Chang[1], Hsi-Chen Hsu[1], Tung-Chuan Yang[1], Wu-Chou Lin[1], Shan-Yu Su[2] and Hsun-Ming Chang[1*]

[1]Department of Obstetrics and Gynecology, China Medical University Hospital, Taichung, Taiwan, [2]Department of Chinese Medicine, China Medical University Hospital, Taichung, Taiwan

Frozen-thawed embryo transfer (FET) has been a viable alternative to fresh embryo transfer in recent years because of the improvement in vitrification methods. Laboratory-based studies indicate that complex molecular and morphological changes in endometrium during the window of implantation after exogenous hormones with controlled ovarian stimulation may alter the interaction between the embryo and endometrium, leading to a decreased implantation potential. Based on the results obtained from randomized controlled studies, increased pregnancy rates and better perinatal outcomes have been reported following FET. Compared to fresh embryo transfer, fewer preterm deliveries, and reduced incidence of ovarian hyperstimulation syndrome were found after FETs, yet there is a trend of increased pregnancy-related hypertensive diseases in women receiving FET. Despite the increased application of FET, the search for the most optimal priming protocol for the endometrium is still undergoing. Three available FET protocols have been proposed to prepare the endometrium: i) natural cycle (true natural cycle and modified natural cycle) ii) artificial cycle (AC) or hormone replacement treatment cycle iii) mild ovarian stimulation (mild-OS) cycle. Emerging evidence suggests that the optimal timing for FET using warmed blastocyst transfer is the LH surge +6 day, hCG administration+7 day, and the progesterone administration+6 day in the true natural cycle, modified natural cycle, and AC protocol, respectively. Although still controversial, better clinical pregnancy rates and live birth rates have been reported using the natural cycle (true natural cycle/modified natural cycle) compared with the AC protocol. Additionally, a higher early pregnancy loss rate and an increased incidence of gestational hypertension have been found in FETs using the AC protocol because of the lack of a corpus luteum. Although the common clinical practice is to employ luteal phase support (LPS) in natural cycles and mild-OS cycles for FET, the requirement for LPS in these protocols remains equivocal. Recent findings obtained from RCTs do not support the routine

application of endometrial receptivity testing to optimize the timing of FET. More RCTs with rigorous methodology are needed to compare different protocols to prime the endometrium for FET, focusing not only on live birth rate, but also on maternal, obstetrical, and neonatal outcomes.

KEYWORDS

in vitro fertilization, frozen embryo transfer, endometrial receptivity, artificial cycle, natural cycle, true natural cycle, modified natural cycle, and mild stimulation cycle

1 Introduction

Since its clinical application in 1978, in vitro fertilization (IVF) has been an efficient procedure of assisted reproductive technology (ART) that has provided a great opportunity for infertile couples worldwide to have children. Despite the development of optimal protocols for personalized, patient-specific stimulation and trigger as well as emerging technologies for improving embryo selection, the success rate for IVF in fresh transfer cycles remains low worldwide (1). With the technical improvement in cryopreservation using vitrification, frozen embryo transfer (FET) has become a viable alternative to fresh embryo transfer (2). The results obtained from laboratory-based studies indicate that the endometria of women in the controlled ovulation stimulation (COS) cycle are not appropriately prepared for embryo implantation (1). Several randomized controlled trials (RCTs) have shown that the clinical pregnancy rates (CPRs) following FET are slightly increased than those following fresh embryo transfer (3, 4). Additionally, elective cryopreservation in IVF cycles can prevent pregnancy-induced late ovarian hyperstimulation syndrome (OHSS) (5). For instance, an RCT study showed that in women with polycystic ovary syndrome (PCOS), FET had a higher live birth rate (LBR), a lower risk of OHSS, and a higher risk of preeclampsia than fresh transfer (6). Women with PCOS who underwent FET also had a lower early pregnancy loss rate compared to those who underwent fresh embryo transfer (6).

Furthermore, the perinatal and obstetric outcomes (including perinatal morbidity, small for gestational age, preterm birth, low birth weight, and antepartum hemorrhage) are less affected following FET (7). Compared to spontaneously conceived newborns, certain birth defects associated with abnormal blastogenesis were increased more than 3-fold in fresh embryo transfers but not in FET (8). However, it may take a longer time to achieve conception, given that embryo transfer is delayed in the FET cycle. Moreover, various obstetric outcomes, including maternal hypertensive disorders of pregnancy, having a large-for-gestational-age baby, and higher birth weight of the children born have been reported in women following FET implantation (1, 9).

During the past decade, cryopreservation of all embryos (or a "freeze-all" protocol) has become increasingly popular worldwide, given that it may overcome the detrimental effects of ovarian stimulation, especially in IVF patients who are at high risk of developing OHSS (5). Using cryoprotectants and rapid freezing, an optimized vitrification technique has become more widely applied for embryo cryopreservation, with a higher survival rate (up to approximately 95%) according to a large cohort study (10). The results obtained from meta-analyses showed that vitrified embryos had higher post-thawed survival rates (both for cleavage and blastocyst stages) than slow freezing embryos (11). Furthermore, clinical outcome comparison results showed that vitrification embryo transfers had higher CPR than slow freezing embryo transfers (12). The increasing number of IVF cycles to perform FET could partly reflect the upward trend in women receiving pre-implantation genetic testing cycles for aneuploidy detection (13, 14). Despite the increased trend in FET, the most optimal priming regimen of the endometrium in the general population during ART remains to be determined. To date, different endometrial preparation strategies have been proposed: i) natural cycle (NC) (true-NC with LH detection in blood or urine and modified-NC in which human chorionic gonadotropin (hCG) is administered to schedule embryo transfer instead of measuring LH); ii) artificial cycle (AC) or hormone replacement treatment using exogenous estradiol and progesterone; and iii) mild ovarian stimulation (mild-OS) cycle using gonadotropins, clomiphene citrate (CC), or letrozole.

With our systematic review, we aim to compare various endometrial preparation protocols for FET regarding reproductive, obstetric, and perinatal outcomes. In addition to the LBR, patient convenience and cost efficiency, pregnancy-related complications and perinatal health are also critical issues for infertile couples undergoing ART treatment.

2 Materials and methods

A comprehensive search was performed in the bibliographic databases PubMed and Embase, and the Cochrane Central Register of Controlled Trials (CENTRAL) from inception to May 31, 2023. Search terms included embryo cryopreservation, frozen embryo transfer, fresh embryo transfer, endometrial preparation, cryo-thawed, natural cycle, modified natural cycle, artificial frozen cycle, hormone replacement treatment, mild ovarian stimulation cycle, clinical outcome comparison, pregnancy outcomes, clinical pregnancy rate, live birth rate, early abortion rate, timing of FET, fresh ET after COS, delayed FET timing, and postpone FET timing. Full-text articles of relevant references were collected and assessed. The impacts of different protocols for FET on reproductive,

obstetric, and perinatal outcomes are discussed based on the evidence derived from large prospective cohort studies RCTs, and meta-analyses. The literature search was restricted to articles published in English. The available articles were identified by manually searching the references of all relevance on this topic.

2.1 When is the optimal timing to perform FET after COS?

Given that there is a residual detrimental effect of COS on the receptivity of the endometrium, it has been suggested to postpone FET timing for at least a menstrual cycle following a successful fresh ET cycle or a free-all cycle (6, 15). Specifically, there is advanced endometrial maturation after COS leading to reduced implantation rates in fresh ET cycles compared to frozen or ET using donor oocytes in a RCT study (15). However, other researchers challenge the speculation that the residual impacts of COS on endometrial receptivity may persist until the next menstrual cycle. Indeed, delay of FET timing may induce emotional stress and anxiety in some patients, resulting in drop-out from their infertility treatment (16). A retrospective cohort study comparing two time points of FET showed that the implantation rates, CPRs, and LBRs were all reduced in the postponed FET compared to the immediate FET cycle (17). Similarly, the results obtained from a meta-analysis showed that the CPR and LBR were slightly higher in immediate FET than in postponed FET (18). A retrospective cohort study compared the immediate and delayed FET in high responder patients undergoing IVF cycles, and the results showed that there was no difference of pregnancy outcomes between two groups (19). Another retrospective study compared three time periods (immediate group < 40 days, delayed group >40 days but < 180 days, and overdue group > 180 days) of FET following freeze-all cycles, and the results showed that the time interval between oocyte retrieval and FET does not impact the pregnancy outcomes (20). However, these results were based on an analysis using retrospective cohort studies with the possible presence of selection bias. A RCT study compared the pregnancy outcomes of immediate FET and delayed FET among patients with a previous failed IVF/ET attempt, and the results showed that immediate FET had higher CPRs and LBRs than delayed FET (21). These findings suggest that unnecessarily delayed timing of FET should be avoid by shortening the time period of live birth during IVF treatment.

2.2 Natural cycle FET

Among the various FET protocols, the NC FET has gained popularity as a more physiological approach. The historical development and refinement of NC FET protocols have evolved over time to optimize success rates and improve patient outcomes (22). Although the concept of relying on a woman's natural menstrual cycle for embryo transfer is not new, the advancements in assisted reproductive medicine have led to the standardization and optimization of NC FET protocols. Early attempts at NC FET were primarily based on spontaneous ovulation without any hormonal interventions, the true-NC FET. However, the lack of control over the timing of ovulation and the inability to accurately predict the optimal timing for embryo transfer limited the success of these early protocols. As a result, modifications were introduced to enhance the timing and precision of embryo transfer in NC FET, the modified-NC FET. Compared to the true-NC, the modified-NC is performed using hCG for ovulation triggering when the dominant follicle is reaching approximately 16-18 mm in diameter, which requires less precise hormonal and ultrasonographic monitoring.

2.3 True-NC FET

One significant advancement in the development of NC FET protocols was the introduction of ultrasound monitoring and hormonal assessments to track follicular development and predict the timing of natural ovulation (23). This allowed for improved timing of embryo thawing and transfer, ensuring synchronization between the embryo and the receptive endometrium. Over time, advancements in ultrasound technology and the development of more sensitive hormonal assays further refined the monitoring and assessment of the natural cycle (23). This improved the accuracy of predicting ovulation and provided a better understanding of the endometrial changes associated with receptivity. In practice, transvaginal ultrasonography is arranged on the cycle day 2 or day 3 to exclude any ovarian cyst or remaining corpus luteum from the previous cycle. Additionally, this baseline ultrasound helps assess the size of ovaries, the number of antral follicles, and the thickness of the endometrium. A baseline hormonal survey has been proposed to evaluate the patient condition and predict IVF outcomes (24), which has been challenged in later studies (23, 25, 26). A retrospective study observed that the acceptable baseline FSH level as a single test to predict IVF treatment failure is only obtained above a high-cutoff level (> 15 IU/L) (Table 1), indicating that the basal FSH level is of limited value in predicting IVF outcomes (26). It has been suggested that the treatment cycle should be canceled when the basal levels of estradiol (E2) and progesterone are elevated (E2 > 80 pg/ml or progesterone > 1.6 ng/ml) because of subsequently decreased pregnancy rates (27). However, this recommendation was based on the observation during the ovarian stimulating IVF cycle (27). The association studies regarding the impact of elevated basal E2/progesterone on the outcomes in NC FET are still pending.

During true-NC preparation, serial ultrasounds are performed every other day (or daily) to monitor follicle size and growth starting on cycle day 8 (22). Transvaginal ultrasonography is a widely used standard method to monitor the development of ovarian follicles and predict ovulation time accurately. The dominant follicle, which is most likely to release the mature oocyte, is closely monitored. Typically, a follicle size of around 18 to 20 mm indicates that it is nearing maturity and ovulation is imminent. The exact size may vary based on individual factors and the specific protocols used by the fertility clinic. The thickness and appearance of the endometrium are also evaluated during transvaginal ultrasound. As ovulation approaches, the endometrium typically thickens (> 7 mm) and shows a triple-line

10.3389/fendo.2023.1250847

TABLE 1 Ultrasonographic and laboratory features of monitoring ovulation in NC FET.

Ultrasonographic features of monitoring ovulation	
Target survey	Image finding
Dominant Follicle	Thinning and stretched appearance *
	Disappearance or sudden decrease in follicle size
	Corpus luteum formation
Douglas pouch	Release of fluid into the pelvic cavity
Endometrium	Homogenous or hyperechoic "luteinized endometrium"
*Impending rupture of the ovarian follicle	
Laboratory features of monitoring ovulation	
E2 (serum)	200-400 pg/ml (peaked 24 to 36 hours prior to ovulation)
LH (serum)	≥180% of last rising LH or ≥ 17 IU/l followed by a ≥ 30% drop in serum estradiol levels (peaked 10 to 12 hours prior to ovulation)
LH (urine)	> 20-40 IU/l (24 to 48 hours prior to ovulation)
Progesterone (serum)	1.5 ng/ml (confirmation of ovulation)

pattern, indicating a good endometrial receptivity for embryo implantation (28). Under the observation using transvaginal ultrasonography, some key indicators can be used to predict or confirm ovulation: 1) the dominant follicle displays a thinning and stretched appearance, indicating impending rupture; 2) disappearance or sudden decrease in follicle size; 3) corpus luteum formation showing increased echogenicity inside the follicle; 4) the release of fluid into the pelvic cavity (Douglas pouch); and 5) Replacement of "triple-line pattern" of the endometrium by homogenous or hyperechoic "luteinized endometrium" (Table 1) (29, 30).

Serum hormonal levels can be utilized to predict ovulation by monitoring the levels of specific key hormones (LH, E2, and progesterone) that play a crucial role in the menstrual cycle. An observation study that included 23 normal endocrine women showed that serum E2 levels rapidly elevated during the late follicular phase, reaching a peak level (at approximately 200-400 pg/ml) 24-36 hours before ovulation (Table 1) (31). The peak level of E2 contributes to the positive feedback control leading to the pre-ovulatory LH surge in the female kisspeptin AVPV/PeN neuron (32). Almost at the time of the peak level of E2, the onset of the LH surge occurs (33). The LH surge triggers the final maturation and release of the oocyte from the follicle. Even in the same woman, variation in the ovulation timing exists from cycle to cycle. In this regard, the estimated timing of ovulation occurs at approximately 10 to 12 hours after the LH peak or 24 to 36 hours after the peak level of E2 (31). The onset of the LH surge seems to be the most reliable timing of impending ovulation, with a duration of 34 to 36 hours before follicle rupture (Table 1) (34). During the true-NC FET, monitoring LH levels through urine-based ovulation predictor kits (detection limits of 20-40 IU/l) or serum LH testing can help predict the timing of ovulation (Table 1). Adequate determination of the LH surge is critical to pinpoint the timing for FET. However, the standard criteria to define the LH surge is still pending without a consensus. The data obtained from a clinical study showed that any serum LH level equal to or exceeding 180% of the latest rising serum LH level is defined as a surge of LH (35). A retrospective study measuring serum levels of LH and estradiol demonstrated that the first detection of serum LH ≥ 17 IU/l followed by a ≥ 30% drop in serum estradiol levels (the next day after LH detection day) may indicate the timing of LH surge (Table 1) (36). Most commonly, a concomitant rise of serum P4 levels above 1.5 ng/ml detected on the day after the LH surge could be utilized to confirm ovulation (22). The appearance of LH detected in the urine is delayed in comparison with its detection in the peripheral blood (37). An observational study demonstrated that the mean time from peak serum LH to positive urine LH was 2 ± 2 hours, while the mean time from positive urine LH to follicular collapse was 20 ± 3 hours (38). Furthermore, the positive predictive values for ovulation within 24 or 48 hours following detecting urine LH were 73% and 92%, respectively (Table 1) (38). Examining serum progesterone levels is an effective method for predicting ovulation. Progesterone is a hormone produced by the corpus luteum, which forms after ovulation. A single serum progesterone level > 3 ng/ml in the mid-luteal phase has been used to retrospectively detect ovulation. Random serum progesterone of > 5 ng/ml has been proposed to confirm ovulation (sensitivity 89.6% and specificity 98.4%) (Table 1) (39).

Adequate secretion of progesterone by the corpus luteum formed from a dominant mature follicle is essential for preparing the receptive endometrium and maintaining a successful conception (40). The application of luteal phase support (LPS) in true-NC FET remained to be elucidated. The available retrospective studies revealed that some studies suggested the application of LPS (41), whereas other studies showed comparable reproductive outcomes in true-NC FET with or without LPS (42–44). A RCT demonstrated that administration of progesterone (vaginal micronized progesterone 400 mg bid) from the day (LH surge+3 day) of true-NC FET (cleavage-stage embryos) had a higher LBR than no LPS (45). Another RCT evaluated women undergoing

cleavage-stage embryos using true-NC FET showed that hCG administration (one on the day of FET and one on the 6th day of FET) for LPS did not increase the ongoing pregnancy rate compared to those without LPS (46).

Additionally, the use of exogenous hormonal agents, such as gonadotropin-releasing hormone (GnRH) agonists or antagonists, was incorporated into NC FET protocols to suppress premature ovulation and provide better control over the timing of ovulation. These medications helped to optimize the endometrial environment and enhance the chances of successful implantation. In recent years, there has been a growing interest in utilizing additional tools, such as endometrial biopsy or molecular markers, to further refine the assessment of endometrial receptivity in NC FET protocols (47, 48). These approaches aim to identify molecular markers or gene expression patterns associated with optimal endometrial receptivity, enhancing the selection of the most favorable timing for embryo transfer. Overall, the historical development of NC FET protocols has involved a gradual progression from spontaneous ovulation to refined approaches that incorporate ultrasound monitoring, hormonal control, and advanced assessment techniques. These advancements have improved the success rates of NC FET and have made it a viable option for patients undergoing assisted reproduction. Continued research and technological advancements are expected to further optimize NC FET protocols and contribute to its ongoing refinement.

In conclusion, the true-NC FET represents a promising alternative to traditional FET protocols, offering a more physiological approach with potential advantages in terms of patient experience, cost-effectiveness, and reduced treatment burden. While challenges and limitations exist, current evidence suggests that true-NC FET can achieve comparable success rates with carefully selected patients. Further research and refinement of the procedure are necessary to optimize patient selection, improve success rates, and ensure long-term safety. The true-NC FET holds great potential for the future of ART and warrants consideration as a valuable option in clinical practice.

2.4 Modified-NC FET

Modified-NC is an evolving technique that is considered more patient-friendly. Compared to the true-NC protocol, modified-NC is more flexible and requires less ultrasonographic and endocrine monitoring (Table 2). The initial monitoring in modified NC is similar to in true-NC; however, in a modified-NC, ovulation is triggered by the injection of hCG when the dominant follicle is between 16-20 mm in diameter. A RCT study including 60 IVF patients showed that the application of modified-NC significantly decreased the number of clinical visits (for cycle monitoring) without affecting the reproductive outcomes (47). To date, there is no consensus regarding the dosage (from 5000 IU to 10000 IU) used to trigger ovulation in the modified-NC protocol. A RCT four-arm study compared three different dosages of hCG (5000, 6500, and 10000 IU) to trigger ovulation during the GnRH antagonist short protocol IVF treatment (49). The results showed that increasing hCG trigger doses (6500-10000 IU) significantly increased endogenous progesterone concentration during the mid- to late-luteal phase (49). These findings suggest that the administration of hCG may act as an ovulation trigger and also as a promotor of luteal phase support. A retrospective cohort study comparing true-NC and modified-NC cycles revealed that modified-NC displayed significantly higher implantation rate,

TABLE 2 Characteristics of different FET protocols.

	True-NC	Modified-NC	Mild-OS	AC
Endometrial preparation	▪ Day 2/3 ultrasonography to exclude ovarian cyst/corpus luteum ▪ Day 2/3: consider cancelation if FSH>15 IU/L ▪ Day 8: monitor follicular growth daily or every other day until features of ovulation	▪ Day 2/3 ultrasonography to exclude ovarian cyst/corpus luteum ▪ Day 2/3: consider cancelation if FSH>15 IU/L ▪ Day 8: monitor follicular growth daily or every other day until dominant follicle 16-20 mm; and triple-line pattern	▪ Day 2/3: CC/letrozole or/and gonadotropin ▪ Day 8: monitor follicular growth daily or every other day until dominant follicle 17-18 mm and EM >7 mm	▪ Day 1/2/3: estradiol step-up/fixed-dose regimen ▪ Day 8: monitor endometrial receptivity by ultrasonography
Timing of embryo transfer	▪ Confirmation of ovulation by ultrasonography and laboratory findings ▪ Blastocyst transfer -LH surge Day+6 -Ovulation Day+5	▪ Ovulation triggered by hCG ▪ Blastocyst transfer -hCG trigger Day+7	▪ Ovulation triggered by hCG ▪ Blastocyst transfer -hCG trigger Day+7 ▪ Day 3 embryo -hCG trigger Day+5	▪ Ultrasonography monitoring for FET -EM >7 mm -EM "triple line" pattern -EM good blood flow ▪ Blastocyst transfer -Progesterone administration Day+6
Luteal support	▪ Might have some benefits, but more studies needed	▪ Might not have benefits, but more studies needed	▪ Might have some benefits, but more studies needed	▪ Continue progesterone administration until luteo-placental shift
Advantages	▪ More physiological approach ▪ Cost-effectiveness ▪ Reduced treatment burden	▪ More flexible ▪ Require less ultrasonographic and endocrine monitoring	▪ More favorable endocrine environment ▪ Improved endometrial receptivity	▪ More convenient and flexible to schedule FET ▪ A lower cycle cancellation rate

CPR, and LBR (50). However, a RCT contradicted this finding and was terminated early because the results obtained from an interim analysis showed a significantly higher ongoing pregnancy rate in the true-NC group than in the modified-NC group (51).

Similar to the situation in true-NC, there is a debate regarding whether LPS is needed in modified-NC. Given its long half-life up to at least 7 days, hCG administration may have an adequate luteotropic effect during the early luteal phase (52). Theoretically, the application of LPS is not required in the modified-NC FET, given that too early progesterone supplementation may cause asynchrony between the embryo and endometrium, leading to adverse reproductive outcomes (42, 53, 54). In this regard, two retrospective studies did not show any improved reproductive outcomes with the application of LPS in modified-NC (55, 56). In line with these results, two RCTs also demonstrated no beneficial effects of LPS on the reproductive outcomes following modified-NC FET (57, 58). Emerging evidence suggests that when LPS is applied in modified-NC, the administration of progesterone should not be started earlier than the LH surge+3 day (42, 45).

2.5 What is the optimal timing for FET in true-NC and modified-NC protocols?

The optimal window of embryo implantation may vary from day 16 to day 20 for cleavage-stage (2-12 cells) embryos in a 28-day cycle (59–62). The thawed embryos may take a longer time to develop into the blastocyst stage because these embryos lose approximately more than half the cell viability of their blastomeres (63). Therefore, the window of embryo transfer in a NC ranges from LH+7 to LH+11 (63). In this regard, there is a time difference in FET between true-NC and modified-NC, given that ovulation occurs 24 to 36 hours after a spontaneous LH surge, while 36 to 48 hours after hCG administration (64). In clinical practice, FET using blastocyst-stage embryos is arranged on ultrasonographic confirmation of ovulation+5 days or LH surge+6 days in the true-NC and hCG administration +7 days in the modified-NC, respectively (22). A retrospective study measured the spontaneous LH surge (≥ 20 mIU/ml) to determine the timing of modified-NC, suggesting that FET was scheduled on hCG+6 days with a documented LH surge, whereas FET was scheduled on hCG+7 days without an LH surge (65). Similarly, a multicenter-RCT evaluated the optimal timing for modified-NC FET indicated that embryo transfer should be arranged on LH+6 days in true-NC and hCG+7 days in modified-NC (Table 2) (65).

In summary, based on the literature review, FET can be scheduled on ovulation+5 day or LH+6 day in true-NC protocol and hCG+7 day in modified-NC protocol using blastocyst-stage embryos (Table 2). For day 3 cleavage-stage embryo transfer, FET can be scheduled on ovulation+3 day or LH+4 day in true-NC protocol and hCG+5 day in modified-NC protocol.

2.6 Mild ovarian stimulation cycle

Mild-OS cycle for FET aims to reduce the supraphysiological levels of hormones observed in high-dosage protocols and improve subtle defects in folliculogenesis, leading to a more favorable endocrine environment and improved endometrial receptivity (Table 2) (66, 67). Appropriate patient selection is crucial for the successful implementation of mild-OS in FET cycles. Factors such as age, ovarian reserve, previous response to ovarian stimulation, and the number and quality of cryopreserved embryos should be considered when determining the suitability of a patient for mild-OS (67). Tailoring mild-OS protocols to individual patient characteristics is essential to optimize outcomes. In mild-OS FET, either oral ovulatory agents (clomiphene citrate or CC and letrozole), exogenous gonadotropins, or in combination, can be used to prepare the endometrium for embryo transfer. Several ovulation induction protocols have been proposed in the mild-OS cycle, which includes CC at a dosage of 50-100 mg per day, letrozole at a dosage of 2.5-5 mg per day, and recombinant/urinary FSH at a low dosage of less than 150 IU per day, starting on the second or third day of the menstruation (22). Letrozole is an aromatase inhibitor that was initially introduced as an alternative ovulation induction agent in the early 2000s. Unlike CC, letrozole has the advantage of without interference with endometrial development and has been reported to be more effective in obese women with PCOS (68). Similar to CC, not all patients respond to a 5-day regimen of letrozole administration. Therefore, extended regimens (7–10 days), stair-step protocols, and in combination with low-dosage recombinant gonadotropins have been proposed (69).

Close monitoring and adjustment of medication dosages based on ovarian response using vaginal ultrasonography and hormonal profiles are then performed after medication. Similar to modified-NC, hCG is administrated when the leading follicle reaches 17-18 mm in diameter, the serum E2 level is more than 150 pg/ml, or the endometrial thickness is more than 7 mm (22). Eventually, FET can be scheduled on hCG+7 day for blastocyst-stage embryos or hCG+5 day for day-3 embryos (Table 2). Although most clinicians are applying LPS in mild-OS cycle FET (70), more well-designed RCTs are needed to evaluate the place of LPS in the mild-OS cycle for FET.

2.7 Artificial cycle or hormone replacement treatment

AC or hormone replacement treatment for FET involves the administration of exogenous estrogen and progesterone to stimulate the growth of the endometrium and inhibit follicular growth. Initially, estrogen is given to stimulate endometrial growth, followed by progesterone administration to prepare the endometrium for embryo transfer. Compared to other protocols, the AC protocol is more convenient and flexible for both patients and physicians to schedule embryo transfers with a lower cycle cancellation rate (Table 2) (71). However, the disadvantages AC FET are some potential detrimental effects induced by the supplementation of exogenous estrogen and the absence of the corpus luteum.

2.8 Administration of estrogen

In AC, estrogen administration is usually started within the first three days of a menstrual cycle to prime the endometrium. Estradiol

can be given as a fixed-dose or in a step-up regimen. The fixed-dose regimen is utilized to prevent follicular growth and ovulation, whereas the step-up regimens can increase estradiol exposure in a more physiologic manner (71). The fixed-dose regimen is given at a dosage of 6 mg per day starting on the first, second, or third day of the cycle. The dosage of estradiol in the step-up regimen varies, but is most commonly given starting at 2 mg per day during the first week, then increased to 4 mg per day for the following 5 days, and finally step-up to 6 mg per day until the day of embryo transfer (72). A large-scale retrospective study included 8254 cycles and compared two regimens (fixed-dose and step-up regimens) in oocyte donation cycles using both oral and transdermal routes (72). The results showed that there was no significant difference between the two groups in terms of LBR (oral: 33% vs 32.5%; transdermal: 35.7% vs 32%, respectively) (72). A more recent retrospective study included 394 cycles and compared three regimens used for estradiol administration: one fixed-dose regimen using 6 mg per day and two step-up regimens (one regimen received 2 mg per day for 6 to 7 days, then 4 mg per day for 4 to 5 days, and 6 mg per day until ET; the other regimen received 4 mg per day for 7 to 8 days, then 6 mg per day until ET) (73). They found that the step-up regimen starting with 4 mg per day resulted in the greatest endometrial thickness, compared to the step-up regimen using 2 mg per day and the fixed-dose regimen using 6 mg per day (10.2 ± 1.3 mm vs. 9.6 ± 1.4 mm vs. 8.6 ± 0.9 mm; $P < 0.001$) (73). Furthermore, the reproductive outcomes also favored the 4 mg step-up regimen, with the highest CPR (55.2% vs. 41.1% vs. 42.2%; P <0.035) and highest LBR (50.9% vs. 40.8% vs. 48.1%; P=0.320) (73). There seems to be a trend favoring the 4 mg step-up regimen over the 2 mg step-up regimen or the fixed-dose regimen in terms of endometrial thickness and reproductive outcomes.

