Observational Study

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Decreasing serum estradiol level on day of progesterone start in programmed frozen embryo transfer cycles and the pregnancy outcomes

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Abstract

High serum estradiol levels may reduce the success of conception and live birth in both in vitro fertilization and frozen embryo transfer (FET). This retrospective study sought to determine whether an association exists between decreasing the serum estradiol level and the clinical outcome following programmed FET cycles. The analysis retrieved the data of patients who underwent programmed FET cycles at the Center of Reproductive Medicine of Weifang People's Hospital from January 2022 to March 2023. The pregnancy outcomes were compared between patient groups with different estradiol levels but otherwise identical profiles. Of all 769 included patients, 188 received 3 mg/d estradiol valerate (group A) and 581 received 4 mg/d 17β-estradiol (group B). Of group B patients, 186 (group BP) with identical baseline clinical data as patients in group A were selected by using propensity score matching. While the serum estradiol level was much lower in those receiving 3 mg/d estradiol valerate (group A) than those receiving 4 mg/d 17β-estradiol (group BP), the endometrial thickness was not affected by the medication regimen. Both groups had comparable pregnancy outcomes, including clinical pregnancy, implantation, early miscarriage, ectopic pregnancy, and live birth rate. In programmed FET cycles, decreasing the serum level of estradiol does not significantly impact the pregnancy outcome.

Abbreviations: FET = frozen embryo transfer, IVF = in vitro fertilization, β-hCG = β-human chorionic gonadotropin.

Keywords: 17β-estradiol, endometrial preparation, estradiol valerate, frozen embryo transfer, live birth rate, pregnancy outcomes

1. Introduction

Frozen embryo transfer (FET), which was first reported in 1983,[1] is now a mature option in assisted reproductive technology. Compared to in vitro fertilization (IVF), it has a lower risk of the ovarian hyperstimulation syndrome and allows the storage of surplus embryos for preimplantation diagnoses. Roque et al[2] concluded in a systematic review and meta-analysis that FET gave significantly higher ongoing pregnancy rates and clinical pregnancy rates than fresh embryo transfer, presumably because a better embryo-endometrium synchrony could be achieved in the endometrium preparation cycles of FET. Nevertheless, controversy exists regarding which endometrial preparation regimen leads to better pregnancy outcomes. When the natural ovulation cycle is used for endometrial preparation, the estradiol and progesterone levels are monitored closely to determine the window of implantation, as the dominant follicle will increase the estradiol level to promote endometrial proliferation and upregulate the expression of progesterone receptors.[3] Alternatively, the programmed cycle (hormone replacement therapy) may be conveniently or preferentially used, especially for patients with ovulation disorders, as it gives a clear date of the transplantation and requires less monitoring. In the programmed cycle, endometrial proliferation is stimulated by exogenous estrogen only, but consensus is currently lacking on how to supplement the exogenous estrogen most effectively for the sake of endometrial preparation. The impact of supraphysiological levels of estradiol on pregnancy outcomes has been reported,[4,5] whereas few studies examined the effects of low estradiol levels on pregnancy outcomes. It has been estimated that an estradiol level of 50 to 100 pg/mL is required to trigger the downstream effects of estradiol action.[6] This study aimed to determine the impact of low estradiol level at the onset of intramuscular progesterone administration in using programmed FET cycles.

2. Materials and methods

2.1. Patients

Patients who underwent programmed FET cycles at the Center of Reproductive Medicine, Weifang People's Hospital, from January 2022 to March 2023 were divided into group A (those without functional cysts and took 3 mg/d estradiol valerate

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

This retrospective study received ethical approval from the Ethics Committee of Weifang People' Hospital. All patient couples provided written informed consent. All methods were carried out in accordance with relevant guidelines and regulations.

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for endometrial growth) and group B (those who took 4 mg/d 17β-estradiol for endometrial growth). Patients were included if they were 21 to 45 years of age, underwent IVF/intracytoplasmic sperm injection with their cryopreserved embryos, and the transfer involved D3 good quality embryos (7–9 cells, <10% fragmentation rate) or D5/D6 blastocysts (4/5 AA, AB, BA, BB, AC, CA, BC, CB). Patients were excluded if they had (1) spontaneous follicular growth and ovulation in programmed cycles, (2) adenomyosis, (3) untreated hydrosalpinx, or (4) lesions in the uterine cavity or endometrium, or if they failed at least 3 transfers previously. Written informed consent was obtained from all patient couples. All related procedures and protocols were approved by the Institutional Review Boards at Weifang People's Hospital.

There were 188 patients in group A and 581 patients in group B. The propensity score matching method was used to select a subset of patients from group B (referred to hereafter as group BP) that were indistinguishable from the patients of group A regarding the baseline clinical data (Table 1). The propensity score of each patient was calculated by the 1:1 nearest matching method, and caliper matching was employed to limit the logarithmic standard deviation of the propensity score to 0.02. Figure 1 illustrates the construction of the patient groups.

