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ORIGINAL ARTICLE

The impact of serum estradiol and progesterone levels during implantation on obstetrical complications and perinatal outcomes in frozen embryo transfer

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ABSTRACT

Background: This study sought to evaluate obstetric complications and perinatal outcomes in frozen embryo transfer (FET) using either a natural cycle (NC-FET) or a hormone therapy cycle (HT-FET). Furthermore, we investigated how serum levels of estradiol (E2) and progesterone (P4) on the day of and 3 days after embryo transfer (ET) correlated with clinical outcomes in the two groups.

Methods: We conducted a retrospective, single-center study from January 1, 2015, to December 31, 2019. The study included couples who underwent NC-FET or HT-FET resulting in a singleton live birth. Serum levels of E2 and P4 were measured on the day of and 3 days after ET. The primary outcomes assessed were preterm birth rate, low birth weight, macrosomia, hypertensive disorders in pregnancy, gestational diabetes mellitus, postpartum hemorrhage, and placenta-related complications.

Results: A total of 229 singletons were included, with 49 in the NC-FET group and 180 in the HT-FET group. There were no significant differences in obstetric complications and perinatal outcomes between the two groups. The NC-FET group had significantly higher serum levels of P4 (17.2 ng/mL vs 8.85 ng/mL; $p < 0.0001$) but not E2 (144 pg/mL vs 147 pg/mL; $p = 0.69$) on the day of ET. Additionally, 3 days after ET, the NC-FET group had significantly higher levels of both E2 (171 pg/mL vs 140.5 pg/mL; $p = 0.0037$) and P4 (27.3 ng/mL vs 11.7 ng/mL; $p < 0.0001$) compared with the HT-FET group.

Conclusion: Our study revealed that although there were significant differences in E2 and P4 levels around implantation between the two groups, there were no significant differences in obstetric complications and perinatal outcomes. Therefore, the hormonal environment around implantation did not appear to be the primary cause of differences in obstetric and perinatal outcomes between the two EM preparation methods used in FET.

Keywords: Frozen embryo transfer; Hormone environment around implantation; Hypertensive disorder in pregnancy; Macrosomia; Neonatal outcome; Obstetric outcome

1. INTRODUCTION

Frozen embryo transfer (FET) was first introduced in 1984 and has become a widely performed procedure. FET allows for the storage of surplus embryos and also reduces the need for repeated oocyte retrievals. Although FET has shown comparable live birth rates to fresh embryo transfer (ET),[1] recent evidence suggests that FET may be associated with certain obstetric and perinatal complications, such as pregnancy-induced hypertension (PIH), large for gestational age (LGA), postpartum hemorrhage (PPH),[2] and preterm delivery.[3]

FET can be performed either after spontaneous ovulation (NC-FET) or following exogenous estradiol (E2) supplementation for endometrial preparation (HT-FET). Previous publications have demonstrated similar clinical pregnancy rates and live birth rates for both methods.[4–6] However, further concerns have been raised regarding the obstetric and perinatal outcomes of HT-FET, with some studies indicating an increased risk of maternal and perinatal morbidity. For instance, Saito et al[7] and Xu et al[8] reported higher risks of hypertensive disorders of pregnancy (HDP) and placenta accreta associated with HT-FET. Similarly, Ginström et al[9] and Busnelli et al[10] suggested that HT-FET is linked to higher rates of PIH, PPH, post-term birth, and macrosomia. Asserhøj et al[11] also found that HT-FET is associated with a higher rate of cesarean section, as well as PIH and PPH, compared to NC-FET. Conversely, numerous publications have shown that NC-FET significantly decreases the risk of HDP, preeclampsia, LGA, macrosomia, preterm birth, post-term birth, low birth weight, cesarean section, PPH, placental abruption, and accreta.[12,13]

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The underlying pathophysiology that contributes to the disparity in maternal and perinatal outcomes between these two FET preparation methods is still being investigated. In this study, we aimed to compare the differences in serum hormone levels between the two methods during the period of ET and examine if these differences play a crucial role in obstetric and perinatal outcomes.