Different routes of estrogen administration (oral, transdermal, and vaginal) have been proposed to employ in AC FET based on individual patient characteristics, with the oral form being the most widely used route (74). The Cochrane Database of Systematic Reviews compared the equivalent of 6 mg estradiol daily in oral and two different transdermal forms and found comparable reproductive outcomes among these different administration routes (75). A prospective study compared the efficacy of transdermal estrogen (gel) with oral estradiol in AC FET and found that there was no difference in endometrial thickness, implantation rates, CPR, and miscarriage rate; however, the transdermal form had a better patient satisfaction rate (8.02 ± 1.07 vs 6.96 ± 0.99, p<0.01), and the related side effects were significantly lower (18.1% vs 55.1%, p<0.01) (76). A retrospective cohort study compared the effects on the endometrial preparation using oral and vaginal tablets of estrogen in AC FET, and they found a thinner endometrium on the day of transfer in the vaginal form group, but the two groups had similar reproductive outcomes (77). A small, randomized study compared the effects of oral and vaginal estrogen administration on endometrial preparation [evaluating endometrial histology by hematoxylin and eosin staining and immunohistochemical analysis of estrogen receptor (ER) expression] in patients with primary ovarian insufficiency (78). The results showed that patients using vaginal estrogen for 14 days had higher E2 concentrations, thicker endometrial thickness, and more pronounced ER expression compared to those who used oral estrogen (78). However, oral estrogen remains the most popular application because of its convenience. Future well-designed studies comparing the effects of different routes of estrogen administration on endometrial proliferation, adverse impacts, and risk of thrombosis will be of great interest.

The range of the duration of estrogen priming varies greatly, from 6 days to 36 days, providing flexibility to extend the length of the follicular phase to achieve adequate endometrium for implantation (79). A retrospective study analyzed 4142 FET cycles and compared the 7-day and 14-day estrogen administration, and the results showed both regimens achieved adequate endometrial preparation with comparable reproductive outcomes (80). However, there might be a negative impact on reproductive outcomes if the duration of estrogen priming is too short. A retrospective study included 835 oocyte donation cycles, which were divided into five groups by 10-day increments according to the length of estrogen priming before implantation (81). The results showed that the implantation rates and pregnancy rates among the five groups were similar (81). However, the duration of estrogen administration that was less than 10 days had a significantly higher miscarriage rate (41%, p=0.04) compared to the other four groups of patients who received estrogen administration for longer duration (81).

2.9 Administration of progesterone

In AC for FET, the optimal timing for progesterone administration is critical to ensure proper endometrial preparation and synchronization with the embryo transfer. The timing varies depending on the specific AC protocol and the preferences of the treating physician. Using various techniques (ultrasonography, hysteroscopy, histology, immunobiological staining, and endometrial receptivity array), several markers of endometrial receptivity have been proposed to evaluate the implantation window (28). Among these tools, transvaginal ultrasonography is the most popular and non-invasive technique to evaluate endometrial receptivity in AC protocol. Associations have been identified between clinical pregnancy and various endometrial receptivity markers including endometrial thickness, endometrial pattern, Doppler indices, endometrial wave-like activity, and various molecules; however, their poor ability to predict clinical pregnancy prevents them from being used as diagnostic tests of endometrial receptivity (28). In AC FET, the endometrial thickness (> 7 mm), endometrial pattern (the triple line pattern), and endometrial blood flow (presence of endometrial blood flow) are the most common markers for good endometrial receptivity evaluated by transvaginal ultrasonography (Table 2) (28). When these markers have been detected, progesterone supplementation is commenced, and the timing of FET is scheduled accordingly.

In patients undergoing AC FET, optimal exposure to progesterone supplementation is critical for a successful conception. However, insufficient data were available to apply the route, dosage, and starting date of progesterone during the AC FET cycle.

Progesterone can be administered through various routes, including oral, rectal, intramuscular, subcutaneous, and vaginal (suppositories or gels). The specific route of administration is determined based on individual patient characteristics and clinic preferences (82–88). Each route has its advantages and considerations, such as convenience, patient comfort, and absorption rates. Additionally, various available forms can be used to support endometrial receptivity, including micronized capsules, tablets, suppositories, and bio-adhesive gels. To date, there is still insufficient comparative data regarding the effects of different routes or dosages of progesterone supplementation on the subsequent reproductive outcomes. Among different routes of progesterone, vaginal administration is the most used route. A retrospective study including 346 patients undergoing AC FET cycles compared two regimens of bio-adhesive progesterone gel (90 mg per day and 180 mg per day) and found that the implantation rates and LBRs were significantly higher in the 180 mg per day group (88). Similarly, a retrospective study including 2100 AC FET cycles compared different dosages (900 mg per day and 1200 mg per day) of oral progesterone capsules and found that patients using the dosage of 1200 mg per day had higher CPR (89). These findings suggest that adequate progesterone supplementation during AC FET can reduce the incidence of early pregnancy loss, leading to a significantly higher LBR. In terms of reproductive outcomes, there is a lack of consensus regarding which progesterone administration route is more efficient. Two RCTs have compared intramuscular and vaginal routes in patients undergoing AC FET and found similar CPRs (90, 91). Some retrospective studies observed improved reproductive outcomes in patients using the intramuscular route than those using the vaginal route (92, 93). However, other retrospective studies reported similar reproductive outcomes comparing intramuscular and vaginal routes (94, 95). Interestingly, a RCT compared three different regimens (50 mg per day intramuscular progesterone only; 400 mg per day vaginal progesterone only; and 400 mg per day vaginal progesterone plus 50 mg intramuscular progesterone every 3rd day) in AC FET cycles using vitrified blastocyst transfer (96). The results obtained from the interim analysis showed that patients receiving vaginal progesterone only had a lower CPR (31% vs. 50% vs. 47%) and LBR (27% vs. 44% vs. 46%) compared to the other two groups (96, 97). Another retrospective study including 1364 AC FET cycles compared reproductive outcomes of two regimens (400 mg twice per day vaginal micronized progesterone plus 10 mg twice per day oral progesterone and 400 mg twice per day vaginal micronized progesterone only) (98). Significantly lower abortion rate (3.4% vs. 6.6%) and higher LBR (46.3% vs. 41.3%) were noted in the group of combined vaginal and oral routes progesterone (98). Without a doubt, more RCTs are needed to clarify the best regimen and optimal dosage of various progesterone supplementation for AC FET.

The supplementation of progesterone for luteal support is particularly crucial in AC FET cycles because of the lack of endogenous progesterone secreted by the corpus luteum. There is a debate regarding the optimal duration of luteal support applied in AC FET cycles after conception (99, 100). Theoretically, progesterone supplementation should be continued until the time of luteo-placental shift (at approximately 10-12 weeks of gestation) when the placental tissue is able to produce enough endogenous progesterone (Table 2) (100).

2.10 Administration of GnRH analogs

In AC FET, GnRH analogs have been proposed to control the hypothalamus-pituitary-ovary axis and optimize the endometrial environment. GnRH analogs, such as GnRH agonists and GnRH antagonists, play a crucial role in suppressing the endogenous hormonal fluctuations and preventing premature ovulation (101). GnRH agonists initially cause a temporary surge in gonadotropin release before desensitizing the pituitary gland. Continuous administration of GnRH agonists results in a decrease in the reaction of GnRH because of an obvious uncoupling of GnRH receptors followed by the downregulation of GnRH receptors (102). Therefore, prolonged exposure to GnRH agonists desensitizes the GnRH receptors, leading to decreased FSH and LH secretion from the pituitary gland. This suppression of gonadotropin secretion prevents the development of the dominant follicle and subsequent ovulation (101). GnRH antagonists competitively bind to and block GnRH receptors on the gonadotroph cells of the pituitary gland. This binding prevents the endogenous GnRH from activating the receptors and inhibits the downstream signaling pathways that lead to the release of FSH and LH (103). A single-center RCT included 473 FET cycles and compared the 7-day dosage of GnRH antagonist with a single dose of long-acting GnRH agonist (104). The authors found that there was no significant difference in ongoing pregnancy rate between the two regimens and concluded that the outcomes are similar between GnRH antagonist and down-regulated hormone replacement protocols for women with ovulatory cycles undergoing oocyte donation (104). Although the addition of GnRH analogs for ovulation suppression is highly efficient, the AC FET protocol without GnRH analogs-induced suppression is more popular because of patient friendly. However, compared to GnRH analog application, AC FET without GnRH analogs has been reported with an incidence of approximately 1.9% to 7.4% of cycle cancellation because of the occurrence of premature ovulation (105, 106). A RCT included 234 patients undergoing FET cycles that compared AC protocols with or without GnRH agonist suppression (107). The results showed that AC with GnRH suppression had a higher LBR per initiated cycle than AC without GnRH suppression (107). However, the results obtained from the Cochrane Review database showed that there was no significant difference between the two protocols (AC with GnRH suppression and AC without GnRH suppression) in terms of CPRs, cycle cancellation rates, miscarriage rates, and endometrial thickness (75). A retrospective cohort study included 9263 women who underwent FET with or without long-acting GnRH agonist administration before AC protocol and compared the pregnancy outcomes (108). The results showed that for those who had no or one failure of embryo implantation, there was no difference in LBR between AC with GnRH agonist pretreatment and AC without GnRH agonist pretreatment (108). However, for those who had multiple failure of embryo implantation, AC protocol with GnRH agonist

10.3389/fendo.2023.1250847

pretreatment resulted in a higher LBR than AC protocol without GnRH agonist pretreatment (108).

2.11 Reproductive outcomes after different FET protocols

2.11.1 Reproductive outcome comparison between NC (true-NC/modified-NC) and AC FET

Based on the results analyzed from available cohort studies (retrospective or prospective), most studies reported similar reproductive outcomes between NC (true-NC and modified-NC) FET compared with AC FET (109–113). However, there were some controversial results given that some studies reported better outcomes (114–116) or worse outcomes (117, 118) in patients receiving true-NC/modified-NC than those receiving AC FET. Among the abovementioned protocols, studies have shown that AC FET resulted in the highest early pregnancy loss (109, 119). A retrospective cohort study included 634 FET cycles and compared the early pregnancy loss incidence in patients using AC FET or true-NC FET (120). The results showed that AC FET protocol had a higher early pregnancy loss rate than true-NC FET (54.7% versus 33%, P<0.0001) (120). Similarly, a meta-analysis comparing the obstetric outcomes after NC versus AC FET showed that patients using AC FET protocol had a higher early pregnancy rate (121). A total of 26 RCTs and 113 cohort studies were included in a network meta-analysis, which compared 7 different FET protocols: true- NC; modified-NC; AC with GnRH agonist suppression; AC without GnRH agonist suppression; aromatase inhibitor (letrozole); clomiphene citrate; and exogenous gonadotropin (122) (Figure 1). Using a network meta-analysis, the results showed that AC ranked as the lowest LBR compared with the other protocols (122) (Figure 1). Using a pairwise meta-analysis of observational studies, AC was associated with significantly lower LBRs compared with true-NC and modified-NC (122) (Figure 1). However, the results obtained from a Cochrane review meta-analysis including 5 RCTs revealed a trend, yet not reaching statistic difference, showing a higher CPR in AC than NC (75). Of note, a RCT included 959 FET cycles showed that patients receiving AC FET had a higher cancellation rate than those receiving modified-NC (124/464 versus 101/495, OR 1.4, 95% CI 1.1–1.9, P = 0.02) (123). In this regard, the increased cancellation rate found in the AC FET protocol was due to insufficient endometrial thickness. However, the analytic data showed a similar cost after receiving AC or modified-NC (123). In summary, dada obtained from available studies indicate that the reproductive outcomes in NC (true-NC and modified NC) were slightly better than those in AC with low-quality evidence.

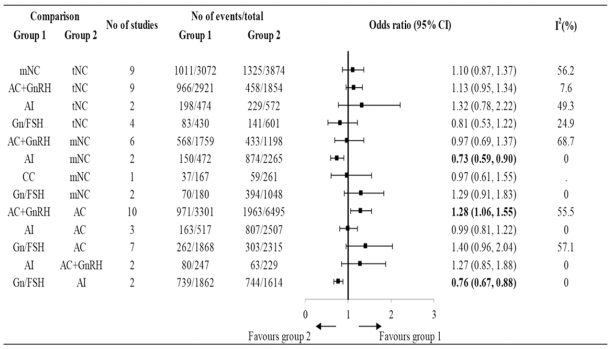


FIGURE 1
The reproductive outcomes of live birth rate among different endometrial preparation protocols in cohort studies. t-NC, true natural cycle; m-NC, modified natural cycle; AC, artificial cycle without suppression; AC+GnRH, artificial cycle with gonadotropin-releasing hormone cycle; Gn/FSH, ovarian stimulation with gonadotropin or follicle stimulating hormone; AI, aromatase inhibitor; CC, clomiphene citrate [Reproduced with permission from SPRINGER/PLENUM PUBLISHERS, Reference (122)].

2.11.2 Reproductive outcome comparison between mild-OS and AC FET

Using transvaginal ultrasonography to measure the endometrial thickness is the most common clinical approach to evaluate endometrial receptivity (28). A retrospective study compared two regimens of endometrial preparation in 2664 women with PCOS undergoing FET (124). The results showed that the endometrial thickness on the day of progesterone supplementation and on the day of embryo transfer was significantly thicker in patients receiving mild-OS with letrozole than in those receiving AC (124). Additionally, after adjusting the related confounding factors, this study demonstrated that LBR was significantly higher, and the early pregnancy loss rate was lower in the letrozole group compared with the AC group (124). In contrast to these results, the other two studies did not show any significant difference in endometrial thickness when letrozole FET was compared with AC FET (125, 126). The endometrial thickness was significantly thinner in mild-OS with CC than in AC (75). Using a pairwise meta-analysis, a Cochrane database showed that the CPR was significantly higher in the mild-OS (letrozole or clomiphene citrate) FET when compared to AC FET (75). However, no significant difference was found in CPR between the mild-OS with clomiphene citrate and AC FET (75). Further meta-analysis showed that the CPR was significantly higher in the mild-OS with letrozole FET when compared to AC FET (75). The reproductive outcome focusing on LBR has been compared between two regimens, mild-OS with letrozole and AC, in an available RCT with a limited sample size (127). The results showed a similar LBR in both mild-OS with letrozole and AC, with low-quality evidence (127). A network meta-analysis including 26 RCTs compared 7 different regimens of FET, and the results showed that significantly higher LBRs were observed in mild-OS with letrozole or mild-OS with gonadotropin compared to the AC (122). More RCTs are required to support the feasible application of mild-OS in patients undergoing FET.

2.11.3 Obstetric and neonatal outcome comparison between NC (true-NC/modified-NC) and AC FET

A most recent meta-analysis included 30 RCTs and cohort studies, which compared the obstetric and neonatal outcomes

following NC FET and AC FET (121). The results obtained from the meta-analyses showed that the average neonatal birthweight was lower following NC FET compared to AC FET (121). Compared to AC FET, newborns delivered from NC FET cycles had a lower risk of various neonatal outcomes, including large for gestational age, low birth weight, and macrosomia (121). Furthermore, pregnant women following NC FET had a lower risk of multiple obstetric outcomes, including early pregnancy loss, preterm birth, very preterm birth, pregnancy-induced hypertension, pre-eclampsia, placenta previa, and postpartum hemorrhage (121). Using a pairwise comparison analysis, a network meta-analysis (including 26 RCTs and 113 cohort studies) that compared different FET protocols showed that infertile patients who achieved pregnancy using AC had an increased risk of developing pregnancy-induced hypertension, postpartum hemorrhage, and preterm labor, compared with those using true-NC (122) (Figure 1). Intriguingly, data obtained from stratified analyses showed that NC FET with LPS significantly decreased the risk of preterm labor (but not other obstetric outcomes) whereas, NC FET without LPS did not decrease this risk (121). Because of the very low quality of evidence, the efficacy of the use of LPS in NC FET remains to be elucidated by using a large-scale RCT.

2.11.4 The application of endometrial receptivity testing in FET cycles

Endometrial receptivity refers to the state of the endometrium that is conducive to embryo implantation and is a complex process involving molecular, cellular, and structural changes in the endometrium (128). The receptive window, known as the window of implantation (WOI), is a limited timeframe during which the endometrium is receptive to embryo attachment and subsequent implantation (129). Deviations from the optimal receptivity window can lead to implantation failure and reduced pregnancy rates. Through the endometrial transcriptomic analyses, researchers have identified several differentially expressed genes among all phases of the menstrual cycle, including the receptive phase (130, 131). In this regard, endometrial receptivity testing is a diagnostic technique that uses transcriptomic analyses to classify the endometrial biopsy samples into pre-receptive, early receptive, receptive, late receptive, or post-receptive (47). The objective of this technique is to determine the optimal for conducting a FET for an individual patient, considering when progesterone exposure begins (47). To date, hundreds of thousands cycles of endometrial receptivity testing have been carried out; however, the results on reproductive outcomes have yielded inconsistent results. Some studies indicate that receptivity-timed FET may lead to better outcomes compared to standardized timing, while others have found no significant difference (47, 132–134). A RCT that included 767 AC FET cycles using at least one euploid blastocyst was conducted to evaluate whether timed FET according to endometrial receptivity testing improves reproductive outcomes relative to standardized FET (135). The primary outcome, LBR, was observed in 58.5% of transfers (223 out of 381) in the intervention group, compared to 61.9% of transfers (239 out of 386) in the control group, with no significant difference between the two groups (p=0.38) (135). No significant differences were found between the intervention and control groups for the predetermined secondary outcomes, including the rate of biochemical pregnancy (77.2% vs 79.5%, p=0.48) and CPR (68.8% vs 72.8%, p=0.25) (135). Taken together, the utilization of endometrial receptivity testing to determine the optimal timing of FET did not result in a significant improvement in the LBR among patients with euploid blastocysts from IVF. These findings do not provide substantial evidence to support the routine use of receptivity testing for guiding the timing of embryo transfer in the context of IVF/ET.

3 Conclusion

Despite the global rise in FET for various indications, there is an ongoing quest to determine the optimal protocol for preparing the endometrium. While NC (true-NC/modified-NC) and AC FET are the commonly utilized protocols, it is crucial to conduct well-designed and powerful RCTs comparing different protocols to optimize endometrial preparation for FET. These trials should not only focus on LBR or CPR but also assess maternal, obstetrical, and neonatal outcomes. Currently, limited-quality evidence indicates that the NC (t-NC/modified-NC) may be superior to AC. Furthermore, caution is advised with AC due to a potential incidence of early pregnancy loss reported in some studies, and recent evidence indicates an increased risk of developing hypertensive disorders in pregnancies because a lack of the corpus luteum. More RCTs are needed to clarify the best regimen and optimal dosage of various estrogen and progesterone supplementation for AC FET and the application of LPS for NC. Regarding the timing of warmed blastocyst transfer, evidence suggests that the optimal timing for FET can be LH surge+6 day, hCG administration+7 day, and the progesterone administration+6 day in the true-NC, modified-NC, and AC protocols, respectively (Table 2). It is important to further explore time adjustments considering individual variations in the WOI or the day of vitrification. Finally, emerging evidence does not support the routine application of endometrial receptivity testing for guiding the timing of FET in different protocols.

Author contributions

Y-WH: Writing – original draft, writing – review, visualization.C-CH: Writing – original draft, Visualization. S-WH: Writing – review, visualization. C-WC: Writing – original

draft, visualization. H-CH: Writing – original draft, visualization. T-CY: Conceptualization, writing – review and editing, visualization. W-CL: Conceptualization, writing – review and editing, visualization. S-YS: Supervision, writing – review and editing, visualization. H-MC: Conceptualization, supervision, writing – review and editing, visualization. All authors contributed to the article and approved the submitted version.

Acknowledgments

We would like to acknowledge participants who generously gave their time and shared their experiences.

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

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Evans J, Hannan NJ, Edgell TA, Vollenhoven BJ, Lutjen PJ, Osianlis T, et al. Fresh versus frozen embryo transfer: backing clinical decisions with scientific and clinical evidence. *Hum Reprod Update* (2014) 20:808–21. doi: 10.1093/humupd/dmu027

2. Wong KM, Mastenbroek S, Repping S. Cryopreservation of human embryos and its contribution to in *vitro* fertilization success rates. *Fertil Steril* (2014) 102:19–26. doi: 10.1016/j.fertnstert.2014.05.027

3. Roque M, Lattes K, Serra S, Sola I, Geber S, Carreras R, et al. Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis. *Fertil Steril* (2013) 99:156–62. doi: 10.1016/j.fertnstert.2012.09.003

4. Roque M, Haahr T, Geber S, Esteves SC, Humaidan P. Fresh versus elective frozen embryo transfer in IVF/ICSI cycles: a systematic review and meta-analysis of reproductive outcomes. *Hum Reprod Update* (2019) 25:2–14. doi: 10.1093/humupd/dmy033

5. Endo T, Honnma H, Hayashi T, Chida M, Yamazaki K, Kitajima Y, et al. Continuation of GnRH agonist administration for 1 week, after hCG injection, prevents ovarian hyperstimulation syndrome following elective cryopreservation of all pronucleate embryos. *Hum Reprod* (2002) 17:2548–51. doi: 10.1093/humrep/17.10.2548

6. Chen ZJ, Shi Y, Sun Y, Zhang B, Liang X, Cao Y, et al. Fresh versus frozen embryos for infertility in the polycystic ovary syndrome. *N Engl J Med* (2016) 375:523–33. doi: 10.1056/NEJMoa1513873

7. Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. *Fertil Steril* (2012) 98:368–377 e361-369. doi: 10.1016/j.fertnstert.2012.05.019

8. Halliday JL, Ukoumunne OC, Baker HW, Breheny S, Jaques AM, Garrett C, et al. Increased risk of blastogenesis birth defects, arising in the first 4 weeks of pregnancy, after assisted reproductive technologies. *Hum Reprod* (2010) 25:59–65. doi: 10.1093/humrep/dep364

9. Zaat T, Zagers M, Mol F, Goddijn M, van Wely M, Mastenbroek S. Fresh versus frozen embryo transfers in assisted reproduction. *Cochrane Database Syst Rev* (2021) 2: CD011184. doi: 10.1002/14651858.CD011184.pub3

10. Cobo A, de los Santos MJ, Castello D, Gamiz P, Campos P, Remohi J. Outcomes of vitrified early cleavage-stage and blastocyst-stage embryos in a cryopreservation program: evaluation of 3,150 warming cycles. *Fertil Steril* (2012) 98:1138–1146 e1131. doi: 10.1016/j.fertnstert.2012.07.1107

11. Loutradi KE, Kolibianakis EM, Venetis CA, Papanikolaou EG, Pados G, Bontis I, et al. Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis. *Fertil Steril* (2008) 90:186–93. doi: 10.1016/j.fertnstert.2007.06.010

12. AbdelHafez FF, Desai N, Abou-Setta AM, Falcone T, Goldfarb J. Slow freezing, vitrification and ultra-rapid freezing of human embryos: a systematic review and meta-analysis. *Reprod BioMed Online* (2010) 20:209–22. doi: 10.1016/j.rbmo.2009.11.013

13. De Geyter C, Wyns C, Calhaz-Jorge C, de Mouzon J, Ferraretti AP, Kupka M, et al. 20 years of the European IVF-monitoring Consortium registry: what have we learned? A comparison with registries from two other regions. *Hum Reprod* (2020) 35:2832–49. doi: 10.1093/humrep/deaa250

14. Wang S, Liu L, Ma M, Wang H, Han Y, Guo X, et al. Preimplantation genetic testing for aneuploidy helps to achieve a live birth with fewer transfer cycles for the blastocyst FET patients with unexplained recurrent implantation failure. *Arch Gynecol Obstet* (2023) 308(2):599–610. doi: 10.1007/S00404-023-07041-5

15. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders. *Fertil Steril* (2011) 96:344–8. doi: 10.1016/j.fertnstert.2011.05.050

16. Huang J, Lu X, Xie Q, Lin J, Cai R, Kuang Y. Timing of frozen-thawed embryo transfer after controlled ovarian stimulation in a non-elective freeze-all policy. *Ann Transl Med* (2019) 7:752. doi: 10.21037/atm.2019.11.74

17. Huang JT, Coughlin CC, Hawryluk EB, Hook K, Humphrey SR, Kruse L, et al. Risk factors and outcomes of nonmelanoma skin cancer in children and young adults. *J Pediatr* (2019) 211:152–8. doi: 10.1016/j.jpeds.2019.04.017

18. Bergenheim SJ, Saupstad M, Pistoljevic N, Andersen AN, Forman JL, Lossl K, et al. Immediate versus postponed frozen embryo transfer after IVF/ICSI: a systematic review and meta-analysis. *Hum Reprod Update* (2021) 27:623–42. doi: 10.1093/humupd/dmab002

19. Zuo N, Gao Y, Zhang N, Li D, Wang X. Effects of immediate versus delayed frozen embryo transfer in high responder patients undergoing freeze-all cycles. *BMC Pregnancy Childbirth* (2021) 21:455. doi: 10.1186/s12884-021-03919-x

20. Huang Y, Gao J, Wang Y, Zhang H, Chen L, Yang Y, et al. The time interval between oocyte retrieval and frozen embryo transfer does not impact reproductive outcomes. *Reprod BioMed Online* (2023) 15:1472-6483(23)00161-X. doi: 10.1016/j.rbmo.2023.03.008

21. Song JY, Dong FY, Li L, Zhang XX, Wang AJ, Zhang Y, et al. Immediate versus delayed frozen embryo transfer in women following a failed IVF-ET attempt: a multicenter randomized controlled trial. *Reprod Biol Endocrinol* (2021) 19:131. doi: 10.1186/s12958-021-00819-9

22. Mumusoglu S, Polat M, Ozbek IY, Bozdag G, Papanikolaou EG, Esteves SC, et al. Preparation of the endometrium for frozen embryo transfer: A systematic review. *Front Endocrinol (Lausanne)* (2021) 12:688237. doi: 10.3389/fendo.2021.688237

23. Erden M, Mumusoglu S, Polat M, Yarali Ozbek I, Esteves SC, Humaidan P, et al. The LH surge and ovulation re-visited: a systematic review and meta-analysis and implications for true natural cycle frozen thawed embryo transfer. *Hum Reprod Update* (2022) 28:717–32. doi: 10.1093/humupd/dmac012

24. Muasher SJ, Oehninger S, Simonetti S, Matta J, Ellis LM, Liu HC, et al. The value of basal and/or stimulated serum gonadotropin levels in prediction of stimulation response and in vitro fertilization outcome. *Fertil Steril* (1988) 50:298–307. doi: 10.1016/S0015-0282(16)60077-8

25. Panaino TR, Silva JB, Lima MA, Lira P, Areas PC, Mancebo AC, et al. High Progesterone levels in the beginning of ICSI antagonist cycles and clinical pregnancy: still a concern? *JBRA Assist Reprod* (2017) 21:11–4. doi: 10.5935/1518-0557.20170004

26. Bancsi LF, Huijs AM, den Ouden CT, Broekmans FJ, LoOman CW, Blankenstein MA, et al. Basal follicle-stimulating hormone levels are of limited value in predicting ongoing pregnancy rates after in vitro fertilization. *Fertil Steril* (2000) 73:552–7. doi: 10.1016/S0015-0282(99)00552-X

27. Kolibianakis EM, Zikopoulos K, Smitz J, Camus M, Tournaye H, Van Steirteghem AC, et al. Elevated progesterone at initiation of stimulation is associated with a lower ongoing pregnancy rate after IVF using GnRH antagonists. *Hum Reprod* (2004) 19:1525–9. doi: 10.1093/humrep/deh272

28. Craciunas L, Gallos I, Chu J, Bourne T, Quenby S, Brosens JJ, et al. Conventional and modern markers of endometrial receptivity: a systematic review and meta-analysis. *Hum Reprod Update* (2019) 25:202–23. doi: 10.1093/humupd/dmy044

