Clinical Outcomes and Placental Pathological Characteristics after Fresh Embryo Transfer and Frozen-Thawed Embryo Transfer with Different Endometrial Preparation Protocols:a retrospective study

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Abstract

Abstract Objective: To analyse the impacts of fresh embryo transfer and frozen-thawed embryo transfer cycles with different endometrial preparation protocols on clinical outcomes and placental pathology. Design: Retrospective case-control study. Setting: Peking University Third Hospital. Population: A total of 3920 Single live birth cases after in vitro fertilization/intracytoplasmic sperm injection and embryo transfer cycles. Method: Cases were divided into the fresh embryo transfer, natural cycle (NC)-frozen-thawed embryo transfer and hormone replacement therapy (HRT)-FET groups, and clinical outcomes and placental pathology characteristics were compared. Main Outcome Measures: preterm birth, preeclampsia, postpartum haemorrhage, placenta implantation, placenta previa, placental accreta, cervical insufficiency, neonatal weight and placental pathology. Result:The risks of preeclampsia, postpartum haemorrhage and preterm birth were significantly higher in the HRT-FET group than the fresh embryo transfer and NC-FET groups (13.42% vs 5.49% vs 5.91%, 21.7% vs 12.1% vs 11.0%, 10.5% vs 7.7% vs 7.6%, $p < 0.05$). Birth weight was lower in the fresh embryo transfer group than the NC-FET and HRT-FET groups ($p<0.05$). There was no statistically significant difference in the incidence of placental structural abnormalities and pathological characteristics among the groups. Conclusion: HRT-FET cycles were associated with increased maternal and foetal complications compared to fresh embryo transfer and NC-HRT cycles. There was no significant difference in the occurrence of placental structural abnormalities or pathological changes among the transfer methods. Keywords: Fresh embryo transfer; Frozen-thawed embryo transfer; Endometrial preparation protocol; Clinical outcome; Placenta Tweetable abstract: Different Embryo Transfer techniques influence the clinical outcomes but not placental pathology .

Introduction

As an important technique in assisted reproductive technology (ART), embryo transfer is widely adopted in clinical practice. As a complementary treatment for fresh embryo transfer, in FET, available embryos are frozen, and then the appropriate time for their transplantation can be selected. Compared with fresh embryo transfer, FET has advantages of reduced trauma from repeated ovulation induction and egg aspiration as well as reduced costs for patients. However, its pregnancy outcomes are of great concern. Although some existing studies point out that FET is not associated with a risk of preeclampsia and is helpful in reducing the incidence of premature birth, low birth weight infants and small for gestational age infants, others argue that FET significantly increases the preeclampsia risk.[1-4]

The placenta is an important organ that connects the mother and the foetus during pregnancy. The formation of the placenta involves the invasion of trophoblast cells and the remodelling of uterine spiral arteries, which can lead to various adverse pregnancy complications. ART techniques, such as the application of ovulation-inducing drugs, in vitro embryo culture, freezing technology, and corpus luteum support during the transplantation cycle, may influence the formation and function of the placenta, lead to structural

abnormalities in placental villi and vascular and inflammatory changes, and eventually affect pregnancy outcomes.

Therefore, understanding the potential impact of different embryo transfer techniques on placenta formation is a guide for clinicians in selecting the appropriate embryo transfer protocol and strengthening the management of pregnancy after successful transfer to minimize pregnancy complications. The aim of this retrospective study was to analyse cases of live births after fresh embryo transfer and frozen-thawed embryo transfer with different endometrial preparation protocols over a 10-year period at our institution and their pregnancy outcomes and placental pathological characteristics.

Materials and methods

Study design

With the approval of the Ethics Committee of the Third Hospital of Peking University (No. 2020-419-01), the clinical data of pregnant women who underwent in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) and embryo transfer and delivered at the Third Hospital of Peking University from January 2012 to February 2022 were collected. The live birth cases were divided into three groups according to the embryo transfer method: the fresh embryo transfer group, natural cycle (NC)-frozen-thawed embryo transfer (FET) group, and hormone replacement therapy (HRT)-FET group.

Inclusion criteria: gestational age [?]24 weeks, live birth, and single pregnancy or single intrauterine foetal demise or reduction before 12 weeks.