2. METHODS

2.1. Study population

This retrospective study was conducted from January 1, 2015, to December 31, 2019, at a single tertiary hospital. This study included infertile couples who underwent frozen FET and successfully achieved live births of singletons. Only cases with complete records of obstetric complications and perinatal outcomes were included in the analysis. The exclusion criteria in this study were as follows: (1) patients with incomplete data and (2) pregnancies that ended in spontaneous abortion or intrauterine fetal demise (IUFD). Patients who underwent a natural cycle for FET (NC-FET) were classified as group I (n = 49), whereas patients who received E2 and progesterone (P4) for endometrium (EM) preparation before FET (HT-FET) were classified as group II (n = 180). No medication was administered to the NC-FET patients.

Ovulation was confirmed by follicle rupture and a serum P4 level >1 ng/mL, and FET was performed 5 days after ovulation. For HT-FET, E2 valerate (2 mg of Estrade; Synmosa, Taipei, Taiwan) was administered at a dose of 2 mg three times daily until the EM reached a thickness of at least 7 mm with a triple-line appearance. Vaginal micronized P4 (100 mg of Utrogestan; Besins) was added at a dose of 200 mg three times a day for 5 days before ET (see Fig. 1). Both day 3 embryos (cleavage stage) and day 5 embryos (blastocyst stage) transfers were performed 5 days after spontaneous ovulation in natural cycles or after 5 days of vaginal P4 supplementation in hormone cycles. Serum E2 and P4 levels were measured on the day of ET and 3 days thereafter. Vaginal micronized P4 (100 mg of Utrogestan; Besins) was continued at a dose of 200 mg three times a day for luteal support in both groups after ET. In addition, all patients in both groups received aspirin from the beginning of endometrial preparation to the day that we checked the pregnancy test to improve the EM pattern and thickness.

2.2. Outcomes

The primary obstetric outcomes assessed were the rates of PIH, gestational diabetes mellitus (GDM), preeclampsia, and PPH (>1000 mL in cesarean section and >500 mL in vaginal delivery). The occurrence of placental accidents, such as placenta previa, placenta accreta, and placental abruption, was also compared between the two groups.

The primary perinatal outcomes examined included the sex of the babies, preterm birth (<37th wk), macrosomia (>4000 g), low birth weight (<2500 g), small for gestational age (SGA), and LGA, of which the latter two were defined as a difference of less than –2 SDs or greater than +2 SDs, respectively, from the expected birth weight for the given gestational age.[14] Neonatal morbidities, such as bronchopulmonary dysplasia, intraventricular hemorrhage grade 3, necrotizing enterocolitis, sepsis, hypoxic-ischemic encephalopathy, and major birth defects (coded according to the *International Classification of Diseases*, 10th Revision, Clinical Modification, Q00-Q99), were also compared. All diagnoses were made by medical doctors, and a single gynecologist reviewed all the outcomes. In cases where the patient did not give birth in our hospital, a telephone interview was conducted to record their obstetric and perinatal outcomes.