29. Su HW, Yi YC, Wei TY, Chang TC, Cheng CM. Detection of ovulation, a review of currently available methods. *Bioeng Transl Med* (2017) 2:238–46. doi: 10.1002/btm2.10058

30. Matijevic R, Grgic O. Predictive values of ultrasound monitoring of the menstrual cycle. *Curr Opin Obstet Gynecol* (2005) 17:405–10. doi: 10.1097/01.gco.0000175360.35341.2b

31. Pauerstein CJ, Eddy CA, Croxatto HD, Hess R, Siler-Khodr TM, Croxatto HB. Temporal relationships of estrogen, progesterone, and luteinizing hormone levels to ovulation in women and infrahuman primates. *Am J Obstet Gynecol* (1978) 130:876–86. doi: 10.1016/0002-9378(78)90264-8

32. Zhang C, Bosch MA, Qiu J, Ronnekleiv OK, Kelly MJ. 17beta-Estradiol increases persistent Na(+) current and excitability of AVPV/PeN Kiss1 neurons in female mice. *Mol Endocrinol* (2015) 29:518–27. doi: 10.1210/me.2014-1392

33. Fritz MA, McLachlan RI, Cohen NL, Dahl KD, Bremner WJ, Soules MR. Onset and characteristics of the midcycle surge in bioactive and immunoactive luteinizing hormone secretion in normal women: influence of physiological variations in periovulatory ovarian steroid hormone secretion. *J Clin Endocrinol Metab* (1992) 75:489–93. doi: 10.1210/jcem.75.2.1639949

34. Hoff JD, Quigley ME, Yen SS. Hormonal dynamics at midcycle: a reevaluation. *J Clin Endocrinol Metab* (1983) 57:792–6. doi: 10.1210/jcem-57-4-792

35. Testart J, Frydman R, Feinstein MC, Thebault A, Roger M, Scholler R. Interpretation of plasma luteinizing hormone assay for the collection of mature oocytes from women: definition of a luteinizing hormone surge-initiating rise. *Fertil Steril* (1981) 36:50–4. doi: 10.1016/S0015-0282(16)45617-7

36. Irani M, Robles A, Gunnala V, Reichman D, Rosenwaks Z. Optimal parameters for determining the LH surge in natural cycle frozen-thawed embryo transfers. *J Ovarian Res* (2017) 10:70. doi: 10.1186/s13048-017-0367-7

37. Roger M, Grenier J, Houlbert C, Castanier M, Feinstein MC, Scholler R. Rapid radioimmunoassays of plasma LH and estradiol-17 beta for the prediction of ovulation. *J Steroid Biochem* (1980) 12:403–10. doi: 10.1016/0022-4731(80)90299-X

38. Miller PB, Soules MR. The usefulness of a urinary LH kit for ovulation prediction during menstrual cycles of normal women. *Obstet Gynecol* (1996) 87:13–7. doi: 10.1016/0029-7844(95)00352-5

39. Ecochard R, Leiva R, Bouchard T, Boehringer H, Direito A, Mariani A, et al. Use of urinary pregnanediol 3-glucuronide to confirm ovulation. *Steroids* (2013) 78:1035–40. doi: 10.1016/j.steroids.2013.06.006

40. Hull MG, Savage PE, Bromham DR, Ismail AA, Morris AF. The value of a single serum progesterone measurement in the midluteal phase as a criterion of a potentially fertile cycle ("ovulation") derived form treated and untreated conception cycles. *Fertil Steril* (1982) 37:355–60. doi: 10.1016/S0015-0282(16)46095-4

41. Veleva Z, Orava M, Nuojua-Huttunen S, Tapanainen JS, Martikainen H. Factors affecting the outcome of frozen-thawed embryo transfer. *Hum Reprod* (2013) 28:2425–31. doi: 10.1093/humrep/det251

42. Montagut M, Santos-Ribeiro S, De Vos M, Polyzos NP, Drakopoulos P, Mackens S, et al. Frozen-thawed embryo transfers in natural cycles with spontaneous or induced ovulation: the search for the best protocol continues. *Hum Reprod* (2016) 31:2803–10. doi: 10.1093/humrep/dew263

43. Lee VC, Li RH, Ng EH, Yeung WS, Ho PC. Luteal phase support does not improve the clinical pregnancy rate of natural cycle frozen-thawed embryo transfer: a retrospective analysis. *Eur J Obstet Gynecol Reprod Biol* (2013) 169:50–3. doi: 10.1016/j.ejogrb.2013.02.005

44. Waldman IN, Racowsky C, Disler ER, Thomas A, Lanes A, Hornstein MD. The clinical relevance of luteal phase progesterone support in true natural cycle cryopreserved blastocyst stage embryo transfers: a retrospective cohort study. *Fertil Res Pract* (2021) 7:4. doi: 10.1186/s40738-021-00096-5

45. Bjuresten K, Landgren BM, Hovatta O, Stavreus-Evers A. Luteal phase progesterone increases live birth rate after frozen embryo transfer. *Fertil Steril* (2011) 95:534–7. doi: 10.1016/j.fertnstert.2010.05.019

46. Lee VCY, Li RHW, Yeung WSB, Pak Chung HO, Ng EHY. A randomized double-blinded controlled trial of hCG as luteal phase support in natural cycle frozen embryo transfer. *Hum Reprod* (2017) 32:1130–7. doi: 10.1093/humrep/dex049

47. Ruiz-Alonso M, Blesa D, Diaz-Gimeno P, Gomez E, Fernandez-Sanchez M, Carranza F, et al. The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. *Fertil Steril* (2013) 100:818–24. doi: 10.1016/j.fertnstert.2013.05.004

48. Ruiz-Alonso M, Valbuena D, Gomez C, Cuzzi J, Simon C. Endometrial Receptivity Analysis (ERA): data versus opinions. *Hum Reprod Open* (2021) 2021:hoab011. doi: 10.1093/hropen/hoab011

49. Svenstrup L, Moller S, Fedder J, Pedersen DE, Erb K, Andersen CY, et al. Does the HCG trigger dose used for IVF impact luteal progesterone concentrations? a randomized controlled trial. *Reprod BioMed Online* (2022) 45:793–804. doi: 10.1016/j.rbmo.2022.04.019

50. Mensing L, Dahlberg ES, Bay B, Gabrielsen A, Knudsen UB. Endometrial preparation methods prior to frozen embryo transfer: A retrospective cohort study comparing true natural cycle, modified natural cycle and artificial cycle. *Arch Gynecol Obstet* (2022) 306:1381–8. doi: 10.1007/s00404-021-06371-6

51. Fatemi HM, Kyrou D, Bourgain C, Van den Abbeel E, Griesinger G, Devroey P. Cryopreserved-thawed human embryo transfer: spontaneous natural cycle is superior to human chorionic gonadotropin-induced natural cycle. *Fertil Steril* (2010) 94:2054–8. doi: 10.1016/j.fertnstert.2009.11.036

52. Fauser BC, de Jong D, Olivennes F, Wramsby H, Tay C, Itskovitz-Eldor J, et al. Endocrine profiles after triggering of final oocyte maturation with GnRH agonist after cotreatment with the GnRH antagonist ganirelix during ovarian hyperstimulation for in vitro fertilization. *J Clin Endocrinol Metab* (2002) 87:709–15. doi: 10.1210/jcem.87.2.8197

53. Casper RF, Yanushpolsky EH. Optimal endometrial preparation for frozen embryo transfer cycles: window of implantation and progesterone support. *Fertil Steril* (2016) 105:867–72. doi: 10.1016/j.fertnstert.2016.01.006

54. Eftekhar M, Aflatoonian A, Mohammadian F, Eftekhar T. Adjuvant growth hormone therapy in antagonist protocol in poor responders undergoing assisted reproductive technology. *Arch Gynecol Obstet* (2013) 287:1017–21. doi: 10.1007/s00404-012-2655-1

55. Kim CH, Lee YJ, Lee KH, Kwon SK, Kim SH, Chae HD, et al. The effect of luteal phase progesterone supplementation on natural frozen-thawed embryo transfer cycles. *Obstet Gynecol Sci* (2014) 57:291–6. doi: 10.5468/ogs.2014.57.4.291

56. Kyrou D, Fatemi HM, Popovic-Todorovic B, Van den Abbeel E, Camus M, Devroey P. Vaginal progesterone supplementation has no effect on ongoing pregnancy rate in hCG-induced natural frozen-thawed embryo transfer cycles. *Eur J Obstet Gynecol Reprod Biol* (2010) 150:175–9. doi: 10.1016/j.ejogrb.2010.02.038

57. Eftekhar M, Rahsepar M, Rahmani E. Effect of progesterone supplementation on natural frozen-thawed embryo transfer cycles: a randomized controlled trial. *Int J Fertil Steril* (2013) 7:13–20.

58. Horowitz E, Mizrachi Y, Finkelstein M, Farhi J, Shalev A, Gold E, et al. A randomized controlled trial of vaginal progesterone for luteal phase support in modified natural cycle - frozen embryo transfer. *Gynecol Endocrinol* (2021) 37:792–7. doi: 10.1080/09513590.2020.1854717

59. Navot D, Laufer N, Kopolovic J, Rabinowitz R, Birkenfeld A, Lewin A, et al. Artificially induced endometrial cycles and establishment of pregnancies in the absence of ovaries. *N Engl J Med* (1986) 314:806–11. doi: 10.1056/NEJM198603273141302

60. Rosenwaks Z. Donor eggs: their application in modern reproductive technologies. *Fertil Steril* (1987) 47:895–909. doi: 10.1016/S0015-0282(16)59220-6

61. Van Steirteghem AC, Van den Abbeel E, Braeckmans P, Camus M, Khan I, Smitz J, et al. Pregnancy with a frozen-thawed embryo in a wOman with primary ovarian failure. *N Engl J Med* (1987) 317:113. doi: 10.1056/NEJM198707093170210

62. Salat-Baroux J, Cornet D, Alvarez S, Antoine JM, Tibi C, Mandelbaum J, et al. Pregnancies after replacement of frozen-thawed embryos in a donation program. *Fertil Steril* (1988) 49:817–21. doi: 10.1016/S0015-0282(16)59890-2

63. Navot D, Scott RT, Droesch K, Veeck LL, Liu HC, Rosenwaks Z. The window of embryo transfer and the efficiency of human conception in vitro. *Fertil Steril* (1991) 55:114–8. doi: 10.1016/S0015-0282(16)54069-2

64. Kosmas IP, Tatsioni A, Fatemi HM, Kolibianakis EM, Tournaye H, Devroey P. Human chorionic gonadotropin administration vs. luteinizing monitoring for intrauterine insemination timing, after administration of clomiphene citrate: a meta-analysis. *Fertil Steril* (2007) 87:607–12. doi: 10.1016/j.fertnstert.2006.10.003

65. Johal JK, Bavan B, Zhang W, Gardner RM, Lathi RB, Milki AA. The impact of timing modified natural cycle frozen embryo transfer based on spontaneous luteinizing hormone surge. *J Assist Reprod Genet* (2021) 38:219–25. doi: 10.1007/s10815-020-01994-1

66. Van der Auwera I, Meuleman C, Koninckx PR. Human menopausal gonadotrophin increases pregnancy rate in comparison with clomiphene citrate during replacement cycles of frozen/thawed pronucleate ova. *Hum Reprod* (1994) 9:1556–60. doi: 10.1093/oxfordjournals.humrep.a138748

67. Peeraer K, Couck I, Debrock S, De Neubourg D, De Loecker P, Tomassetti C, et al. Frozen-thawed embryo transfer in a natural or mildly hormonally stimulated cycle in women with regular ovulatory cycles: a RCT. *Hum Reprod* (2015) 30:2552–62. doi: 10.1093/humrep/dev224

68. Legro RS, Brzyski RG, Diamond MP, Coutifaris C, Schlaff WD, Casson P, et al. Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. *N Engl J Med* (2014) 371:119–29. doi: 10.1056/NEJMoa1313517

69. Thomas S, Woo I, Ho J, Jones T, Paulson R, Chung K, et al. Ovulation rates in a stair-step protocol with Letrozole vs clomiphene citrate in patients with polycystic ovarian syndrome. *Contracept Reprod Med* (2019) 4:20. doi: 10.1186/s40834-019-0102-4

70. Green KA, Zolton JR, Schermerhorn SM, Lewis TD, Healy MW, Terry N, et al. Progesterone luteal support after ovulation induction and intrauterine insemination: an updated systematic review and meta-analysis. *Fertil Steril* (2017) 107:924–933 e925. doi: 10.1016/j.fertnstert.2017.01.011

71. Zhang Y, Fu X, Gao S, Gao S, Gao S, Ma J, et al. Preparation of the endometrium for frozen embryo transfer: an update on clinical practices. *Reprod Biol Endocrinol* (2023) 21:52. doi: 10.1186/s12958-023-01106-5

72. Madero S, Rodriguez A, Vassena R, Vernaeve V. Endometrial preparation: effect of estrogen dose and administration route on reproductive outcomes in oocyte donation cycles with fresh embryo transfer. *Hum Reprod* (2016) 31:1755–64. doi: 10.1093/humrep/dew099

73. Sukur YE, Aslan B, Ozmen B, Sonmezer M, Berker B, Atabekoglu CS, et al. Impact of an estrogen replacement regimen on live birth rate in frozen-thawed good-

quality embryo transfer. *Int J Gynaecol Obstet* (2023) 160:829–35. doi: 10.1002/ijgo.14366

74. Ghobara T, Vandekerckhove P. Cycle regimens for frozen-thawed embryo transfer. *Cochrane Database Syst Rev* (2008) 7(7):CD003414. doi: 10.1002/14651858.CD003414.pub2

75. Glujovsky D, Pesce R, Sueldo C, Quinteiro Retamar AM, Hart RJ, Ciapponi A. Endometrial preparation for women undergoing embryo transfer with frozen embryos or embryos derived from donor oocytes. *Cochrane Database Syst Rev* (2020) 10: CD006359. doi: 10.1002/14651858.cd006359.pub3

76. Garimella S, Karunakaran S, Gedela DR. A prospective study of oral estrogen versus transdermal estrogen (gel) for hormone replacement frozen embryo transfer cycles. *Gynecol Endocrinol* (2021) 37:515–8. doi: 10.1080/09513590.2020.1793941

77. Liao X, Li Z, Dong X, Zhang H. Comparison between oral and vaginal estrogen usage in inadequate endometrial patients for frozen-thawed blastocysts transfer. *Int J Clin Exp Pathol* (2014) 7:6992–7.

78. Feng W, Nie L, Wang X, Yang F, Pan P, Deng X. Effect of oral versus vaginal administration of estradiol and dydrogesterone on the proliferative and secretory transformation of endometrium in patients with premature ovarian failure and preparing for assisted reproductive technology. *Drug Des Devel Ther* (2021) 15:1521–9. doi: 10.2147/DDDT.S297236

79. Devroey P, Pados G. Preparation of endometrium for egg donation. *Hum Reprod Update* (1998) 4:856–61. doi: 10.1093/humupd/4.6.856

80. Jiang WJ, Song JY, Sun ZG. Short (seven days) versus standard (fourteen days) oestrogen administration in a programmed frozen embryo transfer cycle: a retrospective cohort study. *J Ovarian Res* (2022) 15:36. doi: 10.1186/s13048-022-00967-5

81. Borini A, Dal Prato L, Bianchi L, Violini F, Cattoli M, Flamigni C. Effect of duration of estradiol replacement on the outcome of oocyte donation. *J Assist Reprod Genet* (2001) 18:185–90. doi: 10.1023/A:1009472416305

82. Miles RA, Paulson RJ, Lobo RA, Press MF, Dahmoush L, Sauer MV. Pharmacokinetics and endometrial tissue levels of progesterone after administration by intramuscular and vaginal routes: a comparative study. *Fertil Steril* (1994) 62:485–90. doi: 10.1016/S0015-0282(16)56935-0

83. Bulletti C, De Ziegler D, Giacomucci E, Polli V, Rossi S, Alfieri S, et al. Vaginal drug delivery: the first uterine pass effect. *Ann N Y Acad Sci* (1997) 828:285–90. doi: 10.1111/j.1749-6632.1997.tb48549.x

84. Cicinelli E, de Ziegler D, Bulletti C, Matteo MG, Schonauer LM, Galantino P. Direct transport of progesterone from vagina to uterus. *Obstet Gynecol* (2000) 95:403–6. doi: 10.1097/00006250-200003000-00017

85. Zarei A, Sohail P, Parsanezhad ME, Alborzi S, Samsami A, Azizi M. Comparison of four protocols for luteal phase support in frozen-thawed Embryo transfer cycles: a randomized clinical trial. *Arch Gynecol Obstet* (2017) 295:239–46. doi: 10.1007/s00404-016-4217-4

86. Turkgeldi E, Hanege BY, Yildiz S, Keles I, Ata B. Subcutaneous versus vaginal progesterone for vitrified-warmed blastocyst transfer in artificial cycles. *Reprod BioMed Online* (2020) 41:248–53. doi: 10.1016/j.rbmo.2020.04.007

87. Alsbjerg B, Thomsen L, Elbaek HO, Laursen R, Povlsen BB, Haahr T, et al. Can combining vaginal and rectal progesterone achieve the optimum progesterone range required for implantation in the HRT-FET model? *Reprod BioMed Online* (2020) 40:805–11. doi: 10.1016/j.rbmo.2020.02.007

88. Alsbjerg B, Polyzos NP, Elbaek HO, Povlsen BB, Andersen CY, Humaidan P. Increasing vaginal progesterone gel supplementation after frozen-thawed embryo transfer significantly increases the delivery rate. *Reprod BioMed Online* (2013) 26:133–7. doi: 10.1016/j.rbmo.2012.10.012

89. Enatsu Y, Enatsu N, Kishi K, Iwasaki T, Matsumoto Y, Kokeguchi S, et al. Effectiveness of high-dose transvaginal progesterone supplementation for women who are undergoing a frozen-thawed embryo transfer. *Reprod Med Biol* (2018) 17:242–8. doi: 10.1002/rmb2.12096

90. Wang Y, He Y, Zhao X, Ji X, Hong Y, Wang Y, et al. Crinone gel for luteal phase support in frozen-thawed embryo transfer cycles: A prospective randomized clinical trial in the chinese population. *PloS One* (2015) 10:e0133027. doi: 10.1371/journal.pone.0133027

91. Lightman A, Kol S, Itskovitz-Eldor J. A prospective randomized study comparing intramuscular with intravaginal natural progesterone in programmed thaw cycles. *Hum Reprod* (1999) 14:2596–9. doi: 10.1093/humrep/14.10.2596

92. Haddad G, Saguan DA, Maxwell R, Thomas MA. Intramuscular route of progesterone administration increases pregnancy rates during non-downregulated frozen embryo transfer cycles. *J Assist Reprod Genet* (2007) 24:467–70. doi: 10.1007/s10815-007-9168-z

93. Kaser DJ, Ginsburg ES, Missmer SA, Correia KF, Racowsky C. Intramuscular progesterone versus 8% Crinone vaginal gel for luteal phase support for day 3 cryopreserved embryo transfer. *Fertil Steril* (2012) 98:1464–9. doi: 10.1016/j.fertnstert.2012.08.007

94. Shapiro DB, Pappadakis JA, Ellsworth NM, Hait HI, Nagy ZP. Progesterone replacement with vaginal gel versus i.m. injection: cycle and pregnancy outcomes in IVF patients receiving vitrified blastocysts. *Hum Reprod* (2014) 29:1706–11. doi: 10.1093/humrep/deu121

95. Leonard PH, Hokenstad AN, Khan Z, Jensen JR, Stewart EA, Coddington CC. Progesterone support for frozen embryo transfer: intramuscular versus vaginal suppository demonstrates no difference in a cohort. *J Reprod Med* (2015) 60:103–8.

96. Devine K, Richter KS, Widra EA, McKeeby JL. Vitrified blastocyst transfer cycles with the use of only vaginal progesterone replacement with Endometrin have inferior ongoing pregnancy rates: results from the planned interim analysis of a three-arm randomized controlled noninferiority trial. *Fertil Steril* (2018) 109:266–75. doi: 10.1016/j.fertnstert.2017.11.004

97. Devine K, Richter KS, Jahandideh S, Widra EA, McKeeby JL. Intramuscular progesterone optimizes live birth from programmed frozen embryo transfer: a randomized clinical trial. *Fertil Steril* (2021) 116:633–43. doi: 10.1016/j.fertnstert.2021.04.013

98. Vuong LN, Pham TD, Le KTQ, Ly TT, Le HL, Nguyen DTN, et al. Micronized progesterone plus dydrogesterone versus micronized progesterone alone for luteal phase support in frozen-thawed cycles (MIDRONE): a prospective cohort study. *Hum Reprod* (2021) 36:1821–31. doi: 10.1093/humrep/deab093

99. Scott R, Navot D, Liu HC, Rosenwaks Z. A human in vivo model for the luteoplacental shift. *Fertil Steril* (1991) 56:481–4. doi: 10.1016/S0015-0282(16)54544-0

100. Neumann K, Depenbusch M, Schultze-Mosgau A, Griesinger G. Characterization of early pregnancy placental progesterone production by use of dydrogesterone in programmed frozen-thawed embryo transfer cycles. *Reprod BioMed Online* (2020) 40:743–51. doi: 10.1016/j.rbmo.2020.01.019

101. Wu HM, Chang HM, Leung PCK. Gonadotropin-releasing hormone analogs: Mechanisms of action and clinical applications in female reproduction. *Front Neuroendocrinol* (2021) 60:100876. doi: 10.1016/j.yfrne.2020.100876

102. Cheng KW, Nathwani PS, Leung PC. Regulation of human gonadotropin-releasing hormone receptor gene expression in placental cells. *Endocrinology* (2000) 141:2340–9. doi: 10.1210/endo.141.7.7543

103. Coccia ME, Comparetto C, Bracco GL, Scarselli G. GnRH antagonists. *Eur J Obstet Gynecol Reprod Biol* (2004) 115 Suppl 1:S44–56. doi: 10.1016/j.ejogrb.2004.01.033

104. Vidal C, Giles J, Garrido N, Remohi J, Simon C, Bellver J, et al. GnRH antagonist for endometrial priming in an oocyte donation programme: a prospective, randomized controlled trial. *Reprod BioMed Online* (2018) 37:415–24. doi: 10.1016/j.rbmo.2018.06.026

105. Dal Prato L, Borini A, Cattoli M, Bonu MA, Sciajno R, Flamigni C. Endometrial preparation for frozen-thawed embryo transfer with or without pretreatment with gonadotropin-releasing hormone agonist. *Fertil Steril* (2002) 77:956–60. doi: 10.1016/S0015-0282(02)02960-6

106. van de Vijver A, Polyzos NP, Van Landuyt L, De Vos M, Camus M, Stoop D, et al. Cryopreserved embryo transfer in an artificial cycle: is GnRH agonist down-regulation necessary? *Reprod BioMed Online* (2014) 29:588–94. doi: 10.1016/j.rbmo.2014.08.005

107. El-Toukhy T, Taylor A, Khalaf Y, Al-Darazi K, Rowell P, Seed P, et al. Pituitary suppression in ultrasound-monitored frozen embryo replacement cycles. *A randomised study. Hum Reprod* (2004) 19:874–9. doi: 10.1093/humrep/deh183

108. Xia L, Tian L, Zhang S, Huang J, Wu Q. Hormonal replacement treatment for frozen-thawed embryo transfer with or without gnRH agonist pretreatment: A retrospective cohort study stratified by times of embryo implantation failures. *Front Endocrinol (Lausanne)* (2022) 13:803471. doi: 10.3389/fendo.2022.803471

109. Tomas C, Alsbjerg B, Martikainen H, Humaidan P. Pregnancy loss after frozen-embryo transfer–a comparison of three protocols. *Fertil Steril* (2012) 98:1165–9. doi: 10.1016/j.fertnstert.2012.07.1058

110. Kawamura T, Motoyama H, Yanaihara A, Yorimitsu T, Arichi A, Karasawa Y, et al. Clinical outcomes of two different endometrial preparation methods for cryopreserved-thawed embryo transfer in patients with a normal menstrual cycle. *Reprod Med Biol* (2007) 6:53–7. doi: 10.1111/j.1447-0578.2007.00165.x

111. Hancke K, More S, Kreienberg R, Weiss JM. Patients undergoing frozen-thawed embryo transfer have similar live birth rates in spontaneous and artificial cycles. *J Assist Reprod Genet* (2012) 29:403–7. doi: 10.1007/s10815-012-9724-z

112. Lathi RB, Chi YY, Liu J, Saravanabavanandhan B, Hegde A, Baker VL. Frozen blastocyst embryo transfer using a supplemented natural cycle protocol has a similar live birth rate compared to a programmed cycle protocol. *J Assist Reprod Genet* (2015) 32:1057–62. doi: 10.1007/s10815-015-0499-x

113. Alur-Gupta S, Hopeman M, Berger DS, Gracia C, Barnhart KT, Coutifaris C, et al. Impact of method of endometrial preparation for frozen blastocyst transfer on pregnancy outcome: a retrospective cohort study. *Fertil Steril* (2018) 110:680–6. doi: 10.1016/j.fertnstert.2018.05.013

114. Chang EM, Han JE, Kim YS, Lyu SW, Lee WS, Yoon TK. Use of the natural cycle and vitrification thawed blastocyst transfer results in better in-vitro fertilization outcomes: cycle regimens of vitrification thawed blastocyst transfer. *J Assist Reprod Genet* (2011) 28:369–74. doi: 10.1007/s10815-010-9530-4

115. Guan Y, Fan H, Styer AK, Xiao Z, Li Z, Zhang J, et al. A modified natural cycle results in higher live birth rate in vitrified-thawed embryo transfer for women with regular menstruation. *Syst Biol Reprod Med* (2016) 62:335–42. doi: 10.1080/19396368.2016.1199064

116. Liu X, Shi W, Shi J. Natural cycle frozen-thawed embryo transfer in young women with regular menstrual cycles increases the live-birth rates compared with hormone replacement treatment: a retrospective cohort study. *Fertil Steril* (2020) 113:811–7. doi: 10.1016/j.fertnstert.2019.11.023

117. Hill MJ, Miller KA, Frattarelli JL. A GnRH agonist and exogenous hormone stimulation protocol has a higher live-birth rate than a natural endogenous hormone protocol for frozen-thawed blastocyst-stage embryo transfer cycles: an analysis of 1391 cycles. *Fertil Steril* (2010) 93:416–22. doi: 10.1016/j.fertnstert.2008.11.027

118. Givens CR, Markun LC, Ryan IP, Chenette PE, Herbert CM, Schriock ED. Outcomes of natural cycles versus programmed cycles for 1677 frozen-thawed embryo transfers. *Reprod BioMed Online* (2009) 19:380–4. doi: 10.1016/S1472-6483(10)60172-1

119. Veleva Z, Tiitinen A, Vilska S, Hyden-Granskog C, Tomas C, Martikainen H, et al. High and low BMI increase the risk of miscarriage after IVF/ICSI and FET. *Hum Reprod* (2008) 23:878–84. doi: 10.1093/humrep/den017

120. Aslih N, Dorzia D, Atzmon Y, Estrada D, Ellenbogen A, Bilgory A, et al. Ovulatory-based FET cycles may achieve higher pregnancy rates in the general population and among anovulatory women. *J Clin Med* (2021) 10. doi: 10.3390/jcm10040703

121. Zaat TR, Kostova EB, Korsen P, Showell MG, Mol F, van Wely M. Obstetric and neonatal outcomes after natural versus artificial cycle frozen embryo transfer and the role of luteal phase support: a systematic review and meta-analysis. *Hum Reprod Update* (2023). doi: 10.1093/humupd/dmad011

122. Wu H, Zhou P, Lin X, Wang S, Zhang S. Endometrial preparation for frozen-thawed embryo transfer cycles: a systematic review and network meta-analysis. *J Assist Reprod Genet* (2021) 38:1913–26. doi: 10.1007/s10815-021-02125-0