Exclusion criteria: 1) ART with donor sperm , 2) conception after preimplantation genetic diagnosis, 3) definite foetal malformation before delivery, and 4) intrauterine foetal death or reduction after12 weeks.

Embryo transfer protocol and luteal support

Fresh embryo transfer: The ovulation induction protocol was chosen according to the clinical characteristics of the patients. When the dominant follicle diameter was [?] 17 mm, recombinant human chorionic gonadotropin (hCG) was injected, and ultrasound-guided transvaginal oocyte retrieval was performed 36-38 hours after the injection.

HRT-FET cycle: For women with a previous history of a thin endometrium or an irregular menstrual cycle, oral oestradiol valerate (Progynova, Schering, Germany) 4-6 mg/d was administered from day 3 of menstruation, and the dose was adjusted according to endometrial thickness. When endometrial thickness reached 8 mm or more (with oestradiol valerate continued for at least 10-12 days), progesterone gel (Serotonin, Merck Serono, 90 mg/d) combined with dydrogesterone tablets (Duphaston, Solvay Netherlands, 10 mg bid in the first two days, 20 mg bid later) was administered prior to endometrial transformation. The Day 3 cleavage embryo or the Day 7 blastocyst was thawed and transferred.

NC-FET cycle: For patients with regular menstrual cycles, ovulation was monitored by transvaginal ultrasound, and cleavage-stage embryos were transferred on the 3rd day after ovulation, whereas blastocysts were transferred on the 5th day after ovulation. Dydrogesterone tablets (Duphaston, Solvay Netherlands, 20-40 mg/d) were taken from the day of ovulation until the 12th-14th day after embryo transfer.

After fertilization, the cleavage and development of embryos were evaluated. Embryos available for transfer contained 4-8 blastomeres on the 3^{rd} day after fertilization and were graded 2 or above. No more than 2 emtryos could be transferred and the remaining embryos were cryopreserved. Embryos with more than 50% viable cells after thawing could be transferred. The level of β-hCG in serum was checked on the 12^{th}-14^{th} day after embryo transfer. The appearance of an intrauterine gestational sac in vaginal ultrasonography performed 4 weeks after embryo transfer was diagnosed as clinical pregnancy. Progesterone gel (Serotonin, Merck Serono, 1 time/d, 90 mg/d) was used for luteal support, fresh embryo transfer and natural cycle medications were administered until the 8^{th}-10^{th} week of pregnancy, and the artificial cycle was performed until the 12^{th} week of pregnancy.

Clinical outcomes and placental pathological characteristics

We retrieved data on the following aspects:

1.Basic characteristics: maternal age, fertilization method, height, prepregnancy body mass index (BMI), and mode of delivery.

2.Pregnancy outcomes and complications: gestational age, preterm birth, preeclampsia, gestational hypertension, gestational diabetes mellitus (GDM), postpartum haemorrhage, placenta increta, placenta previa, placental accreta, premature rupture of membranes, placental abruption , placenta weight, position of cord attachment (velamentous placenta, battledore placenta, circumvallate placenta, etc.), and placenta morphology (placenta multipartita, pseudoleaf placenta).

3. Placental pathology: All placentas that met the inclusion criteria and submitted for pathological examination were analysed. The observation indicators included placental calcification, chorioamnionitis, chorionic infarction, haemorrhage, syncytiotrophoblastic nodules hyperplasia, and cellulose deposition.

4. Neonatal outcomes: neonatal malformations, macrosomia (birth weight greater than 4000 g), large for gestational age (a birth weight more than the 90^{th} percentile for gestational age), small for gestational age (a birth weight less than the 10^{th}percentile for gestational age), neonatal asphyxia, neonatal intensive care unit (NICU) referral rate.

Statistical analysis

The data were analysed by SPSS 28.0 statistical software. Normally distributed measurement data are presented as ?x±s, and an independent-sample t test was used for comparisons between groups. Quantitative data with a skewed distribution are presented as the median and quartile, and the Mann-Whitney test was used for comparisons among groups. Enumerated data are presented as percentiles; the $\chi 2$ test was used for comparisons among three groups, and the corrected Bonferroni test was used for pairwise comparisons between groups. Differences were considered statistically significant at $P < 0.05$.