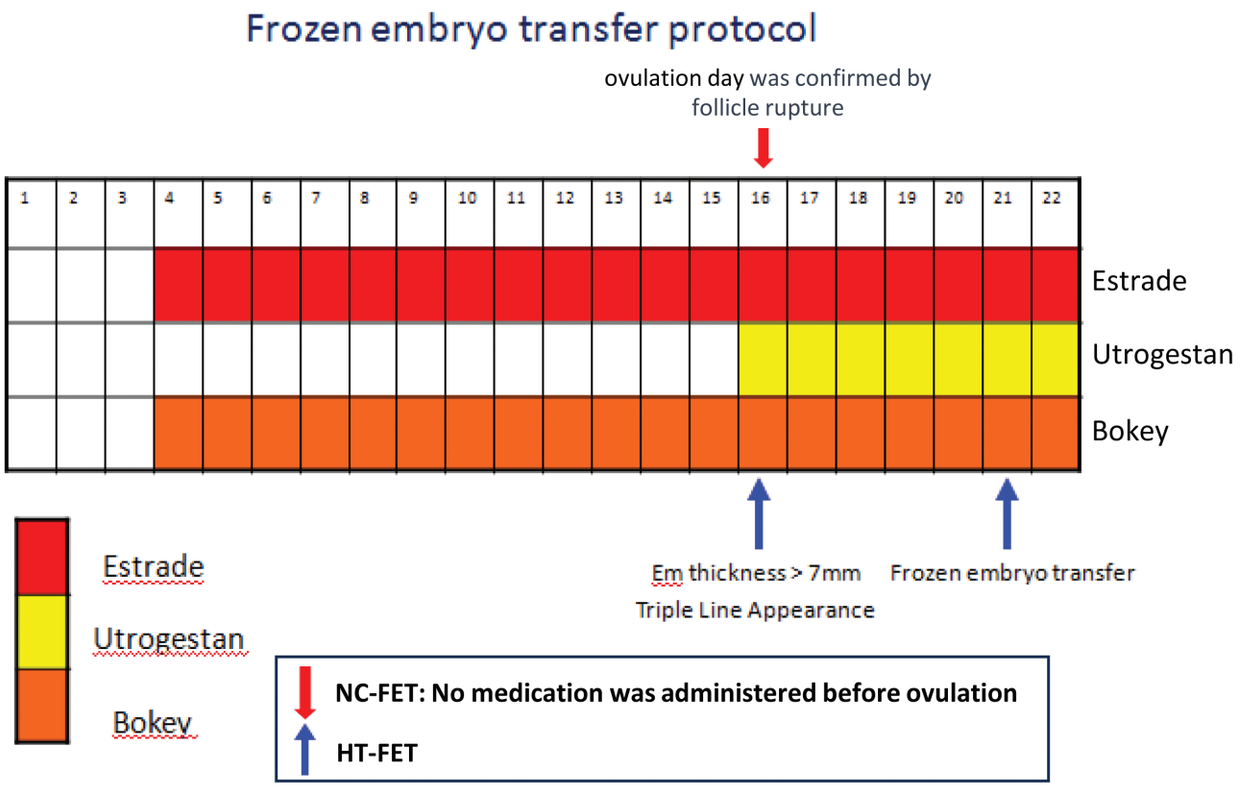

Fig. 1 Frozen embryo transfer protocol. FET = frozen embryo transfer; HT-FET = frozen embryo transfer using hormone therapy cycle; NC-FET = frozen embryo transfer using natural cycle.

2.3. Statistical analysis

Baseline information was presented as frequencies with percentages for categorical variables and as means with SDs or medians with quartiles for continuous variables. Differences in baseline information between natural cycles and programmed cycles were compared using Pearson chi-square test or Fisher exact test for categorical variables and Student *t*-test or Wilcoxon rank sum test for continuous variables.

Furthermore, logistic regression models were used to estimate the crude and multivariable odds ratios (mORs) with 95% CIs for the hormone therapy (HT) cycle for each variable of interest. To identify potential critical risk factors associated with the programmed cycle, multiple logistic regression analyses were performed using both the full model and the reduced model, which were established based on crude ORs with $p < 0.05$.

Additionally, logistic regression with the Firth approach was used to estimate the risk of certain maternal and perinatal outcomes between the natural cycle and programmed cycle, considering the rarity of events. All analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC), and statistical significance was set at $p < 0.05$.

3. RESULTS

A total of 229 singletons were included in the study, with 49 in the NC-FET group and 180 in the HT-FET group. Characteristics of maternal and treatment factors are summarized in Table 1.

Table 1

Maternal and treatment characteristics of singleton pregnancies after frozen embryo transfer from 2015 to 2019

	Natural cycle	Programmed cycle	*p*
Number of deliveries	49	180	…
Maternal age	37.00 ± 4.80	35.77 ± 4.52	0.0963
Body mass index (kg/m^2)	21.88 ± 2.84	22.48 ± 3.82	0.2283
<20	11 (22.45)	52 (28.89)	0.2478
20-24	29 (59.18)	82 (45.56)	…
24	9 (18.37)	46 (25.56)	…
Nulliparous	36 (73.47)	136 (75.56)	0.8523
Smoking	0	0	…
Insemination type			0.4644
IVF	24 (48.98)	89 (49.44)	
ICSI	17 (34.69)	49 (27.22)	…
IVF/ICSI	8 (16.33)	42 (23.33)	…
Embryo stage			0.5966
Cleavage stage	16 (32.65)	51 (28.33)	
Blastocyst stage	33 (67.35)	129 (71.67)	
Number of embryos transferred			0.8388
1	10 (20.41)	34 (18.89)	…
2	39 (79.59)	146 (81.11)	…
Number of gestational sacs			1.0000
1	42 (85.71)	152 (84.44)	…
2	7 (14.29)	28 (15.56)	…
Years of involuntary childlessness			0.8066
1-2	21 (42.86)	81 (45.00)	…
3-4	11 (22.45)	47 (26.11)	…
5	17 (34.69)	50 (27.78)	…
Not report	0	2 (1.11)	…
Cause of infertility			0.7806
Female factor	26 (53.06)	98 (54.44)	…
Polycystic ovarian syndrome	5 (10.20)	31 (17.22)	…
Tubal factor	6 (12.24)	18 (10.00)	…
Endometriosis	2 (4.08)	15 (8.33)	…
Other female factor	13 (26.53)	34 (18.89)	…
Male factor	8 (16.33)	34 (18.89)	…
Mixed (male/female)	7 (14.29)	28 (15.56)	…
Unexplained	8 (16.33)	20 (11.11)	…
E2 value at ET day (pg/mL)	144 (107-181)	147 (108.50-196)	0.6909
<108	13 (26.53)	44 (24.44)	0.8329
108-195	25 (51.02)	88 (48.89)	…
195	11 (22.45)	48 (26.67)	…
P4 value at ET day (ng/mL)	17.20 (12.90-23.20)	8.85 (6.55-11.60)	<0.0001
E2 value 3 d after ET (pg/mL)	171 (133-258)	140.50 (110.50-179.50)	0.0037
<112	9 (18.37)	47 (26.11)	0.0009
112-202	17 (34.69)	98 (54.44)	…
202	23 (46.94)	35 (19.44)	…
P4 value 3 d after ET (ng/mL)	27.30 (22.00-37.60)	11.70 (9.00-15.95)	<0.0001

ET = embryo transfer; E2 = estradiol; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; P4 = progesterone.

Statistical analysis revealed no significant differences in background characteristics between the two groups. The mean maternal age was 37.0 years in the NC-FET group and 35.77 years in the HT-FET group. Most of the participants in both groups were nulliparous (73.47% in NC-FET, 75.56% in HT-FET), and the cause and duration of infertility were similar between the two groups. Single embryo transfer was performed in 20.41% of NC-FET cases and 18.89% of HT-FET cases.

Regarding obstetric and perinatal outcomes, there were no statistically significant differences in PIH, preeclampsia, placenta previa, placenta accreta, PPH, or GDM between the two groups (Table 2). However, there was a higher risk of macrosomia (>4000 g) in newborns after NC-FET (p = 0.03). No statistically significant differences were observed in terms of sex, preterm birth rate, low birth weight rate, neonatal morbidity, or major birth defects between the two groups. Three instances of major birth defects were reported exclusively in the HT-FET group (Table 3).

Logistic regression analysis demonstrated that the risk of placenta previa, placenta accreta, and PIH was potentially higher in HT-FET pregnancies than in NC-FET pregnancies, although the differences did not reach statistical significance (Table 4). The univariate odds ratios (uORs) and mORs for these outcomes showed a trend towards increased risk in the programmed FET group, but the CIs were wide. As for neonatal outcomes, logistic regression analysis showed that the risk of neonatal morbidity was increased in programmed FET pregnancies, although the differences did not reach statistical significance (uOR = 1.99; CI, 0.34-11.53 and model 1 mOR = 1.55; CI, 0.26-9.38 and model 2 mOR = 2.77; CI, 0.42-18.18). On the other hand, the risk of macrosomia (>4000 g) was significantly lower in HT-FET pregnancies (uOR = 0.11; CI, 0.02-0.78; p = 0.02 and model 1 mOR = 0.21; CI, 0.03-1.34; p = 0.09 and model 2 mOR = 0.09; CI, 0.01-0.61; p = 0.01) (Table 1). However, after adjusting for all variables in the logistic regression analysis, there was no significant difference in the risk of macrosomia between the two groups (Table 4). Therefore, it can be concluded that there was no significant difference in the risk of obstetric complications and neonatal outcomes between the two methods.

Regarding serum hormone levels, P4 levels on both the day of ET and 3 days after ET were significantly higher in the NC-FET group than in the HT-FET group. On the day of ET, the P4 level was 17.2 ng/mL in the NC-FET group and 8.85 ng/mL in the HT-FET group (p < 0.0001). Similarly, 3 days after ET, the P4 level was 27.3 ng/mL in the NC-FET group and 11.7 ng/mL in the HT-FET group (p < 0.0001). The E2 level was significantly higher in the NC-FET group than in the HT-FET group 3 days after ET (171 pg/mL vs 140.5 pg/mL; p = 0.0037), but there was no such significant difference between the two groups on the day of ET (144 pg/mL vs 147 pg/mL; p = 0.69).

4. DISCUSSION

Although our study observed a higher tendency for the development of placenta previa, placenta accreta, and PIH in the HT-FET group, these differences did not reach statistical significance (Table 4). Additionally, overall obstetric complications and perinatal outcomes were found to be comparable between the two groups, except for a higher risk of macrosomia in the NC-FET group. However, after adjusting for all variables using logistic regression analysis, no significant difference was found between the two groups.

Von Versen-Höynck et al proposed that the absence of corpus luteum (CL) formation in HT-FET may be a key factor contributing to the differences in maternal and perinatal outcomes between the two methods. CL, which is responsible for producing vasoactive agents such as relaxin and vascular endothelial growth factor, is believed to play a crucial role in initial placentation and subsequent development of maternal and neonatal complications.[15–18]

While some studies have suggested that E2 and P4 exposure around implantation does not directly influence pregnancy outcomes,[19,20] some evidence indicates that excessive hormone

Table 2

Comparison of maternal outcome between two groups

	Natural cycle (n = 49)	Programmed cycle (n = 180)	*p*
Placenta previa	3 (6.12%)	16 (8.89%)	0.7710
Placenta abruptio	1 (2.04%)	4 (2.22%)	1.0000
Placenta accreta	0	5 (2.78%)	0.5873
Pregnancy-induced hypertension	1 (2.04%)	10 (5.56%)	0.4645
Gestational diabetes mellitus	9 (18.37%)	22 (12.22%)	0.3447
Preeclampsia	2 (4.08%)	9 (5.00%)	1.0000
Postpartum hemorrhage	6 (12.24%)	19 (10.56%)	0.7964
Hypertensive disorder of pregnancy	3(6.12%)	14 (7.78%)	1.0000

Statistical methods: two-sample *t*-tests, Mann-Whitney *U* tests, χ^2 tests, and Fisher exact tests.

Table 3

Comparison of perinatal outcome between two groups

	Natural Cycle (n = 49)	Programmed Cycle (n = 180)	*p*
Number of deliveries	49	180	…
Sex			0.3334
Male	30 (61.22%)	95 (52.78%)	…
Female	19 (38.78%)	85 (47.22%)	…
Preterm deliveries (<37 wk)	5 (10.20%)	27 (15.00%)	0.4901
Low birth weight (<2500 g)	4 (8.16%)	16 (8.89%)	1.0000
Macrosomia (>4000 g)	3 (6.12%)	1 (0.56%)	0.0316
Neonatal morbidity	1 (2.04%)	10 (5.56%)	0.4645
Major birth defects	0	3 (1.67%)	1.0000

Statistical methods: two-sample t-tests, Mann-Whitney *U* tests, 2 tests, and Fisher exact tests.
$p < 0.05$.

Table 4

Logisitic regression for risk of maternal and perinatal outcomes between natural cycle and programmed cycle

	Univaritate OR (95% CI)	*p*	Mutivariable OR (95% CI) Model 1 (Full Model)	*p*	Mutivariable OR (95% CI) Model 2 ($p < 0.05$)	*p*
Maternal outcomes						
Placenta previa	1.33 (0.40-4.47)	0.6414	2.59 (0.64-10.47)	0.1819	3.01 (0.68-13.30)	0.1471
Placental abruption	0.83 (0.12-5.46)	0.8415	1.40 (0.22-9.09)	0.7216	1.68 (0.20-13.82)	0.6291
Placenta accreta	3.10 (0.16-58.69)	0.4504	2.52 (0.28-22.62)	0.4085	2.11 (0.15-36.57)	0.5515
Pregnancy-induced hypertension	1.99 (0.34-11.53)	0.4421	1.99 (0.38-10.55)	0.4171	1.47 (0.23-9.26)	0.6802
Gestational diabetes mellitus	0.61 (0.26-1.40)	0.2413	0.80 (0.27-2.35)	0.6830	0.75 (0.27-2.09)	0.5877
Preeclampsia	1.05 (0.25-4.45)	0.9444	1.49 (0.31-7.24)	0.6221	1.26 (0.25-6.24)	0.7805
Postpartum hemorrhage	0.81 (0.31-2.10)	0.6627	1.05 (0.33-3.36)	0.9380	0.76 (0.25-2.33)	0.6309
Hypertensive disorder of pregnancy	1.16(0.34-3.93)	0.8150	1.51(0.36-6.22)	0.5721	1.20(0.30-4.76)	0.8007
Neonatal outcomes						
Neonatal morbidity	1.99 (0.34-11.53)	0.4421	1.55 (0.26-9.38)	0.6330	2.77 (0.42-18.18)	0.2892
Major birth defects	1.95 (0.10-39.51)	0.6629	6.23 (0.50-85.05)	0.1522	3.62 (0.19-69.62)	0.3932
Sex (male)	0.71 (0.38-1.36)	0.3058	0.60 (0.26-1.39)	0.2315	0.68 (0.31-1.53)	0.3527
Mode of delivery (vaginal)	0.87 (0.45-1.66)	0.6645	0.67 (0.28-1.57)	0.3525	0.77 (0.35-1.69)	0.5098
Preterm deliveries (<37 wk)	1.45 (0.54-3.88)	0.4590	1.85 (0.55-6.30)	0.3231	1.62 (0.52-5.03)	0.4036
Low birth weight (<2500 g)	1.01 (0.34-3.05)	0.9797	1.06 (0.29-3.88)	0.9321	0.86 (0.24-3.02)	0.8104
Macrosomia (>4000 g) or large for gestational age	0.11 (0.02-0.78)	0.0271	0.21 (0.03-1.34)	0.0981	0.09 (0.01-0.61)	0.0145

ET = embryo transfer; E2 = estradiol; OR = odds ratio; P4 = progesterone.

The OR reference is natural cycle. The OR adjust by firth logistic regression. Model 1: mutivariable OR adjust by all variable in Table 1 (maternal age, body mass index, nulliparous, insemination type, embryo stage, number of embryos transferred, number of gestational sacs, years of involuntary childlessness, cause of infertility, ET day E2, ET day P4, E2 level 3 d after ET, and P4 level 3 d after ET). Model 2: multivariable OR adjust by significant variable in Table 1 (ET day P4, E2 level 3 d after ET and P4 level 3 d after ET).

levels can impact the quality of placentation and increase the risk of certain complications. Certain studies have reported associations between excessive hormone exposure and adverse outcomes such as preterm labor, SGA, and placenta accreta.[2,19,21,22]

Merviel et al[23] concluded that the pathophysiology of preeclampsia is linked to implantation disorder. Estrogens, particularly E2, which have a local vasodilating effect on the uterine arteries during implantation, might play a critical role in the development of these implantation disorders. Bourdon et al found an inverse correlation between the duration of E2 priming and the live birth rate, while Sekhon et al reported that the duration of estrogen administration was associated with a shorter duration of pregnancy.[19,22] These findings suggest that excessive E2 exposure during implantation may lead to epigenetic changes in the developing embryo and affect the quality of placentation.[21,24–26]

Conversely, previous studies have reported that a higher risk of placenta accreta after FET may be associated with low E2 levels around implantation.[27] In our study, a notable correlation was observed between patients diagnosed with HDP and E2 levels 3 days after ET. Specifically, when compared to E2 values below 112, E2 values within the range of 112 to 202 exhibited a significantly lower odds ratio (mOR: 0.20; 95% CI, 0.05-0.75; $p = 0.0179$), as did E2 values above 202 (mOR: 0.14; 95% CI, 0.02-0.76; $p = 0.0234$) (Supplementary Table 1, http://links.lww.com/JCMA/A204).

These findings suggest that relatively low E2 values 3 days after ET may contribute to the development of hypertensive disorders during pregnancy. Low P4 levels in early pregnancy have been reported to induce excessive invasion of the trophoblast and the subsequent development of placenta accreta.[28] However, in our study, though we observed significantly higher P4 levels on the day of and 3 days after ET in the NC-FET group, the incidence of placenta accreta and previa was comparable between the two groups. We did notice an increase in the incidence of macrosomia in the NC-FET group, but further investigation is needed to determine whether this increased incidence is related to higher levels of P4 around implantation.

Notably, we did not find a correlation between low levels of P4 and SGA in our study.

FET, particularly HT-FET, has been reported to be associated with an increased risk of PIH.[3,29] Previous studies have identified several risk factors for PIH, such as advanced maternal age (>40), high body mass index (BMI) (>35), multiple pregnancies, and polycystic ovary syndrome.[30] In our study, these factors were comparable between the two groups, and the mOR (model 1) did not change significantly after adjusting for these factors.

To our surprise, the mOR of PIH even decreased after adjusting for significant variables (which include E2 and P4 serum levels around ET; Table 4). Therefore, low serum E2 and P4 levels around implantation might be causes of PIH, as reported in previous studies, though this cannot be justified by our results. Instead, our study demonstrated that E2/P4 levels around implantation do not appear to be the primary cause of differences in obstetric and perinatal outcomes between the two EM preparation methods used in FET.

Our study examined the correlation between obstetric and perinatal outcomes and serum E2 and P4 levels around implantation in FET with different EM preparation methods. We did observe a significant difference in P4 levels around implantation, as well as a significant difference in E2 levels 3 days after ET, but not on the day of ET. However, these differences did not seem to impact obstetric complications and perinatal outcomes in FET cycles. Although our study found that E2/P4 levels around implantation may not affect obstetric and neonatal outcomes in FET, it is likely that higher doses of E2 and P4 used in HT-FET may lead to poorer obstetric and perinatal outcomes. Therefore, we assume that there is a safe range of serum E2/P4 levels for FET that minimizes obstetric and neonatal complications. The clinical implications of these

findings require further investigation. In particular, large prospective randomized clinical trials will be necessary to address these questions effectively.

The strengths of this study are that our data included all assisted reproductive technology (ART) cycles in our hospital and that all ART-related characteristics and parameters that might have affected obstetric and perinatal outcomes, such as duration of infertility, parity, cause of infertility, BMI, smoking habit, insemination type, number of ETs, number of gestational sacs, and embryo quality, were all reviewed and collected. In this way, missing data were limited. In addition, to compare obstetric and neonatal outcomes, only infertile couples who received FET and had live singletons were recruited; pregnancies that ended with spontaneous abortion or IUFD were not included. Additionally, all decisions regarding EM preparations and protocol were made by an experienced physician, and it was based solely on whether a patient could normally ovulate. Our protocol for programmed FET was standardized, and we exclusively used true NC-FET rather than modified NC-FET for normal-ovulatory patients, meaning that we avoided triggering ovulation with human chorionic gonadotropin. Therefore, due to the similar basic characteristics shared between the two groups, the relationships between the different methods of EM preparation and clinical outcomes that we identified here can be considered more precise. This is also the first study to analyze the correlation between serum E2 and P4 levels around implantation and how these levels relate to obstetric and perinatal outcomes in FET with different EM preparation methods. However, this study is limited by its retrospective nature and small sample size originating from a single medical center. Our first true NC-FET began in 2015, but because live birth data from 2020 was incomplete, expansion of this cohort was not possible.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://links.lww.com/JCMA/A204.

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