123. Groenewoud ER, Cohlen BJ, Al-Oraiby A, Brinkhuis EA, Broekmans FJ, de Bruin JP, et al. A randomized controlled, non-inferiority trial of modified natural versus artificial cycle for cryo-thawed embryo transfer. *Hum Reprod* (2016) 31:1483–92. doi: 10.1093/humrep/dew120

124. Zhang J, Liu H, Wang Y, Mao X, Chen Q, Fan Y, et al. Letrozole use during frozen embryo transfer cycles in women with polycystic ovary syndrome. *Fertil Steril* (2019) 112:371–7. doi: 10.1016/j.fertnstert.2019.04.014

125. Aleyasin A, Aghahosseini M, Safdarian L, Noorzadeh M, Fallahi P, Rezaeian Z, et al. Can letrozole plus HMG protocol improve pregnancy outcomes in frozen-thawed embryo transfer? An RCT. *Int J Reprod BioMed* (2017) 15:83–6. doi: 10.29252/ijrm.15.2.83

126. Peigne M, Devouche E, Ferraretto X, Gricourt S, Luton D, Patrat C, et al. Higher live birth rate with stimulated rather than artificial cycle for frozen-thawed embryo transfer. *Eur J Obstet Gynecol Reprod Biol* (2019) 243:144–9. doi: 10.1016/j.ejogrb.2019.10.040

127. Sheikhi O, Golsorkhtabaramiri M, Esmaeilzadeh S, Mahouti T, Heidari FN. Reproductive outcomes of vitrified blastocyst transfer in modified natural cycle versus mild hormonally stimulated and artificial protocols: A randomized control trial. *JBRA Assist Reprod* (2018) 22:221–7. doi: 10.5935/1518-0557.20180040

128. Miravet-Valenciano JA, Rincon-Bertolin A, Vilella F, Simon C. Understanding and improving endometrial receptivity. *Curr Opin Obstet Gynecol* (2015) 27:187–92. doi: 10.1097/GCO.0000000000000173

129. Cha J, Sun X, Dey SK. Mechanisms of implantation: strategies for successful pregnancy. *Nat Med* (2012) 18:1754–67. doi: 10.1038/nm.3012

130. Horcajadas JA, Pellicer A, Simon C. Wide genomic analysis of human endometrial receptivity: new times, new opportunities. *Hum Reprod Update* (2007) 13:77–86. doi: 10.1093/humupd/dml046

131. Diaz-Gimeno P, Horcajadas JA, Martinez-Conejero JA, Esteban FJ, Alama P, Pellicer A, et al. A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature. *Fertil Steril* (2011) 95:50–60, 60 e51-15. doi: 10.1016/j.fertnstert.2010.04.063

132. Tan J, Kan A, Hitkari J, Taylor B, Tallon N, Warraich G, et al. The role of the endometrial receptivity array (ERA) in patients who have failed euploid embryo transfers. *J Assist Reprod Genet* (2018) 35:683–92. doi: 10.1007/s10815-017-1112-2

133. Neves AR, Devesa M, Martinez F, Garcia-Martinez S, Rodriguez I, Polyzos NP, et al. What is the clinical impact of the endometrial receptivity array in PGT-A and oocyte donation cycles? *J Assist Reprod Genet* (2019) 36:1901–8. doi: 10.1007/s10815-019-01535-5

134. Bassil R, Casper R, Samara N, Hsieh TB, Barzilay E, Orvieto R, et al. Does the endometrial receptivity array really provide personalized embryo transfer? *J Assist Reprod Genet* (2018) 35:1301–5. doi: 10.1007/s10815-018-1190-9

135. Doyle N, Jahandideh S, Hill MJ, Widra EA, Levy M, Devine K. Effect of timing by endometrial receptivity testing vs standard timing of frozen embryo transfer on live birth in patients undergoing in vitro fertilization: A randomized clinical trial. *JAMA* (2022) 328:2117–25. doi: 10.1001/jama.2022.20438

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 19 October 2023
DOI 10.3389/fendo.2023.1293576

OPEN ACCESS

EDITED BY
Emre Pabuccu,
Ufuk University, Türkiye

REVIEWED BY
Yavuz Emre Şükür,
Ankara University, Türkiye
Sabri Cavkaytar,
Üsküdar University, Türkiye

*CORRESPONDENCE
Ismail Guler
driguler@yahoo.com

RECEIVED 13 September 2023
ACCEPTED 03 October 2023
PUBLISHED 19 October 2023

CITATION
Guler I, Demirdag E, Akdulum MFC, Polat M, Erdem A and Erdem M (2023) Serum LH level prior to progestin administration is significant on pregnancy and live birth in programmed frozen-thawed embryo transfer cycles. *Front. Endocrinol.* 14:1293576. doi: 10.3389/fendo.2023.1293576

Serum LH level prior to progestin administration is significant on pregnancy and live birth in programmed frozen-thawed embryo transfer cycles

Ismail Guler*, Erhan Demirdag, Munire F. C. Akdulum, Mert Polat, Ahmet Erdem and Mehmet Erdem

Gazi University School of Medicine, Department of Obstetrics and Gynecology, Ankara, Türkiye

Purpose: To evaluate the impact of serum LH levels prior to progestin administration on the outcomes of programmed frozen-thawed embryo transfer (FET) cycles.

Methods: Retrospective cohort study was conducted to compare the treatment outcomes between four groups based on the 25 percentiles of serum LH levels before progestin administration in 596 cycles of 518 patients undergoing artificial endometrial preparation protocols for FET. Primary outcome measures were ongoing and live birth rates. Secondary outcome measures were the pregnancy rates, clinical pregnancy rates, and pregnancy loss rates.

Results: The trends in clinical pregnancy (CPR) and live birth rates (LBR) increased from the first to the fourth quartile (Q1 to Q4) of serum LH levels prior to progestin administration (37,0% to 48,3%, p = 0.042, and 22.6% to 39.5%, respectively, p = 0.003). Pregnancy loss rates (PLR) were higher in group Q1, although the difference was not statistically significant. Based on a multivariate logistic regression analysis, a low serum LH level before progestin initiation was found to be the most significant predictor associated with a negative effect on live birth (OR: 0,421, 95% CI 0,178 – 0,994, p=0,048). The day of estrogen initiation was significantly correlated with serum LH levels and quartiles of serum LH levels before progestin administration (r=0,200, p=0,015 and r=0,215, p=0,009, respectively).

Conclusion: The serum LH level prior to progestin administration significantly affects pregnancy and live birth rates in patients undergoing an artificial endometrial preparation protocol for FET. LH monitoring should be incorporated into the follow-up, in addition to assessing endometrial thickness and morphology in artificial FET cycles.

KEYWORDS

frozen-thawed embryo transfer, luteinizing hormone, estrogen, artificial endometrial preparation, programmed cycle

Introduction

Due to advances in vitrification methods, the elimination of OHSS, and increased rates of preimplantation genetic testing, frozen-thawed embryo transfers (FETs) are being performed more prevalent than ever worldwide (1). So, ensuring that the endometrium is ready for the implantation of frozen-thawed embryos is crucial. Natural cycles or modified natural cycles by the trigger of ovulation, letrozole ovulation induction, and artificial endometrial preparation protocols can be used for the timing of FET. The artificial FET cycle, using estrogen alone first and then its combination with progestins to represent proliferative and secretory phases of the endometrium, is a suitable protocol, especially for oligo-anovulatory patients. It provides comparable pregnancy rates and flexibility in clinics dealing with a high volume of IVF cycles (2). FET in an artificial protocol is usually scheduled after the endometrium with a trilaminar pattern is detected to be more than 7mm thick with estrogen treatment (3). In addition, some variations of the artificial protocol to optimize treatment outcomes include down-regulation with GnRH agonists and starting doses of estrogen in a fixed or incremental fashion, with or without monitoring levels of serum steroid hormones.

In physiology, FSH selects the follicular cohort in the last few days of the previous cycle, and then stimulates follicular growth and estrogen synthesis from granulosa cells, which helps to proliferate the endometrium (4). In the follicular phase, increased estrogen levels first inhibit luteinizing hormone (LH) and then stimulate an LH surge after reaching and remaining above a critical level. Increased LH around midcycle has several well-known gonadal effects by binding to its LH/hCG receptor (LHCG-R), including ovulation of a mature oocyte, stimulation of androgen synthesis in theca cells, and production of progesterone from the corpus luteum to promote secretory changes in the endometrium for possible embryo implantation. LHCG-Rs have also been shown in many extragonadal tissues, including the endometrium in both its epithelial and stromal cells, although their exact roles have yet to be identified. However, preliminary studies suggest that LH and endometrial LHCG-R may affect implantation, pregnancy maintenance (5), endometrial vascular development, and the modulation of uterine receptivity (6).

In some artificial FET cycles without desensitization, synthetic estrogen increases serum LH levels just before progestin initiation, like what happens in a natural midcycle. Only a few clinical trials have assessed the importance of increased LH levels before progestin supplementation in artificial FET cycles, revealing that this is associated with similar (7, 8) or improved outcomes (9). This context may also explain better pregnancy outcomes in artificial FET cycles with unplanned, spontaneous follicular growth and ovulation (10).

Although the timing of progestin initiation and FET in artificial cycles after a period of estrogen treatment were mainly based on the detection of sufficient endometrial thickness and trilaminar morphology, the importance of LH levels just before the progestin initiation may change daily practice. Therefore, we aimed to investigate the effect of serum LH levels before progestin administration on the outcomes of artificial FET cycles.

Materials and methods

We reviewed the records of 2508 THAW – ET cycles in the IVF Unit of Gazi University School of Medicine and Novaart IVF Center between January 28, 2014, and October 14, 2022. In this retrospective study, we included 596 cycles of 518 patients with an artificial endometrial preparation protocol without desensitization. Exclusion criteria included having protocols for frozen embryo transfer (FET) other than programmed cycles. Cycles involving hypogonadotropic patients, as well as those that underwent desensitization and were canceled before embryo transfer due to endometrium-related factors such as polyps, fluid, or irregularities, elevated progesterone levels, embryo degeneration after thawing, and any patient-related factors identified during the treatment follow-up period, were also excluded. Treatment outcomes were compared between four groups regarding 25 percentiles of serum LH levels before progestin administration. Institutional Review Board (IRB) approval was obtained from The Institutional Review Board and Ethics Committee of Gazi University School of Medicine.

In the study, all oocytes were fertilized with intracytoplasmic sperm injection (ICSI), and embryos were cryopreserved with the vitrification method. In the artificial endometrial preparation protocol for transferring vitrified and thawed embryos, patients started estradiol treatment (Estrofem 2mg, Novo Nordisk®, Istanbul, Turkey) two or three times daily, depending on the clinician's preference, during their 2^{nd}-4^{th} of menstrual cycles after the baseline evaluation. Patients were re-evaluated by transvaginal ultrasonography 6-7 days later to check the endometrium. If endometrial thickness was detected as ≥7mm with a trilaminar pattern, serum LH, estradiol, and progestin levels were measured. Then, initiation of vaginal progestin (Crinone 8% gel 90mg, qd, Merck®, İstanbul, Turkey, or Progestan capsule 200mg, 2q12h, Koçak Farma ®, İstanbul, Turkey) was planned within a day. If the thickness and morphology of the endometrium were insufficient, the dose and duration of estradiol were readjusted accordingly. Patients continued to be followed at 2-3 days intervals until the endometrium reached the ≥7mm thickness for the initiation of progestin. Although estrogen therapy was extended with the goal of achieving an endometrial thickness of ≥7mm before initiating progesterone, in cases where the endometrium did not reach 7mm, embryo transfer was also performed for patients who achieved an endometrial thickness of 6mm or more. If serum progesterone level was already elevated at the time of the decision to start progestin administration or if the endometrial thickness remained too thin < 6mm, the cycles were canceled. The duration of progestin before FET was 3 or 5 days based on the age of cleavage or blastocyst stage embryos, respectively. On the day of planned FET, after the quality of thawed embryos was re-assessed based on the Istanbul consensus (11), embryo transfer was performed with a catheter (Wallace; CooperSurgical®, Ballerup, Denmark) under the guidance of suprapubic pelvic ultrasonography. The maximum number of transferred embryos was 2, depending on legal regulation. A pregnancy test was measured on the 14^{th} day after FET. The rate of positivity of pregnancy test and visible intrauterine gestational sac with ultrasonography or pathology result of products

10.3389/fendo.2023.1293576

TABLE 1 Clinical and hormonal characteristics and cycle outcomes of cases in the study.

Variable		Data
Age (years)		31,9 ± 5,1
Duration of infertility (years)		5,05 ± 3,75
BMI (kg/m^2)		24,4 ± 4,5
Diagnosis of Infertility	Unexplained	40,5%
	Female Factor	27%
	Male Factor	26,1%
	Both Female & Male Factor	6,4%
Etiology of Female Infertility	Unexplained	67,6%
	Ovulatory Factor	24,9%
	Tubal Factor	4,8%
	Decreased Ovarian Reserve	2,7%
Duration of Estrogen administration before progestin initiation (days)*		8 (7, 10)
On the day of Progestin initiation,	serum LH (mIU/mL)	17,17 ± 9,41
	serum Estradiol (pg/ml)	228,45 ± 108,18
	endometrial thickness	10,08 ± 3,95
Number of Transferred Embryos		1,6 ± 0,4
Age of Transferred Embryos	Cleavage	29,5%
	Blastocyst	70,5%
Quality of Transferred Embryos	Moderate & Good	83,7%
	Poor Quality	16,3%
Outcomes per cycle (n, Rate)	Pregnancy Rate	318, 53,4%
	Clinical Pregnancy Rate	245, 41,1%
	Ongoing Pregnancy Rate	193, 32,4%
	Live Birth Rate	172, 28,9%

Data are mean ± standard deviation, * median (Q1, Q3) or rate %, BMI, body mass index; AFC, antral follicle count; FSH, follicle stimulating hormone; E2, estradiol; LH, Luteinizing hormone.

of conception after an abortion indicate pregnancy and clinical pregnancy rates, respectively. An ongoing pregnancy is defined as a live fetus beyond the 20th gestational week, and a live birth refers to a baby who has survived for at least one week after birth.

The data were analyzed with Statistical Package for Social Sciences (SPSS, Version 28, IBM, Chicago, IL, USA). Skewness, kurtosis, and Kolmogorov-Smirnov tests were used as normality tests. One-Way ANOVA and chi-squared tests were used to compare parametric and categorical data between groups based on 25 percentiles of serum LH levels before progestin initiation. Multivariate logistic regression analysis was performed to identify the most significant variables in predicting live birth. Primary outcome measures were live birth rate (LBR) and ongoing pregnancy rate (OPR). Secondary outcome measures included pregnancy rate (PR), clinical pregnancy rate (CPR), biochemical pregnancy loss rate, and clinical pregnancy loss rate. $p < 0.05$ was used for statistical significance.

TABLE 2 Values of 25 percentiles of serum LH prior to the progestin initiation.

	25th	50th	75th
Serum LH (mIU/mL)	10	15,88	22,21

Results

Within the study period, among the 599 cases who received programmed frozen-thawed embryo transfer cycle, we identified 55 cycles out of 741 (7,4%) that were canceled for various reasons. Causes of cycle cancellation included endometrium-related factors (such as a remaining thickness <6mm, polyp, fluid, or irregularity) (n=22, 2,9%), elevated progesterone (n=11, 1,4%), degeneration of embryos after thawing (n=8, 1%), and various patient-related factors (n=14, 1,8%) detected during the follow-up period of treatment. Cycles in which embryo transfer was canceled for these reasons, as well as those with an absence of LH levels before progesterone initiation, were excluded from the analysis. Finally, data from 596 cycles of 518 patients were included for analysis in this study.

The baseline characteristics of cases and cycle outcomes of the FET with artificial endometrial preparation are listed in Table 1. The median duration of Estrogen administration before progestin initiation was 8 days.

Table 2 presents the 25 percentiles of serum LH values prior to progestin initiation, while Table 3 compares characteristics and cycle outcomes between groups based on these values. The group with serum LH <25th percentile had significantly lower rates of live birth, ongoing pregnancy, clinical pregnancy, and pregnancy, compared to other quartiles. Rates of PR, CPR, OPR, and LBR increased from Q1 to Q4 of serum LH level before progestin administration. However, biochemical, and clinical pregnancy loss rates were similar among groups. Early initiation of estrogen treatment was significantly associated with a higher likelihood of being in the first quartile of serum LH before progestin administration. There was a significant correlation between the day of estrogen initiation and both the serum LH levels and quartiles of serum LH level prior to progestin administration (r=0,200, p=0,015 and r=0,215, p=0,009, respectively).

According to the results of the multivariable logistic regression analysis, the most significant variable for predicting live birth was a low level of serum LH (<25th percentile) prior to progestin start (OR: 0,421, 95% CI 0,178 – 0,994, p=0,048), which outperformed other factors, including age, BMI, endometrial thickness prior to starting progestin, number of transferred embryos, embryo age, and quality, Table 4.

10.3389/fendo.2023.1293576

TABLE 3 Comparison of demographic variables and cycle outcomes between quartiles based on 25 percentiles of serum LH prior to the progestin initiation.

		1ST quartile	2nd quartile	3rd quartile	4th quartile	*p*
Age (years)		31,3 ± 4,8	31,8 ± 5,1	33,0 ± 5,3	31,3 ± 5,0	**0,011**
BMI (kg/m^2)		25,8 ± 4,9	24,0 ± 4,2	23,9 ± 4,5	23,6 ± 4,2	**0,000**
Infertility period (years)		5,2 ± 3,7	5,4 ± 4,4	4,9 ± 3,4	4,5 ± 3,2	0,224
Basal AFC		20,0 ± 8,0	17,5 ± 6,8	18,5 ± 6,5	18,6 ± 6,7	0,506
Cycle day of estrogen initiation	Day 2	39,1%	31,6%	31%	20%	**0,009**
	Day 3	34,8%	31,6%	31%	25,6%	
	Day 4	26,1%	36,8%	37,9%	45,7%	
Prior to starting progestin,	LH (mIU/mL)	7,09 ± 2,0	12,6 ± 1,7	18,7 ± 1,9	30,07 ± 7,3	**0,000**
	E2 (pg/ml)	233,0 ± 118,8	218,8 ± 100,9	235,9 ± 108,4	226,3 ± 104,3	0,525
	Progesterone (ng/mL) *	0,26 (0,13, 0,50)	0,3 (0,13, 0,70)	0,3 (0,15, 0,76)	0,3 (0,16, 0,60)	0,393
	Endometrial Thickness	9,9 ± 1,7	10,0 ± 1,5	10,4 ± 7,3	9,8 ± 1,74	0,649
Number of transferred embryos	1 (n)	36,3% (53/146)	34,9% (53/152)	36,2% (54/149)	40,3% (60/149)	0,790
	2 (n)	63,7% (93/146)	65,1% (99/152)	63,8% (95/149)	59,7% (89/149)	
Age of transferred embryos	Cleavage (n)	29,9% (43/144)	34,7% (52/150)	26,0% (38/146)	27,3% (39/143)	0,373
	Blastocyst (n)	70,1%(101/144)	65,3% (98/150)	74,0% (108/146)	72,7%(104/143)	
Quality of transferred embryos	Moderate & Good (n)	84,1% (69/82)	85,7% (72/84)	81,5% (66/81)	83,3% (60/72)	0,905
	Poor (n)	15,9% (13/82)	14,3% (12/84)	18,5% (15/81)	16,7% (12/72)	
Primary Outcomes						
Live Birth Rate, (n)		22,6%, (33/146)	26,3%, (40/152)	28,2%, (42/149)	39,5%, (57/149)	**0,003**
Ongoing Pregnancy Rate, (n)		24%, (35/146)	32,9%, (50/152)	30,9%, (46/149)	41,6%, (62/149)	**0,003**
Secondary Outcomes						
Clinical Pregnancy Rate, (n)		37,0%, (54/146)	38,2%, (58/152)	40,9%, (61/149)	48,3%, (72/149)	**0,042**
Pregnancy Rate, (n)		45,9%, (67/146)	49,3%, (75/152)	59,7%, (89/149)	58,4%, (87/149)	**0,009**
Biochemical Pregnancy Loss Rate, (n)		7,5%, (11/146)	9,2%, (14/152)	14,8%, (22/149)	8,7%, (13/149)	0,166
Clinical Pregnancy Loss Rate,		12,3%, (18/146)	5,9%, (9/152)	10,1%, (15/149)	7,4%, (11/149)	0,216

Data are mean ± standard deviation, * median (Q1, Q3) or rate (%). One Way ANOVA or chi-squared test was used for comparisons as appropriate. BMI, body mass index; AFC, antral follicle count; FSH, follicle stimulating hormone; LH, luteinizing hormone; E2, estradiol. Statistically significant *p* values were written bold.

Discussion

Observations from daily clinical practice suggest that higher levels of LH upon progestin initiation may result in increased pregnancy rates during frozen embryo transfer cycles with an artificial endometrial preparation protocol, despite a limited number of relevant reports in the literature. Therefore, this study was conducted to evaluate the impact of serum LH levels prior to progestin administration on the outcomes of FET cycles that use an artificial endometrial preparation protocol without desensitization. Our data indicates that higher serum LH levels prior to progestin initiation for FET were associated with increased rates of clinical pregnancy and live birth. Furthermore, being in the first quartile of LH levels prior to progestin initiation was found to be a significant negative predictor for live birth in FET cycles with artificial endometrial preparation protocol. Additionally, this study is unique in that it evaluates the impact of the cycle day of estrogen therapy initiation on LH levels prior to progestin administration. Our findings suggest that initiating estrogen therapy on cycle day 2 or 3, as opposed to cycle day 4, was associated with lower serum LH levels prior to progestin administration.

The role of LH levels on FET outcomes during artificial cycles has been evaluated in only a few studies. In the first study, which evaluated the role of LH levels on FET outcomes during artificial cycles, three study groups were established based on the 0-25th, 25th-75th, and 75th-100th percentiles of LH levels on day 14 of the artificial cycle. The results showed statistically similar pregnancy rates per ET among the groups, but CPRs increased from 12% to 16% from group 1 to group 3 (7). In the second study, which had a different design, the authors examined the role of LH rise, as measured by a doubling of LH levels on the 10th day of estrogen administration, in artificial FET cycles. They found that LH rise

10.3389/fendo.2023.1293576

TABLE 4 Multivariate logistic regression analysis of possible predictors for live birth in frozen thawed embryo transfer cycles with artificial endometrial preparation.

Variable		Odds Ratio	95% Confidence Interval	*p*
Age		1,006	0,950 – 1,065	0,838
BMI		0,957	0,890 – 1,029	0,237
LH levels prior to starting progestin*	1st quarter	0,421	0,178 – 0,994	**0,048**
	2nd quarter	0,719	0,334 – 1,547	0,410
	3rd quarter	0,649	0,297 – 1,419	0,184
	4th quarter	reference		
Embryo Quality		1,384	0,582 – 3,294	0,462
Embryo Age		1,270	0,694 – 2,325	0,438
Endometrial thickness prior to starting progestin		1,027	0,855 – 1,232	0,779
Number of transferred Embryos		1,367	0,721 – 2,591	0,338

*Categorical Covariate, BMI, body mass index.
Statistically significant *p* value was written bold.

occurred in approximately 2/3 of cycles, but this did not significantly impact cycle outcomes (8). The third study reported that the group in the first quartile, based on the 25th percentile of serum LH levels before the initiation of progestin, had a significantly lower live birth rate (LBR) as the primary outcome after an artificial (FET) cycle (9). Additionally, pregnancy loss rates were found to be significantly higher in the first and second quartiles compared to the other quartiles. Consistent with the previous study, both pregnancy and live birth rates increased from the first through fourth quartile of serum LH levels prior to the day of progestin initiation in our study. Also, being in the first quartile predicted a negative effect on live birth when other confounders were considered in the regression analysis. However, in contrast, pregnancy loss rates did not differ significantly among the study groups.

This study and the previous trial mentioned above showed that LH levels before the day of progestin administration may have implications for implantation in women undergoing FET cycles. Although we do not know exactly how the mid-cycle increase in serum LH affects implantation, in addition to its well-known effects on ovulation and oocyte maturation, there is evidence suggesting possible mechanisms. In molecular studies, LH has been shown to modulate implantation by binding to endometrial LHCG-Rs that are strongly expressed during the implantation window in mice (12). Increased expression of human endometrial LHCG-R in the mid-luteal phase has also been demonstrated, suggesting cross-talk between hCG and LH to enhance endometrial receptivity (13). LH and LHCG-R may also regulate endometrial angiogenesis, which is important for uterine receptivity and implantation (5, 6).

A recent systematic review on endometrial preparation for FET showed that natural or modified natural cycles are superior to artificial FET protocols (14). A significantly lower pregnancy loss rate was also found in natural and letrozole FETs compared to programmed FETs (15). It can be proposed that the superiority of natural and modified natural protocols over artificial FET cycles is related to LH function. All natural, modified natural, and letrozole FETs depend on the LH rise, but not all artificial FET cycles are associated with an LH rise. In a study based on the theory of endometrial LHCG-R, subcutaneous injections of low-dose hCG during the proliferative phase in artificial FET cycles, with and without desensitization, were successful in increasing endometrial thickness and receptivity in patients with a history of recurrent implantation failure and thin endometrium (16). These clinical results also indirectly support the effectiveness of LH in implantation. In this context, it can be speculated that some methods that reduce LH, such as pre-cycle oral contraceptive use or starting the second artificial cycle without a break, may also be associated with adverse pregnancy outcomes. Low pregnancy rates in GnRH agonist-triggered fresh cycles may be related to an early and rapid rise and fall in endogenous LH levels, which could be directly detrimental to the endometrium in addition to causing early luteolysis (17).

To improve outcomes based on the significant findings of this study, we can consider avoiding desensitization protocols, which can suppress LH levels. Additionally, initiating estrogen on day 4 instead of day 2 or 3 may be helpful in achieving the desired serum LH levels prior to progestin initiation, as early onset of estrogen administration may be associated with a rapid rise and subsequent fall of serum LH levels. Patients with a history of recurrent implantation failure and low serum LH levels prior to progestin initiation with preferred endometrial thickness and morphology may benefit from natural, modified natural, or letrozole ovulation induction protocols in FET cycles. Another strategy is initiating low-dose subcutaneous hCG injections concomitantly with progestin administration to activate LHCG-R in the endometrium. While no study in the literature have evaluated this, step-up estrogen administration may also offer a potential alternative to the late initiation of estrogen therapy (on the 4th day instead of the 2nd or 3rd day of menstruation) to achieve higher levels of LH before progestin initiation. Although a recent study compared two step-up estrogen protocols to a fixed-dose

estrogen regimen without desensitization, it did not assess LH levels before progestin initiation and estrogen was started on the 2nd or 3rd day of menstruation (18). Nevertheless, the pregnancy rates were comparable among all three groups.

In conclusion, while this study did not evaluate serum LH levels throughout the period of estrogen supplementation, low LH levels at the start of progestin administration may be indicative of an early rise in LH, which may be in a downward trend or may have already fallen and not yet risen. Nevertheless, this study, which is the second of its kind in the literature, revealed a significant association between low serum LH levels at the onset of progestin and reduced pregnancy and live birth rates in programmed frozen embryo transfer (FET) cycles. Given that early initiation of estrogen therapy may be associated with lower LH levels prior to progestin administration, delaying estrogen initiation until the 4th day of the cycle may be a beneficial strategy for improving pregnancy and live birth outcomes in frozen-thawed embryo transfer cycles.

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 humans were approved by The Institutional Review Board and Ethics Committee of Gazi University School of Medicine. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

IG: Writing – original draft, Writing – review & editing. ED: Data curation, Writing – review & editing. MA: Data curation, Writing – review & editing. MP: Data curation, Writing – review & editing. AE: Methodology, Supervision, Writing – review & editing. ME: Data curation, Methodology, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We thank Gazi University for supporting this study.