2.2. Endometrial preparation and thawed embryo transfer

Patients started to receive Progynova® (estradiol valerate; Bayer, Leverkusen, Germany; 1 mg t.i.d., group A) or Femoston® (17β-estradiol; Abbott, Abbott Park, IL; 2 mg b.i.d., group B) orally at the start of their menstrual cycle (day 2–4), and their serum levels of estradiol and progesterone and endometrial thickness were then monitored. After more than 10 days of medication, once the endometrial thickness reached 7 mm, they were given intramuscular injections of progesterone (Xianju Pharma, Zhejiang, China; 40 mg q.d.) to induce endometrial transformation until FET. FET was performed 4 days after endometrial transformation if cleavage-stage embryos were transferred and 6 days after endometrial transformation if blastocysts were transferred.

The medications for luteal support after the embryo transfer were the following: group A, progesterone injection (Xianju Pharma, Zhejiang, China; 40 mg q.d., i.m.), Progynova® (1 mg t.i.d.), Duphaston® (dydrogesterone; Abbott, Abbott Park, IL; 10 mg t.i.d.), and Utrogestan® (vaginal micronized progesterone; Besins, Le Concorde, Monaco; 200 mg q.d.); group B, progesterone injection (Xianju Pharma, Zhejiang, China; 40 mg q.d., i.m.), Femoston® (2 mg 17β-estradiol/10 mg dydrogesterone, b.i.d.), Duphaston® (10 mg b.i.d.), and Utrogestan® (200 mg q.d.). Two weeks after the FET, the serum β-human chorionic gonadotropin (β-hCG) was measured to identify biochemical pregnancy, and if elevated levels of serum β-hCG were identified, a transvaginal ultrasound was performed 2 weeks later to confirm the clinical pregnancy and determine the number of implanted embryos. The supplementation of exogenous hormone was terminated after 10 weeks of gestation. The patient was monitored regularly until giving birth.

2.3. Statistical analysis

Data analysis was performed using SPSS 25.0 (IBM Corp., Armonk, NY). Continuous variables were presented as mean ± standard deviation if they had a normal distribution and as median (25% quartile, 75% quartile) if otherwise, and analyzed by the Kruskal–Wallis test and the Mann–Whitney *U* test. Dichotomous variables were shown as frequencies (%) and analyzed by the χ^2 test.

3. Results

The patients in group A had significantly lower ($P < .05$) anti-müllerian hormone (2.89 ng/mL) than those in group B (3.62 ng/mL), and they also tended to use blastocyst for FET and transferred fewer embryos ($P < .05$). Other aspects of the patient data were indistinguishable ($P > .05$) between group A and group B. Through propensity score matching, 186 patients were selected from group B and paired with the patients in group A. This set of selected patients, referred to as group BP, was indistinguishable from group A (Table 1).

Table 2 shows the endometrial thickness and the blood parameters of the patients in group A and group BP. Although the patients in group A had significantly lower levels ($P < .001$) of estradiol both on the day of starting progesterone and on the day of FET, they had identical endometrial thickness and progesterone level on the day of FET compared to the patients in group BP ($P > .05$). Table 3 shows that the 2 groups had

Table 1

Comparison of patient data before and after propensity score matching.

Group		A	B*	*P*-value†	BP*	*P*-value†
Patient						
Female	Number	188	581		186	
	Age (yr)	34 (30, 36)	33 (30, 36)	.353	33 (30, 36.25)	.578
	BMI (kg/m^2)‡	23.84 (21.60, 26.22)	23.70 (20.85, 26.22)	.398	23.44 (20.57, 25.86)	.132
	AMH (ng/mL)‡	2.89 (1.44, 4.50)	3.62 (1.78, 5.70)	.009	3.38 (1.37, 5.14)	.537
Male	Age (yr)	34 (31, 37)	33 (31, 36)	.219	33 (31, 36)	.474
Previous protocols				.068		.139
GnRH-ant‡		120 (63.83%)	404 (69.54%)		102 (54.84%)	
GnRH-a‡		41 (21.81%)	127 (21.86%)		56 (30.11%)	
Mild stimulation		27 (14.36%)	50 (8.61%)		28 (15.05%)	
Fertilization method				.369		.793
IVF‡		153 (81.40%)	455 (78.31%)		149 (80.11%)	
ICSI‡		35 (18.60%)	126 (21.69%)		37 (19.89%)	
Type of embryo transferred				.014		.108
Blastocyst		141 (75.00%)	380 (65.40%)		126 (67.74%)	
Cleavage-stage embryo		47 (25.00%)	201 (34.60%)		60 (32.26%)	
Number of embryos transferred		1.1	1.21	.001	1.13	.418

* B, before propensity score matching; BP, after propensity score matching.