Results

A total of 3920 cases met the inclusion and exclusion criteria, including 1822 cases in the fresh embryo transfer group and 2098 cases in the FET group. In the FET group, there were 1353 cases of NC-FET and 745 cases of HRT-FET. S1 shows that the differences in prepregnancy BMI, the number of pregnancies and deliveries, the endometrial thickness on the day of hCG injection/transformation, and whether women had polycystic ovarian syndrome (PCOS) or a history of previous uterine operations were statistically significant among the three groups ($P<0.001$). The prepregnancy BMI and proportion of women with PCOS in the FET group were significantly higher than those in the fresh embryo transfer group and the HRT-NC group ($P < 0.001$). The proportions of blastocyst transfer and ICSI in the FET group were significantly higher than those in the fresh embryo transfer group ($P<0.001$ and $P=0.001$, respectively). The proportion of first-pregnancy and primipara was higher in the fresh embryo transfer group than in the FET group, and more people in the FET group had a history of uterine manipulation than those in the fresh embryo transfer group ($P < 0.001$). The comparison of endometrial thickness on the day of hCG injection/transformation showed that the endometrium of the fresh embryo transfer group was the thickest, followed by that of the NC-FET group, and the endometrium of the HRT-FET group was the thinnest.

There was a significant difference in the incidence of preeclampsia among the three groups, and further comparison indicated that the incidences of severe preeclampsia and early-onset preeclampsia were also statistically significantly different. The risks of preeclampsia, severe preeclampsia and early-onset preeclampsia were significantly higher in the HRT-FET group than in the fresh embryo and NC-FET groups ($P>0.05$) .The rates of postpartum haemorrhage and caesarean section among the three groups were statistically significantly different ($P < 0.05$), with the rate of the HRT-FET group being significantly higher than those of the fresh embryo and NC-FET groups ($P < 0.001$). Further analysis of the causes of postpartum haemorrhage in the three groups showed that the most common cause was uterine inertia, followed by placental factors.

In the fresh embryo transfer group, 28% developed postpartum haemorrhage due to placental factors, with placenta previa being the most common placental factor. In the NC-FET group and HRT-FET group, the incidence of postpartum haemorrhage due to placental factors was 18.1% and 27.2%, respectively, of which placental implantation was the most common cause. When comparing the proportions of women with cervical insufficiency, the data revealed that the proportion of women with cervical insufficiency in the HRT-FET group was significantly higher than that in the fresh embryo transfer group (P<0.05). The preterm birth rate was higher in the HRT-FET group than in the fresh embryo transfer group (P=0.022) and the NC-FET group (P=0.026) (Table 1).

Table 2 shows that the rate of neonatal transfer to the NICU in the HRT-FET group was higher in the fresh embryo transfer group and NC-FET groups (P < 0.001), whereas there was no difference between the fresh embryo and NC-FET groups (P > 0.05). The most common reasons for neonatal transfer to the NICU in the fresh embryo, NC-FET and HRT-FET groups were suspected neonatal infection, neonatal jaundice and neonatal respiratory distress, respectively. The three groups were statistically significantly different (p<0.05) in the delivery rates of large for gestational age and small for gestational age neonates and in neonatal birth weight, with the neonatal birth weight being significantly lower in the fresh embryo transfer group than in the NC-FET group (p=0.004) and HRT-FET group (P=0.001). The proportion of large for gestational age neonates was higher in the HRT-FET group than in the fresh embryo transfer group (Table 2).

Table 3 demonstrates that there were no statistically significant differences in the incidences of placental structural abnormalities (velamentous placenta, accessory placenta, battledore placenta, circumvallate placenta, etc.), premature rupture of membranes or placental abruption among the three groups (P > 0.05). In terms of placental adhesion and placental implantation, the incidence was significantly different among the three groups (P < 0.001), in which the rate was higher in the HRT-FET group than in the fresh embryo transfer group. In women with no previous uterine operation, the incidence of placental adhesions was highest in the HRT-FET group (4.3%), followed by the fresh embryo transfer group (2.2%) and the NC-FET group (0.8%), and the incidence of placental implantation was significantly lower in the NC-FET group than in the HRT-FET group (p=0.001). In addition, the difference in the proportion of women who developed placenta previa among the three groups was statistically significant (P=0.020), and the incidence of placenta previa was higher in the fresh embryo transfer group than in the HRT-FET group (P=0.007).