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

References

1. Fu Y, Chen D, Cai B, Xu Y, Zhu S, Ding C, et al. Comparison of two mainstream endometrial preparation regimens in vitrified-warmed embryo transfers after PGT. *Reprod BioMed Online* (2022) 44(2):239–46. doi: 10.1016/j.rbmo.2021.09.009

2. Roelens C, Blockeel C. Impact of different endometrial preparation protocols before frozen embryo transfer on pregnancy outcomes: a review. *Fertil Steril* (2022) 118 (5):820–27. doi: 10.1016/j.fertnstert.2022.09.003

3. Lensen S, Lantsberg D, Gardner DK, Sophian AD, Wandafiana N, Kamath MS. The role of timing in frozen embryo transfer. *Fertil Steril* (2022) 118(5):832–38. doi: 10.1016/j.fertnstert.2022.08.009

4. Macklon NS, Fauser BC. Follicle-stimulating hormone and advanced follicle development in the human. *Arch Med Res* (2001) 32(6):595–600. doi: 10.1016/s0188-4409(01)00327-7

5. Han SW, Lei ZM, Rao CV. Treatment of human endometrial stromal cells with chorionic gonadotropin promotes their morphological and functional differentiation into decidua. *Mol Cell Endocrinol* (1999) 147(1-2):7–16. doi: 10.1016/s0303-7207(98)00240-8

6. Filicori M, Fazleabas AT, Huhtaniemi I, Licht P, Rao Ch V, Tesarik J, et al. Novel concepts of human chorionic gonadotropin: reproductive system interactions and potential in the management of infertility. *Fertil Steril* (2005) 84(2):275–84. doi: 10.1016/j.fertnstert.2005.02.033

7. Griesinger G, Weig M, Schroer A, Diedrich K, Kolibianakis EM. Mid-cycle serum levels of endogenous LH are not associated with the likelihood of pregnancy in artificial frozen-thawed embryo transfer cycles without pituitary suppression. *Hum Reprod* (2007) 22(10):2589–93. doi: 10.1093/humrep/dem207

8. Khoury S, Kadour-Peero E, Calderon I. The effect of LH rise during artificial frozen-thawed embryo transfer (FET) cycles. *Reprod Fertil* (2021) 2(3):231–35. doi: 10.1530/RAF-21-0017

9. Zhou R, Zhang X, Dong M, Huang L, Zhu X, Wang S, et al. Association between endogenous LH level prior to progesterone administration and live birth rate in artificial frozen-thawed blastocyst transfer cycles of ovulatory women. *Hum Reprod* (2021) 36(10):2687–96. doi: 10.1093/humrep/deab172

10. Su Y, Ji H, Jiang W, Xu L, Lu J, Zhao C, et al. Effect of unplanned spontaneous follicular growth and ovulation on pregnancy outcomes in planned artificial frozen embryo transfer cycles: a propensity score matching study. *Hum Reprod* (2021) 36 (6):1542–51. doi: 10.1093/humrep/deab059

11. Alpha Scientists in Reproductive M and Embryology E.S.I.G.O. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. *Hum Reprod* (2011) 26(6):1270–83. doi: 10.1093/humrep/der037

12. Gridelet V, Tsampalas M, Berndt S, Hagelstein MT, Charlet-Renard C, Conrath V, et al. Evidence for cross-talk between the LH receptor and LH during implantation in mice. *Reprod Fertil Dev* (2013) 25(3):511–22. doi: 10.1071/RD11241

13. Choi J, Smitz J. Luteinizing hormone and human chorionic gonadotropin: distinguishing unique physiologic roles. *Gynecol Endocrinol* (2014) 30(3):174–81. doi: 10.3109/09513590.2013.859670

14. Mumusoglu S, Polat M, Ozbek IY, Bozdag G, Papanikolaou EG, Esteves SC, et al. Preparation of the endometrium for frozen embryo transfer: A systematic review. *Front Endocrinol (Lausanne)* (2021) 12:688237. doi: 10.3389/fendo.2021.688237

15. Godiwala P, Makhijani R, Bartolucci A, Grow D, Nulsen J, Benadiva C, et al. Pregnancy outcomes after frozen-thawed embryo transfer using letrozole ovulation induction, natural, or programmed cycles. *Fertil Steril* (2022) 118(4):690–98. doi: 10.1016/j.fertnstert.2022.06.013

16. Papanikolaou EG, Kyrou D, Zervakakou G, Paggou E, Humaidan P. "Follicular HCG endometrium priming for IVF patients experiencing resisting thin endometrium. A proof of concept study". *J Assist Reprod Genet* (2013) 30(10):1341–5. doi: 10.1007/s10815-013-0076-0

17. Humaidan P, Kol S, Papanikolaou EG. GnRH agonist for triggering of final oocyte maturation: time for a change of practice? *Hum Reprod Update* (2011) 17 (4):510–24. doi: 10.1093/humupd/dmr008

18. Sukur YE, Aslan B, Ozmen B, Sonmezer M, Berker B, Atabekoglu CS, et al. Impact of an estrogen replacement regimen on live birth rate in frozen-thawed good-quality embryo transfer. *Int J Gynaecol Obstet* (2023) 160(3):829–35. doi: 10.1002/ijgo.14366

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 20 October 2023
DOI 10.3389/fendo.2023.1224574

OPEN ACCESS

EDITED BY
Zhen-Gao Sun,
Affiliated Hospital of Shandong University of Traditional Chinese Medicine, China

REVIEWED BY
Xiushan Feng,
Fujian Medical University, China
Qingzhen Xie,
Renmin Hospital of Wuhan University, China

*CORRESPONDENCE
Sijia Lu
lusijia@yikongenomics.com
Yanping Li
liyanp@csu.edu.cn

†These authors have contributed equally to this work and share first authorship

RECEIVED 17 May 2023
ACCEPTED 28 August 2023
PUBLISHED 20 October 2023

CITATION
Huang X, Fu J, Zhang Q, Zhao J, Yao Z, Xia Q, Tang H, Xu A, He A, Liang S, Lu S and Li Y (2023) Integrated treatment guided by RNA-seq–based endometrial receptivity assessment for infertility complicated by MEN1. *Front. Endocrinol.* 14:1224574. doi: 10.3389/fendo.2023.1224574

Integrated treatment guided by RNA-seq–based endometrial receptivity assessment for infertility complicated by MEN1

Xi Huang[1,2†], Jing Fu[1,2†], Qiong Zhang[1,2], Jing Zhao[1,2], Zhongyuan Yao[1,2], Qiuping Xia[1,2], Hongying Tang[1,2], Aizhuang Xu[1,2], Aihua He[3], Shaolin Liang[4,5,6], Sijia Lu[7]* and Yanping Li[1,2]*

[1]Department of Reproductive Medicine, Xiangya Hospital, Central South University, Changsha, Hunan, China, [2]Clinical Research Center for Women's Reproductive Health in Hunan Province, Changsha, Hunan, China, [3]Department of Reproductive Medicine Center, The Third Xiangya Hospital, Central South University, Changsha, Hunan, China, [4]National Comprehensive Utilization of Science and Technology Information Resources and Public Service Center, Scientific and Technical Information (STI)-Zhilian Research Institute for Innovation and Digital Health, Beijing, China, [5]"Mobile Health" Ministry of Education-China Mobile Joint Laboratory, Xiangya Hospital, Central South University, Changsha, China, [6]Institute for Six-sector Economy,Fudan University, Shanghai, China, [7]Department of Clinical Research, Yikon Genomics Company, Ltd., Suzhou, Jiangsu, China

Background: Preimplantation genetic testing (PGT) serves as a tool to avoid genetic disorders in patients with known genetic conditions. However, once a selected embryo is transferred, implantation success is attained independent of embryo quality. Using PGT alone is unable to tackle implantation failure caused by endometrial receptivity (ER) abnormalities in these patients.

Methods: We validated our newly developed RNA-seq–based ER test (rsERT) in a retrospective cohort study including 511 PGT cycles and reported experience in treating an infertile female patient complicated by multiple endocrine neoplasia type 1 (MEN1).

Results: Significant improvement in the clinical pregnancy rate was found in the performed personalized embryo transfer (pET) group (CR, 69.7%; $P = 0.035$). In the rare MEN1 case, pET was done according to the prediction of the optimal time of window of implantation after unaffected blastocysts were obtained by PGT-M, which ultimately led to a healthy live birth. However, none of the mRNA variants identified in the patient showed a strong association with the MEN1 gene.

Conclusions: Applying the new rsERT along with PGT improved ART outcomes and brought awareness of the importance of the ER examination in MEN1 infertile female patients. MEN1-induced endocrine disorder rather than MEN1 mutation contributes to the ER abnormality.

Trial Registration: Reproductive Medicine Ethics Committee of Xiangya Hospital Registry No.: 2022010.

KEYWORDS

endometrial receptivity, RNA-seq, PGT-M, personalized embryo transfer, MEN1

Introduction

Preimplantation genetic testing (PGT) is an important technique developed to select embryos during artificial reproduction technology (ART) and avoid embryonic genetic abnormalities that lead to miscarriage or the inheritance of genetic diseases. However, once a single unaffected embryo is transferred, high levels of implantation and live birth success are attained independent of patient age and embryo quality. Patients with known genetic conditions may still fail to establish a successful pregnancy due to damaged endometrial receptivity (ER) although they obtained normal embryos by PGT (1). As a relatively expensive process in China, ART treatment following multiple PGT attempts may place a significant financial burden on patient couples (2).

Better technologies for personalized embryo transfer (pET), which not only consider applying PGT to select the "right" embryo but also try to find the "right" time for implantation, may contribute to the improvement of implantation and live birth outcomes for patients with genetic disorders. To define the "right" time, there is a certain period of endometrial maturation, called ER (3), during which the trophectoderm of the blastocyst can attach to the endometrial epithelial cells and subsequently proceed to invade the endometrial stroma. In 2009, ER array (ERA) was developed to detect the specific time point in the endometrial cycle in which ER is optimal and embryo implantation is possible, so-called window of implantation (WOI) (4). The reliability and reproducibility of the ERA test for determining the exact time of the WOI, which can be used with better results than histological dating of ER, showed that it was accurate and consistent (5). However, a decade has passed since the launch of ERA, and there remains limited evidence of the optimal indication of ERA as conflicting effects are reported on obstetric outcomes (6, 7). To develop a more Asian-specific ER assessment tool with better clinical performance, our group applied an improved endometrial biopsy sampling scheme with a machine learning algorithm to construct a novel RNA-seq–based ER test (rsERT) consisting of ER-specific marker genes. The new method was initially validated in a cohort with 142 patients diagnosed with repeat implantation failure (8). This gives us the basic technology that we need to study the use of rsERT on a larger group of patients and to focus on some patients with rare genetic diseases like multiple endocrine neoplasia type 1 (MEN1).

MEN1 is a rare autosomal dominant condition (prevalence 3–20/100,000) resulting from mutations in the tumor suppressor gene *MEN1* and characterized by various neuroendocrine tumors such as parathyroid hyperplasia, pancreatic endocrine tumors, and pituitary adenomas. Patients with MEN1 may have amenorrhea and reproductive disorders due to hormonal abnormalities, but, currently, there are limited studies discussing the direct impact of *MEN1* mutation on fertility. There are only a few case reports that describe patients with MEN1 developing infertility as a further symptom of the disease (9–11), whereas a multigenerational cohort study of the MEN1 population (Tasman 1 MEN1 kindred) controversially indicated no adverse impact of MEN1 on patient fertility overall, but MEN1 may impair the reproductive potential of individuals with pituitary disease (12). Because it is unclear how MEN1 affects fertility, clinical guidelines for the disease, particularly therapy of patients with MEN1 with infertility, are restricted. In the current clinical setting, PGT-M is recommended to block birth defects such as monogenic gene diseases. However, the only reported successful PGT-M treatment for the MEN1 condition is for male patients (13). In terms of female patients, embryo transplantation may still fail because of defected ER and displacement of the time of ER considering the level of endocrine complication in patients with MEN1. In addition, even if the embryo is implanted, multiple endocrine disorders induced by MEN1 can have a long-term impact on maternal–fetal safety. Several attempts have been made to use multidisciplinary team (MDT) management for pregnant women who have a MEN1 diagnosis (14–16). Therefore, a combination of pET to ensure "successful" transplantation of *MEN1* mutation–negative embryo and MDT management to maintain homeostatic balance during the whole ART treatment may contribute to a positive treatment scheme for patients with MEN1.

To this end, we first further evaluated our newly developed rsERT in a retrospective cohort study including 511 PGT frozen embryo transfer (FET) cycles to see whether improved patient outcomes can be obtained by the combination of PGT with the rsERT application. Second, we applied the integrated treatment scheme to an infertile female patient complicated by MEN1 who had a failed pregnancy after PGT-M embryo transfer. A wide range of hormone levels in this patient were monitored and analyzed before and after we applied the treatment. Bioinformatic studies were performed to see whether any mRNA variants detected in this patient via rsERT are associated with the MEN1 mutation.

Methods

Subjects

In this retrospective cohort study, we evaluated the PGT FET cycle baseline characteristics and results in our center between April 2019 and May 2022. The main end-points were as follows: no pregnancy; blood β-human chorionic gonadotropin (β-HCG) below 10, 12 days after embryo transferred; and clinical pregnancy, accessed by ultrasound 28 days after transferred in β-HCG positive patients. In total, 511 FET cycles were included into the study and further divided into three groups: control group: only performed PGT, n = 432 cycles; pET group: the rsERT showed WOI displaced and pET performed, n = 33 cycles; and non-displaced group: rsERT showed normal WOI, n = 46 cycles. Among the three groups, the number of retrieval cycles in each group was 347, 31, and 35, respectively. Time to pregnancy (TTP) was defined as the duration (months) from patients obtained unaffected embryos to clinical pregnancy. In our retrospective study, we compared the cycles with clinical pregnancy rate (CPR), live birth rate (LBR), cumulative CPR (CCPR), and cumulative LBR (CLBR).

Abbreviations: PGT, preimplantation genetic test; ART, artificial reproduction technology; ER, endometrial receptivity; rsERT, RNA-seq–based endometrial receptivity test; pET, personalized embryo transfer; WOI, window of implantation; MEN1, multiple endocrine neoplasia type 1; MDT, multidisciplinary team; FET, frozen embryo transfer; DEGs, differentially expressed genes; CR, clinical pregnancy; TTP, time to pregnancy.

This study was conducted at the Department of Reproductive Medicine, Xiangya Hospital, Central South University. The study was approved by the Reproductive Medicine Ethics Committee of Xiangya Hospital (registration no. 2022010).

Endometrium sampling

The patient provided written informed consent that she understood that the endometrium biopsies were performed for research purposes. We sampled the endometrium on the sixth day of progesterone supplementation during the hormone replacement therapy cycle (defined as P + 5 day, where the first day as P + 0 day) (Figure 1B). The collected samples were immediately placed in 1.5 mL of RNAlater buffer, sealed, and cryopreserved at −80°C. Sequencing analysis was performed within 7 days of sampling.

rsERT-guided personalized embryo transfer protocol

We described our development and how we guided pET according to the test result of rsERT in a previously published paper (8); pET was performed at the timing of optimal WOI predicted by rsERT. In brief, the endometrium RNA sequencing was put into the rsERT, and the method would predict the ER status of the sampling time. The timing of optimal WOI could be calculated according to the time of sampling time. The sample collected on P + 5 from this patient was predicted by rsERT model as pre-receptivity, and the optimal period of ER for her is 20 h after sampling. Subsequently, the corresponding frozen-thawed blastocysts transfer would be performed on the basis of this predicted time.

Bioinformatics analysis

Z-values were calculated to analyze the differentially expressed genes (DEGs) between the patient's endometrial mRNA and the background genes in the receptive phase of the rsERT. A *MEN1*-related gene list was obtained from STRING database. ToppGene Functional Annotation tool (ToppFun) was used to analyze the priority of DEGs based on the list. STRING was also used to analyze the protein–protein interaction (PPI) network among variants with *MEN1*. The sequence data reported in this study were archived in the Sequence Read Archive (SRA) with the accession number SUB13725948.

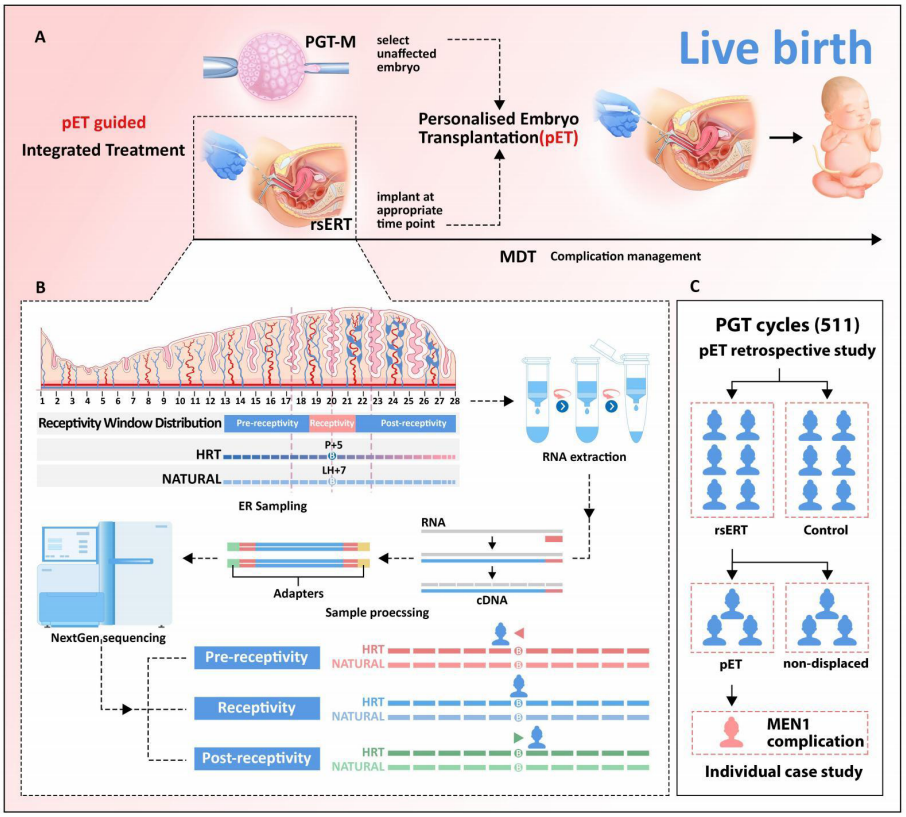


FIGURE 1
Validation of rsERT-guided integrated treatment scheme. **(A)** rsERT-guided integrated treatment scheme. The integrated treatment scheme is based on the combination of pET (PGT with rsERT) and MDT. **(B)** pET will be performed at the timing of optimal WOI predicted by rsERT, MDT will also be performed to ensure complication management if needed. **(C)** Data collected from 511 cycles treated in our center were divided into two major groups: performed PGT alone (control group) and rsERT group (further divided into pET and non-displaced groups).

Endocrine function monitoring

A systematic evaluation and adjustment of multiple endocrine functions by MDT management were performed, including patient's pituitary function, thyroid function, parathyroid function, blood glucose, and lipid profile, by reproductive center physicians and endocrinologists before entering the cycle. Hormone level datasets before the treatment were obtained from the Department of Gynecology and Obstetrics in our affiliated hospital.

Statistics

Continuous data subject to a normal distribution are presented as means ± SD and were compared using ANOVA analysis. Categorical data are expressed as counts and percentages and were compared using the Chi-square test or the Fisher's exact test. A two-sided P-value equal to or less than 0.05 was considered to be statistically significant. Statistical analysis was performed using IBM SPSS software (version 25.0, IBM Corp.).

Results

Integrated treatment scheme and cycle characteristics

Here, we demonstrated a rsERT-guided integrated treatment scheme based on the combination of pET (PGT with rsERT) and MDT (Figure 1A). In this scheme, pET will be performed at the timing of optimal WOI predicted by rsERT, and MDT will also be performed to ensure complication management if needed. The predictive model of rsERT was developed and trained utilizing DEGs among the pre-receptive, receptive, and post-receptive endometrium collected from Asian female group (8) (Figure 1B). To validate the patient outcomes for the pET, we retrospectively analyzed the data collected from 511 PGT FET cycles treated in our center (Table 1). They were divided into two major groups: those who only performed PGT (control group) and those who also performed rsERT, with the rsERT group further subdivided into the pET group (rsERT result showed WOI displace, so PGT was also performed for pET) and the non-displace group (Figure 1C). There were no significant differences in the couple mean age, infertility type, infertility duration, PGT type, Anti Miillerian Hormone (AMH), endometrial thickness, endometrial pattern, and the percent of high-quality blastocysts. The number of oocytes retrieved in non-displaced group was more than pET group and control group (P = 0.026). The CPR in the pET group was significantly higher than that in the control group and non-displaced group (P = 0.035). The previous cycle numbers in non-displaced group were more than that in the pET group and the control group (P < 0.001), whereas there was no significant difference in TTP (P = 0.550) (Table 1). Multiple comparisons tests (pairwise group comparisons used a Bonferroni-adjusted significance level of.017) were also performed; the result showed the CPR in the pET group was significantly higher than that in the control group (P < 0.011 for pET vs. control) and that in the non-displaced group did not differ significantly with both control and pET (P = 0.484 for non-displaced vs. control, P = 0.118 for non-displaced vs. pET).

Improvement of patient outcome

There was no significant statistical difference in the baseline clinical characteristics among the three groups. rsERT was performed in some patients to accurately determine whether the endometrium was in the WOI and, after pET, was performed in patients with abnormal ER, and the CPR was obviously improved. The LBR in the pET group was 8.6% higher than that in the control group, and the CCPR and CLPR were both higher in the pET group than that in the control group, although the difference was not statistically significant (Table 2). Furthermore, there was not a statistically significant difference in TTP among groups with CPR, which means that there was not a delay in getting patients ready for pregnancy; although the pET technique took slightly longer, it increased the probability that they would get pregnant.

Rare MEN1 patient complication

Within the 511 PGT FET cycles cohort, there was a rare infertility patient case complicated by MEN1. The patient was a 26-year-old Chinese woman. She discovered a large prolactinoma in her brain in 2004 (age 10), which later received radiotherapy and oral bromocriptine treatment after the resection was completed. She was diagnosed with pituitary amenorrhea in 2009 (age 15) and was given oral medication to establish an artificial menstrual cycle. In 2018 (age 25), she developed symptoms of central hypothyroidism, hyperparathyroidism (HPT), and a parathyroid nodule. Later, she was diagnosed with MEN1 after a mutation of the MEN1 gene c1268G>A (p.Trp423Term) was identified by whole exon sequencing, which also indicated the mutation came from her father. The patient then underwent parathyroidectomy twice and received postoperative hormone replacement therapy. Since her marriage in 2017 (age 24), she has not been able to conceive. Therefore, she decided to accept PGT-M treatment in 2019 (age 25) with her husband. The couple got three blastocysts after intracytoplasmic sperm injection (ICSI) and blastocyst culture. PGT-M revealed that only one of them did not carry pathogenic gene mutation, but biochemical pregnancy occurred after the normal blastocyst was implanted (Figure 2A). Endocrine abnormalities including hypopituitarism, obesity, central hypothyroidism, primary HPT, and hyperlipidemia were found at the time that the patient was admitted to our center in 2020 (age 26) for in vitro fertilization (IVF) treatment. As a first step in this case, we managed the patient's weight loss and restored her hormone balance. A controlled ovarian hyperstimulation protocol without pituitary downregulation was used, and 11 oocytes were obtained. Four blastocysts formed after ICSI, and two of them were found to be euploid embryos without maternal pathogenic genes after PGT-M. The

10.3389/fendo.2023.1224574

TABLE 1 Characteristics and clinical pregnancy rates of the rsERT and control groups.

Characteristics	Control (n = 432)	rsERT pET (n = 33)	rsERT Non-displaced (n = 46)	*P*-value
Female age (means ± SD), years	32.10 ± 4.56	31.91 ± 4.79	31.91 ± 4.63	*P* = 0.943
Male age (means ± SD), years	35.7 ± 7.05	36.03 ± 7.52	34.65 ± 7.15	*P* = 0.590
Infertility type, N (%)				
Primary infertility	109 (25.2%)	14 (42.4%)	15 (32.6%)	*P* = 0.067
Secondary infertility	323 (74.8%)	19 (57.6%)	31 (67.4%)	
Infertility duration (means ± SD), years	3.14 ± 3.00	3.70 ± 3.45	3.04 ± 2.65	*P* = 0.567
PGT type, N (%)				
PGT-A	220 (50.9%)	13 (39.4%)	25 (54.3%)	*P* = 0.628
PGT-SR	143 (33.1%)	15 (45.5%)	15 (32.6%)	
PGT-M	69 (16.0%)	5 (15.2%)	6 (13.0%)	
AMH (means ± SD), ng/mL	3.91 ± 2.98	4.59 ± 3.08	3.40 ± 2.15	*P* = 0.219
Number of oocytes retrieved (means ± SD)	14.4 ± 7.758	16.727 ± 6.8706	17.087 ± 7.4441	***P* = 0.026**
				P = 0.094[a]
				P = 0.837[b]
				***P* = 0.024**[c]
Endometrial thickness, (means ± SD), mm	9.394 ± 1.7819	9.403 ± 1.6124	9.791 ± 2.0764	*P* = 0.363
Endometrial pattern, N (%)				
A	100 (23.1%)	12 (36.4%)	15 (32.6%)	*P* = 0.069
B	296 (68.5%)	21 (63.6%)	25 (54.3%)	
C	36 (8.3%)	0 (0.0%)	6 (14.3%)	
Proportion of high-quality blastocysts, N (%)	414 (95.8%)	33 (100.0%)	45 (97.8%)	*P* = 0.402
Previous cycle numbers, (means ± SD)	2.72 ± 1.101	3.48 ± 1.787	3.59 ± 1.833	***P* = 0.000**
				P = 0.062[a]
				P = 0.992[b]
				***P* = 0.009**[c]
CPR, N (%)	202 (46.8%)	23 (69.7%)	24 (52.2%)	***P* = 0.035**
				***P* = 0.011**[a]
				P = 0.118[b]
				P = 0.484[c]
TTP, means ± SD, month	6.05 ± 6.54 (n = 202)	6.91 ± 3.91 (n = 23)	7.38 ± 6.45 (n = 24)	*P* = 0.550
LBR, N (%)	172 (39.9%)	16 (48.5%)	20 (43.5%)	*P* = 0.581

Bold P-value indicates statistical significance; CPR, clinical pregnancy rate; TTP, time to pregnancy; LBR, live birth rate; a, indicating the p-value of the pET group compared with the control group; b, indicating the p-value of the pET group compared with the non-displaced group; c, indicating the p-value of the non-displaced group compared with control group.

