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Endometrial preparation methods prior to frozen embryo transfer: a retrospective cohort study comparing true natural cycle, ovulation induction, hormone replacement treatment and GnRHa pretreatment

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Abstract

Purpose To compare pregnancy outcomes of four endometrial preparation methods prior to frozen embryo transfer (FET).

Methods A total of 3,030 programmed cycles were analyzed: 302 with natural cycle (NC), 131 with ovulation induction (OI), 1,078 with hormone replacement treatment (HRT), and 1,519 with GnRHa pretreatment (GnRHa + HRT). Primary outcomes investigated were positive human chorionic gonadotropin (hCG), chemical pregnancy, clinical pregnancy, abortion, and live birth. Additionally, the impact of age, body mass index (BMI), embryo number, high-quality embryo, and endometrial thickness on pregnancy outcomes were analyzed.

Results The positive hCG rates for NC, OI, HRT, and GnRHa + HRT groups were 63.4%, 62.6%, 68.3%, and 71.7%, respectively ($P = 0.004$). Clinical pregnancy rates were 50.4%, 54%, 57.5%, and 61.8%, respectively ($P = 0.004$). Live birth rates were 38.2%, 45%, 46.5%, and 50.9%, respectively ($P = 0.007$). No significant differences were found in abortion and chemical pregnancy rates among the four protocols. NC showed significantly higher positive hCG ($p = 0.044$), live birth ($p = 0.005$), and clinical pregnancy rates ($p = 0.010$) compared to other methods. Compared to HRT, GnRHa + HRT displayed significantly higher live birth ($p = 0.027$) and clinical pregnancy rates ($p = 0.027$). Multiple logistic regression showed that the number of embryos and high-quality embryos increased HCG positivity, clinical pregnancy, and live birth rates, while age reduced these rates. BMI increased the abortion rate, and endometrial thickness increased the live birth rate. Chemical pregnancy was unaffected by these factors.

Conclusion NC offers improved outcomes compared to other methods. Additionally, specific factors such as embryo quality and embryo number significantly influence pregnancy outcomes.

Clinical trial number Not applicable.

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Keywords Frozen embryo transfer, Endometrial preparation, Pregnancy outcomes

Introduction

The frozen embryo transfer (FET) technique has evolved rapidly since its first successful application in 1983 [1]. This method addresses issues such as ovarian hyperstimulation syndrome in fresh cycles and delayed embryo transfer due to inadequate endometrial preparation [2]. With advancements in cryopreservation techniques and the development of effective ovarian stimulation protocols, various FET protocols have been established. These protocols allow for different endometrial preparation methods tailored to the patient's specific case, ensuring optimal endometrial receptivity and hormone levels before transfer. Consequently, this approach reduces the impact of steroid hormones on embryos during ovarian stimulation and egg retrieval compared to fresh embryo transfers [2], significantly enhancing the success rate of in vitro fertilization (IVF).

To maximize the success of assisted reproductive technology, selecting the appropriate endometrial preparation regimen during an FET cycle is crucial [3]. There are four common endometrial preparation regimens: ovulation-inducing cycles, natural cycles (NCs), hormone replacement therapy, and GnRH agonist (GnRHa) pretreatment therapy. NCs are suitable for women with normal menstrual cycles or ovulation but present clinical limitations, including frequent clinic visits, less flexibility, and a higher risk of cycle cancellation [4]. Ovulation assisted cycles are applicable to a broader population but share the same limitations as NCs.

Artificial cycles, however, can be used by women with regular or irregular menstrual cycles and normal or abnormal ovulation. These cycles offer greater flexibility, fewer check-ups, and a low cycle cancellation rate, making them widely used in clinical practice [5]. Nonetheless, studies have indicated an increased risk of pre-eclampsia and postpartum hemorrhage with HRT regimens compared to NCs [6–8]. Despite this, no significant difference in pregnancy outcomes has been observed between HRT and NC regimens [4, 9].

GnRHa pretreatment therapy suppresses ovarian hormone production, preventing natural ovulation and reducing cycle cancellation. However, the effectiveness of combining HRT with GnRHa pretreatment on reproductive outcomes remains unclear. GnRHa + HRT has been shown improved pregnancy outcomes in women with endometriosis and adenomyosis [10, 11], but it is ineffective for women with polycystic ovary syndrome and increases treatment costs [12]. GnRHa pretreatment enhances endometrial avβ3 integrin expression, improving endometrial receptivity. Additionally, the GnRHa + HRT regimen reduces the expression of the pro-inflammatory factors IL-6 and IL-11 during transplantation, potentially improving birth rates over pregnancy rates [13]. For the general population, excluding those with adenomyosis and endometriosis, GnRHa pretreatment improves pregnancy outcomes. As such, the effect of different endometrial preparation regimens on pregnancy outcomes is of great interest to clinicians.

In FET, pregnancy outcomes are influenced by embryo treatment, endometrial receptivity, synchronization of endometrial growth, and embryo development, in addition to the endometrial preparation regimen. Factors such as patient age, body mass index (BMI), embryo quality, and endometrial condition also impact pregnancy outcomes. This study aims to provide clinicians with evidence for selecting FET protocols by comparing pregnancy outcomes of four different endometrial preparation regimens at our center and conducting a multifactorial analysis of how individual characteristics may affect pregnancy outcomes.

Materials and methods

Study and patients

This retrospective analysis included FET cycles from the Reproductive Hospital of Jiangxi University of Traditional Chinese Medicine, covering data from January 2020 to December 2023. The study comprised 3,030 thaw cycles (NC-FET = 302, Ovulation Induction = 131, GnRHa + HRT = 1,519, and HRT = 1,078), all performed at a single site utilizing the same laboratory. The primary outcomes of interest were biochemical pregnancy (beta-human chorionic gonadotropin (beta-hCG) > 5 IU), ultrasound-diagnosed clinical pregnancy (gestational sac seen on ultrasound), miscarriage rate, and live birth. Patient characteristics were prospectively recorded in the clinic database and extracted for analysis.

Inclusion criteria were: (1) patients under 35 years of age, and BMI less than 24. (2) patients with vitrified embryos derived from IVF/ICSI cycles. Exclusion criteria included: (1) cycles with preimplantation genetic testing (PGT), (2) patients with chronic hypertension or diabetes mellitus before the index pregnancy, and (3) patients with congenital or secondary uterine abnormalities (e.g., unicornuate uterus, didelphys uterine, septate uterus, adenomyosis, endometrial polyps, uterine fibroids, or intrauterine adhesions).

Ovarian stimulation and IVF/ICSI

All patients received control ovulation stimulate (COS) treatment and monitoring. COS protocols, laboratory procedures, and luteal phase support were fully described in publications. When at least three follicles reached

a diameter ≥ 18 mm, human chorionic gonadotrophin (hCG) was administered to induce follicular maturation. Oocytes were retrieved under transvaginal ultrasound (TVS) guidance 36 h later. Hyaluronidase was used to remove the granulosa cells after incubating for 3–4 h, followed by IVF/ICSI. Embryos were cultured to D3, D5 or D6 and then vitrified.

Endometrial preparation and FET

Women were assigned to different endometrial preparation groups based on their preference, schedule, or the habitual practice of their physicians.

NC-FET

Patients in the NC-FET group underwent transvaginal ultrasound on approximately day 10–12 of their menstrual cycle to determine the size of the dominant follicle. Once the dominant follicle reached approximately 16 mm, patients were required to visit the hospital daily for TVS testing. Serum hormone levels of luteinizing hormone (LH), progesterone, and estrogen were assessed when the dominant follicle reached a mean diameter of > 18 mm, after monitored daily until follicle rupture. The embryo was transferred on day 3 or day 5 after the follicle discharged. A maximum of two embryos were thawed on the day of FET.

Ovulation induction

In the ovulation stimulation cycle, preparation began with oral letrozole on days 3–5 of menstruation for 5 days. On the 10th day of menstruation, hMG (75–150 IU) was administered based on the follicular response. When the dominant follicle reached a diameter of 18 mm, hCG (2000–8000 IU, Lizhu, Guangdong, China) was injected to trigger final oocyte maturation. Ovulation was confirmed by TVS. The embryo was transferred on day 3 or day 5 after the follicle discharged. A maximum of two embryos were thawed on the day of FET.

HRT

In the HRT group, women underwent serum sex hormone level tests for LH, follicle stimulation hormone (FSH), progesterone, and estrogen and TVS on day two of the menstrual cycle. Approximately 12 days after beginning estradiol treatment (6 mg/d, Baier, Beijing, China), patients were scanned by TVS to assess endometrial thickness. If the endometrium did not reach a satisfactory thickness, the estradiol dosage was adjusted or supplemented. Once the endometrial thickness reached 8 mm, dydrogesterone tablets (80 mg/d, Xiangju, Zhejiang, China) were added for endometrial transformation. The embryo was transferred on day 3 or day 5. Progesterone support was continued until 10 weeks of gestation.

GnRHa + HRT

In the GnRHa + HRT group, routine vaginal ultrasonography and basal sex hormone tests were performed on the 2nd day of the menstrual cycle. Long-acting GnRHa (3.75 mg, Boente, Beijing, China) was intramuscularly injected. Vaginal ultrasound and sex hormone levels were reexamined 30 days later, followed by the start of estradiol treatment. Approximately 12 days after beginning estradiol treatment, patients were scanned by TVS to determine endometrial the thickness of the endometrium. If the endometrium did not reach a satisfactory thickness, the estradiol dosage was adjusted or supplemented. Once the endometrial thickness reached 8 mm, dydrogesterone tablets were added for endometrial transformation. The embryo was transferred on day 3 or day 5. Progesterone support was continued until 10 weeks of gestation.

Embryo grading

Cleavage-stage embryos were classified as high-quality embryos if they had seven to nine cells on Day 3, fewer than 20% anucleate fragments, equal-sized blastomeres in the major of cells, and no multinucleation according to the ASEBIR embryo assessment criteria, with minor modifications [14]. The morphological evaluation of blastocysts was performed according to the Gardner and Schoolcraft grading system [15].The blastocysts were graded according to the following three morphological parameters: inner cell mass (ICM), trophectoderm, and the degree of expansion. At our center, blastocysts of grade ≥ 4BB were defined as high-quality blastocysts on days 5 or 6.

Statistical analysis

Data are presented as mean ± standard deviation for continuous variables or as median and interquartile range. Counts and proportions were used for categorical variables (%). Comparisons between groups were performed using the Pearson X^2 test. Categorical variables were subjected to univariate analysis, and variables with $P < 0.05$ were included in a logistic regression analysis for multifactorial conditional analysis. An OR value > 1 indicated a positive effect on pregnancy outcome, while an OR value < 1 indicated a negative effect. A difference of $P < 0.05$ was considered statistically significant.

Results

Population characteristics

A total of 3,030 FET cycles were analyzed. Of these, 302 patients underwent the ovulation induction protocol, 131 patients the natural protocol, 1,219 patients the GnRHa + HRT protocol, and 1,078 patients underwent the HRT protocol. Population characteristics of the included patients are shown in Table 1. High-quality

Table 1 Demographical characteristics by treatment

Variables		OI	NC	GnRHa + HRT	HRT	*P*
Embryos Number	1	118 (39.1%)	55 (42.0%)	627 (41.3%)	393 (36.5%)	NS
	2	184 (60.9%)	76 (58.0%)	892 (58.7%)	685 (63.5%)	
High Quality Embryo	No	8 (2.6%)	6 (4.6%)	47 (3.1%)	58 (5.4%)	0.016
	Yes	294 (97.4%)	125 (95.4%)	1472 (96.9%)	1020 (94.6%)	
Endometrial Morphology	A	70 (23.2%)	40 (30.5%)	271 (17.8%)	195 (18.1%)	0.002*
	B	44 (14.6%)	18 (13.7%)	205 (13.5%)	128 (11.9%)	
	C	188 (62.3%)	73 (55.7%)	1043 (68.7%)	755 (70.0%)	
BMI		21.71 (20.03 ~ 23.93)	21.09 (19.72 ~ 23.00)	21.64 (19.77 ~ 24.03)	21.48 (19.53 ~ 23.73)	NS
Progesterone Day (E2)		246.50 (166.00 ~ 352.00)	210.00 (149.00 ~ 329.00)	257.00 (173.00 ~ 396.00)	279.00 (188.00 ~ 456.00)	< 0.001*
Progesterone Day (P)		0.75 (0.43 ~ 1.25)	0.79 (0.50 ~ 1.42)	0.29 (0.17 ~ 0.52)	0.35 (0.19 ~ 0.60)	< 0.001*
Age (years)		31.00 (28.00 ~ 34.00)	32.00 (29.00 ~ 35.00)	31.00 (28.00 ~ 34.00)	30.00 (27.00 ~ 33.00)	< 0.001*
Endometrial Thickness (mm)		9.00 (8.00 ~ 11.00)	10.00 (8.00 ~ 11.00)	10.00 (9.00 ~ 11.00)	9.00 (8.00 ~ 11.00)	< 0.001*

For normally distributed continuous variables, data are expressed as mean ± SD, otherwise data are expressed as median interquartile range. OI ovulation induction, NC natural cycle, GnRHa+HRT GnRHa pretreatment, HRT hormone replacement

Treatment. BMI body mass index. NS, not statistically significant

Table 2 Comparison of outcomes by treatment group

Pregnancy outcomes	Variables	OI	NC	GnRHa+ HRT	HRT	*P*
HCG Positive	No	113 (37.4%)	48(36.6)	430 (28.3%)	342 (31.7%)	0.004*
	Yes	189 (62.6%)	83(63.4)	1089 (71.7%)	736 (68.3%)	
Chemical Pregnancy	No	276 (91.4%)	114(87%)	1369 (90.1%)	962 (89.2%)	0.478
	Yes	26 (8.6%)	17(13.0%)	150 (9.9%)	116 (10.8%)	
Clinical Pregnancy	No	139 (46.0%)	65(49.6%)	580 (38.2%)	458 (42.5%)	0.004*
	Yes	163 (54.0%)	66(50.4%)	939 (61.8%)	620 (57.5%)	
Abortion	No	276 (91.4%)	116(88.5%)	1363 (89.7%)	962 (89.2%)	0.714
	Yes	26 (8.6%)	15 (11.5%)	156 (10.3%)	116 (10.8%)	
Live Birth	No	166 (55.0%)	81 (61.8%)	746 (49.1%)	577 (53.5%)	0.007*
	Yes	136 (45.0%)	50 (38.2%)	773 (50.9%)	501 (46.5%)	

OI ovulation induction, NC natural cycle, GnRHa+HRT GnRHa pretreatment, HRT hormone replacement Treatment. (*$P<0.05$)

embryos, endometrial morphology, estrogen day (E2), progesterone day (P), age, and endometrial thickness were significantly different among the four protocols. There were no significant differences in the number of embryos and BMI among the four protocols.

