The relationship between serum oestrogen levels and clinical outcomes of hormone replacement therapy-frozen embryo transfer: a retrospective clinical study

Na Kong1,2,3†, Jingyu Liu1,2†, Chunxue Zhang1,2†, Yue Jiang1,2, Yingchun Zhu1,2, Guijun Yan1,2, Haixiang Sun1,2,3* and Chenyang Huang1,2*

Abstract

Background: This study aimed to explore the relationship between serum oestrogen (E2) levels before endometrial transformation and pregnancy outcomes of hormone replacement therapy-frozen embryo transfer (HRT-FET) cycles, which has been investigated for years without any consensus.

Methods: A retrospective cohort study of 10,209 cycles HRT-FET cycles was conducted at the Reproductive Medicine Center of Nanjing Drum Tower Hospital from March 2017 to December 2020. A smooth fitting curve was constructed to identify the relationship between serum E2 levels before endometrial transformation and the clinical pregnancy rate. Then, threshold and saturation effect analysis was employed to explore the cut-off value of serum E2 levels. In addition, patients were divided into 2 groups based on their levels of serum E2 measured before progesterone-induced endometrial transformation: Group 1, < 300 pg/mL (n = 6251) and Group 2, ≥ 300 pg/mL (n = 3958). The clinical pregnancy and miscarriage rates of all groups were compared. Further smooth fitting curve analysis was employed by different subgroups segmented according to different endometrial thicknesses.

Results: When the serum E2 level was greater than 300 pg/mL, the clinical pregnancy rate decreased significantly (62.9% vs. 59.8%, p < 0.01), but the miscarriage rates were similar (13.5% vs. 15.6%, p = 0.14). While serum E2 level reached or exceeded 1400 pg/mL, there was no significant correlation between the clinical pregnancy rate and E2 level. The clinical pregnancy rate reached its higher level at lower E2 levels, regardless of the different endometrial thicknesses.

Conclusions: Patients with a lower pretransformation serum E2 level (less than 300 pg/mL) have a higher clinical pregnancy rate and there was no correlation between the clinical pregnancy rate and a higher serum E2 level (greater than 1400 pg/mL) in HRT-FET cycles.

Keywords: Clinical pregnancy rate, Endometrial transformation, Endometrial thicknesses, Hormone replacement therapy-frozen embryo transfer, Serum oestrogen levels
pregnancy rates compared with fresh embryo transfer cycles [1–5]. The artificial hormone replacement therapy (HRT) cycle for FET is widely used for its stable clinical pregnancy rate and more convenient time schedule [6, 7]. However, several studies suggested that HRT-FET could increase the risk of pregnancy-related complications (hypertension disorders, placenta accrete and intrahepatic cholestasis of pregnancy), as well as the risk of low birth weight and small for gestational age, which was speculated to be related to the abnormal oestrogen (E2) level of HRT-FET cycles [8, 9]. Therefore, the control of serum E2 level in HRT-FET cycles needs further discussing.

Serum E2 is essential for endometrial receptivity, myometrial spiral artery remodelling, and placental development [10]. Studies have shown that higher serum E2 levels lead to impaired endometrial receptivity and reduced clinical pregnancy rates [11]. In addition, high serum E2 levels before embryo transfer in fresh in vitro fertilization (IVF) cycles are strongly associated with decreased embryo implantation rates [12]. Furthermore, high serum E2 levels in IVF cycles increase the incidences of preeclampsia, foetal growth restriction and low-birthweight infants [13, 14]. Therefore, serum E2 levels during the HRT cycle may be closely related to pregnancy outcomes.

Hormone replacement cycles are commonly employed in our reproductive medicine centre, and some patients have serum E2 levels that are obviously higher than the natural physiologic levels before endometrial transformation. However, the number of relevant studies on the relationship between serum E2 levels before endometrial transformation and pregnancy outcomes of FET cycles is limited to date [11, 15, 16], and these studies have failed to reach a consensus. Therefore, we conducted a retrospective review of recent hormonal replacement FET cycles at the reproductive medicine centre of Nanjing Drum Tower Hospital to explore the relationships between serum E2 levels before endometrial transformation and clinical pregnancy outcomes.

**Methods**

**Patients**
From March 2017 to December 2020, all patients who underwent frozen thawed embryo transfer with artificial hormone replacement (Femoston, 2 mg oestradiol; 2 mg oestradiol with 10 mg dydrogesterone, Abbott, USA) cycles at the reproductive medicine centre of Nanjing Drum Tower Hospital were included in this retrospective study. Patients were 20 to 39 years of age when undergoing HRT-FET and had a body mass index (BMI) of 20.5 to 30.4 kg/m2. All patient couples provided written informed consent. All patients received a comprehensive prepregnancy physical examination to exclude drugs and pregnancy contraindications before FET cycles. The general health status of the patients involved in this study are normal. The exclusion criteria for this study were as follows: (1) Use of other hormonal replacement drugs; (2) More than three transfer cycles; (3) Use of gonadotrophin-releasing hormone agonist (GnRHa) pre-treatment; (4) Combined hydrosalpinx or lesions of the uterine cavity and endometrium; or (5) Endometriosis or adenomyosis.

**Endometrial preparation and thawed embryo transfer**
Patients without abnormalities (sex hormone levels, uterus and adnexa) in HRT-FET cycles were started with oral oestriadiol (Femoston, Abbott, USA, 2 mg oestradiol t.i.d. × 14 days) on the second day of their menstrual cycle. Serum E2 and P levels and endometrial thickness were monitored. Oestradiol tablets were additionally administered vaginally (Femoston, 2 mg oestradiol q.d.) according to cases of a lower endometrial thickness. When the endometrial thickness met a certain standard, oral oestradiol combined with dydrogesterone compound tablets (Femoston, 2 mg oestradiol and 10 mg dydrogesterone t.i.d. × 5 or 6 days) were administered, along with intramuscular injection of progesterone (P) at 60 mg q.d. for 5 or 6 days to induce endometrial transformation. The serum E2 and P values mentioned in this manuscript were measured 1 or 2 days before initiating endometrial transformation. At the fifth day of endometrial transformation, cleavage-stage embryos were thawed and transferred. Blastocysts were thawed and transferred at the 6th day of endometrial transformation. All medications were maintained at original doses after embryo transfer. Patients usually take Femoston (2 mg oestradiol and 10 mg dydrogesterone, t.i.d.) and progesterone sustained-release vaginal gel (90 mg, q.d.) for luteal support. Serum β-human chorionic gonadotropin (β-hCG) levels were detected 2 weeks after embryo transfer to determine biochemical pregnancy. Patients with elevated serum β-hCG levels were examined by transvaginal ultrasound 4 weeks after embryo transfer to confirm the clinical pregnancy and number of implanted embryos. Luteal support was maintained for 2 months after transfer when the patient became pregnant. The patient was continuously followed up to identify any abnormalities in pregnancy.

**Statistical analysis**
To analyse the effect of serum E2 levels before endometrial transformation on the clinical outcomes of HRT-FET cycles, we performed a smooth curve fit analysis. Then, threshold and saturation effect analysis were employed to explore the cut-off value of serum E2 levels. Two cut-off values of E2 levels were
identified. When the serum $E_2$ level was less than 300 pg/mL, the clinical pregnancy rate was at a high level and was not related to the serum $E_2$ level. When the serum $E_2$ level is greater than 300 pg/mL, the clinical pregnancy rate continues to decline. Therefore, cycles were divided into the following 2 groups based on their levels of serum $E_2$ measured before endometrial transformation: Group 1, $< 300$ pg/mL and Group 2, $\geq 300$ pg/mL. We used the Kolmogorov-Smirnov normality test to detect the normal distribution of the variables. T-test was employed for the normally distributed variables and Mann Whitney-U test was employed for the non-normally distributed variables. For the statistical analysis for categorical variables, the variables in Table 1 were tested by chi-squared test (meeting the requirements of chi-square test: theoretical frequency ($T$) > 5 and sample number ($n$) > 40). The parameters distributed with normally distribution were explained as Mean $\pm$ Standard Deviation (SD) and the parameters distributed with non-normally distribution was explained as Median (25th-75th percentiles). A smooth curve fit analysis of serum $E_2$ levels and clinical pregnancy was employed by different endometrial thicknesses (< 8.4 mm and $\geq$ 8.4 mm). All the analyses were performed with the R package (version 3.6.0) and EmpowerStats (X&Y Solution, Inc., Boston, MA). A $p$ value $< 0.05$ was considered statistically significant.

### Results

#### Smooth fitting curve of serum $E_2$ levels and clinical pregnancy rates

As shown in Fig. 1, the clinical pregnancy rate of the patients decreased obviously as the serum $E_2$ level gradually increased. When serum $E_2$ levels were less than 300 pg/mL, clinical pregnancy rates were maintained at a higher level. When the serum $E_2$ level reached or exceeded 1400 pg/mL, there was no significant change in the clinical pregnancy rate, which was at a lower level (Fig. 1).

#### Threshold effect analysis of serum $E_2$ levels on the clinical pregnancy rate

In order to clarify the fluctuation of the clinical pregnancy rate with serum $E_2$ levels observed in Fig. 1, the results of the threshold effect analysis suggested that there was a curvilinear relationship between serum $E_2$ levels and the clinical pregnancy rate (Table 2, logarithmic likelihood ratio $= 0.042$). When the serum $E_2$ level was less than 1400 pg/mL, the clinical pregnancy rate decreased with the increase of the serum $E_2$ level (Table 2, OR: 0.975, 95%CI: 0.960–0.989, $p < 0.001$). When the serum $E_2$ level reached or exceeded 1400 pg/mL, the clinical pregnancy rate is not affected by the serum $E_2$ level (Table 2, OR: 0.999, 95%CI: 0.983–1.015, $p = 0.907$). Therefore, we further performed a threshold effect analysis for the cycles with serum $E_2$ levels less than 1400 pg/mL, which suggested that there was no relationship between the clinical pregnancy rate and

### Table 1 Comparison of general characteristics and FET outcomes data between different $E_2$ level groups

| Variable                                      | Group 1     | Group 2     | $p$ value |
|-----------------------------------------------|-------------|-------------|-----------|
| Cases (n)                                     | 6251        | 3958        |           |
| Baseline characteristics                      |             |             |           |
| Age, median (25th-75th percentiles), years   | 30.0 (28.0–33.0) | 30.0 (28.0–34.0) | 0.06      |
| Body mass index, mean $\pm$ SD, kg/m$^2$      | 24.0 $\pm$ 3.3 | 24.4 $\pm$ 3.1 | 0.42      |
| No. of cycles, mean $\pm$ SD                 | 2.6 $\pm$ 0.9 | 2.6 $\pm$ 0.9 | 0.43      |
| FET outcomes                                  |             |             |           |
| $E_2$, median (25th-75th percentiles), pg/mL  | 193.00 (149.00–238.52) | 510.07 (371.06–1473.14) | $< 0.01$ |
| $P$, median (25th-75th percentiles), ng/mL   | 0.17 (0.07–0.40) | 0.34 (0.16–0.59) | $< 0.01$ |
| Endometrial thickness, median (25th-75th percentiles), mm | 9.50 (8.80–10.50) | 9.00 (8.50–10.00) | $< 0.01$ |
| No. of transfer embryos, mean $\pm$ SD       | 1.40 $\pm$ 0.49 | 1.42 $\pm$ 0.49 | 0.06      |
| Transfer blastocyst rate (%)                 | 62.3 (3896/6251) | 61.5 (2436/3958) | 0.43      |
| Clinical pregnancy rate (%)                  | 62.9 (3930/6251) | 59.8 (2365/3958) | $< 0.01$ |
| Implantation rate (%)                        | 54.6 (4775/8751) | 51.3 (2881/5620) | $< 0.01$ |
| Miscarriage rates (%)                        | 13.5 (529/3930) | 15.6 (369/2365) | 0.14      |
| Live birth rate (%)                          | 52.8 (3299/6251) | 48.8 (1930/3958) | $< 0.01$ |

Patients had a better clinical outcome (pregnancy rate and implantation rate) with a lower $E_2$ level (<300 pg/mL)

$E_2$, oestrogen, No. number, $P$, progesterone, SD, standard deviation
the serum E₂ level (Table 3, OR: 1.074, 95%CI: 1.000-1.153,  \( p = 0.051 \)) when the serum E₂ level was less than 300 pg/mL. However, when the serum E₂ level was between 300 and 1400 pg/mL, the clinical pregnancy rate decreased with the increase of the serum E₂ level (Table 3, OR: 0.944, 95%CI: 0.919–0.970,  \( p < 0.001 \)). A further smooth curve fitting analysis with different serum E₂ levels also confirmed the results (Fig. S1).

**Table 2** Threshold effect analysis of E₂ level (100pg/mL) on the clinical pregnancy rate

| Outcome                              | Clinical pregnancy |
|--------------------------------------|--------------------|
| Model I (linear)                     | OR  0.986 (0.980, 0.992)  < 0.001 |
| Linear effect                        |                   |
| Model II (polynomial)                | OR  0.950 (0.948, 0.953)  < 0.001 |
| Predicted threshold (K, E₂ level, 100pg/mL) | 14                |
| Effect 1 (< K)                       | 0.975 (0.960, 0.989)  < 0.001 |
| Effect 2 (> K)                       | 0.999 (0.983, 1.015)  0.907 |
| Variability of effectiveness         | 0.172 (0.019, 0.324) |
| Logarithmic likelihood ratio test    | 0.042              |

When the E₂ level was less than 1400 pg/mL, there was a negative relationship between E₂ levels and the clinical pregnancy rate. E₂, oestrogen; OR, odds ratio; CI, confidence interval; K, predicted threshold.