10.3389/fendo.2023.1224574

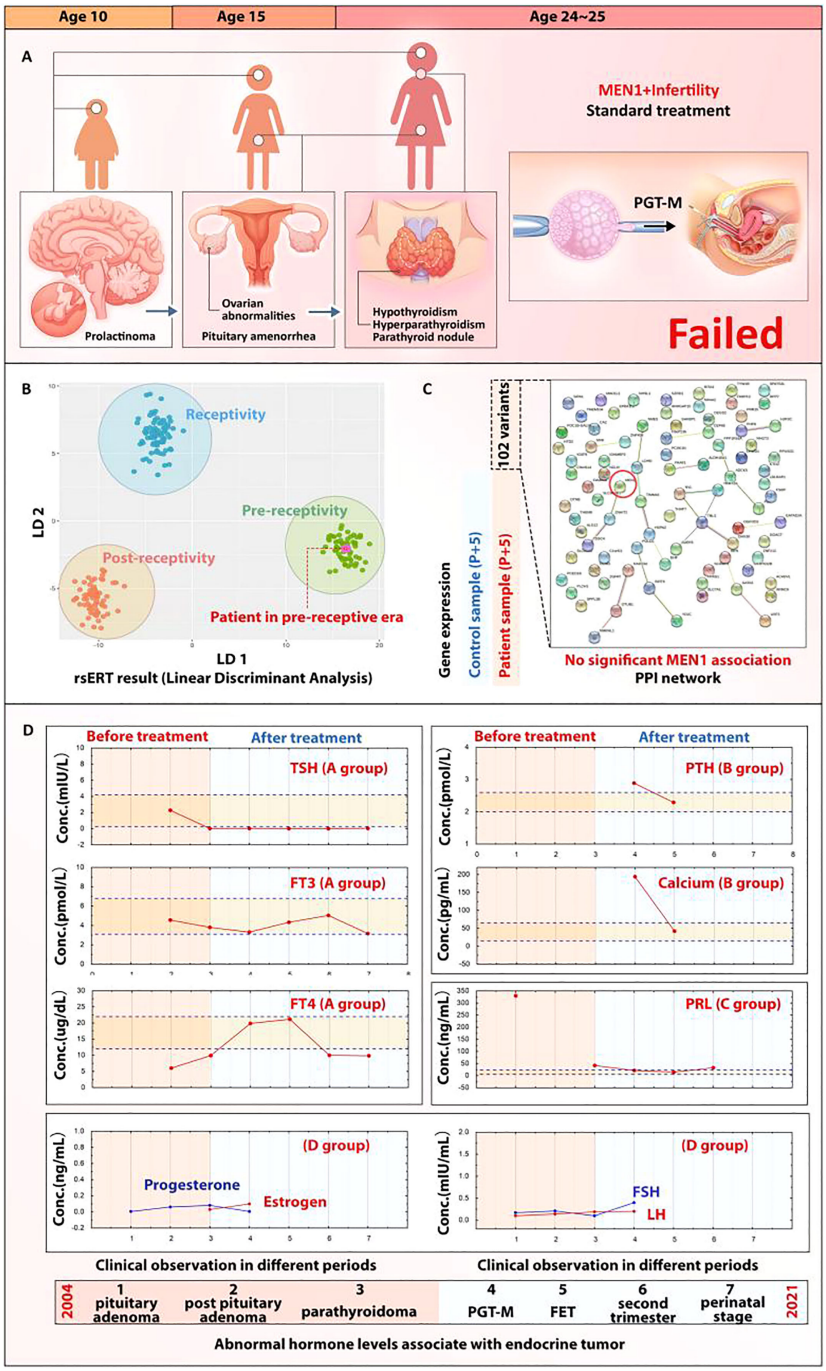


FIGURE 2
MEN1 and ER abnormality. ER abnormality in this reported case may be induced by the MEN1-associated endocrine conditions but not MEN1 mutation. **(A)** Indication of the rsERT biopsy day and presentation of rsERT result; the purple dot shows that the patient's sample was in the pre-receptive area. **(B)** rsERT result for the patient with MEN1. **(C)** PPI analysis of MEN1 with the 102 variants identified; no strong association can be observed. **(D)** Restoration of endocrine homeostasis of the patient; hormones maintained a normal level under MDT management.

patient's ER was examined using rsERT, and a pET for the unaffected embryo to the patient was performed on the basis of the rsERT result (Figure 2B). After pET, progesterone for luteal support was administered daily, including 600 mg intravaginal and 200 mg oral. Oral medications, such as Bromocript 2.5 mg quaque die (Qd) for hyperprolactinemia, Euthyrox (Merck) 112.5 μg (Qd) for hypothyroidism, metformin 0.5 g bis in die (Bid) for insulin resistance, were used long-term under MDT monitoring. Twenty-eight days later, the ultrasound result confirmed the intrauterine pregnancy. The patient had a cesarean section at 38 + 2 weeks' Gestational age (GA) and delivered a live male infant with a weight of 3,850 g. Apgar scores were 10 for 1 min, 5 min, and 10 min after birth.

Characterization of MEN1 mutation association

To further study whether the *MEN1* mutation directly impacts the ER at the genetic level, we identified 102 mRNA variants in the patient's mRNA profile and found out that *GATA2* and *NR4A2* ranked the top two genes (**Additional file 1**). However, when we queried the STRING database for PPI analysis, no strong association of *MEN1* with the profile (weak association score of 0.621) was found for *ZNHIT2*, ranking the 41st in the gene cluster (Figure 2C). As no association could be found with the MEN1 mutation, we posited that the ER abnormality may be induced by the long-term impact on the endocrine disorders in this patient caused by MEN1, as pituitary prolactinoma and parathyroid adenoma were two of the major clinical phenotypes. The patient's historical clinical data showed a serious hormonal imbalance since the age of 10. By applying MDT management after the admission to the center, most of the hormones' level were restored to a normal range (Figure 2D) and the restoration contributed to the success rate of personalized embryo transplantation.

Live birth achieved by integrated treatment

We performed pET with MEN1 mutation–negative blastocyst (Figure 3B) based on the estimated WOI. Thirty-five days after transplantation, the patient was sent to the Department of Obstetrics for further MDT management. No abnormalities of fetal nuchal translucency were observed at 12 weeks' GA, and then oral metformin of the patient was discontinued. Amniocentesis, which was performed in the same month, showed no abnormalities in fetal chromosomes, and no MEN1 c.1268G>A mutation was detected (Figure 3A). The patient had a cesarean section at 38 + 2 weeks' GA and delivered a live male infant with a weight of 3,850 g (Figure 3C).

Discussion

Considering that the clinical outcomes of the application of ERA remain under debate, we aim at developing a more suitable ER assessment method for Asians, so we established the rsERT by utilizing Chinese patient samples collected in our center. In our study, PGT for aneuploidy (PGT-A) was the most component of PGT type. It analyzes the chromosome copy number to diagnose the aneuploids embryos. In ART, PGT-A is usually applied to patients with advanced maternal age, recurrent pregnancy loss, and recurrent implant failure. PGT-M helps select embryos free from monogenic disorders, reducing the risk of transferring embryos with genetic issues that could hinder implantation. The combination of rsERT and PGT creates more personalized and precise treatment plans for each individual patient. This approach considers both the genetic health of embryos and the receptivity of the endometrium, potentially leading to improved outcomes.

To further explore the patient benefit within the group of people who underwent PGT, our retrospective study result indicates performing rsERT along with PGT can largely increase patient benefit as transferable embryos are difficult to obtain (especially considering the MEN1 case): CPR was significantly higher compared to the control group; the LBR, CCPR, and CLPR were all higher in the pET group than that in the control group. Although statistical significance of CPR may not have been observed in the pET group and the non-displaced group in certain cases, LBR, CCPR, and CLPR showed no statistical difference; it is possible that the limited sample size could have resulted in insufficient statistical efficiency. We assumed that this still be an optimistic tendency. In the future study, we plan to enhance statistical efficiency by increasing the sample size, optimizing experimental design, and considering other factors that might influence statistical efficiency. This will enable us to detect potential differences and provide more convincing support for the research findings more accurately.

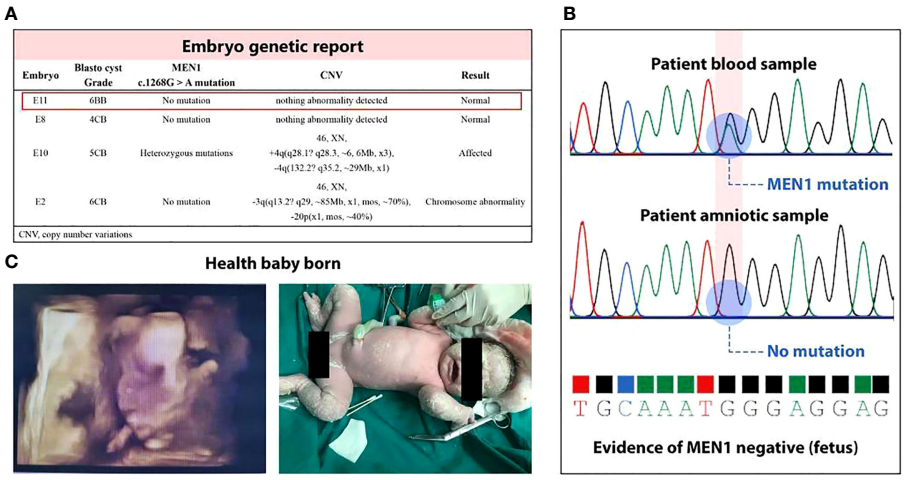


FIGURE 3
Health live birth. **(A)** Unaffected blastocysts were obtained by PGT-M; E11 was selected on the basis of its higher blastocyst grade in Gardner score system. **(B)** Amniocentesis showed no abnormalities in fetal chromosomes and no MEN1 c.1268G>A mutation was detected. **(C)** A baby was born with Apgar score 9-10-10, without positive airway pressure support for respiratory distress. The infant reached his developmental milestones by his age.

TABLE 2 CCPR and CLBR of the rsERT and control groups.

Characteristics	Control	rsERT		*P*-value
		pET	Non-displaced	
	(n = 347)	(n = 31)	(n = 35)	
CCPR, N (%)	200 (57.6%)	23 (74.2%)	22 (62.9%)	P = 0.180
CLBR, N (%)	172 (49.6%)	16 (51.6%)	16 (54.3%)	P = 0.855

CCPR, cumulative clinical pregnancy rate; CLBR, cumulative liver birth rate.

Actually, we are already performing a multi-center randomized clinical trial to obtain more credible data.

Although performing rsERT slightly increases the TTP, it could be saved by increasing the success rate of implantation. The findings of the retrospective analysis give us the confidence to administer the integrated treatment to the patient with unique MEN1.

There are a limited number of clinical guidelines covering the treatment and management of patients with MEN1 with infertility. The current MEN1 clinical practice, which is generally similar to tumors occurring in non-MEN1 patients, suggests performing genetic tests for index patients with MEN1 and their first-degree relatives (17). Therefore, the application of PGT-M can be seen in a few MEN1-related IVF treatment cases to block the MEN1 mutation inheritance. However, the carriers of MEN1 syndrome in those reports were male patients. Whereas in our case, the 25-year-old female patient received an unaffected blastocyst via PGT-M but underwent an unsuccessful embryo transfer, which brings our awareness of exploring further treatment schemes in addition to PGT-M for patients with MEN1 with infertility.

This patient harbored a mutation of the *MEN1* gene c.1268G>A and presented with pituitary adenoma as well as parathyroid. A similar mutation variant has been reported in an Australian case, who presented with clinical phenotypes including lung and thymic carcinoids, prolactinoma, nonfunctioning pituitary adenomas, insulinomas, and HPT (18). The literature search indicated that *MEN1* may affect ER via decidualization by interfering with estrogen alpha receptor *ESR1* (19) and nuclear factor–κappa B (NF-κB) (20). This finding encouraged us to investigate the patient's ER condition, and, thus, we applied rsERT, an RNA-seq–based ER tool that is capable of identifying ER-related genes and predicting the optimal WOI for pET.

The rsERT result showed that the WOI of the patient was delayed, but, interestingly, both *ESR1* and NF-κB could not be found in the patient's mRNA variants, whereas two other genes, *NR4A* and *GATA2*, were found to rank the top two in the gene cluster. Our expectation might have been that MEN1 may have had a direct impact on ER. In such a case, either *ESR1* and NF-κB can be found in the mRNA variants or the association of *MEN1* with *NR4A* and *GATA2* can be identified. However, the PPI analysis showed a negative result in all the cases mentioned. Given the achievement of clinical pregnancy via pET, which is dependent on ER prediction and WOI identification, our finding adds to the scientific debate on whether MEN1 affects infertility by providing limited evidence that MEN1 has no direct effect on ER but is impaired ER by endocrine disorders. In addition, this finding also emphasizes the importance of performing MDT management for patients with MEN1 with infertility to maintain endocrine homeostasis. It also provides us the new research idea and the clinical application of rsERT in the future.

Conclusions

The combination of rsERT and PGT contributes to a pET that assists clinicians in selecting the "right" blastocyst and performing embryo transfer at the "right" time, and the MDT management plays an important role in maintaining endocrine homeostasis. The case elucidates a new angle for developing treatment guidelines for infertile patients with MEN1 by applying an integration of multiple techniques, including rsERT, PGT-M, and MDT management.

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 authors.

Ethics statement

The studies involving human participants were reviewed and approved by the Reproductive Medicine Ethics Committee of Xiangya Hospital (Registry No.2022010). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) and minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

YL, XH, and JF formulated the study and collected the data. SSL, XH, and JF analyzed the data, edited the manuscript, and organized the figures and tables. YL, QZ, JZ and AX were involved in the clinical treatment team. SJL and AH performed the endometrium sampling and RNA sequencing. ZY and QX performed the embryos PGT. HT was involved in following-up the patient. XH and JF are co-first authors; the two authors contributed to this article equally. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by grants from the National Natural Science Foundation of China (grant no. 81873858), the key Project of Research and Development Plan in Hunan Province (grant no. 2021SK2028), and the National Key Research and Development Program of China (grant nos. 2018YFC1004800 and 2021YFC2700404).

Acknowledgments

The authors thank the patient and her family members; Zhongwei Xu from Shenzhen Children's Hospital; Jun Ren from Yikon Genomics Company; Jinjing Shen, Yu Jian, and Qiaoqiao Feng from the Department of Obstetrics, Xiangya Hospital, Central South University; and Jiannan Xiong, Yuan Sheng, and Jianjuan Zhao from STI-Zhilian Research Institute for Innovation and Digital Health.

Conflict of interest

Author SJL is employed by Yikon Genomics Company, Ltd.

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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1224574/full#supplementary-material

References

1. Troncoso C, Bosch E, Rubio C, Remohí J, Simón C, Pellicer A. The origin of biochemical pregnancies: lessons learned from preimplantation genetic diagnosis. *Fertil Steril* (2003) 79:449–50. doi: 10.1016/s0015-0282(02)04670-8

2. Tatum M. China's fertility treatment boom. *Lancet* (2020) 396:1622–3. doi: 10.1016/S0140-6736(20)32475-2

3. Barbieri RL. Yen & Jaffe's reproductive endocrinology : physiology, pathophysiology, and clinical management. In: Strauss JF, Barbieri RL, editors. *Yen and Jaffe's Reproductive Endocrinology*. Philadelphia: Elsevier (2019). p. 556–581.e7. doi: 10.1016/B978-0-323-47912-7.00022-6

4. Díaz-Gimeno P, Horcajadas JA, Martínez-Conejero JA, Esteban FJ, Alamá P, Pellicer A, et al. A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature. *Fertility Sterility* (2011) 95:50–60.e15. doi: 10.1016/j.fertnstert.2010.04.063

5. Díaz-Gimeno P, Ruiz-Alonso M, Blesa D, Bosch N, Martínez-Conejero JA, Alamá P, et al. The accuracy and reproducibility of the endometrial receptivity array is superior to histology as a diagnostic method for endometrial receptivity. *Fertil Steril* (2013) 99:508–17. doi: 10.1016/j.fertnstert.2012.09.046

6. Ben Rafael Z. Endometrial Receptivity Analysis (ERA) test: an unproven technology. *Hum Reprod Open* (2021) 2021:hoab010. doi: 10.1093/hropen/hoab010

7. Ruiz-Alonso M, Valbuena D, Gomez C, Cuzzi J, Simon C. Endometrial Receptivity Analysis (ERA): data versus opinions. *Hum Reprod Open* (2021) 2021: hoab011. doi: 10.1093/hropen/hoab011

8. He A, Zou Y, Wan C, Zhao J, Zhang Q, Yao Z, et al. The role of transcriptomic biomarkers of endometrial receptivity in personalized embryo transfer for patients with repeated implantation failure. *J Transl Med* (2021) 19:176. doi: 10.1186/s12967-021-02837-y

9. Brandi ML, Weber G, Svensson A, Falchetti A, Tonelli F, Castello R, et al. Homozygotes for the autosomal dominant neoplasia syndrome (MEN1). *Am J Hum Genet* (1993) 53:1167–72.

10. Schuppe H-CS. Case Report: Secondary infertility as early symptom in a man with multiple endocrine neoplasia-type 1. *Hum Reprod* (1999) 14:252–4. doi: 10.1093/humrep/14.1.252

11. Juodelė L, Serapinas D, Sabaliauskas G, Krasauskienė A, Krasauskas V, Verkauskienė R, et al. Carcinoma of two parathyroid glands caused by a novel MEN1 gene mutation – a rare feature of the MEN 1 syndrome. *Medicina* (2011) 47:92. doi: 10.3390/medicina47110092

12. Thompson M, Burgess J. No evidence of adverse fertility and pregnancy outcomes in patients with unrecognized and untreated multiple endocrine neoplasia type 1. *Clin Endocrinol (Oxf)* (2019) 90:312–9. doi: 10.1111/cen.13890

13. Lima AD, Alves VR, Rocha AR, Martinhago AC, Martinhago C, Donadio N, et al. Preimplantation genetic diagnosis for a patient with multiple endocrine neoplasia type 1: case report. *JBRA Assist Reprod* (2018) 22:67–70. doi: 10.5935/1518-0557.20180010

14. Daglar HK, Kirbas A, Biberoglu E, Laleli B, Danisman N. Management of a multiple endocrine neoplasia type 1 during pregnancy: A case report and review of the literature. *J Exp Ther Oncol* (2016) 11:217.

15. Dhiman D, Bloria SD, Sabharwal P, Sarna R, Chauhan R. Management of a multiple endocrine neoplasia 1 patient in pregnancy. *J Hum Reprod Sci* (2020) 13:349–51. doi: 10.4103/jhrs.JHRS_164_19

16. Li J, Xu D, Liu X. Management of a multiple endocrine neoplasia type 1 during pregnancy: a case with primary hypertension. *Gynecol Endocrinol* (2021) 38:1–3. doi: 10.1080/09513590.2021.1974382

17. Thakker RV, Newey PJ, Walls GV, Bilezikian J, Dralle H, Ebeling PR, et al. Clinical practice guidelines for multiple endocrine neoplasia type 1 (MEN1). *J Clin Endocrinol Metab* (2012) 97:2990–3011. doi: 10.1210/jc.2012-1230

18. Bergman L, Teh B, Cardinal J, Palmer J, Walters M, Shepherd J, et al. Identification of MEN1 gene mutations in families with MEN 1 and related disorders. *Br J Cancer* (2000) 83:1009–14. doi: 10.1054/bjoc.2000.1380

19. Dreijerink KMA, Mulder KW, Winkler GS, Höppener JWM, Lips CJM, Timmers HTM. Menin links estrogen receptor activation to histone H3K4 trimethylation. *Cancer Res* (2006) 66:4929–35. doi: 10.1158/0008-5472.CAN-05-4461

20. Heppner C, Bilimoria KY, Agarwal SK, Kester M, Whitty LJ, Guru SC, et al. The tumor suppressor protein menin interacts with NF-κB proteins and inhibits NF-κB-mediated transactivation. *Oncogene* (2001) 20:4917–25. doi: 10.1038/sj.onc.1204529

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 23 October 2023
DOI 10.3389/fendo.2023.1278042

OPEN ACCESS

EDITED BY
Emre Pabuccu,
Ufuk University, Türkiye

REVIEWED BY
Meltem Sönmezer,
Memorial Ankara Hospital, Türkiye
Mehmet Erdemom,
Gazi University, Türkiye

*CORRESPONDENCE
Pınar Özcan
drpinarozcan@hotmail.com

RECEIVED 15 August 2023
ACCEPTED 29 September 2023
PUBLISHED 23 October 2023

CITATION
Demirel C, Özcan P, Tülek F, Timur HT and Pasin Ö (2023) Initiating luteal phase support with sc progesterone based on low serum progesterone on the transfer day in true natural cycle frozen embryo transfers.
Front. Endocrinol. 14:1278042.
doi: 10.3389/fendo.2023.1278042

Initiating luteal phase support with sc progesterone based on low serum progesterone on the transfer day in true natural cycle frozen embryo transfers

Cem Demirel [1], Pınar Özcan [2*], Fırat Tülek [1], Hikmet Tunç Timur [3] and Özge Pasin [4]

[1]Department of Obstetrics and Gynaecology, Acibadem University School of Medicine, Istanbul, Türkiye, [2]Department of Obstetrics and Gynaecology, Bezmialem University School of Medicine, Istanbul, Türkiye, [3]Department of Obstetrics and Gynecology, Dokuz Eylul University School of Medicine, Izmir, Türkiye, [4]Department of Biostatistics and Medical Informatics, Bezmialem University School of Medicine, Istanbul, Türkiye

Introduction: Concerning contemporary *in-vitro* fertilisation (IVF) practice, the use of frozen embryo transfer (FET) cycles has become more common than fresh transfers. Natural cycle (NC), programmed artificial cycle and mild stimulation cycle are primary endometrium preparation cycles. Monitoring serum progesterone levels in FET cycles are in the scope of current research focus. Low progesterone levels on the day of embryo transfer is presumed to negatively affect pregnancy outcomes, while progesterone supplementation may improve pregnancy rates. The purpose of our trial is to evaluate whether initiating subcutaneous (SC) progesterone supplementation on the day of embryo transfer when serum progesterone levels are below 10 ng/mL in tNC-FET will result in pregnancy rates comparable to those of patients with sufficient serum progesterone.

Methods: Retrospective single centre study was conducted between August 2022 and April 2023 with 181 tNC-FETs. Patients were separated into groups according to serum progesterone concentrations (≥10 ng/mL and <10 ng/mL) on embryo transfer (ET) day. S.c progesterone (25 mg) was given on the day of ET when serum progesterone was <10 ng/mL, continuing until the 10th gestational week. Blood samples for pregnancy tests were collected 12 days after ET. Outcome parameters were pregnancy rate, clinical pregnancy rate (CPR), miscarriage rate, multiple pregnancy rate, biochemical pregnancy, and ongoing pregnancy rate (OPR).

Results: About half (49.7%) had adequate progesterone concentrations (≥10ng/mL) on ET day. There was no significant difference between the groups regarding positive pregnancy test, OPR, multiple pregnancies, and miscarriage rates (57.8% versus 52.7%; 34.4% versus 29.7%, 1.1% versus 2.2%; 7.8% versus 5.5%; respectively, for progesterone concentrations on ET day ≥10 ng/mL and <10 ng/mL). With 55.2% of transfers leading to clinical pregnancy, significant differences emerged in biochemical pregnancy and CPR (3.3% vs 12.1%,

P=0.02; 54.4% vs 40.7%, P=0.03, for ≥10 ng/mL and <10 ng/mL progesterone concentrations on ET day).

Discussion: This study indicates that nearly half of the tNC-FETs may need luteal phase support due to low progesterone. However, 25 mc sc progesterone rescued the luteal support and yielded similar OPR as compared to normal progesterone group. Further studies are needed for understanding optimal progesterone levels, supplementation effectiveness, and potential benefits of earlier supplementation in FETs.

KEYWORDS

luteal phase support, *in-vitro* fertilisation, progesterone, frozen embryo transfer, natural cycle

Introduction

In today's IVF practice, frozen embryo transfer (FET) cycles have started to override fresh transfers, owing to the improvements in vitrification techniques along with the added benefits of elimination of ovarian hyperstimulation syndrome (OHSS) and improved success rates in hyper-responders when freeze-all strategy is used. Increased utilisation of preimplantation genetic screening of embryos further augments the switch to frozen transfer cycles (1, 2). According to a United States nationwide database, FET cycles have recently become approximately 77.0% of all ETs (3). There are mainly three strategies utilised, each having further modifications within, for endometrium preparation during FET, namely natural cycle, programmed artificial cycle, and mild stimulation cycle FETs. Until very recently, there had been no clear-cut consensus yet for the optimal FET strategy, but the recent trend is more towards the use of a natural cycle (4–7). The reasons are primarily two folds, one being a higher ongoing pregnancy rate compared to artificial cycles due to a lower incidence of early pregnancy loss and the other one being the pregnancy complications like pregnancy induced hypertension and significant for gestational age fetuses that are unique to artificial cycles, most probably due to the absence of corpus luteum (8). Nowadays, the main research topic is focused on monitoring FET cycles best and finding out the confounding factors that may affect success rates. Monitorisation of serum progesterone levels is a central topic of interest in FET cycles, and it has been documented in various reports that serum progesterone levels below a certain threshold around the time of ET result in decreased odds for live birth rates. This has been verified in artificial FET cycles where serum progesterone levels lower than 8-10 ng/mL on the day or preceding day of ET are shown to adversely affect the live birth rates (9, 10). Rescue strategies in the form of adding 25 mg of supplemental subcutaneous (SC) progesterone are shown to restore live birth rates to those of patients with adequate serum progesterone levels in artificial FET cycles, to overcome the deleterious effect of the insufficient progesterone support of the endometrium (11, 12).

The corpus luteum (CL) is involved in a natural cycle FET. Nevertheless, progesterone production by CL in some cycles may be insufficient to provide adequate support for implantation and continuation of pregnancy. A very recent meta-analysis of randomised controlled trials for luteal phase support (LPS) in natural cycle FET (NC-FET) has shown that progesterone supplementation for LPS was associated with increased LBR and CPR in NC-FET cycles, giving additional evidence for the insufficiency of corpus luteum in some of the natural cycles (13). In the same meta-analysis, LPS improves LBR only in true NC-FET but not in modified NC-FET, where hCG used to trigger dominant follicles must act as LPS per se. A recent retrospective study has shown that patients with low serum progesterone (< 10 ng/mL) on the day before NC-FET have reduced live birth rates compared to those who have > 10 ng/mL progesterone (14). Of note, these NC-FET cases were not using any LPS, solely depending on progesterone production by CL. Since lower progesterone concentrations on ET day during natural cycles FET may negatively influence pregnancy outcomes, progesterone supplementation for the restoration of serum progesterone to adequate concentrations may be an opportunity to improve PRs (9, 14–16).

The purpose of our trial is to evaluate whether initiating SC progesterone supplementation on the day of embryo transfer when serum progesterone levels are below 10 ng/mL in tNC-FET will result in pregnancy rates comparable to those of patients with sufficient serum progesterone. In brief, to our knowledge, this is the first study to evaluate the feasibility of a rescue progesterone supplementation strategy in tNC-FET cases.

Materials and methods

This retrospective cohort study in a single centre was carried out at the IVF centre of Acibadem Ataşehir Hospital between August 2022 and April 2023. A total of 181 true natural cycles vitrified–warmed FETs (tNC-FET) were evaluated. The Ethical Committee of

10.3389/fendo.2023.1278042

Bezmialem University approved the protocol of our study (Approval no. 2023/165). Exclusion criteria were patients with cycle cancellation due to lack of a viable embryo or who had Mullerian anomalies not corrected by surgery, such as bicornuate, unicornuate, or didelphic uterus, those with a history of recurrent miscarriage, and those with a presence of hydrosalpinx. Those who had corrected uterine anomalies such as uterine septum, submucosal fibroids, or endometrial polyps were not excluded.

The collected data consisted of demographic characteristics of the patients, including age at oocyte pick up (year), IVF indications (tubal, male, unexplained, diminishing ovarian reserve, ovulatory, mixed), serum LH concentration (IU/L) on the day of LH surge, serum progesterone concentration (ng/mL) on the day of LH surge and the day of embryo transfer, endometrial thickness (mm) on the day of embryo transfer, stage of transferred embryo (Day 3/ blastocyst) and number of embryos transferred. Outcome parameters analysed are pregnancy rate, clinical pregnancy rate (CPR) (fetal heartbeat by transvaginal ultrasound), miscarriage rate (any clinical pregnancy lost before the 12th gestational week), multiple pregnancy rate, biochemical pregnancy, and ongoing pregnancy rate (OPR) (pregnancies beyond 12 weeks of pregnancy).

All women underwent a transvaginal ultrasound scan on the second or third day of menstruation to confirm the absence of any ovarian cyst or CL. The second control was scheduled on the eighth day of the cycle to evaluate the emergence of the dominant follicle by transvaginal ultrasonography. Endocrine monitoring with serum LH and progesterone measurements was initiated everyday at 9 am once the leading follicle attained a mean diameter of approximately 15 mm. An increase of at least 180% compared to the previous serum LH level was taken as consistent with LH surge. FETs were performed four days after the LH rise for day three embryos and six days after the blastocyst stage. Blood samples for serum progesterone concentrations were collected on ET day at 9 am for all patients. 25 mg of SC progesterone (Prolutex; IBSA, Switzerland) per day was initiated at 11 am in patients with serum progesterone concentrations <10 ng/mL on ET day. The blood samples for pregnancy tests were collected 12 days after ET. Progesterone supplementation was discontinued if there was no pregnancy. We hypothesized that patients with serum progesterone concentrations <10 ng/mL in tNC-FET on the day of embryo transfer may have insufficiency of corpus luteum. We would like to continue SC progesterone until the luteo-placental shift (10th weeks of pregnancy). Therefore, progesterone supplementation at the same dose was continued until the 10th gestational week for viable pregnancies. Serum analysis and hormone measurement, vitrification and warming procedure, and ET were performed as described in our previous study (17).