Of the live birth cases enrolled, 521 placentas were examined for placental pathology after delivery. An analysis of the pathological reports revealed that there was no statistically significant difference in the incidence of infarction, calcification, interstitial haemorrhage, syncytiotrophoblastic nodule hyperplasia, fibrin deposition or chorionic villus infarction among the three groups (all P > 0.05).The incidence of chorioamnionitis was significantly different among the three groups (P=0.020), with a lower incidence of chorioamnionitis in the HRT-FET group than in the NC-FET group (P<0.05) (Table 4). After adjusting for potential complications, including premature rupture of membranes, gestational diabetes, cervical insufficiency, premature birth, urogenital system infection, and a history of intrauterine operation, it was evident that there was no significant association between the different embryo transfer methods and chorioamnionitis (odds ratio (OR)=1.117, 95% confidence interval (CI): 0.899-1.387, p=0.320).

Discussion

Main finding

From January 2012 to February 2022, a total of 3,920 cases were collected, including 1822 fresh embryo transfer cases, 1353 NC-FET cases and 745 HRT-FET cases. The HRT-FET group had the highest risk of preeclampsia, cervical insufficiency and postpartum haemorrhage among the three groups.The birth weight of neonates was higher in the FET group than that of neonates in the fresh embryo transfer group. However, the incidence of placental histological abnormalities in the fresh embryo transfer, NC-FET, and HRT-FET groups was similar. The placental pathological analysis of the submitted placentas showed that there was no significant difference in the incidence of placental infarction, calcification, syncytiotrophoblastic nodule hyperplasia or fibrin deposition.

Strengths and limitations

Our study has several strengths. First, all the patients were have their prenatal examination and delivered at our institution, where the clinical data were relatively complete. Second, our research had a large sample and a long research time span, which would be more representative. However, there are some limitations to this study. Due to the impact of retrospective analysis, the three groups of people had heterogeneity, and there is subjectivity in the selection of placental pathology, which may lead to bias. In addition, this study lacks a normal placental control group , and further prospective randomized controlled trials are still needed in the future.

Interpretation

In the present study, patients in the HRT-FET group were more likely to have a higher prepregnancy BMI and complicate with PCOS, which would increase the probability of combining insulin resistance, metabolic abnormalities and obesity, and lead to preeclampsia. According to Versen-Höynck,[5] the risk of preeclampsia and severe preeclampsia would increase in pregnant women without a corpus luteum compared with those with one or more corpus lutea; moreover, a subgroup analysis of patients receiving FET indicated that an HRT cycle may lead to an increased risk of preeclampsia compared with a natural cycle. Compared NC-FET with a normal corpus luteum, HRT-FET cases are at increased risk of preeclampsia due to suppression of their own follicular function, resulting in lack of many vasoactive substances secreted by the corpus luteum, and reduced vascular compliance.[6] The results of the present study indicate that for patients who have hypertension diagnosed before pregnancy, a previous history of preeclampsia or a family history of hypertension, the selection of fresh embryo transfer and NC-FET rather than HRT-FET is recommended. For those who have already received HRT-FET, their blood pressure, urine protein and weight gain need to be well monitored to prevent preeclampsia.

Studies have shown that high levels of oestradiol are a risk factor for low birth weight infants. Animal experiments have also found that high levels of oestrogen in early pregnancy inhibit extravillous trophoblasts from infiltrating the spiral arterioles of the uterus, resulting in poor placental angiogenesis, which eventually leads to adverse pregnancy outcomes.[7] It is speculated that the weight of newborns may be related to the use of a super physiological dose of oestradiol for ovarian hyperstimulation. In addition, previous studies have reported that the embryo freezing process of FET may affect the early epigenetic changes in embryos and then affect the growth potential of newborns. Mainigi et al. used mouse models to study the effect of ovulation induction on the growth of the placenta and foetus and suggested that VEGF affects the formation of the placenta. VEGF also upregulates the expression of the negative growth regulatory gene Grb10, a differentially methylated gene of the placenta, whose expression is markedly upregulated in trophoblasts in the labyrinth zone of the mouse placenta (the major site of maternal-foetal material exchange in the mouse placenta), but there was no significant difference in the foetal mouse.[8,9] It is speculated that multiple factors may be jointly involved in influencing foetal weight in different transplantation protocols.

Previous studies have shown that among singleton pregnant women, patients with PCOS have a higher risk of cervical insufficiency than non-PCOS patients. Higher prepregnancy BMI increased the risk of cervical insufficiency.[10] At the same time, cervical insufficiency may be related to insulin resistance and hyperandrogenism. In this study, compared with the NC-FET and fresh embryo transfer groups, the HRT-FET group had a higher prepregnancy BMI , a higher proportion of PCOS and intrauterine operation histories. These factors increased the risk of cervical insufficiency in this group of patients. The results of this study suggest that in the choice of embryo transfer method, for the combination of PCOS, obesity, insulin resistance and other risk factors for cervical insufficiency, the prioritization of fresh embryo transfer or NC-FET can reduce the risk of premature birth and cervical insufficiency. Ultrasound regularly monitors the length of the cervix uteri during pregnancy and obstetric examination to alert patients to the occurrence of cervical insufficiency.

It is speculated that the incidences of placental increta and placenta accreta may be related to the supra-physiological dose of oestrogen caused by controlled ovarian hyperstimulation (COH) in the fresh embryo transfer protocol and the thinner endometrium on the day of hCG injection/transformation in the HRT-FET

group. Aberdeen et al indicated that supraphysiological doses of oestrogen lead to a significant reduction in the pregnancy-associated protein A (PAPP-A) produced by the placenta and decidua, affecting the infiltration of trophoblasts and embryonic development and adhesion.[11] Senapati et al showed that COH affects the expression of key genes that mediate endometrial remodelling in early embryo implantation, which may affect the infiltration of trophoblasts and vascular remodelling. In this research, the incidence of placenta previa was higher in the fresh embryo transfer group, which may be related to asynchrony in endometrial development and interference with placenta formation caused by the effect of supraphysiological doses of oestrogen on the endometrium. The postpartum haemorrhage rate of the HRT-FET group was higher than that of the fresh embryo transfer and NC-FET group, which may be related to the higher proportion of placenta accreta and placental adhesion in the FET hormone replacement cycle. After the foetus is delivered, due to placental adhesion and placenta accreta, the placenta cannot be delivered smoothly, which affects uterine contraction and increases the risk of postpartum haemorrhage. Therefore, for women who undergo HRT-FET, clinicians should be more vigilant about postpartum haemorrhage during delivery, the maternal physical condition should be fully assessed and blood resources should be prepared; for patients with previous intrauterine operations, especially those with a history of multiple intrauterine operations, HRT-FET needs to be selected with caution. If HRT-FET treatment is performed, more attention should be given to evaluating the severity of placenta accreta, and the mode of delivery should be fully evaluated to reduce the risk of postpartum haemorrhage and the serious maternal and foetal complications caused by placenta accreta.

Conclusion

In conclusion, as a supplementary treatment for fresh embryo transfer, FET is a relatively safe transplantation scheme. Compared with HRT-FET, the NC-FET is more in line with the internal milieu, and the risk of obstetric complications lower. In cases where fresh embryos have failed repeatedly or are not suitable for fresh embryo transfer, NC is recommended for FET to reduce the risk of complications. In this study, there was no significant effect of the three embryo transfer methods on placental structural and pathological abnormalities.

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Disclosure of interests

All authors declare no competing interests.Completed disclosure of interests form available to view online as supporting information.

Contribution to authorship

YQW and RL conceived the study, provided overall guidance, edited and reviewed the final version.YYL collected data, performed the statistical analysis and drafted the first version of the manuscript. All authors contributed to the interpretation of data and read and approved the final manuscript.

Ethics approval

The study was approved by the Ethics Committee of the Peking University Third Hospital ,China.(No. 2020-419-01).

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Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Table 1 Comparison of obstetric outcomes of three groups

	Fresh embryo transfer group N=1822	Frozen-thawed embryo transfer group N=2098 NC-FET group N=1353	Frozen-thawed embryo transfer group N=2098 HRT-FET group N=745	Frozen-thawed embryo transfer group N=2098 Total	χ^2/U value	P value	χ^2/U value*	P value
Gestational age(week)	39.14(38.29,40.00)	39.14(38.43,40.14)	39.14(38.21,40.14)	39.14(38.29,40.14)	2.47	0.25	-1.47	0.141

Preterm birth	140(7.7%)	103(7.6%)	78(10.5%)	181(8.63%)	6.37	0.041	1.15	0.283
24-27^{+6}week	7(5.00%)	7(6.80%)	2(2.56%)	9(4.97%)	7.99	0.239	3.65	0.310
28-31^{+6}week	26(18.57%)	10(9.71%)	15(19.23%)	25(13.81%)	Ref	Ref	Ref	Ref
32-33^{+6}week	14(10.00%)	18(17.48%)	12(15.38)	30(16.57%)	Ref	Ref	Ref	Ref
34-36^{+6}week	93(66.43)	68(66.02%)	49(62.82%)	117(64.64%26)	Ref	Ref	Ref	Ref
Placental weight	520(500,600)	520(500,600)	540(500,600)	530(500,600)	4.50	0.106	-2.00	0.046
Preeclampsia	100(5.49%)	80(5.91%)	100(13.42%)	180(8.58%)	54.90	¡0.001	14.05	¡0.001
Preeclampsia	52(2.85%)	37(2.73%)	48(6.44%)	85(4.05%)	23.74	¡0.001	4.15	0.042
Severe preeclampsia	48(2.63%)	43(3.18%)	52(6.98%)	95(4.53%)	29.70	¡0.001	9.95	0.002
Gestational hypertension	47(2.58%)	34(2.51%)	43(5.77%)	77(3.67%)	20.44	¡0.001	3.79	0.052
Early-onset preeclampsia	36(1.98%)	28(2.07%)	32(4.30%)	60(60/180)	13.15	0.001	3.19	0.074
Gestational diabetes mellitus	583(32.0%)	436(32.2%)	236(31.7%)	672(32.03%)	0.07	0.967	0.000	0.983
Type A1	544	388	209	597				
Type A2	39	48	27	75				
Cervical insufficiency	39(2.1%)	37(2.7%)	33(4.4%)	70(3.34%)	10.27	0.006	5.16	0.023
Postpartum haemorrhage	221(12.1%)	149(11.0%)	162(21.7%)	311(14.82%)	53.21	¡0.001	6.03	0.014
uterine inertia	163	122	124	246				
placental factors	46	27	44	71(22.83%)				
placenta increta	20	7	20	27				
placenta accreta	12	9	17	26				
Placenta previa	14	11	7	18				
Soft birth canal la	aceration	7	14	21				
Coagulation dysfunction	0	1	2	3				
Mode of delivery					57.30	¡0.001	12.88	¡0.001
Vaginal delivery	985(54.1%)	714(57.0%)	300(40.3%)	1014(48.33%)				
Caesarean section	837(45.9%)	639(43%)	445(59.7%)	1084(51.67%)				

Notes:indicates P < 0.05 for the fresh embryo transfer group compared with the NC-FET group; indicates P < 0.05 for the fresh embryo transfer group compared with the HRT-FET group; indicates P < 0.05 for the NC-FET group compared with the HRT-FET group; * indicates the fresh embryo group compared with the frozen-thawed embryo transfer group

Table 2 Comparison of neonatal outcomes of three groups

	Fresh embryo transfer group N=1822	Frozen-thawed embryo transfer group N=2098 NC-FET group N=1353	Frozen-thawed embryo transfer group N=2098 HRT-FET group N=745	Frozen-thawed embryo transfer group N=2098 Total	χ^2/U value	P value	χ^2/U value*	P value
Neonatal weight	3260(2990,3580)	3330(3030,3590)	3350(3060,3625)	3340(3040,3880)	15.07	0.001	-3.73	¡0.001
Macrosomia	100(5.5%)	74(5.5%)	59(7.9%)	133(6.34%)	6.42	0.040	1.26	0.261
Large for gestational age	267(14.7%)	238(17.6%)	142(19.1%)	280(18.11%)	9.22	0.010	1.39	0.238
Small for gestational age	95(5.2%)	65(4.8%)	28(3.8%)	93(4.43%)	2.45	0.293	1.30	0.254
Neonatal malformation	49(2.7%)	39(2.9%)	19(2.6%)	58(2.76%)	0.22	0.896	0.02	0.885
Neonatal asphyxia	29(1.6%)	12(0.9%)	14(1.9%)	26(1.24%)	4.30	0.177	0.88	0.349
the rate of neonatal transfer to the NICU	250(13.7%)	174(12.9%)	148(19.9%)	322(15.35%)	20.99	¡0.001	2.07	0.150
the reason for neonatal transfer to the NICU								
Suspected neonatal infection	72(28.80%)	39(22.41%)	34(22.97%)	73(22.67%)				
neonatal jaundice	61(24.4%)	48(27.59%)	29(19.59%)	77(23.91%)				

neonatal respiratory distress	31(12.40%)	33(18.97%)	50(33.78%)	83(25.77%)
Preterm birth	49(19.6%)	34(19.54%)	18(12.16%)	52(16.15%)
Neonatal hypoglycemia	11(4.3%)	8(4.60%)	6(4.05%)	14(4.35%)
High-risk infants	9(3.6%)	3(1.72%)	5(3.39%)	8(2.48%)
Scalp hematoma	5(2%)	1(0.57%)	4(2.70%)	5(1.55%)
Vomiting	2(0.8%)	1(0.57%)	1(0.68%)	2(0.62%)
High hemoglobin or anemia	6(2.4%)	3(1.72%)	1(0.68%)	4(1.24%)
Meconium aspiration	1(0.4%)	0(0%)	1(0.68%)	1(0.31%)
Others	2(0.8%)	2(1.15%)	2(1.35%)	4(1.24%)

Notes:indicates P < 0.05 for the fresh embryo transfer group compared with the NC-FET group; indicates P < 0.05 for the fresh embryo transfer group compared with the HRT-FET group; indicates P < 0.05 for the NC-FET group compared with the HRT-FET group; * indicates the fresh embryo group compared with the frozen-thawed embryo transfer group

Table 3 Comparison of structural characteristics of the placenta and associated complications of three groups

	Fresh embryo transfer group N=1822	Frozen-thawed embryo transfer group N=2098 NC-FET group N=1353	Frozen-thawed embryo transfer group N=2098 HRT-FET group N=745	Frozen-thawed embryo transfer group N=2098 Total	χ^2/U value	P value	χ^2/U value*	P value
Placental structural abnormalities								
Velamentous placenta	55(3.0%)	45(3.3%)	27(3.6%)	72(3.43%)	0.67	0.716	0.53	0.466
Battledore placenta	93(5.1%)	80(5.9%)	43(5.8%)	123(5.86%)	1.10	0.578	1.08	0.299
others	24(1.3%)	13(1.0%)	10(1.3%)	23(1.10%)	0.99	0.609	0.40	0.526
placenta accreta	73(4.0%)	48(3.5%)	79(10.6%)	127(6.05%)	57.85	¡0.001	8.44	0.004
A history of intrauterine operation	32(1.7%)	37(2.7%)	47(6.3%)	84(4.00%)				

No uterine operations	41(2.3%)	11(0.8%)	32(4.3%)	43(2.05%)	27.97	¡0.001	0.19	0.665
Placenta increta	66(3.6%)	35(2.6%)	66(8.9%)ab	101(4.81%)	49.74	¡0.001	3.40	0.065
A history of intrauterine operation	38(2.1%)	23(1.7%)	47(6.2%)	70(3.34%)				
No uterine operations	28(1.5%)	12(0.9%)	20(2.7%)	32(1.53%)	10.30	0.006	0.00	0.977
Placenta previa	238(13.1%)	152(11.2%)	69(9.3%)	221(10.53%)	7.84	0.020	6.03	0.014
Premature rupture of membranes	394(21.6%)	329(24.3%)	159(21.3%)	488(23.26%)	3.93	0.140	1.50	0.221
At term	327	283	129	412				
Preterm	67	46	30	76				
Placental abruption	10(0.6%)	7(0.5%)	7(0.9%)	14(0.67%)	1.50	0.473	0.23	0.635

Notes:indicates P < 0.05 for the fresh embryo transfer group compared with the NC-FET group; indicates P < 0.05 for the fresh embryo transfer group compared with the HRT-FET group; indicates P < 0.05 for the NC-FET group compared with the HRT-FET group; * indicates the fresh embryo group compared with the frozen-thawed embryo transfer group