**Table 3** Threshold effect analysis of E₂ level (100pg/mL) in cycles with lower E₂ level (< 1400 pg/mL)

| Outcome                              | Clinical pregnancy |
|--------------------------------------|--------------------|
| Model I (linear)                     | OR  0.970 (0.950, 0.990)  0.004 |
| Linear effect                        |                   |
| Model II (polynomial)                | OR  0.950 (0.948, 0.953)  < 0.001 |
| Predicted threshold (K, E₂ level, 100pg/mL) | 3                |
| Effect 1 (< K)                       | 1.074 (1.000, 1.153)  0.051 |
| Effect 2 (> K)                       | 0.944 (0.919, 0.970)  < 0.001 |
| Variability of effectiveness         | 0.572 (0.499, 0.646)  |
| Logarithmic likelihood ratio test    | 0.004              |

When the E₂ level was between 300 to 1400 pg/mL, there was a negative relationship between E₂ levels and the clinical pregnancy rate. E₂, oestrogen; OR, odds ratio; CI, confidence interval; K, predicted threshold.

**General situations of these 2 groups**

Furthermore, we divided all enrolled cycles into 2 groups according to different serum E₂ levels before endometrial transformation: Group 1 - E₂ < 300 pg/mL and Group 2 - E₂ ≥ 300 pg/mL. Serum P levels were lower in group 1, and the endometrium was thicker in the first group. There were no significant differences in the number or stages of embryos transferred between the two groups. The clinical pregnancy and embryo implantation rates were much higher in group 1. The early miscarriage rate
was similar in these 2 groups. This result suggested that patients might have a better clinical outcome with a lower serum E₂ level (<300 pg/mL), but there might be some indefinite factors which might have influence on HRT-FET outcomes, such as patient age, serum P level, and endometrial thickness, among others.

Analysis of the different endometrial thicknesses affecting the clinical outcomes of HRT-FET
To evaluate whether the endometrial thickness affected the clinical outcomes of HRT-FET, we conducted a threshold effect analysis of the endometrial thickness (Table 4). The results suggested a predicted threshold of endometrial thickness associated with a clinical pregnancy rate of 8.4 mm. In addition, we performed a smooth fitting curve for the relationship between pre-transformation serum E₂ level and clinical pregnancy rate according to the different endometrial thicknesses (<8.4 mm and ≥ 8.4 mm) of HRT-FET cycles (Fig. S2). Regardless of the levels of endometrial thickness, a decreasing trend in the clinical pregnancy rate was also detected with increasing E₂ levels before endometrial transformation. This result suggested that the different endometrial thickness levels might not affect the adverse effect of increased serum E₂ levels on clinical pregnancy of HRT-FET cycles.

Discussion
In our study, the clinical pregnancy rate maintained a high level when the serum E₂ level before endometrial transformation was less than 300 pg/mL. However, the serum E₂ level couldn’t affect the clinical pregnancy rate of HRT-FET when the serum E₂ level is more than 1400 pg/mL.

In 1983, Trounson A first reported a successful pregnancy by FET. FET is currently widely used in clinical human-assisted reproductive technology (ART) [17], mainly for patients who have a previously failed fresh embryo transfer, whose fresh embryo transfer was cancelled due to the risk of OHSS, or who require embryo storage for other reasons [18]. Artificial hormone replacement cycles have been more widely employed because of their convenience. Exogenous oestrogen and progesterone are orally administered to change the endometrium to achieve synchronization with the embryos, which is necessary for embryo implantation in HRT-FET cycles. Embryo implantation is one of the central factors in ART and depends mainly on the ability of the endometrium to receive the embryo for implantation and on the quality of the embryo. Endometrial growth in HRT-FET cycles relies on exogenous oestrogens taken by the patients, and there are two regimens: fixed or escalating doses. Considering patient compliance, the HRT-FET cycles we included in this study were all performed with a fixed oestrogen dose. The serum E₂ and P levels and endometrial thickness of the patients were monitored regularly, and progesterone was administered to transform the endometrium when the endometrium reached the expected thickness. However, there are differences in the absorption and metabolism of exogenous oestrogens among different individuals. Therefore, there are also differences in serum E₂ levels among different individuals. Of interest is whether the difference in serum E₂ levels influenced the clinical outcomes of HRT-FET cycles. Some studies have explored the association between serum E₂ levels before endometrial transformation and the clinical outcomes of HRT-FET cycles, but they remain inconclusive [11, 15, 16, 19]. In our retrospective study, the results suggested that the levels of serum E₂ before endometrial transformation were closely related to the clinical pregnancy rates of patients with HRT-FET cycles when they were less than 1400 pg/mL. Higher clinical pregnancy and embryo implantation rates were achieved when serum E₂ levels were less than 300 pg/mL.

Although sustained elevations in oestrogen in the follicular phase are indispensable for endometrial growth, previous studies have suggested that excessive oestrogen may have adverse effects. In vitro studies reported that oestrogen overexpression in first-trimester human trophoblast cells and the first-trimester placenta is able to inhibit trophoblast invasion by inducing apoptosis, potentially leading to abnormal pregnancy outcomes [20]. In addition, a number of in vivo studies have explored the effects of serum E₂ levels on endometrial function. In mouse models, oestrogen should be maintained in a certain range to enable the uterus to be receptive, and properly increased serum E₂ levels are closely associated with altered expression of genes involved in embryo implantation [21]. At the same time, in a baboon

Table 4 Threshold effect analysis of the endometrial thickness on the clinical pregnancy rate

| Outcome | Clinical pregnancy |
|---------|--------------------|
| Model I (linear) | OR 95% CI p value |
| Linear effect | 1.058 (1.029, 1.088) < 0.01 |
| Model II (polyline) | OR 95% CI p value |
| Predicted threshold (K, Endometrial thickness, mm) | 8.4 |
| Effect 1 (< K) | 1.757 (1.494, 2.065) < 0.01 |
| Effect 2 (> K) | 1.010 (0.979, 1.042) 0.52 |
| variability of effectiveness | 0.575 (0.483, 0.684) |
| Logarithmic likelihood ratio test | < 0.01 |

The predicted cut-off value of endometrial thickness was 8.4 mm

OR odds ratio, CI confidence interval, K predicted threshold
higher serum E\(_2\) levels during early pregnancy (first 60 days) allowed extra villous trophoblast invasion and uterine artery-related functions affecting the development of pregnancy [21]. It follows that appropriate serum E\(_2\) levels have important effects on both embryo implantation and ongoing pregnancy but that serum E\(_2\) levels that are too high may adversely affect it. Finding a reasonable range of serum E\(_2\) levels before endometrial transformation in HRT-FET cycles is essential to improve the pregnancy rate of ART cycles [22]. Clinically, excessive serum E\(_2\) levels after controlled ovarian hyperstimulation (COH) during IVF cycles may lead to a reduced clinical pregnancy rate [23] or adverse pregnancy outcomes [13, 24]. In a previous study, when serum E\(_2\) levels reached 3560 ± 1233 pg/mL or even higher [25] on the hCG trigger day in fresh transplant cycles, the clinical pregnancy rate was significantly lower. However, serum E\(_2\) levels in fresh transplant cycles are much higher than those in HRT-FET cycles in most situations. Therefore, this serum E\(_2\) limit is of limited significance for artificial hormone replacement cycle guidance. There are many studies focusing on HRT-FET cycles that have set cut-off values for serum E\(_2\) levels before endometrial transformation: 299, 400, 600 or 689 pg/mL [11, 16, 26]. Our study was not a direct equivalent of previous studies. In our retrospective study, a smooth curve fitting model was innovatively used to analyse HRT-FET data from our reproductive medicine centre over a period of nearly 3 years, suggesting that the peak clinical pregnancy rate occurs when the serum E\(_2\) level before endometrial transformation is less than 300 pg/mL. Regarding this, we grouped serum E\(_2\) [12, 27, 28] levels before endometrial transformation, and further statistical analysis suggested that we could achieve better clinical pregnancy outcomes when serum E\(_2\) levels were lower than 300 pg/mL in HRT-FET cycles. It is generally accepted that a high serum E\(_2\) level in an FET cycle refers to more than a peak (284.5 ± 77.9 pg/mL) value in the physiological state of the natural cycle [21]. In addition, the serum E\(_2\) level of patients in group 1 (E\(_2\) < 300 pg/mL) of our enrolled cycle was 194.11 ± 56.84 pg/mL, which was closer to the natural cycle situation, which had less of an effect on endometrial receptivity.