The data were analysed using SPSS Statistics for Windows, Version 26 (IBM Corp, Armonk, NY, USA). Data were reported as mean ± SD or number and percentage. The Pearson chi-squared test, Fisher's exact and Fisher Freeman Halton tests were used to compare categorical variables. The Kolmogorov–Smirnov test was used to test the normal distribution of continuous variables. The homogeneity of variance was evaluated with Levene's test. Student's t-test was used to compare two independent groups regarding the means of normally distributed variables. The Mann–Whitney U-test was used to compare two independent groupsregardingf the means of non-normally distributed variables. P<0.05 was considered significant. As this is a retrospective study, no power analysis was performed prior to the study, and we included all women who underwent natural cycles FET during the period.

Results

A total of 181 tNC- FET were analysed. The mean age was 35.37 ± 4.72 in patients with serum progesterone concentrations ≥10 ng/mL on the day of ET and 35.35 ± 5.3 years in patients with serum progesterone concentrations <10 ng/ml on ET day. Overall, 49.7% (90/181) of patients had adequate serum progesterone concentrations on ET day (≥10 ng/mL). Patients with serum progesterone concentrations <10 ng/mL and ≥10 ng/mL were similar in terms of age at oocyte pick up, IVF indications, serum LH concentration on the day of LH surge, serum progesterone concentration on the day of LH surge, endometrial thickness on the day of embryo transfer and number of embryos transferred. (Table 1). There was a significant difference between groups

TABLE 1 The characteristics of patients.

Variables	Progesterone ≥10 ng/mL (n=90)	Progesterone <10 ng/mL (n=91)	P value
Age at oocyte pick up (years)	35.37 ± 4.72	35.35 ± 5.3	0.98
Indication of IVF (n. (%)) Tubal Male Unexplained Ovulatory Mixed	 21.1 (19) 1.1 (1) 2.2 (2) 1.1 (1) 74.4 (67)	 24.2 (22) 4.4 (4) 4.4 (4) 3.3 (3) 63.7 (58)	0.22
Serum LH (IU/L) concentration at the day of LH surge	19.57 ± 9.88	19.22 ± 7.55	0.78
Serum progesterone concentration (ng/mL) at the day of LH surge	2.33 ± 10.6	1.34 ± 6.11	0.18
Serum progesterone concentration (ng/mL) on embryo transfer day	14.63 ± 5.57	7.29 ± 1.93	<0.001*
Endometrial thickness (mm) on embryo transfer day	9.05 ± 1.6	9.29 ± 1.54	0.19
Number of embryos transferred (n. (%)) 1 2	 65 (72.2%) 25 (27.8%)	 60 (65.9%) 31 (34.1%)	0.42
Embryo stage at transfer Day 3 blastocyst	 5.6 (5) 94.4 (85)	 18.7 (17) 81.3 (74)	0.01*

Values are expressed as mean± standard deviation for continuous variables and (n. (%)) for categorical variables. *P<0.05. significant difference.

regarding serum progesterone concentrations on ET day (14.63 ± 5.57 vs 7.29 ± 1.93 ng/mL; P < 0.001, respectively, for ≥10 ng/mL and <10 ng/mL serum progesterone concentration). There was a statistically significant difference in terms of the distribution of day three embryos and blastocysts in patients with serum progesterone concentrations ≥10 ng/mL and patients with <10 ng/mL on the day of ET. The number of day three embryo transfers was significantly higher in patients with serum progesterone concentrations <10 ng/mL on the day of ET (5.6% versus 18.7%; P =0.01). There was a statistically significant difference in serum progesterone concentrations between those undergoing day 3 and day 5 frozen thawed embryo transfers (7.99 ± 6.86 vs 11.34 ± 5.23 ng/mL; P<0.001, respectively, for day three and blastocyst stages). However, there was no significant difference between the patients in terms of clinical pregnancy rates according to the day of embryo transfer (2% versus 8.1%; P=0.31).

Of all transfers, 55.2% (100/181) of all transfers resulted in a clinical pregnancy. There was no significant difference between the groups regarding positive pregnancy test, OPR, multiple pregnancies, and miscarriage rates (57.8% versus 52.7%; 34.4% versus 29.7%, 1.1% versus 2.2%; 7.8% versus 5.5%; respectively, for progesterone concentrations on ET day ≥10 ng/mL and <10 ng/mL). Biochemical pregnancy rates (3.3% versus 12.1%, P = 0.02) was lower and clinical pregnancy rates (54.4% versus 40.7%, P = 0.03) was higher in patients with ≥10 ng/mL P concentrations on the day of ET. Serum progesterone concentrations on ET day were evaluated by percentiles (<10%, 10–49%, 50–90%, and >90%). The threshold for <10%, 10– 49%, 50–90%, and >90% were 0–5.9 (11%), 5,9–9,96 (39.2%), 9.96–16.54 (39.8%) and >16.64 ng/mL (9.9%), respectively. However, the positive pregnancy test of serum progesterone percentiles on ET day was 8% in the <10% percentile, 40% in the 10–49% percentile, 39% in the 50–90% percentile, and 13% in the >90% percentile. The CPR of serum progesterone percentiles on ET day was 5.8% in the <10% percentile, 37.2% in the 10–49% percentile, 43% in the 50–90% percentile, and 14% in the >90% percentile. There was no significant difference between the patients in terms of CPR in all percentiles when evaluating separately according to the day of embryo transfer. The CPR of serum progesterone percentiles for day three embrio on ET day was 35.3% in the <10% percentile, 41.2% in the 10–49% percentile, 17.6% in the 50–90% percentile, and 5.9% in the >90% percentile (P=0.41). The CPR of serum progesterone percentiles for blastocyst stages on ET day was 9.4% in the <10% percentile, 37.5% in the 10–49% percentile, 46.9% in the 50–90% percentile, and 6.3% in the >90% percentile (P=0.14). 10 ng/mL was represented as the threshold of the 51st percentile in this study. A total of 78.5% of biochemical pregnancies were under the 50 percentile.

Discussion

The correlation between low serum progesterone levels around the timing of embryo transfer and decreased LBR has been demonstrated in artificial and natural frozen embryo transfer cycles (9, 10, 14). The necessity of exogenous luteal phase support has been debated in NC-FET cycles since there is a corpus luteum to produce endogenous progesterone. However, the adequacy of CL function is not absolute, and it has been shown in a recent study that low serum progesterone (<10 ng/mL) on the day before NC-FET has been associated with reduced live birth rates compared to cycles with ≥ 10 ng/mL progesterone (14). A recent meta-analysis to evaluate whether LPS is beneficial in NC-FET also supports the findings of this study, giving additional evidence for the insufficiency of the corpus luteum in some of the natural cycles. This meta-analysis showed that progesterone supplementation for LPS was associated with increased LBR and CPR in NC-FET cycles (13). Stavridis et al., have recently presented another meta-analysis. The results of this meta-analysis suggested that rescue progesterone in patients with lower serum progesterone levels results in similar CPR, OPR and LBRs to patients with sufficient progesterone levels in artificial FET (18).

The lower limit of serum progesterone concentration indicative of an adequate luteal phase in natural cycles is not yet clear. However, mid-luteal serum concentrations of about 10 ng/mL are mostly accepted as adequate progesterone production by the corpus luteum during a natural cycle (14, 19, 20). Therefore, we took 10 ng/mL as the threshold serum progesterone concentration on the ET day in our study. In our study, only 49.7% of patients who are undergoing an NC-FET reached the cutoff serum progesterone concentration (≥10 ng/mL) on ET day, but 50.3% of patients fell short of this range (<10 ng/mL). Therefore, nearly half the NC-FET cycles might have benefitted from some LPS. LPS might be routinely implemented one day after LH surge in a standard fashion or reserved for cases with low levels of serum progesterone around the time of embryo transfer/implantation. Meanwhile, the number of day three embryo transfers was significantly higher in patients with serum progesterone concentrations <10 ng/mL on the day of ET and serum progesterone concentrations on ET day were significantly lower in day three embryo transfers. Therefore, 10 ng/mL as the threshold concentration of serum progesterone on the day of ET may not be suitable for day three embryo transfers. The importance of endocrine monitoring in t-NC is still a matter of debate. A retrospective study including 610 patients underwent t-NC FET found a 28.4% incidence of serum P4 elevation before the LH surge but there was no significant difference in terms of OPR between patients with or without P4 elevation on the day of LH surge (32.5% vs 31.7%) (21). The results of subgroup analysis demonstrated that, not the level, but the duration of P4 exposure before the LH surge was associated with the lower pregnancy rates.

Our study is the first study in patients undergoing t-NC FET to evaluate the feasibility of the alternative approach, reserving the initiation of progesterone support when the serum levels of progesterone are below 10 ng/mL on the day of ET. If this approach had been practical, it might have rescued nearly half of the tNC-FET cycles with adequate serum progesterone concentrations from unnecessary progesterone supplementation.

The major finding of our study is that rescue protocol by adding SC progesterone is effective and as a result no significant difference could be shown in terms of OPR. At the same time, CPR in patients

with low serum progesterone concentrations on ET day was lower despite LPS with sc progesterone supplementation. Our study has found that although pregnancy rates are similar, CPR is inferior in cycles with low serum progesterone despite the initiation of SC progesterone on ET day because of a higher incidence of biochemical pregnancies in this group. Therefore, SC progesterone supplementation can not sustain the essential luteal phase support when initiated on the day of ET if the endogenous CL function is inadequate. There are two possible explanations for this finding: Either we are too late to initiate luteal phase rescue, or the support dose/mode (sc) is inadequate. According to our best knowledge, our study is the first study to evaluate the effect of sc progesterone supplementation on PRs in tNC-FET based on serum progesterone level on ET day. Our previous study in programmed artificial FET cycles evaluated the effect of additional rescue. SC progesterone supplementation for restoring serum progesterone level in patients with low serum progesterone concentration on ET day, two days after the rescue treatment (17). However, in current study, we did not evaluate whether adequate serum progesterone level was reached with SC progesterone supplementation or not, due to its retrospective nature.

Bjuresten et al. reported an improvement in LBRs with 400 mg twice daily of vaginal progesterone in NC-FETs in their RCT, including 435 women (30% vs. 20%, P = 0.02), compared to those without supplementation (16). Likewise, in a prospective randomised controlled trial, Wanggren et al. evaluated the effect of LPS with 100 mg twice daily vaginal progesterone tablet in NC-FET cycles. They demonstrated the beneficial effect of vaginal progesterone supplementation on PRs (OR: 1.465, 95% CI 1.012–2.108, P=0.049), CPRs (OR: 1.497, 95% CI 1.024–2.188, P=0.043) and LBRs (OR:1.635, 95% CI 1.102–2.428, P=0.017) (22). In a retrospective cohort study including 228 consecutive patients who underwent NC-FET, Kim et al. reported improved LBR and reduced miscarriage rate with vaginal progesterone gel supplementation (23). Moreover, two recent meta-analyses strongly demonstrated the association between LPS with vaginal progesterone in NC-FET and higher LBRs (24, 25). In fact, according to our results, individualised rescue protocol using SC progesteron is effective since OPR is similar between groups. The primary difference was that we used an individualised LPS with SC progesterone by using serum progesterone levels on the day of ET. The optimal dosage of sc progesterone supplementation as LPS in tNC-FET has not been thoroughly studied, and further studies are needed on this aspect. Furthermore, in all those studies mentioned, vaginal progesterone as LPS was initiated before the transfer day, a RCT comparing rescue LPS with SC progesterone with no support is necessary to find out the exact role of this protocol.

We have previously mentioned a debate in the literature related to the beneficial effect of progesterone supplementation as LPS in tNC-FET. For tNC-FETs, it remains an open question about how to support the luteal phase, which dosages and agents to use, and the LPS's efficacy for these selected agents in clinical practice. In this regard, our study may shed more light on our knowledge to affect the current practice for NC-FET.

There are few points related to the current study as limitations. The first limitation is that it is a retrospective study with a relatively small sample size and that we did not report LBRs. We do not think patients with low serum progesterone concentrations on ET day can be refrained from progesterone supplementation because of the well-documented strong relationship between adequate serum progesterone concentrations and successful implantation and pregnancy outcome. Furthermore, the design of our practice to add LPS rescue with SC 25 mg progesterone is a clinically proven mode of action in artificial FET cycles when serum progesterone levels are found in a suboptimal range for a successful outcome on the day of ET. The only difference was that this strategy was never validated in tNC-FET cases. We were expecting the same beneficial effect of this strategy in tNC-FET cases since we have an additional functioning CL in a natural cycle. The second limitation is that serum progesterone concentrations were not measured after starting progesterone supplementation to confirm the attainment of adequate levels with sc progesterone. The design of our study, SC progesterone supplementation as LPS in natural cycles FET based on serum progesterone level on ET day, might be considered as the strength of our study.

In conclusion, according to our results, almost half of the patients who undergo tNC-FET might need LPS because of low serum progesterone concentrations on ET day. The measurement of serum progesterone level on ET day may create the opportunity for progesterone supplementation for patients with low serum progesterone concentrations. On the other hand, daily 25 mg sc progesterone supplementation may achieve similar OPR. Starting luteal phase support earlier than the day of embryo transfer in all NC-FET cases may be a better clinical decision in this setting until cut-off levels of P on the day of ET in tNC-FET is established and the benefit of rescue protocol is proven by RCTs. Further well-designed randomised controlled trials with large samples of patients are needed to evaluate the cutoff level of serum progesterone, which determine the necessity of LPS and the effect of sc progesterone supplementation as LPS on LBR during NC-FET.

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

Ethics statement

The studies involving humans were approved by The Ethical Committee of Bezmialem University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

CD: Conceptualization, Methodology, Writing – review & editing. PÖ: Conceptualization, Formal Analysis, Methodology, Supervision, Writing – original draft. FT: Data curation, Formal Analysis, Methodology, Writing – original draft. HT: Data curation, Formal Analysis, Writing – review & editing. ÖP: Data curation, Formal Analysis, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

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

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Roque M, Haahr T, Geber S, Esteves SC, Humaidan P. Fresh versus elective frozen embryo transfer in IVF/ICSI cycles: a systematic review and meta-analysis of reproductive outcomes. *Hum Reprod Update* (2019) 25(1):2–14. doi: 10.1093/humupd/dmy033

2. Saket Z, Källén K, Lundin K, Magnusson Å, Bergh C. Cumulative live birth rate after IVF: trend over time and the impact of blastocyst culture and vitrification. *Hum Reprod Open* (2021) 2021(3):1–9. doi: 10.1093/hropen/hoab021

3. Department of Health U and Services Centers for Disease Control H. *Assisted Reproductive Technology Fertility Clinic Success Rates Report* (2018). Available at: http://www.cdc.gov/art/reports.

4. Ghobara T, Vandekerckhove P. Cycle regimens for frozen-thawed embryo transfer. *Cochrane Database Syst Rev* (2008) 1). doi: 10.1002/14651858.CD003414.pub2

5. Glujovsky D, Pesce R, Sueldo C, Quinteiro Retamar AM, Hart RJ, Ciapponi A. Endometrial preparation for women undergoing embryo transfer with frozen embryos or embryos derived from donor oocytes. *Cochrane Database Syst Rev* (2020) 10(10). doi: 10.1002/14651858.CD006359.pub3

6. Groenewoud ER, Cantineau AEP, Kollen BJ, Macklon NS, Cohlen BJ. What is the optimal means of preparing the endometrium in frozen-thawed embryo transfer cycles? A systematic review and meta-analysis. *Hum Reprod Update* (2013) 19 (5):458–70. doi: 10.1093/humupd/dmt030

7. Groenewoud ER, Cohlen BJ, Al-Oraiby A, Brinkhuis EA, Broekmans FJM, De Bruin JP, et al. A randomized controlled, non-inferiority trial of modified natural versus artificial cycle for cryo-thawed embryo transfer. *Hum Reprod* (2016) 31(7):1483–92. doi: 10.1093/humrep/dew120

8. Wu H, Zhou P, Lin X, Wang S, Zhang S. Endometrial preparation for frozen-thawed embryo transfer cycles: a systematic review and network meta-analysis. *J Assist Reprod Genet* (2021) 38(8):1913–26. doi: 10.1007/s10815-021-02125-0

9. Gaggiotti-Marre S, Martinez F, Coll L, Garcia S, Álvarez M, Parriego M, et al. Low serum progesterone the day prior to frozen embryo transfer of euploid embryos is associated with significant reduction in live birth rates. *Gynecol Endocrinol* (2019) 35 (5):439–42. doi: 10.1080/09513590.2018.1534952

10. Labarta E, Mariani G, Holtmann N, Celada P, Remohí J, Bosch E. Low serum progesterone on the day of embryo transfer is associated with a diminished ongoing pregnancy rate in oocyte donation cycles after artificial endometrial preparation: a prospective study. *Hum Reprod* (2017) 32(12):2437–42. doi: 10.1093/humrep/dex316

11. Álvarez M, Gaggiotti-Marre S, Martínez F, Coll L, García S, González-Foruria I, et al. Individualised luteal phase support in artificially prepared frozen embryo transfer cycles based on serum progesterone levels: a prospective cohort study. *Hum Reprod* (2021) 36(6):1552–60. doi: 10.1093/humrep/deab031

12. Labarta E, Mariani G, Rodríguez-Varela C, Bosch E. Individualized luteal phase support normalizes live birth rate in women with low progesterone levels on the day of embryo transfer in artificial endometrial preparation cycles. *Fertil Steril* (2022) 117 (1):96–103. doi: 10.1016/j.fertnstert.2021.08.040

13. Jiang Y, Wang L, Shen H, Wang B, Wu J, Hu K, et al. The effect of progesterone supplementation for luteal phase support in natural cycle frozen embryo transfer: a systematic review and meta-analysis based on randomized controlled trials. *Fertil Steril* (2023) 119(4):597–605. doi: 10.1016/j.fertnstert.2022.12.035

14. Gaggiotti-Marre S, Alvarez M, González-Foruria I, Parriego M, Garcia S, Martínez F, et al. Low progesterone levels on the day before natural cycle frozen embryo transfer are negatively associated with live birth rates. *Hum Reprod* (2020) 35 (7):1623–9. doi: 10.1093/humrep/deaa092

15. Montagut M, Santos-Ribeiro S, De Vos M, Polyzos NP, Drakopoulos P, Mackens S, et al. Frozen-thawed embryo transfers in natural cycles with spontaneous or induced ovulation: the search for the best protocol continues. *Hum Reprod* (2016) 31(12):2803–10. doi: 10.1093/humrep/dew263

16. Bjuresten K, Landgren BM, Hovatta O, Stavreus-Evers A. Luteal phase progesterone increases live birth rate after frozen embryo transfer. *Fertil Steril* (2011) 95(2):534–7. doi: 10.1016/j.fertnstert.2010.05.019

17. Ozcan P, Cetin C, Okten B, Tanoglu FB, Taha HS, Pasin O, et al. The importance of serum progesterone concentration at embryo transfer day and effect of rescue additional progesterone during programmed artificial frozen embryo transfer cycles. *Reprod BioMed Online* (2022) 45(4):785–92. doi: 10.1016/j.rbmo.2022.05.023

18. Stavridis K, Kastora SL, Triantafyllidou O, Mavrelos D, Vlahos N. Effectiveness of progesterone rescue in women presenting low circulating progesterone levels around the day of embryo transfer: a systematic review and meta-analysis. *Fertil Steril* (2023) 119(6):954–63. doi: 10.1016/j.fertnstert.2023.02.007

19. Hull MGR, Savage PE, Bromham DR, Ismail AA, Morris AF. The value of a single serum progesterone measurement in the midluteal phase as a criterion of a potentially fertile cycle ("ovulation") derived form treated and untreated conception cycles. *Fertil Steril* (1982) 37(3):355–60. doi: 10.1016/S0015-0282(16)46095-4

20. Cédrin-Durnerin I, Isnard T, Mahdjoub S, Sonigo C, Seroka A, Comtet M, et al. Serum progesterone concentration and live birth rate in frozen-thawed embryo transfers with hormonally prepared endometrium. *Reprod BioMed Online* (2019) 38 (3):472–80. doi: 10.1016/j.rbmo.2018.11.026

21. Lee VC, Li RH, Chai J, Yeung TW, Yeung WS, Ho PC, et al. Effect of preovulatory progesterone elevation and duration of progesterone elevation on the pregnancy rate of frozen-thawed embryo transfer in natural cycles. *Fertil Steril* (2014) 101(5):1288–93. doi: 10.1016/j.fertnstert.2014.01.040

22. Wånggren K, Dahlgren Granbom M, Iliadis SI, Gudmundsson J, Stavreus-Evers A. Progesterone supplementation in natural cycles improves live birth rates after embryo transfer of frozen-thawed embryos-a randomized controlled trial. *Hum Reprod* (2022) 37(10):2366–74. doi: 10.1093/humrep/deac181

23. Kim C-H, Lee Y-J, Lee K-H, Kwon S-K, Kim S-H, Chae H-D, et al. The effect of luteal phase progesterone supplementation on natural frozen-thawed embryo transfer cycles. *Obstet Gynecol Sci* (2014) 57, 291. doi: 10.5468/OGS.2014.57.4.291

24. Mizrachi Y, Horowitz E, Ganer Herman H, Farhi J, Raziel A, Weissman A. Should women receive luteal support following natural cycle frozen embryo transfer? A systematic review and meta-analysis. *Hum Reprod Update* (2021) 27(4):643–50. doi: 10.1093/humupd/dmab011

25. Seol A, Shim YJ, Kim SW, Kim SK, Lee JR, Jee BC, et al. Effect of luteal phase support with vaginal progesterone on pregnancy outcomes in natural frozen embryo transfer cycles: A meta-analysis. *Clin Exp Reprod Med* (2020) 47(2):147–52. doi: 10.5653/cerm.2019.03132

frontiers | Frontiers in Endocrinology

TYPE Study Protocol
PUBLISHED 09 November 2023
DOI 10.3389/fendo.2023.1285040

OPEN ACCESS

EDITED BY
Zhen-Gao Sun,
Affiliated Hospital of Shandong University of Traditional Chinese Medicine, China

REVIEWED BY
Fu-Jen Huang,
Specialist Hospital, Poland
Tian Xia,
First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, China

*CORRESPONDENCE
Juan Carlos Castillo-Farfán
jcastillo@institutobernabeu.com

†These authors have contributed equally to this work

RECEIVED 29 August 2023
ACCEPTED 26 October 2023
PUBLISHED 09 November 2023

CITATION
De la Torre Perez E, Carratalá-Munuera MC, Castillo-Farfán JC, Lledó-Bosch B, Moliner-Renau B, Bernabeu-García A and Bernabeu-Pérez R (2023) Impact of endometrial compaction on reproductive outcomes after cryotransfer of euploid embryos in a modified natural cycle: protocol for a prospective cohort study.
Front. Endocrinol. 14:1285040.
doi: 10.3389/fendo.2023.1285040

Impact of endometrial compaction on reproductive outcomes after cryotransfer of euploid embryos in a modified natural cycle: protocol for a prospective cohort study

Esperanza De la Torre Perez[1†], Maria Concepción Carratalá-Munuera[2†], Juan Carlos Castillo-Farfán[3,4*], Belén Lledó-Bosch[3], Belén Moliner-Renau[3], Andrea Bernabeu-García[3,4] and Rafael Bernabeu-Pérez[3,4]

[1]Medical Department, Bernabeu Institute, Madrid, Spain, [2]Clinical Medicine Department, School of Medicine. University Miguel Hernández de Elche, Alicante, Spain, [3]Medical Department, Bernabeu Institute, Alicante, Spain, [4]University Chair of Community Medicine and Reproductive Health, Miguel Hernandez University of Elche, Alicante, Spain

Introduction: Embryo implantation is a complex and poorly understood process. Most studies to date have focused on the analysis of the endometrium at the end of the estrogenic phase, while the available data on its importance after secretory transformation are limited and inconsistent. Current evidence does not allow for a conclusive interpretation of the changes observed in the pre-implantation endometrium, whether in the natural or replacement cycle, and their relevance in the development of a pregnancy or the implications for clinical practice.

Methods: Multicenter prospective observational cohort study. Based on our sample size calculation, the study group will consist of 206 women (exposed or "compaction" group: 103 women with a decrease of ≥ 5% in endometrial thickness between the estrogenic phase and the day of embryo transfer; non-exposed "non-compaction" group: 103 women with similar or greater endometrial thickness between these time points). The main objective of this study is to compare the ongoing pregnancy rates in natural cycles for euploid embryo transfer in patients who present endometrial compaction at the time of transfer versus those who with a stable or greater endometrial thickness with respect to the estrogenic phase. The estimated duration of the study is 30 months. Inclusion criteria are: 18 to 50 years of age, with primary or secondary infertility, subjected to endometrial preparation in a modified natural cycle for transfer of a genetically euploid blastocyst, from their own oocyte or oocyte donation, with a normal uterine cavity. Exclusion criteria are: uterine or endometrial disease (e.g., multiple myomatosis, severe adenomyosis, Asherman syndrome, refractory endometrium), conditions that prevent correct ultrasound assessment (tilted uterus), or a history of recurrent implantation failure or repeated miscarriages.

Discussion: The findings from this study will provide valuable insights into the potential influence of the "endometrial compaction" phenomenon on reproductive outcomes during natural cycle endometrial preparation. By examining this aspect, we aim to contribute to a better understanding of the factors that may impact successful outcomes in fertility treatments.

KEYWORDS

assisted reproduction technology, ectopic pregnancy, endometrial compaction, *in vitro* fertilization-embryo transfer, endometrial thickness, IVF, placental complications

1 Introduction

Embryo implantation is a complex and poorly understood process, in which critical cross-talk must be established between the developing embryo and the receptive endometrial surface (1). Various hypotheses have been put forward about the conditions necessary for a receptive endometrium, among which is endometrial thickness. In that line, ultrasound monitoring of the endometrial cycle is currently the most widely used method to pinpoint the ideal moment for embryo transfer in the so-called "implantation window" (2).

Most studies to date have analyzed the endometrium at the end of the estrogenic phase, accepting that trilaminar morphology with a thickness of 7 mm to 12 mm is associated with a higher pregnancy rate (3–9). In contrast, an endometrial thickness under 7 mm compromises the prognosis of the transfer (3–5) and reduces the odds of implantation, clinical pregnancy, and a live birth (6, 10), along with increasing the risk of adverse obstetric outcomes derived from deficient placentation (11–19). However, the available data on the importance of the endometrium after secretory transformation are limited and inconsistent (20, 21).

Approximately half of women present reduced endometrial thickness around embryonic implantation relative to that measured in the estrogenic phase, a phenomenon known as endometrial compaction. In recent years, several studies have tried to determine if this event is a factor in reproductive outcomes (22–32). Some authors have found no differences associated with endometrial compaction in Frozen embryo transfer (FET) during a replacement cycle with estro-progesterone therapy (22–24), whereas others have observed a positive association between compaction and pregnancy rates (25–27). Regarding the natural cycle, research interest has been increasing significantly, but so far, the information is even more limited and discordant. One retrospective study from 2015 to 2019 (28) described compaction as more frequent in natural cycles than in replacement ones, associating it with a negative impact on the pregnancy rate. However, another study (29) found more compaction in the replacement cycle, but no significant association with the pregnancy rate. Subsequently, a prospective investigation (30) analyzed euploid embryo transfers in replacement, stimulated, and natural cycles, finding no differences in endometrial compaction. Two studies have recently been carried out during natural cycles (31, 32). In one (31), endometrial expansion was associated with a slight increase in clinical pregnancy, which was not reflected in changes in live births. However, a later study (32) reported that endometrial compaction was associated with a better pregnancy rate.

One reason for the incomplete transformation of the endometrium in the secretory phase may originate in an alteration in the estradiol-progesterone ratio, which occurs in certain situations such as ovarian hyperstimulation. One study (33) found that compaction was inversely proportional to the response to ovarian stimulation, although authors did not find a clear association between these changes and the pregnancy rate.