† Compared to group A.

‡ AMH = anti-müllerian hormone, BMI = body mass index, GnRH-a = GnRH-agonist, GnRH-ant = GnRH-antagonist, ICSI = intracytoplasmic sperm injection, IVF = in vitro fertilization.

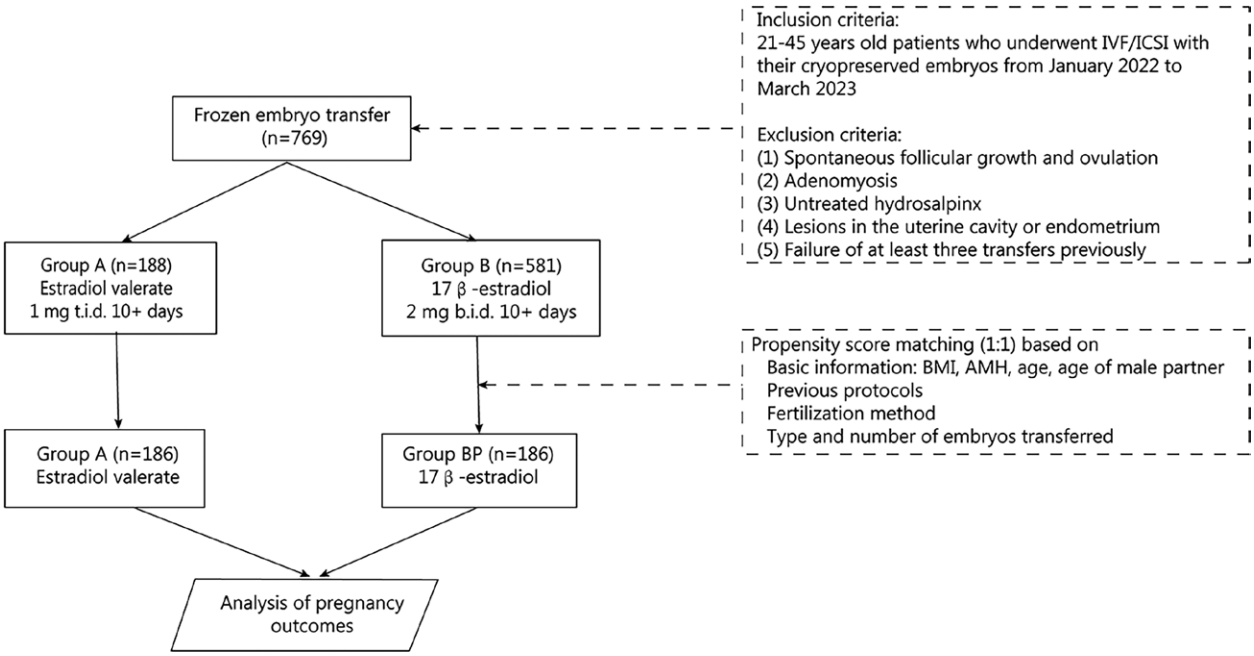

Figure 1. Construction of patient groups for analysis.

Table 2

Comparison of blood parameters between patient groups.

	Group A	Group BP*	*P*-value
Number of patients	186	186	
Endometrial thickness (cm)	9 (8, 10)	9 (8, 10)	.368
Estradiol level on day of progesterone start (pg/mL)	106.74 (85.67, 143.35)	158.72 (120.08, 201.92)	<.001
Estradiol level on day of embryo transfer (pg/mL)	109.96 (84.77, 146.32)	149.73 (111.14, 200.22)	<.001
Progesterone on day of embryo transfer (ng/mL)	8.33 (6.43, 10.80)	8.64 (6.81, 10.44)	.337

* After propensity score matching.

Table 3

Comparison of pregnancy outcome between patient groups.

	Group A	Group BP*	*P*-value
Pregnancy			
Biochemical pregnancy	102 (54.84%)	106 (56.99%)	.676
Implantation	102 (49.76%)	102 (48.57%)	.809
Clinical pregnancy	95 (51.08%)	96 (51.61%)	.917
Early miscarriage	15 (15.79%)	11 (11.46%)	.383
Ectopic pregnancy	1 (1.05%)	3 (3.13%)	.621
Live birth	76 (40.86%)	75 (40.32%)	.916
Complications			
Gestational hypertension	6 (6.32%)	9 (9.38%)	.432
Gestational diabetes mellitus (GDM)	18 (18.95%)	10 (10.42%)	.096

* After propensity score matching.

identical ($P > .05$) pregnancy outcomes, including biochemical pregnancy, implantation, clinical pregnancy, early miscarriage, ectopic pregnancy, and live birth. The slight differences in complications (gestational hypertension and gestational diabetes mellitus) were not statistically significant ($P > .05$).

4. Discussion

When using fresh embryo transfer for IVF, the elevated level of estradiol obstructs the embryo implantation as it decreases the receptivity of endometrium.[7] Patients in FET cycles have lower serum estradiol level than those attempting fresh embryo transfer. During programmed FET cycles, ovulation is inhibited, and patients rely on exogenous estradiol and progesterone for the endometrial/embryo synchronization. The serum estradiol level can thus vary greatly depending on the dose and/or route of estradiol administration. Endometrial preparation is often achieved by either a fixed-dose regimen (4–6 mg) or a step-up regimen (from 2 to 6 mg) of estradiol orally,[8] and progesterone is typically started when the endometrial thickness reaches

7 mm. Supraphysiologic estradiol levels (>300–500 pg/mL) during programmed cycles have a negative correlation with pregnancy rates and live birth rates.[4,5,9,10] Ma et al[11] showed in a mouse model that the duration of the window of uterine receptivity is drastically curtailed at higher estrogen levels. At our center, patients are typically given estradiol orally at a fixed dose (4 mg/d), which is simpler than the step-up regimen and allows good patient compliance, and this approach has achieved a stable pregnancy rate. In this work, patients in group A received an even lower dose of 3 mg/d estradiol but obtained comparable pregnancy outcome, suggesting that the "threshold" of low estradiol levels may be reevaluated in programmed FET cycles.

Estradiol and its receptor are a major factor that plays an important role in preparing the "window of implantation." Romanski et al[12] suggested that, for patients undergoing the first natural FET, the estradiol level over the "threshold" (100 pg/mL) must be maintained for a long enough time (>4 days) for the endometrium to proliferate and mature adequately and upregulate enough progesterone receptors. However, few studies looked at the effects of low estradiol levels on programmed FET cycles. In donation programmed cycles,[13] serum estradiol concentrations <100 pg/mL were found enough to induce changes to sustain normal implantation. Low estrogen levels may lead to a thinner endometrium, but had similar outcomes. Garimella et al[14] divided 509 patients, which is a small sample size, who went through hormone replacement FET cycles into 6 groups based on their serum estradiol level and found that, except that the miscarriage rate was higher for the >500 pg/mL group and the <100 pg/mL group, the outcomes were otherwise similar between groups. Sekhon et al[15] found in a study involving 1439 patients undergoing euploid blastocysts transfer that the duration of estrogen administration (10–36 days) did not impact pregnancy outcomes. In this work, a fixed dose of 3 mg/d estradiol for 10 or more days for the patients in group A, which attained a median estradiol level of 106.74 pg/mL (25% quartile 85.67 pg/mL, 75% quartile 143.35 pg/mL), was considered to cross the threshold necessary to initiate the morphological and biological alterations for endometrial receptivity.

Compared to women with a normal pregnancy, women experiencing miscarriage after spontaneous pregnancy have markedly lower serum estradiol levels,[16,17] and the deviating estradiol trajectory is indicative of an inadequate ovarian response to rising hCG levels. However, ovaries do not need to provide the corpus luteum function in programmed FET, and there is no well-established relationship between low estradiol levels and early miscarriage in programmed FET.[13,14] We did not find a significant rise in the rate of miscarriage when estradiol decreased. Albrecht et al[18] argued that during early primate pregnancy, elevated estradiol levels adversely affect extravillous trophoblast invasion and uterine artery remodeling. Hsieh et al[19] noted that at the fifth gestational week in programmed FET cycles, women who later developed preeclampsia had significantly higher estradiol levels, and affirmed the importance of having low but adequate levels of estradiol in early pregnancy. In our study, lower levels of estradiol (group A vs group BP) were not related to higher rates of pregnancy complications. Of the 6 women in group A who experienced gestational hypertension, 2 had a late-term miscarriage, and others proceeded to give a live birth.

This study is a retrospective analysis and has some limitations. For example, we excluded patients who experienced unexpected follicle development and ovulation, a situation that can occur during programmed FET when the estradiol level is low. Further in-depth and high-quality studies are still needed to understand the implications of low estradiol level in programmed FET. Nevertheless, within the scope of the current study, we noted that decreasing the serum estradiol level did not reduce the pregnancy rate or increase the risk of an adverse pregnancy outcome in programmed FET. The findings here may help in deciding on the appropriate dosage of estradiol in clinical settings, especially in patients combined with endometrial cancer, endometrial polyps, and endometrial hyperplasia.

Author contributions

Conceptualization: Na Sun, Hua-Gang Ma.
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Validation: Na Sun, Ping-Ping Sun, Hai-Ru Cao, Hua-Gang Ma.
Writing – original draft: Na Sun.
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