According to further observation of the initial fitting curve result, we found that when the serum E\(_2\) level gradually increased to a certain extent, the clinical pregnancy rate had a slight fluctuation. There was a slightly positive correlation between increasing serum E\(_2\) levels and the clinical pregnancy rate when the serum E\(_1\) level was less than 300 pg/mL and within a certain range (approximately 1400–2400 pg/mL). Conversely, when the serum E\(_2\) level was in a certain range (300–1400 pg/mL) and more than 2400 pg/mL, the expected clinical pregnancy rate decreased progressively with increasing serum E\(_2\) levels. The serum E\(_2\) increase in the HRT-FET cycles of this study far exceeded the normal physiological category, which was due mainly to 2 mg of oestradiol for the vaginal plug added in patients with unexpected endometrial thickness. It has been reported that in vaginal plugs with oestradiol 4 mg/day, the serum E\(_2\) concentration can reach a maximum of 4800 pg/mL; in addition, the combination of oral and vaginal oestradiol can achieve better endometrial thickness and improve endometrial receptivity [29]. Therefore, most of the patients in the HRT-FET cycles with higher serum E\(_2\) levels (>1400 pg/mL) had oestradiol medication vaginally because of an unexpected endometrial thickness. Higher serum E\(_2\) levels may be better for endometrial proliferation, such that clinical pregnancy rates improve with higher serum E\(_2\) levels when E\(_2\) levels are between approximately 1400–2400 pg/mL. When serum E\(_2\) levels reach a certain range, they will have a limited effect on the improvement of endometrial thickness. When serum E\(_2\) levels are greater than approximately 2400 pg/mL, the clinical pregnancy rate will decrease with higher serum E\(_2\) levels. These results are valuable for the regulation of oestrogen dosage during HRT-FET cycles in our reproductive medicine centre.

In IVF-ET cycles, endometrial thickness can reflect the functional status of the endometrium to a certain degree [30]. Appropriate endometrial thickness is an essential condition for embryo implantation. Endometrial thickness is a routine detection index to evaluate the ability to accept embryo implantation because of the convenience and maturity of the measuring procedure. At present, most studies believe that endometrial thickness less than 6–8 mm may lead to adverse clinical outcomes [31]. In our study, there was a significant difference in endometrial thickness between the two groups. Patients with high serum E\(_2\) levels were mainly caused by vaginal medication, and the main reason for vaginal medication is the thin endometrial thickness. Therefore, the endometrial thickness of group 2 was slightly lower than that of group 1. To exclude the influence of endometrial thickness, we further used the threshold prediction model to calculate the cut-off value (8.4 mm) that might affect the clinical outcomes. On this basis, we divide the research data into two subgroups for smoothing curve fitting again. The results showed that the clinical pregnancy rate decreased with increasing serum E\(_2\) levels before endometrial transformation, regardless of endometrial thickness. In addition, we also found the difference of serum P levels between these two groups. There are few studies on the impact of serum P level on the clinical pregnancy outcome of HRT-FET cycles, which mainly discuss the impact of serum P level after endometrial transformation
or on the day of embryo transfer. Furthermore, we conducted a univariate analysis of the serum P level before transformation, and the results showed that it has no significant effect on the clinical pregnancy rate (Table. S1).

The results of our study are not identical to those of some previous studies. Niu et al. [16] retrospectively reviewed 274 FET cycles. Patients with different serum E2 levels on the start day of progesterone had similar pregnancy rates. However, the higher E2 level in their study (299 ± 48.9 pg/mL) was much lower than the higher serum E2 level in our study. Moreover, the previous study included only the outcomes of cleavage-stage embryo transfer. Celik et al. conducted a retrospective study [28] of 468 patients in 2019: Serum E2 monitoring prior to progesterone administration could not predict patient live birth rates. A novel retrospective study [32] suggested no significant difference in FET clinical outcomes when serum E2 levels were between 100 and 500 pg/mL before endometrial transformation but that the spontaneous abortion rate was significantly increased when the serum E2 level was below 100 pg/mL or over 500 pg/mL. However, the highest serum E2 cut-off value of this study was only 500 pg/mL, which was much lower than the high serum E2 cut-off value of our study, and this study included only the outcomes of blastocyst transfer. The retrospective analysis in our centre has a larger sample size than previous studies and incorporates different numbers and types of embryos transferred. To control for the influence of embryonic factors on the clinical outcomes of HRT-FET cycles, we further stratified the statistics for the different types of embryos transferred. A smooth curve fit was employed between the level of serum E2 before endometrial transformation and the clinical pregnancy rate of patients with different numbers and types of embryos transferred, and we found that the clinical pregnancy rates of the different numbers and types of embryos transferred decreased gradually as the level of serum E2 increased (Fig. S3). The results suggested that the increased serum E2 level before endometrial transformation impaired the clinical pregnancy rate regardless of the number and types of embryos transferred.

Our study was limited to HRT-FET cycles without pre-treatment. However, a larger number of patients were pretreated with GnRHa before oral exogenous oestrogen and progesterone at our centre, and the relationship between pretransformation serum E2 levels and clinical outcomes in such patients needs further exploration. In addition, we didn’t include the duration of oestrogen used of patients in HRT-FET cycles, which might has influence on the clinical pregnancy outcomes. The main drawback of this study is its retrospective design. To further clarify the effect of serum E2 levels before endometrial transformation on clinical outcomes in HRT-FET cycles, higher-quality and large-scale randomized controlled trials are needed. We can further design clinical randomized controlled studies to clarify the impact of serum E2 level on clinical outcome under different method and dose of exogenous oestrogen administration. Therefore, the dosage of exogenous oestrogen can be reduced to avoid drug abuse and drug-related risks while maintaining a high clinical pregnancy rate.