Furthermore, it is unclear whether the endometrial changes that occur before implantation are relevant to gestational complications. To date, only one study has identified compaction as a protective factor for ectopic pregnancy (34). However, a subsequent study (35) found no association between endometrial compaction prior to embryo transfer and preterm birth or placenta-mediated pregnancy complications. Current evidence does not allow for a conclusive interpretation of the changes observed in the pre-implantation endometrium, whether in the natural or replacement cycle, and their relevance in the development of a pregnancy or the implications for clinical practice. Therefore, the main objective of this study is to compare reproductive outcomes (ongoing pregnancy rate) in a homogeneous sample of patients who undergo euploid embryo transfer in a modified natural cycle, according to whether they present endometrial compaction at the time of transfer or show a stable or greater endometrial thickness relative to the estrogenic phase. Likewise, we will analyze whether endometrial compaction is associated with serum progesterone levels on the day of the transfer, and we will assess the variation in serum progesterone on the day of the pregnancy test and its impact on reproductive outcomes: biochemical pregnancy, clinical pregnancy, ongoing pregnancy, and early pregnancy loss rates.

2 Methods

2.1 Design

This is a multicenter, prospective observational cohort study, which will be performed at the different centers of the Bernabeu

Institute in Spain, specifically in Alicante, Madrid, Albacete, Cartagena, Elche, and Mallorca. The ethics committee of the Alicante General University Hospital approved the study (committee code 22/053Tut, Supplementary Material 1). A flowchart of this study design can be seen in Figure 1.

2.2 Study population

The sample will be drawn from patients at the Bernabeu Institute in Alicante, Madrid, Albacete, Cartagena, Elche, and Mallorca, undergoing fertility treatment that includes endometrial preparation in a modified natural cycle for transfer of previously analyzed frozen and euploid embryos, and who meet the inclusion criteria: aged 18 to 50 years, with primary or secondary infertility, with a normal uterine cavity, undergoing endometrial preparation in a modified natural cycle for single embryo transfer in the blastocyst state from own oocyte or oocyte donation cycles, who had normal results on preimplantation genetic testing for aneuploidy (PGT-A) via trophectoderm biopsy. Exclusion criteria were: uterine or endometrial disease (multiple myomatosis [>3 fibroids of > 3 cm], adenomyosis, Asherman syndrome); difficulties in correctly measuring endometrial thickness due to a retroverted or tilted uterus; a history of recurrent implantation failure (3 or more transferred blastocysts of good quality, from their own oocyte [<35 years] or oocyte donation); recurrent early Pregnancy Loss (the loss of two or more pregnancies before 10 weeks of gestational age (36) and suboptimal endometrial response (endometrium < 6 mm on the day of ovulation triggering). (Table 1)

Eligible patients who sign informed consent will be divided into two cohorts: the exposed (or compaction) group and the non-exposed (non-compaction) group, depending on the endometrial thickness on the day of embryo transfer, as measured by transvaginal ultrasound:

- The compaction group will comprise patients who present a decrease of 5% or more in endometrial thickness on the day of embryo transfer with respect to the estrogenic phase.
- The non-compaction group will be made up of women presenting similar or greater endometrial thickness on the day of the transfer with respect to the estrogenic phase, measured with transvaginal ultrasound.

We defined compaction percentage to avoid minor measurement variations and according to previous studies using similar values (24, 25, 28, 32).

2.3 Sampling

Researchers at the assisted reproduction services of the Bernabeu Institute centers will be responsible for recruitment during their clinical practice, through opportunistic sampling of the patients undergoing frozen embryo transfer after PGT-A. Together with the embryo transfer consent, patients will receive information on the purpose of the study and be asked to sign informed consent as a condition for participating (Supplementary Material 2). The endometrial preparation treatment will not differ from usual practice.

2.4 Variables

Data collection will commence after both the patient and the researcher have signed informed consent. A purpose-designed data collection notebook will be designed for the study. The variables under study will be incorporated into an anonymized and encrypted database for subsequent statistical analysis. The main explanatory (exposure) variable is endometrial compaction, defined as a

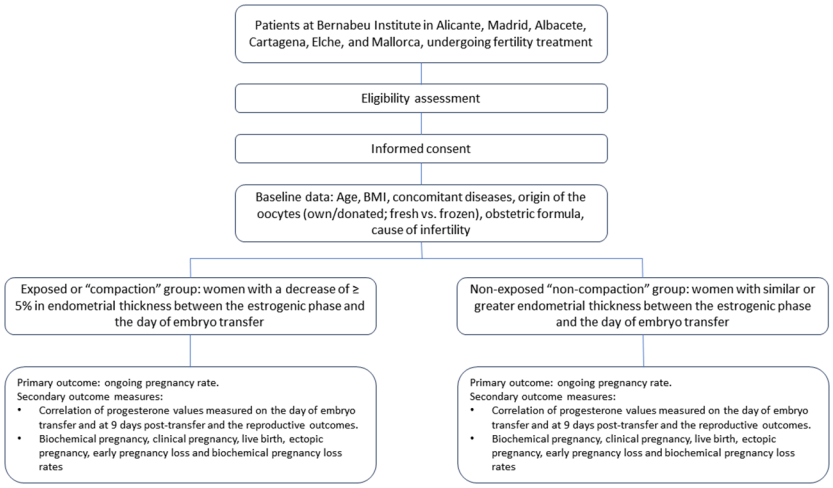


FIGURE 1
Flowchart of the study design.

TABLE 1 Inclusion/exclusion criteria.

Inclusion criteria
Age 18 to 50 years
Primary or secondary infertility
Normal uterine cavity
Endometrial preparation in a modified natural cycle
Undergoing single embryo transfer in the blastocyst state from their own oocyte or oocyte donation
Normal results on preimplantation genetic testing for aneuploidy (PGT-A) via trophectoderm biopsy
Exclusion criteria
Uterine or endometrial disease (myomatosis [>3 fibroids of > 3 cm], adenomyosis, Asherman syndrome)
Difficulties in correctly measuring endometrial thickness (e.g. due to a retroverted or tilted uterus)
History of recurrent implantation failure (3 or more transferred blastocysts of good quality)
History of recurrent early pregnancy loss
Suboptimal endometrial response (endometrium < 6 mm on the day of ovulation triggering)

reduction in the thickness of the endometrium of 5% or more from the day of ovulation induction to the day of embryo transfer. Other variables include:

2.4.1 Baseline patient parameters from the electronic medical record

- Age at the time of transfer.
- Standardized body mass index.
- Concomitant diseases (hypertension, hypothyroidism, diabetes, autoimmune diseases…).
- Origin of the oocytes: own gametes/donated gametes; fresh vs. frozen.
- Obstetric formula.
- Cause of infertility:
    - Male factor: male diagnosed with seminal or urological problems causing infertility.
    - Uterine factor: presence of uterine disease (fibroids, synechiae, adenomyosis) causing infertility.
    - Tubal factor: obstruction of fallopian tubes.
    - Ovarian factor: endometriosis, low ovarian reserve, previous ovarian surgery.
    - Unknown cause: not included in any of the above.
    - Mixed cause: presence of 2 or more factors.

2.4.2 Prospective variables: cycle follow-up

- Follicular phase length until ovulation induction (days).
- Endometrial thickness (mm), as measured by vaginal ultrasound in the follicular phase prior to ovulation induction (recombinant hCG 6500 subcutaneous IU).
- Endometrial thickness (mm) in the secretory phase (7 days after administration of recombinant hCG), at the time of embryo transfer, as measured by vaginal ultrasound*.
- Serum progesterone on the day of embryo transfer and at 9 days post-transfer.
- Serum b-hCG at 9 days after the embryo transfer, according to standard protocol.

2.4.3 Definitions for reproductive outcomes

Definitions for reproductive outcomes were (36, 37):

- Biochemical pregnancy: A pregnancy diagnosed only by the detection of beta hCG in serum 9 days after the embryo transfer.
- Clinical pregnancy: A pregnancy diagnosed by ultrasonographic visualization of one or more gestational sacs or definitive clinical signs of pregnancy. In addition to intra-uterine pregnancy, it includes a clinically documented ectopic pregnancy.
- Ongoing pregnancy: the presence of positive heartbeat as seen by sonography at 10 weeks gestational age.
- Live birth: 22 completed weeks of gestational age.
- Ectopic pregnancy: ultrasonic or surgical visualization of a pregnancy outside of the endometrial cavity.
- Early pregnancy loss: spontaneous pregnancy demise before 10 weeks of gestational age (before 8th developmental week).
- Biochemical pregnancy loss: spontaneous pregnancy demise based on decreasing serum b-hCG levels, without an ultrasound evaluation.

*Both ultrasounds will be performed by the attending gynecologist. In the cases of international patients who perform the follicular faze scan outside the center, a standardized data collection sheet (Supplementary Material 3) will be used, and the attending gynecologist will evaluate both this and the ultrasound images according to standard protocols.

2.5 Natural cycle monitoring

Women with regular menstrual cycles (28 ± 7 days) will undergo transvaginal ultrasound between days 7 to 10 of their menstrual cycle, adjusted based on cycle length. This procedure aims to monitor endometrial and follicular growth and will be repeated every two days as required. Ovulation will be induced using 6500 IU of hCGr (Ovitrelle®; NV Organon) when ultrasound reveals an endometrial thickness of 7 mm or more and a follicle measuring 17-20 mm, aligning with standard clinical practice (38–

40). Patients will receive a daily vaginal dose of 400 mg progesterone pessaries at bed time (Cyclogest®; Gedeon Richter,Budapest, Hungary) starting two-days after hCG administration and continued until 7 weeks of gestation if pregnancy is achieved (41).

2.5.1 Outcome measures

2.5.1.1 Primary outcome measure

The primary outcome for the comparison of the two groups is the ongoing pregnancy rate.

2.5.1.2 Secondary outcome measures

Correlation of progesterone values measured on the day of embryo transfer and at 9 days post-transfer and the reproductive outcomes.

Biochemical pregnancy, clinical pregnancy, live birth, ectopic pregnancy, early pregnancy loss and biochemical pregnancy loss rates.

2.5.2 Sample size calculation and statistical analysis

With an expected proportion of live births in the non-compaction group of 50% and in the compaction group of 70%, and taking into account a two-sided significance level of 0.05 and a power of 80%, the number of women required in each group would be 93. Assuming an attrition rate of 15%, 103 women are needed in each group, for a total sample size of 206.

In the descriptive analysis, qualitative variables will be expressed as frequency and percentage, and quantitative variables as measures of central tendency and dispersion. For the univariable analysis, the Chi-square test or Fisher's exact test will be used to compare qualitative variables. The normality of the quantitative variables will be checked using the Kolmogorov-Smirnov test, and in the case of a non-parametric distribution, a log transformation will be performed. If the distribution is normal, the student's t test will be used for comparison. P values of less than 0.05 will be considered statistically significant. Variables that do not meet the criterion of normality will be analyzed using the Wilcoxon-Mann-Whitney test. Multivariable analyses will be carried out using linear or binary logistic regression to control for potential confounders. Cases will be entered into a database and analyzed using the statistical package SPSS version 20.0 for Windows (SPSS Inc. Chicago. IL).

3 Discussion

Currently, there is no solid evidence that allows us to interpret whether the changes observed in the pre-implantation endometrium influence pregnancy outcomes. Although scientific interest in this area has increased dramatically in recent years, the results published to date are highly heterogeneous, probably due to differences in the study population; endometrial preparation protocols; type of ultrasounds used for the assessment of the endometrium (abdominal vs. vaginal); and number, quality and stage of the transferred embryos; among other differences. Nevertheless, it is plausible that the pre-implantation endometrium may play a role in the subsequent development of gestational complications related to placentation. To date, only two retrospective studies have looked into this question: the first (34) identified compaction as a protective factor for ectopic pregnancy, while the second (35) found no association between this event and preterm birth or placenta-mediated pregnancy complications. The present study would be the first to provide prospective evidence on the possible impact of endometrial compaction on obstetric complications such as early miscarriage, biochemical pregnancy loss, and ectopic pregnancy, including detailed baseline and clinical data from the patients. In addition, an exploratory study of complications in advanced pregnancy and childbirth could be considered.

One potential limitation of this study resides in the fact that different professionals will perform the ultrasound scans, and in the case of international patients, these will be professionals outside the center, which could introduce a measurement bias. However, measures will be taken to minimize this bias by requesting imaging results from patients whose ultrasound is performed outside the center, as done in routine practice. Images of poor quality that cannot be evaluated will be excluded from the study.

Regarding the strengths, this study will be the first prospective analysis of reproductive outcomes from euploid embryos in natural cycles, comparing patients who present endometrial compaction versus stable or increased endometrial thickness at the time of transfer. In addition, we will assess the association between endometrial compaction and serum progesterone levels, variations in serum progesterone and its impact on pregnancy outcomes, implantation rate, clinical pregnancy, clinical abortion, and biochemical abortion. The prospective design will allow a careful selection of the sample and rigorous collection of patient variables. In addition, its multicenter nature will favor the generalizability of the results. Furthermore, the sample will include national and international patients, and all necessary resources are available, with no need for modifying usual clinical practice.

The primary purpose of this study is to assess reproductive outcomes, specifically the ongoing pregnancy rate, in a homogeneous sample of patients undergoing euploid embryo transfer using a modified natural cycle. A prospective analysis will be performed to investigate whether the observed changes in endometrial development and serum progesterone levels in the natural cycle are relevant to the development of a pregnancy. This approach will contribute to improving our understanding of the ideal circumstances for embryo implantation and its application in clinical practice. In addition, the correlation between these factors and serum progesterone levels will be discussed, which could provide an additional avenue for outcome evaluation. The results obtained from this clinical research will be reviewed and discussed by the research team for subsequent publication and dissemination.

Ethics statement

The ethics committee of the Alicante General University Hospital (22/053Tut) approved this study. The results obtained as a result of clinical research will be reviewed and discussed by the research team for subsequent publication and dissemination. The study will be carried out in strict compliance with international

research ethics norms. Before including any study participant, the ethics committee of Alicante General University Hospital approved the protocol, the information sheet that will be given to the participants, and the informed consent form that will be used.

Author contributions

EP: Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review and editing. MC-M: Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review and editing. JC-F: Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review and editing. BL-B: Investigation, Writing – original draft, Writing – review and editing. MB-R: Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review and editing. A-G: Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review and editing. RBP: Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The development of this study was empowered by a grant specifically dedicated to financing R+D+I (Research + Development + Investigation) projects, with a focus on generating an industrial.

Acknowledgments

We acknowledge the contribution of the colleagues from Miguel Hernandez University, which assisted on giving scientific consistency to the study, and to all the gynecologists and assistants at Bernabeu Institute that helped in data collection.

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

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1285040/full#supplementary-material

References

1. Bergh PA, Navot D. The impact of embryonic development and endometrial maturity on the timing of implantation. *Fertil Steril* (1992) 58:537–42. doi: 10.1016/S0015-0282(16)55259-5

2. Shapiro H, Cowell C, Casper RF. The use of vaginal ultrasound for monitoring endometrial preparation in a donor oocyte program. *Fertil Steril* (1993) 59:1055–58. doi: 10.1016/S0015-0282(16)55927-5

3. Weissman A, Gotlieb L, Casper RF. The detrimental effect of increased endo- metrial thickness on implantation and pregnancy rates and outcome in an *in vitro* fertilization program. *Fertil Steril* (1999) 71:147–9. doi: 10.1016/S0015-0282(98)00413-0

4. Wu Y, Gao X, Lu X, Xi J, Jiang S, Sun Y, et al. Endometrial thickness affects the outcome of in *vitro* fertilization and embryo transfer in normal responders after GnRH antagonist administration. *Reprod Biol Endocrinol* (2014) 9:12–96. doi: 10.1186/1477-7827-12-96

5. Isaacs JD Jr, Wells CS, Williams DB, Odem RR, Gast MJ, Strickler RC. Endometrial thickness is a valid monitoring parameter in cycles of ovulation induc-tion with menotropins alone. *Fertil Steril* (1996) 65:262–6. doi: 10.1016/S0015-0282(16)58082-0

6. Gao G, Cui X, Li S, Ding P, Zhang S, Zhang Y. Endometrial thickness with *in-vitro* fertilization: a meta analysis. *Reprod BioMed Online* (2020) 40(1):124–33. doi: 10.1016/j.rbmo.2019.09.005

7. Gallos ID, Khairy M, Chu J, Rajkhowa M, Tobias A, Campbell A, et al. Optimal endometrial thickness to maximize live births and minimize pregnancy losses: analysis of 25,767 fresh embryo transfers. *Reprod BioMed Online* (2018) 37(5):542–8. doi: 10.1016/j.rbmo.2018.08.025

8. Yuan X, Saravelos SH, Wang Q, Xu Y, Li TC, Zhou C. Endometrial thickness as a predictor of pregnancy outcomes in 10787 fresh IVF-ICSI cycles. *Reprod BioMed Online* (2016) 33:197–205. doi: 10.1016/j.rbmo.2016.05.002

9. Kasius A, Smit JG, Torrance HL, Eijkemans MJ, Mol BW, Opmeer BC. Endometrial thickness and pregnancy rates after IVF: a systematic review and meta-analysis. *Hum Reprod Update* (2014) 20:530–41. doi: 10.1093/humupd/dmu011

10. Liu KE, Hartman M, Hartman A, Luo ZC, Mahutte N. The impact of a thin endometrium on fresh and frozen -thaw IVF outcomes: an analysis of over 40000 embryo transfers. *Hum Reprod* (2018) 33(10):1883–88. doi: 10.1093/humrep/dey281

11. Liu H, Zhang J, Wang B, Kuang Y. Effect of endometrial thickness on ectopic pregnancy in frozen embryo transfer cycles: an analysis including 17244 pregnancy cycles. *Fertil Steril* (2020) 113(1):131–9. doi: 10.1016/j.fertnstert.2019.09.003

12. Liu X, Qu P, Bai H, Shi W, Shi J. Endometrial thickness as a predictor of ectopic pregnancy in 1125 in *vitro* fertilization – embryo transfer cycles: a matched case – control study. *Arch Gynecol Obstet* (2019) 300(6):1797–803. doi: 10.1007/s00404-019-05353-z

13. Rombauts L, McMaster R, Motteram C, Fernando S. Risk of ectopic pregnancy is linked to endometrial thickness in a retrospective cohort study of 8120 assisted reproduction technology cycles. *Hum Reprod* (2015) 30:2846–52. doi: 10.1093/humrep/dev249

14. Jing S, Li X, Zhang S, Gong F, Lu G, Lin G. The risk of placenta previa and cesarean section associated with a thin endometrial thickness: a retrospective study of 5251 singleton births during frozen embryo transfer in China. *Arch Gynecol Obstet* (2019) 300(5):1227–37. doi: 10.1007/s00404-019-05295-6

15. Rombauts L, Motteram C, Berkowitz E, Fernando S. Risk of placenta praevia is linked to endometrial thickness in a retrospective cohort study of 4537 singleton assisted reproduction technology births. *Hum Reprod* (2014) 12:2787–93. doi: 10.1093/humrep/deu240

16. Guo Z, Xu X, Zhang L, Zhang L, Yan L, Ma J. Endometrial thickness is associated with incidence of small-for-gestational age infants in fresh in *vitro* fertilization-intracytoplasmic sperm injection and embryo transfer cycles. *Fertil Steril* (2020) 113 (4):745–52. doi: 10.1016/j.fertnstert.2019.12.014

17. Zhang J, Liu H, Mao X, Chen Q, Si J, Fan Y, et al. Effect of endometrial thickness on birthweight in frozen embryo transfer cycles: an analysis including 6181 newborns. *Hum Reprod* (2019) 34(9):1707–15. doi: 10.1093/humrep/dez103

18. Moffat R, Beutler S, Schötzau A, De Geyter M, De Geyter C. Endometrial thickness influences neonatal birth weight in pregnancies with obstetric complications achieved after fresh IVF-ICSI cycles. *Arch Gynecol Obstet* (2017) 296(1):115–22. doi: 10.1007/s00404-017-4411-z

19. He L, Zhang Z, Li H, Li Y, Long L, He W. Correlation between endometrial thickness and perinatal outcome for pregnancies achieved through assisted reproduction technology. *J Perinat Med* (2019) 48(1):16–20. doi: 10.1515/jpm-2019-0159

20. Griesinger G, Trevisan S, Cometti B. Endometrial thickness on the day of the embryo transfer is a poor predictor of IVF treatment outcome. *Hum Reprod Open* (2018) 2018(1):hox031. doi: 10.1093/hropen/hox031

21. Bu Z, Wang K, Dai W, Sun Y. Endometrial thickness significantly affects clinical pregnancy and live birth rates in frozen-thawed embryo transfer cycles. *Gynecol Endocrinol* (2016) 32(7):524–8. doi: 10.3109/09513590.2015.1136616

22. Colombo S, Franasiak JM, Kaser DJ, Molinaro TA, et al. Significance of endometrial lining decrease after progesterone start in synthetic frozen embryo transfer (FET) cycles. *Fertil Steril* (2017) 108:304–5. doi: 10.1016/j.fertnstert.2017.07.900

23. Olgan S, Dirican EK, Sakinci M, Caglar M, Ozsipahi AC, Gul SM, et al. Endometrial compaction does not predict the reproductive outcome after vitrified-warmed embryo transfer: a prospective cohort study. *Reprod BioMed Online* (2022) 45 (1):81–7. doi: 10.1016/j.rbmo.2022.02.025

24. Riestenberg C, Quinn M, Akopians A, et al. Endometrial compaction does not predict live birth rate in single euploid frozen embryo transfer cycles. *J Assist Reprod Genet* (2021) 38(2):407–12. doi: 10.1007/s10815-020-02043-7

25. Haas J, Smith R, Zilberberg E, Nayot D, et al. Endometrial compaction (decreased thickness) in response to progesterone results in optimal pregnancy outcome in frozen embryo transfers. *Fertil Steril* (2019) 112:503–9. doi: 10.1016/j.fertnstert.2019.05.001

26. Forman EJ. The receptive endometrium: the thinner the better? *Fertil Steril* (2019) 112:468–9. doi: 10.1016/j.fertnstert.2019.05.037

27. Zilberberg E, Smith R, Nayot D, Haas J, et al. Endometrial compaction before frozen euploid embryo transfer improves ongoing pregnancy rates. *Fertil Steril* (2020) 113:990–5. doi: 10.1016/j.fertnstert.2019.12.030

28. Bu Z, Yang X, Song L, Kang B, Sun Y. The impact of endometrial thickness change after progesterone administration on pregnancy outcome in patients transferred with single frozen thawed blastocyst. *Reprod Biol Endocrinol* (2019) 17 (1):99. doi: 10.1186/s12958-019-0545-0

29. Ye J, Zhang J, Gao H, Zhu Y, et al. Effect of endometrial thickness change in response to progesterone administration on pregnancy outcomes in frozen-thawed embryo transfer: analysis of 4465 cycles. *Front Endocrinol* (2020) 11:546232. doi: 10.3389/fendo.2020.546232

30. Shah JS, Vaughan DA, Dodge LE, et al. Endometrial compaction does not predict live birth in single euploid frozen embryo transfers: a prospective study. *Hum Reprod* (2022) 37(5):980–7. doi: 10.1093/humrep/deac060

31. Jin Z, Li J, Yang E, et al. Effect of endometrial thickness changes on clinical pregnancy rates after progesterone administration in a single frozen-thawed euploid blastocyst transfer cycle using natural cycles with luteal support for PGT-SR- and PGT-M-assisted reproduction: a retrospective cohort study. *Reprod Biol Endocrinol* (2021) 19(1):154. doi: 10.1186/s12958-021-00841-

32. Youngster M, Mor M, Kedem A, Gat I, et al. Endometrial compaction is associated with increased clinical and ongoing pregnancy rates in unstimulated natural cycle frozen embryo transfers: a prospective cohort study. *J Assist Reprod Genet* (2022) 39(8):1909–16. doi: 10.1007/s10815-022-02544-7

33. Lam MT, Li HWR, Ng EHY. Impact of endometrial thickness and volume compaction on the live birth rate following fresh embryo transfer of *in vitro* fertilization. *J Ultrasound Med* (2022) 41(6):1455–63. doi: 10.1002/jum.15830

34. Li Q, Liu A, Shen H, Zhang X. Endometrial compaction after human chorionic gonadotrophin administration reduces ectopic pregnancy rate following fresh embryo transfer in *vitro* fertilization/intracytoplasmic sperm injection cycles in patients with non-thin endometrium: a retrospective cohort study. *Reprod Biol Endocrinol* (2022) 20 (1):151. doi: 10.1186/s12958-022-01020-2

35. Gill P, Melamed N, Barrett J, Casper RF. A decrease in endometrial thickness before embryo transfer is not associated with preterm birth and placenta-mediated pregnancy complications. *Reprod BioMed Online* (2023) 46(2):283–8. doi: 10.1016/j.rbmo.2022.11.010

36. Kolte AM, Bernardi LA, Christiansen OB, Quenby S, et al. ESHRE Special Interest Group, Early Pregnancy. Terminology for pregnancy loss prior to viability: a consensus statement from the ESHRE early pregnancy special interest group. *Hum Reprod* (2015) 30(3):495–8. doi: 10.1093/humrep/deu299

37. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, et al. The international glossary on infertility and fertility care, 2017. *Hum Reprod* (2017) 32(9):1786–801. doi: 10.1093/humrep/dex234

38. Groenewoud ER, Cohlen BJ, Al-Oraiby A, Brinkhuis EA, et al. A randomized controlled, non-inferiority trial of modified natural versus artificial cycle for cryo-thawed embryo transfer. *Hum Reprod Oxf Engl* (2016) 31(7):1483–92. doi: 10.1093/humrep/dew120

39. Saupstad M, Freiesleben NLC, Skouby SO, Andersen LF, et al. Preparation of the endometrium and timing of blastocyst transfer in modified natural cycle frozen-thawed embryo transfers (mNC-FET): a study protocol for a randomised controlled multicentre trial. *BMJ Open* (2019) 9(12):e031811. doi: 10.1136/bmjopen-2019-031811

40. Fatemi HM, Kyrou D, Bourgain C, Van den Abbeel E, et al. Cryopreserved-thawed human embryo transfer: spontaneous natural cycle is superior to human chorionic gonadotropin-induced natural cycle. *Fertil Steril* (2010) 94(6):2054–8. doi: 10.1016/j.fertnstert.2009.11.036

41. Kyrou D, Fatemi HM, Popovic-Todorovic B, Van den Abbeel E, et al. Vaginal progesterone supplementation has no effect on ongoing pregnancy rate in hCG-induced natural frozen-thawed embryo transfer cycles. *Eur J Obstet Gynecol Reprod Biol* (2010) 150:175–9. doi: 10.1016/j.ejogrb.2010.02.038

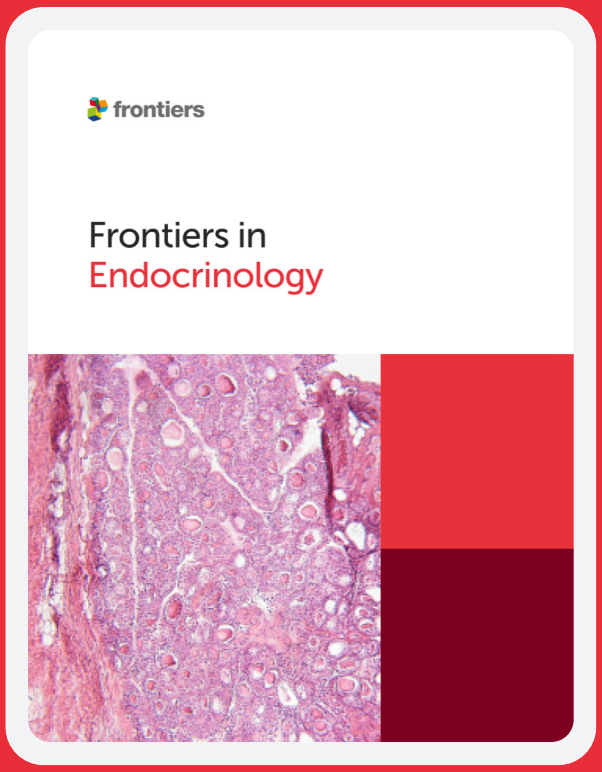
Frontiers in Endocrinology

Explores the endocrine system to find new therapies for key health issues

The second most-cited endocrinology and metabolism journal, which advances our understanding of the endocrine system. It uncovers new therapies for prevalent health issues such as obesity, diabetes, reproduction, and aging.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact