Original Article

Effect of different endometrial preparation methods on pregnancy outcome of FET in women with a normal menstrual cycle

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Abstract

Objective: This study aims to explore the relationship between different endometrial preparations and pregnancy outcomes among patients with regular ovulatory cycles in order to find the best endometrial preparation methods in the freeze–thaw embryo transfer (FET) cycle. **Materials and Methods:** This is a retrospective study to investigate FET pregnancy outcomes in women who had a regular menstrual cycle, were younger than 35 years old, and underwent a modified natural cycle (mNC), ovulation induction (OI), or a hormone replacement treatment (HRT) cycle. A total of 1071 frozen cycles were included for analysis. **Results:** The implantation rate and live birth rate (LBR) in the OI group show a significant difference when compared to the mNC and HRT groups ($P < 0.01$). After adjusting for confounding factors, the logistic regression analysis revealed that the number of embryos transferred, the embryo stage, and quality were significantly associated with clinical pregnancy rate and LBR. The LBR was additionally affected by the mode of the endometrial preparation; the OI cycle could increase LBR. **Conclusions:** Endometrial preparation methods affect the LBR in women with a regular menstrual cycle. The OI cycle had an advantage in the LBR of FET.

Keywords: Endometrial preparation, frozen–thawed embryo transfer, live birth rate, normal menstrual cycle

Introduction

Frozen–thawed embryo transfer (FET) is an essential technology in *in vitro* fertilization (IVF) or intra-cytoplasmic sperm injection (ICSI) nowadays,[1] which provides many clinical advantages. FET can freeze the excess embryos retrieved in IVF cycles to be stored and transfer them to the "physiologic environment" at a later date,[2] which increases the cumulative pregnancy rate.[3] It also helps to reduce the incidence of ovarian hyperstimulation (OHSS) and multiple pregnancies.[4] FET is easy to conduct, is economic, and can be completed in a shorter time than the repeated fresh cycles.[5]

Improving the pregnancy rate of FET is a fundamental problem in assisted reproductive technology (ART).[6] After balancing the differences in embryo quality, endometrial receptivity becomes a pre-condition for embryo implantation.[7] Appropriate endometrial preparation methods are critical factors that affect endometrial receptivity.[6]

Modified natural cycle (mNC), ovulation induction (OI), and hormone replacement treatment (HRT) cycle are widely used endometrial preparation methods in FET. However, there was no unified optimal endometrial preparation method in patients

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with regular menstrual cycles. To the best of our knowledge, some researchers[3,8-13] who evaluated the effects of natural and HRT cycling on pregnancy outcomes had no consistent conclusion. Most studies documented that the pregnancy outcome after FET was similar to that of the natural and HRT cycles. Levron *et al.*[14] revealed that natural cycling yielded a better clinical pregnancy rate (CPR) than HRT cycling, whereas others found the opposite conclusion.[15] Most studies focused on OI and HRT cycle in women with ovulatory dysfunction. Parts of the previous literature that studied the pregnancy outcome between natural, HRT, and OI cycles in FET do not limit the inclusion criteria.[7,16,17]

The study was designed as retrospective research to compare the pregnancy outcomes after FET cycles with endometrial preparation methods of mNC, OI, and HRT cycle in patients with normal menstrual cycle patients. The aim is to explore the relationship between different endometrial preparations and pregnancy outcomes among patients with regular ovulatory cycles in order to find the best endometrial preparation methods.

Materials and Methods

Study population

This retrospective analysis selected the patients with regular menstrual cycles who underwent FET in Lianyungang Maternal and Child Health Hospital between January 2012 and August 2022. The study was approved by the Lianyungang Maternal and Child Health Hospital Research Ethics Board. As a retrospective study, patients were not asked to participate actively. The inclusion criteria included (I) patients with a normal ovarian reserve function and menstrual cycle; (II) age ≤35 years old; (III) patients who choose IVF/ICSI because of infertility, which excluded the patients with diseases such as uterus, hydrosalpinx and intrauterine effusion without operation, ovulation disorders, and decreased ovarian reserve function; (IV) patients who had one or two transferred cycles; and (V) No smoking or quit smoking for 3 months. According to the doctor's preferences, the participants were allocated to different FET protocols. Each treatment has advancements, such as the mNC with less medication, the OI cycle with a reducing cycle cancellation rate, and the HRT cycle with easy scheduling. The HRT cycle includes HRT with or without gonadotropin-releasing hormone (GnRH) agonist pre-treatment. GnRH agonists have been mainly used in people with repeated implantation failure, and the overall data were relatively few in the center. Therefore, the study excluded the HRT cycle with GnRH agonists. All patients who performed FET have tested the serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E_2) on day 2 or 3 of the menstrual cycle. The primary sex hormones of these patients were within the normal range before starting FET. The thickness of the endometrium before transplantation needs to reach 7 mm or more, and the endometrial classification on the conversion day needs to be type A or B;[18] therefore, the study did not count the primary sex hormone values, endometrial thickness, and type.

Embryo culture, cryopreservation, and thawing

All patients were subjected to controlled ovarian hyperstimulation (COH), which was triggered with a GnRH antagonist or human chorionic gonadotropin (hCG). Oocyte retrieval was done 34–36 h after the trigger. Embryos were cultured at 37°C in an atmosphere of 5% CO_2, 5% O_2, and 90% N_2. According to the morphological standard of cleavage embryo,[19] the blastomere's size, number, uniformity, and fragment ratio were rated. The high-quality cleavage embryos include the embryo of grades I and II. The cleavage embryo's scoring criteria were as follows: Grade I: average cleavage speed, the number of cells consistent with the time, large blastomeres, uniform and transparent cytoplasm, no vacuoles, and no or less than 10% fragments. Grade II: the embryonic development speed is expected, the blastomere is uniform or roughly uniform, the number is equal or approximately equal, the cytoplasm is uniform, there are no vacuoles, and the fragments are 10–25%. The blastocyst score is according to the Gardner scoring standard.[20] A good-quality embryo was defined as ≥3BB (AA, AB, BA, and BB). We used vitrification for cryopreservation. Briefly, embryo vitrification is carried out using a kitazato open vitrification system and DMSO-EG as a cryoprotectant. When thawing, we use the kitazato thawing kit. Criteria for embryo resuscitation to be successful: At least half of the blastomeres of cleavage embryos survived, and the blastocyst cavity expanded after 2 hours of blastocyst resuscitation. The utilization rate of embryo resuscitation was about 90% in the center.

Endometrial preparation

In the OI cycle, letrozole (2.5 mg: LE, Jiangsu Hengrui Pharmaceutical Company, Jiangsu province, China) was administered for 5 days and started at day 3 to day 5 of the menstrual cycle; then human menopausal gonadotropin (75IU: hMG, Livzon Pharmaceutical Co, Guangzhou province, China) was injected every 2 days from day 5 to day 10 of the cycle. Medication was adjusted according to the body mass index (BMI). After 1 week of initiation, the transvaginal ultrasound (TVUS) monitored the follicle's diameter and endometrium. Medication was adjusted according to the follicle diameter and endometrial thickness. If necessary, add oral estradiol to increase the endometrial thickness. If the follicular diameter was >18 mm, 5000–10,000 IU of hCG (Livzon Pharmaceutical Co, Guangzhou province, China) was carried out. We adjusted the FET time following the ovulation time. We performed luteal phase support with 200 mg soft progesterone capsules (Besins Healthcare Benelux, Emiliano Revilla Sanz Avenida de, Spain) vaginal suppository twice a day. The cleavage embryo was performed on the third day of progesterone exposure, and the blastocyst was transferred on the fifth day. The progesterone supplementation was continued until the 14 days of transfer. If the pregnancy test was positive, the luteal phase support was continued.

The center adopts the mNC cycle because of the better control of ovulation time compared with the natural cycle. In the mNC group, TVUS monitored follicle development on the 11th day of

menstruation. The serum levels of LH, E_2, and progesterone (P) were measured when the follicle diameter reached 18 mm. If the endometrial thickness was less than 7 mm as the follicle diameter was more than 14 mm, oral estradiol (Estradiol valerate tablets, DELPHARM Lille S.A.S, France) was administered. If the follicle diameter was >18 mm and the peal value of E_2 was >200 pg/ml, hCG (5000–10,000 IU) was given. The timing of ET and the luteal phase supported was the same as the OI cycle.

The HRT cycle was administrated 4 mg oral estradiol for 3 days, starting from the 3rd day of the menstruation, and then increased to 6 mg one day after 3 days. TVUS monitored the endometrial thickness when the estradiol was administrated for 1 week. The transfer of endometrium would be started as the endometrial thickness reaches 7 mm, and the timing is about 10 to 14 days after taking estrogen. The luteal phase support included the soft progesterone capsules (200 mg) triple a day or progesterone injected daily (60 mg; Tianjin Jinyao Pharmaceutical Co., Lt, Tianjin, China). Furthermore, oral dydrogesterone (10 mg; Abbott Healthcare Products B.V. The Netherlands) was given three times a day. The timing of ET was the same as the mNC and OI cycle.

Outcome assessment and follow-up visit

The primary endpoint measure was the live birth rate (LBR). The secondary outcome included implantation rate (IR), positive hCG rate, CPR, ectopic pregnancy rate (EPR), miscarriage rate (MR), and multiple pregnancy rate (MPR). The intra-uterine gestational sac was seen by TVUS and is defined as clinical pregnancy during ET after 28–30 days, and ectopic pregnancy refers to the presence of a gestational sac outside the uterine cavity. Newborns who delivered after 28 weeks and survived

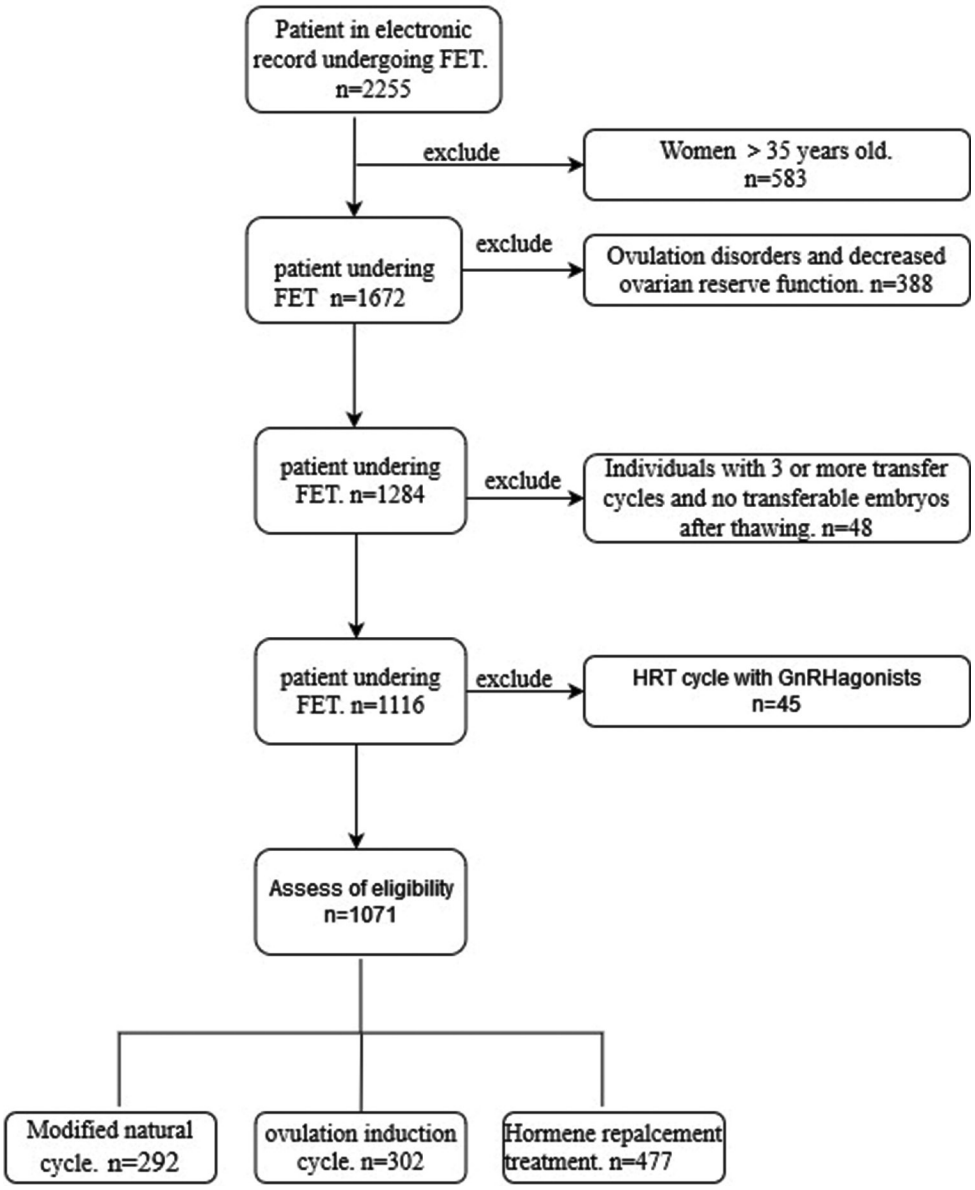

Figure 1: Flow chart of patient selection

were described as a live birth. Those who terminate the pregnancy after less than 28 weeks of pregnancy and less than 1000 g of fetal body weight were called miscarriages. IR is the ratio of intra-uterine gestational sacs seen under TVUS to the number of transferred embryos. Trained nurses obtained all pregnancy information through medical records or telephone follow-ups.

Statistical analysis

Data were transferred to Microsoft Excel for analysis. SPSS 22.0 software (IBM, NY, USA) performed the statistical analysis. Data were presented as mean ± atandard deviation (SD) if the data conform to the normality; otherwise, they were described as a median. Categorical variables were described as percentage numbers and compared with those of Pearson's Chi-square test or Fisher's exact test. Logistic regression analyses were used to evaluate the effect of potential confounders. *P*-value < 0.05 was considered a significant difference.

Ethics approval

The study was approved by the Lianyungang Maternal and Child Health Hospital Research Ethics Board (LYG-MER2021022). As the manuscripts do not include information or images that could lead to identification of a study participant, informed consent was not applicable under it. The need for written informed consent was waived by the Lianyungang Maternal and Child Health Hospital Research Ethics Board ethics committee due to the retrospective nature of the study.

Results

A total of 1071 FET patients fulfilled the inclusion criteria, and their data were analyzed [Figure 1].

The baseline characteristics of the participants are presented in Table 1. There were no differences in the couples of years, infertility duration and type, COH protocol, and fertilization method among the three groups. The patients in the OI group had a lower BMI compared with OI and HRT cycles ($P = 0.012$). The embryo stage includes cleavage embryos and blastocyst. The cleavage embryos in the OI cycles were less than those of the HRT and mNC cycles, and the blastocysts were more than the HRT and mNC groups ($P < 0.001$). It was defined as high-quality embryos as at least one Grade I or Grade II embryo in the transferred embryos; otherwise, it was specified as non-quality embryos. The high-quality embryo in the OI group was more than the mNC and HRT groups ($P = 0.013$). The number of embryos transferred in the OI group was less than the mNC and HRT groups ($P < 0.001$).

The pregnancy outcome among the different endometrial preparation methods:

Table 2 shows the pregnancy outcome among the different endometrial preparation methods. No differences were observed in positive hCG, CPR, and EPR among the three groups. The IR and LBR in the OI group were higher than those in the mNC and HRT groups ($P < 0.01$). The MCR in the mNC cycle had

Table 1: Comparison of baseline characteristics of the population

Variables	mNC (*n*=292)	OI (*n*=302)	HRT (*n*=477)	*P*
Women (y)	29.92±3.18	30.45±2.87	29.74±3.20	0.080
Men (y)	31.37±4.66	31.37±3.93	31.07±4.40	0.526
Infertility type, *n* (%)				
Primary	120 (41.11%)	131 (43.38%)	235 (49.27%)	0.062
secondary	172 (58.90%)	171 (56.62%)	242 (50.73%)	
Duration of infertility (y)	3.78±2.61	3.92±2.43	4.05±2.69	0.359
BMI (Kg/m^2)	22.77±3.21	22.78±3.27*	23.00±3.29	0.012
COH protocol, *n* (%)				
Stimulation	258 (88.36%)	276 (91.39%)	412 (86.37%)	0.079
Microstimulation	34 (11.64%)	26 (8.61%)	65 (13.36%)	
Infertility type, *n* (%)				
Pelvic factors	201 (68.84%)	209 (69.21%)	349 (73.17%)	
Men	44 (15.07%)	41 (13.58%)	55 (11.53%)	0.578
Unexplained	47 (16.10%)	52 (17.22%)	73 (15.30%)	
No. of embryos	1.65±0.58	1.34±0.49#	1.59±0.55	0.000
Fertilization method				
IVF	242 (82.88%)	258 (85.43%)	411 (86.16%)	0.453
ICSI	50 (17.12%)	44 (14.57%)	66 (13.84%)	
Embryos stage, *n* (%)				
Cleavage embryo	224 (82.88%)	121# (40.07%)	340 (71.28%)	
Blastocyst	68 (23.29%)	181# (59.93%)	137 (28.72%)	0.000
Embryo quality, *n* (%)				
High-quality embryo	194 (66.44%)	228* (75.50%)	323 (67.71%)	0.013
Non-quality embryo	48 (16.44%)	29* (9.60%)	87 (18.24%)	

, #: OI cycle group significantly different from mNC and HRT groups (: *P*<0.05. #: *P*<0.001). OI=Ovulation induction, mNC=Modified natural cycle. HRT=Hormone replacement treatment, Years, BMI=Body mass index, COH=Controlled ovarian hyperstimulation, No=number

a significant difference to the OI and HRT cycles. The MR was lower in the OI group than in the mNC and HRT groups, whereas the data had no significant difference.

The data were grouped according to the number of embryos transferred and embryo stage. The CPR and LBR had no significant difference among the three endometrial preparation methods in the same embryo stage. The mNC group had lower CPR and LBR than OI and HRT groups in the same number of embryos transferred (P<0.05), which is presented in Figure 2.

After adjusting for the age of women, BMI, the number of embryos transferred, embryo stage, and quality, the logistic regression analysis revealed that the number of embryos transferred, the embryo stage, and quality were significantly associated with CPR and LBR. The LBR was additionally affected by the mode of the endometrial preparation as already seen in the univariate analysis; the OI cycle could increase LBR compared to the mNC group, which is presented in Figure 3.

Discussion

FET has increased significantly because of the wildly acceptance and application of the single embryo transfer strategy.[21] Improving the pregnancy outcome of FET is closely related to the interests of many patients. Embryo implantation is associated with embryo quality and endometrial receptivity.[22] Endometrial preparation methods are a critical factor that affect endometrial receptivity in FET.[6] Our retrospective study was carried out in women with a regular menstrual cycle and ≤35 years old. The purpose of age restriction was to reduce the effect of age on embryo development potential. The analysis found that the endometrial preparation methods, the number of embryos transferred, the embryo stage, and quality

Table 2: Pregnancy outcomes among the different endometrial preparation methods

Pregnancy outcome	mNC	OI	HRT	*P*
Implantation rate(%)	32.62 (152/466)	42.43* (171/403)	31.54 (235/745)	0.003
Positive ß-hCG rate (%)	44.52 (130/292)	51.66 (156/302)	45.91 (219/477)	0.168
Clinical Pregnancy rate(%)	40.07 (117/292)	49.01 (148/302)	42.14 (201/477)	0.064
Ectopic pregnancy rate(%)	0.88 (1/114)	1.99 (3/151)	1.49 (3/201)	0.763
Miscarriage rate(%)	15.79 (18/114)	8.61 (13/151)	13.43 (27/201)	0.184
Live birth rate(%)	32.88 (96/292)	43.38* (131/302)	35.43 (169/477)	0.019
Multiple pregnancy rate(%)	28.95# (33/114)	15.89 (24/151)	16.91 (34/201)	0.014

*: OI cycle group significantly different from mNC and HRT groups, P<0.05. #: mNC cycle group significantly different from OI and HRT groups, P<0.05. OI=Ovulation induction, mNC=Modified natural cycle, HRT=Hormone replacement treatment

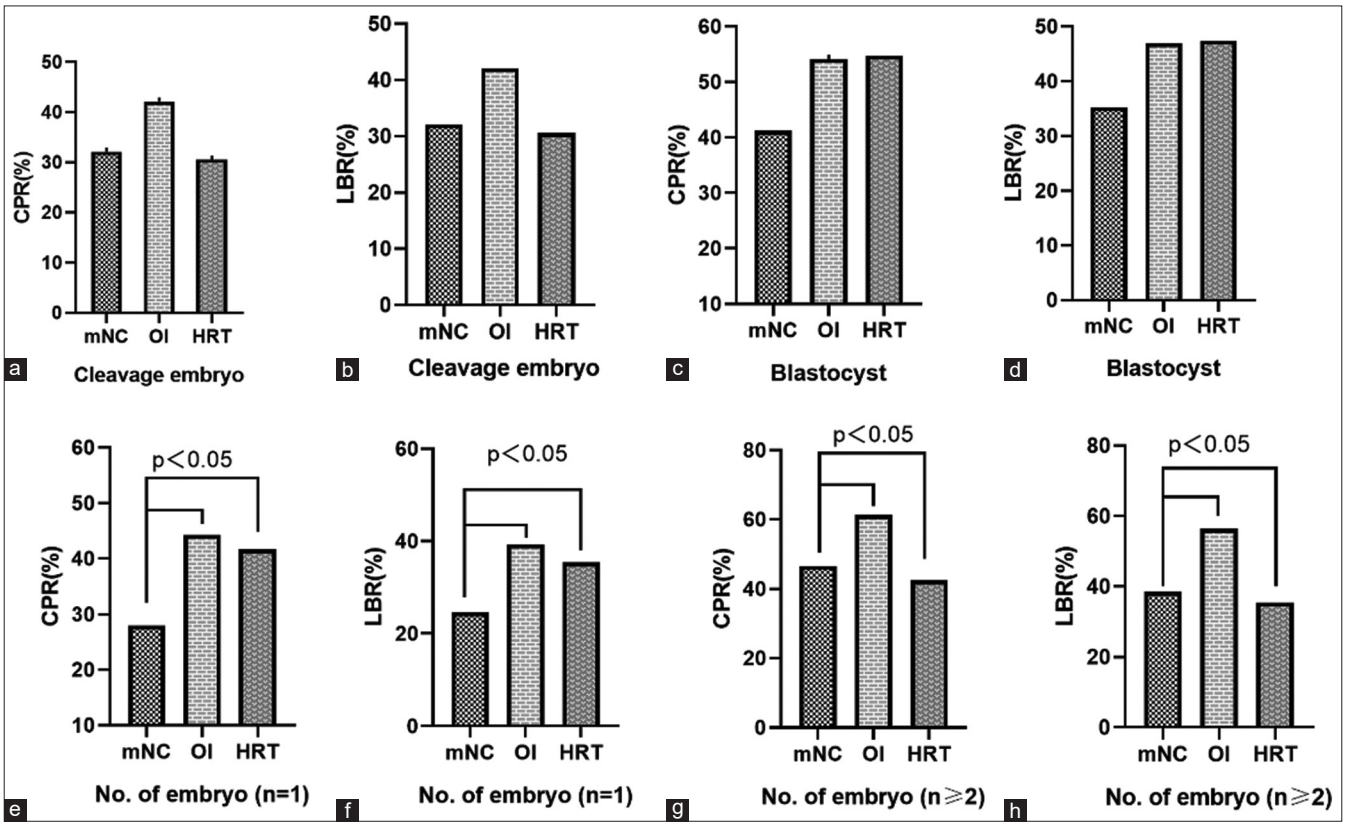

Figure 2: Analyzing the pregnancy outcomes of different endometrial preparation schemes under the same embryo stage and the number of embryo transfered. (a-d) No significant difference in the same embryo stage. (e-h) mNC cycle group significantly different from OI and HRT groups in the same number of embryos transferred, P<0.05. OI=Ovulation induction, mNC=Modified natural cycle. HRT=Hormone replacement treatment

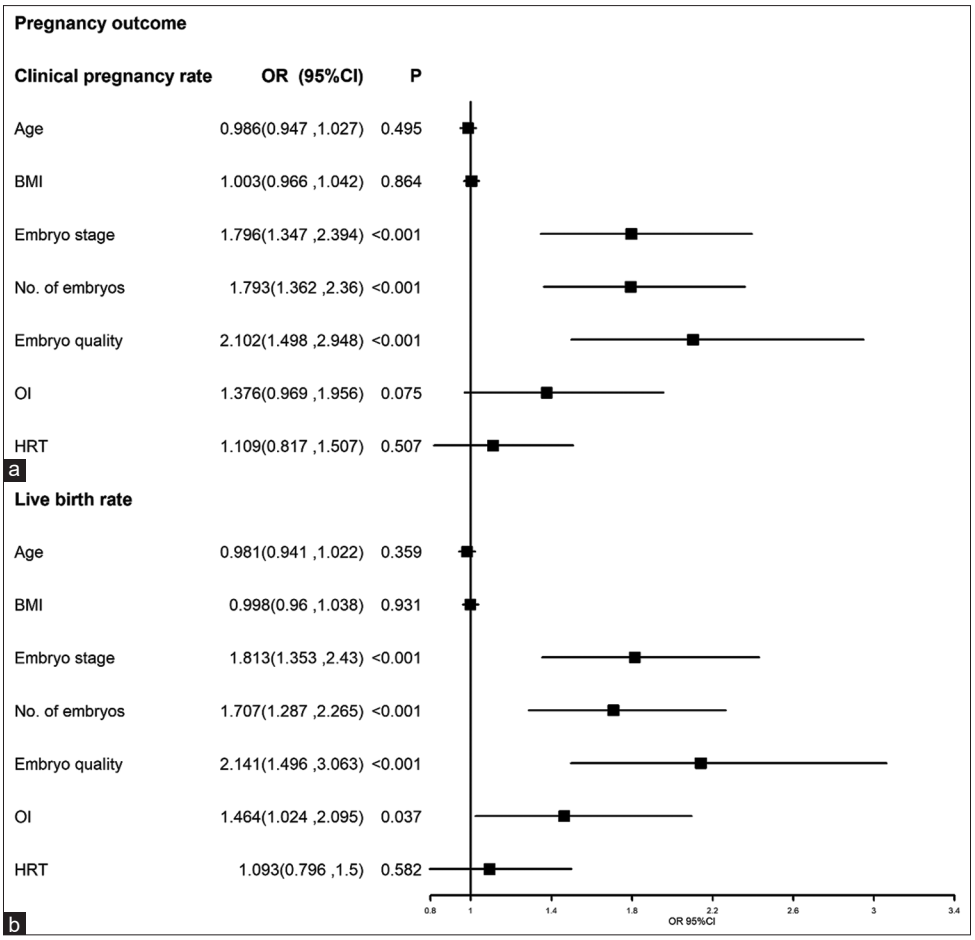

Figure 3: (a) Forest plot of the logistic regression analysis: Clinical pregnancy rate as outcome. (b) Forest plot of the logistic regression analysis: Live birth rate as outcome. OI: Ovulation induction; HRT: Hormone replacement treatment

affect the pregnancy outcome in FET among these people with a normal menstrual cycle. After adjusting for confounding factors, the impact of endometrial preparation methods still existed on LBR.

Comparison of pregnancy outcomes between OI and mNC cycles

There was no unified conclusion on whether the OI cycle positively affected endometrial receptivity. Some studies suggested that the IR and CPR had no significant differences among NC, HRT, and OI cycles.[3,17] On the other hand, other studies[13,23] found that the IR, CRP, and LBR were higher in the OI cycle with low-dose hMG than in the NC group in regular ovulation patients. Peeraer *et al.*[24] also concluded that the LBR was higher in the OI cycle than in the NC group, although there was no statistical difference. The study showed that the IR and LBR were higher in the OI cycle than in the mNC and HRT groups ($P < 0.05$). The logistic regression analysis revealed that the OI cycle also had an effect on the LBR. This study used LE combined with hMG in the OI cycle. There were two main reasons why the OI cycle could increase the pregnant rate; on the one hand, LE could reduce the E_2 level, upregulate the E_2 receptor, and then increase the sensitivity to the E_2.[25] This process increases endometrial blood flow and proliferation, positively impacting pregnancy outcomes.[26] The OI cycle under LE increased the expression of endometrial receptivity factors, including integrin and leukemia inhibitory factors.[27] Integrin is involved in the primary attachment between embryo and endometrium. The decreased integrin expression in the luteal phase affects embryo implantation.[28] On the other hand, hMG can improve luteal function and endometrial receptivity before embryo implantation.[24]

The pregnancy outcome of OI and HRT cycle

Hosseini-Najarkolaei *et al.*[21] revealed that the OI and HRT cycles had similar pregnancy outcomes in patients with polycystic ovary syndrome (PCOS). Zhang *et al.*[29] reported that letrozole used during OI cycles significantly improved LBR than the HRT cycle. The study revealed that the LBR in the OI cycle was higher than in the HRT cycle, and the MR was lower than the HRT group. However, there was no statistical difference. The HRT cycle could arrange the transplantation time more flexibly and controllably, reduces patients' visits, and decreases the cancellation rate.[4] In contrast, the high-dose drug increases the economic burden on patients. The HRT cycle increases the risk of luteal dysfunction. The demand for luteal phase support drugs was higher than the mNC and OI cycle, which increases the risk of thrombosis and cancer.[30]

Comparison of pregnancy outcomes between HRT cycle and mNC

There were many studies on the pregnancy outcomes of NC and HRT cycles in FET. Previous studies[10,31,32] believed that the pregnancy outcome was similar between NC and HRT cycles. Givens CR *et al.*[15] thought that the pregnancy rate in the HRT cycle was higher than in the NC group. Our results showed that the CPR and LBR in the mNC cycle were lower than those in the OI and HRT groups, and the MR was higher than that in the other groups, which may be related to the decrease of the endometrial receptivity in the mNC cycle. The mNC group could reduce the application of medicine and aligns with the physiological state.[14] However, patients need to repeatedly seek medical attention to monitor follicular development and hormone levels. When the follicle matures and E_2 does not meet the standard, the ET plan is canceled.[33,34]

To sum up, the OI cycle had advantages in the LBR of FET.

Limitations

When interpreting our results, both advantages and limitations need to be considered. The main limitation was that the study was a retrospective design with an extended study period.

Recommendation for further studies

A multi-center prospective study should be conducted, and the mechanisms that affect pregnancy from the perspective of basic research should be further explored.

Conclusion and global health implications

Endometrial preparation methods affect the LBR in women with a regular menstrual cycle. The OI cycle had advantages in the LBR of FET. Clinicians at all levels should refocus on the impact of endometrial preparation on pregnancy rate during the treatment of FET. Attempting to improve the pregnancy rate of FET with endometrial preparation of OI cycle in patients with normal ovulation.

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Conflicts of interest

There are no conflicts of interest.

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