Table 4 Comparison of placental pathological characteristics of three groups

	Fresh embryo transfer group N=197	Frozen-thawed embryo transfer group N=324 NC-FET group N=175	Frozen-thawed embryo transfer group N=324 HRT-FET group N=149	Frozen-thawed embryo transfer group N=324 Total	χ^2/U value	P value	χ^2/U value*	P value
Chorioamnionitis	129(65.6%)	122(69.7%)	82(55.5%)	204(62.96%)	7.86	0.020	0.34	0.561
infarction	47(23.9%)	45(25.7%)	29(19.5%)	74(22.84%)	1.84	0.400	0.07	0.790
Calcification	55(27.9$)	57(32.6%)	46(30.9%)	103(31.79%)	0.98	0.613	0.87	0.351
Interstitial haemorrhage	11(5.6%)	7(4.0%)	2(1.3%)	9(2.78%)	4.15	0.125	2.61	0.106
Syncytiotrophoblastic nodule hyperplasia	4(2.0%)	5(2.9%)	4(2.7%)	9(2.78%)	0.29	0.865	0.06	0.810
Fibrin deposition	4(2.0%)	4(2.3%)	3(2.0%)	7(2.16%)	0.04	0.981	0.00	1.000
Chorionic villus infarction	1(0.5%)	5(2.9%)	6(4.0%)	11(3.40%)	5.03	0.081	Ref	0.036#

Notes:indicates P < 0.05 for the fresh embryo transfer group compared with the NC-FET group; indicates P < 0.05 for the fresh embryo transfer group compared with the HRT-FET group; indicates P < 0.05 for the NC-FET group compared with the HRT-FET group; * indicates the fresh embryo group compared with the frozen-thawed embryo transfer group;# indicates fisher's probability metho

S1 Comparison of basic characteristics of the patients received delivery

	Fresh embryo transfer group N=1822	Frozen-thawed embryo transfer group N=2098 NC-FET group N=1353	Frozen-thawed embryo transfer group N=2098 HRT-FET group N=745	Frozen-thawed embryo transfer group N=2098 Total	χ^2/U value	P value	χ^2/U value*	P value
Age(year)	34(32,37)	35(32,37)	34(32,37)	35(32,37)	4.14	0.126	-0.36	0.717
Prepregnancy BMI(kg/m2)	22.31(20.42,24.22)	23.29(20.31,25.53)	23.21(20.96,25.39)	23.62(20.55,25.70)	25.70	¡0.001	-2.10	0.036
the number of pregnancies					213.42	¡0.001	213.08	¡0.001
1	1166(64.0%)	607(44.9%)	344(46.2%)	951(45.33%)				
2	486(26.7%)	392(29.0%)	209(28.1%)	601(28.65%)				
[?]3	170(9.3%)	354(26.2%)	192(25.8%)	546(26.02%)				
Parity					50.67	¡0.001	49.92	¡0.001
1	1723(94.6%)	1187(87.7%)	662(88.9%)	1849(88.13%)				
[?]2	99(5.4%)	166(12.3%)	83(11.1%)	249(11.87%)				
PCOS	86(4.7%)	60(4.4%)	170(22.8%)	230(10.96%)	270.37	¡0.001	51.28	¡0.001
History of intrauterine operation	735(40.3%)	664(49.1%)	384(51.5%)	1048(49.95%)	37.51	¡0.001	36.33	¡0.001
Endometrial thickness(mm)	11(10,12)	10(9,11)	10(9,11)	10(9,11)	291.95	¡0.001	-16.15	¡0.001
D3 No. of embryos transferred	1735(95.22%)	538(39.76%)	299(40.13%)	837(39.90%)	1323.148	¡0.001	1323.118	0.001
D5 No. of blastocysts transferred	87(4.77%)	815(60.24%)	446(59.87%)	1261(60.10%)	Ref	Ref	Ref	Ref
Rate of ICSI	656(36.00%)	566(41.83%)	313(42.01%)	879(41.90%)	14.22	0.001	14.21	¡0.001

Notes:indicates P < 0.05 for the fresh embryo transfer group compared with the NC-FET group; indicates P < 0.05 for the fresh embryo transfer group compared with the HRT-FET group; indicates P < 0.05 for the NC-FET group compared with the HRT-FET group; * indicates the fresh embryo group compared with the frozen-thawed embryo transfer group