Conclusions
In summary, when the serum E2 level before endometrial transformation was less than 1400 pg/mL, the serum E2 level affects the clinical pregnancy rate in the HRT-FET cycle. When the pretransformation serum E2 level is less than 300 pg/mL, patients with HRT-FET cycles may achieve a higher possibility of clinical pregnancy.

Abbreviations
E2: oestrogen; HRT-FET: hormone replacement therapy-frozen embryo transfer; P: progesterone; IVF: in vitro fertilization; BMI: body mass index; GnRHa: gonadotrophin-releasing hormone agonist; β-hCG: β-human choric gonadotropin, ART: assisted reproductive technology; COH: controlled ovarian hyperstimulation; SD: Standard Deviation.

Supplementary information
The online version contains supplementary material available at https://doi.org/10.1186/s12884-022-04605-2.
Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Reproductive Medicine Center, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, 210008 Nanjing, People’s Republic of China. 2Center for Molecular Reproductive Medicine, Nanjing University, 210008 Nanjing, China. 3Drum Tower Clinic Medical College of Nanjing Medical University, 210008 Nanjing, China.

Received: 2 August 2021   Accepted: 22 March 2022
Published online: 29 March 2022

References
1. European IVFMedC, European Society of Human R, Embryology, Calhaz- Jorge C, De Geyter C, Kupka MS, de Mouzon J, Erb K, Mocanu E, Motrenko T et al. Assisted reproductive technology in Europe, 2013: results generated from European registers by ESHRE. Human Reprod 2017, 32(10):1957–1973.
2. European IVFMedCHeSoHR, Embryology, Calhaz-Jorge C, de Geyter C, Kupka MS, de Mouzon J, Erb K, Mocanu E, Motrenko T, Scaravelli G et al. Assisted reproductive technology in Europe, 2012: results generated from European registers by ESHRE. Human Reprod. 2016;31(8):1638–52.
3. Corbett S, Shmorogn D, Claman P. Reproductive Endocrinology Infertility C, Special C. The prevention of ovarian hyperstimulation syndrome. J Obstrct Gynaecol Can. 2014;36(11):1024–33.
4. Groenewoud ER, Cantineau AE, 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. Human Reprod Update. 2013;19(5):458–70.
5. Shapiro BS, Daneshmand ST, Garnier 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. Fertility Sterility. 2011;96(2):344–8.
6. Mackens S, Santos-Ribeiro S, van de Vljer A, Racca A, Van Landuyt L, Tournaye H, Blockeel C. Frozen embryo transfer: a review on the optimal endometrial preparation and timing. Human Reprod. 2017;32(11):2342–4.
7. Gobhara T, Gelbaya TA, Ayeleke RO. Cycle regimens for frozen-thawed embryo transfer. Cochrane Database Syst Reviews. 2017;7:CD003414.
8. Saito K, Kuwahara A, Ishikawa T, Morisaki N, Miyado M, Miyado K, Fukami M, Miyasaki N, Ishihara O, Irahara M, 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(8):1567–75.
9. Li C, He YC, Xu JJ, Wang Y, Liu H, Duan CC, Shi CY, Chen L, Wang J, Sheng JZ, 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(1):341.
10. Albrecht ED, Aberdeen GW, Pepe GJ. The role of estrogen in the maintenance of primate pregnancy. Am J Obstet Gynecol. 2000;182(2):432–8.
11. Fritz R, Jindal S, Fei H, Buyuk E. Elevated serum estradiol levels in artificial autologous frozen embryo transfer cycles negatively impact ongoing pregnancy and live birth rates. J Assisted Reprod Genet. 2017;34(12):1633–8.
12. Aşlan M, Bocca S, Aşlan EO, Duran HE, Stadtmaler L, Oehninger S. Cumulative exposure to high estradiol levels during the follicular phase of IVF cycles negatively affects implantation. J Assisted Reprod Genet. 2007;24(4):111–7.
13. Imudia AN, Awoyugna AO, Doyle JO, Kaimal AJ, Wright DL, Toth TL, Styer AK. Peak serum estradiol level during controlled ovarian hyperstimulation is associated with increased risk of small for gestational age and preeclampsia in singleton pregnancies after in vitro fertilization. Fertility Sterility. 2012;97(6):1374–9.
14. Pereira N, Elias RT, Christos PJ, Petroni AC, Hancock K, Lekovich JP, Rosen-waks Z. Supraphysiologic estradiol is an independent predictor of low birth weight in full-term singletons born after fresh embryo transfer. Human Reprod. 2017;32(7):1410–7.
15. Remohi J, Ardiles G, Garcia-Velasco JA, Gaian P, Simon C, Pellicer A. Endometrial thickness and serum oestradiol concentrations as predictors of outcome in oocyte donation. Human Reprod. 1997;12(10):2271–6.
16. Niu Z, Feng Y, Sun Y, Zhang A, Zhang H. Estrogen level monitoring in artificial frozen-thawed embryo transfer cycles using step-up regime without pituitary suppression: is it necessary? J Experiment Clin Assisted Reprod. 2008;5:6.
17. Shen C, Shu D, Zhao X, Gao Y. Comparison of clinical outcomes between fresh embryo transfers and frozen-thawed embryo transfers. Iran J Reprod Med. 2014;12(6):409–14.
18. Weinerman R, Mainini M. Why we should transfer frozen instead of fresh embryos: the translational rationale. Fertility Sterility. 2014;102(1):10–8.
19. Groenewoud ER, Macklon NS, Cohlen BJ, group Ats. Cryo-thawed embryo transfer: natural versus artificial cycle. A non-inferiority trial. (ANTARCTICA trial). BMC Women’s health. 2012;12:27.
20. Patel S, Kilburn B, Imudia A, Arman DR, Skafar DF. Estradiol Elcts Prog- poptotic and Antiproliferative Effects in Human Trophoblast Cells. Biol Reprod. 2015;93(3):74.
21. Ma WG, Song H, Das SK, Paris BC, Dey SK. Estrogen is a critical determin- ant that specifies the duration of the window of uterine receptivity for implantation. Proc Natl Acad Sci USA. 2003;100(5):2963–8.
22. Kutlu T, Ozkaya E, Ayvaci H, Devranoglu B, Sanverdi I, Sahin Y, Senol T, Karaeteke A. Under curve of temporal estradiol measurements for prediction of the detrimental effect of estrogen exposure on implanta- tion. Int J Gynaecol Obstetrics. 2016;135(2):168–71.
23. Forman R, Fries N, Testart J, Belaisch-Allart J, Hazout A, Frydman R. Evidence for an adverse effect of elevated serum estradiol concentrations on embryo implantation. Fertility Sterility. 1988;49(1):118–22.
24. Farhi J, Ben-Haroush A, Andrawus N, Pinkas H, Sapir O, Fisch B, Ashkenazi J. High serum oestradiol concentrations in IVF cycles increase the risk of pregnancy complications related to abnormal placentaion. Reprod Biomed Online. 2010;21(3):331–7.
25. Adejogie Erasihin A, Acrat M, Erasihin SS, Dokuzeylubun Gungor N. Frozen embryo transfer prevents the detrimental effect of high estrogen on endometrium receptivity. J Turkish German Gynecol Assoc. 2017;18(1):38–42.
26. Deng L, Chen X, Ye DS, Chen SL. Effect of serum estradiol level before progesterone administration on pregnancy outcomes of frozen-thawed embryo transfer cycles. Nan Fang Yi Ke Da Xue Xue Bao. 2018;38(5):601–5.
27. Joo BS, Park SH, An BM, Kim KS, Moon SE, Moon HS. Serum estradiol levels during controlled ovarian hyperstimulation influence the pregnancy outcome of in vitro fertilization in a concentration-dependent manner. Fertility Sterility. 2010;93(2):442–6.
28. Celik C, Asoglu MR, Karakis LS, Findikli N, Gultomruk M, Cavkaytar S, Bah- ceci M. The impact of serum oestradiol concentration prior to progester- one administration on live birth rate in single vitrified-warmed blastocyst transfer cycles. Reprod Biomed Online. 2019;39(6):1026–33.
29. Mattsson LA, Cullberg G. Vaginal absorption of two estradiol preparations. A comparative study in postmenopausal women. Acta Obstetica et Gynecologica Scand. 1986;62(3):395–6.
30. Kasius A, Smit JG, Torrance HL, Eijkemans MJ, Mol BW, Opmeer BC, Broek- mans EJ. Endometrial thickness and pregnancy rates after IVF: a systemic review and meta-analysis. Human Reprod Update. 2014;20(4):530–41.
31. 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. Reproduct Biomed Online. 2016;33(2):197–205.
32. Garmiella S, Karunakaran S, Gedela DR. Does serum estradiol level have an impact on outcomes in hormonal replacement frozen-warmed embryo transfer cycles? J Gynecol Endocrinol. 2021;1:1–4.