Outcomes

The outcome of hCG positivity, chemical pregnancy, clinical pregnancy, abortion, and live birth after different endometrial preparation protocols are shown in Table 2. The positive hCG rates were the highest in GnRHa + HRT as 71.7%, followed by HRT (68.3%), NC (63.4%) and Ovulation Induction (62.6%). The differences of positive hCG rate were statistically significantly ($P = 0.004$). The clinical pregnancy rates were also highest in GnRHa + HRT as 61.8%, late HRT (57.5%), Ovulation Induction (54%) and NC (50.4%). The differences of clinical pregnancy rate were statistically significantly ($p = 0.004$). Most of the Live Brith rate were GnRHa + HRT(50.9%), then HRT(46.5%), Ovulation Induction (45%) and NC (38.2%), the rate were statistically significant ($p = 0.007$). There were no significant differences in the abortion rate ($p = 0.714$), the rates were NC (11.5%), HRT (10.8%), GnRHa + HRT(10.3%), and Ovulation Induction (8.6%). In the chemical pregnancy rate, there were also no significant differences ($p = 0.478$), the rates were NC (13%), HRT (10.8%), GnRHa + HRT(9.9%), and Ovulation Induction (8.6%).

Comparative analysis

Table 3 displays the outcome measures following FET in the Ovulation Induction, NC, GnRHa + HRT, and HRT groups. There were no significant differences in pregnancy outcomes when comparing ovulation induction with NC and HRT. Compared to GnRHa + HRT, ovulation induction displayed significantly higher hCG positivity ($p = 0.002$), clinical pregnancy rates ($p = 0.011$), and NC displayed significantly higher hCG positivity ($p = 0.044$), live birth ($p = 0.005$), clinical pregnancy rates ($p = 0.010$). Compared to HRT, GnRHa + HRT showed significantly higher live birth ($p = 0.027$) and clinical pregnancy rates ($p = 0.027$).

Table 3 Treatment two by two comparison for outcomes

Pregnancy outcomes	OI vs. NC	OI vs. GnRHa + HRT	OI vs. HRT	NC vs. GnRHa + HRT	NC vs. HRT	GnRHa + HRT vs. HRT
HCG	0.878	0.002*	0.063	0.044*	0.256	0.060
Clinical pregnancy	0.492	0.011*	0.272	0.010*	0.120	0.027*
Chemical Pregnancy	0.163	0.497	0.270	0.259	0.444	0.463
Pregnancy clinical	0.492	0.011*	0.272	0.010*	0.120	0.027*
abortion	0.354	0.380	0.277	0.671	0.810	0.687
Birth	0.185	0.063	0.657	0.005*	0.071	0.027*

OI ovulation induction, NC natural cycle, GnRHa+HRT GnRHa pretreatment, HRT hormone replacement Treatment. (*P<0.05)

Table 4 Multiple logistic regression of HCG by treatment group

factors	OR (95% CI)	*P*
BMI	1.01 (0.99, 1.04)	0.309
Progesterone Day (E2)	1.00 (1.00, 1.00)	0.091
Progesterone Day (P)	0.93 (0.84, 1.02)	0.140
Age (Years)	0.97 (0.96, 0.99)	0.007*
Embryos Number	1.37 (1.17, 1.61)	< 0.001*
High Quality Embryo (Yes vs. No)	1.62 (1.10, 2.37)	0.014*
Treatment (GnRHa + HRT vs. NC)	1.28 (0.86, 1.90)	0.220
Treatment (HRT vs. NC)	1.09 (0.73, 1.62)	0.678
Treatment (Ovulation Induction vs. NC)	0.89 (0.58, 1.38)	0.613
Endometrial Morphology (A vs. C)	0.84 (0.69, 1.03)	0.099
Endometrial Morphology (B vs. C)	0.87 (0.69, 1.10)	0.252
Endometrial Thickness (mm)	1.03 (0.99, 1.08)	0.117

OI ovulation induction, NC natural cycle, GnRHa+HRT GnRHa pretreatment, HRT hormone replacement Treatment. BMI body mass index. (*P<0.05)

Table 5 Multiple logistic regression of chemical pregnancy by treatment group

Factors	OR (95% CI)	*P*
BMI	1.00 (0.97, 1.04)	0.934
Progesterone Day (E2)	1.00 (1.00, 1.00)	0.504
Progesterone Day (P)	0.97 (0.81, 1.15)	0.691
Age (Years)	1.00 (0.97, 1.03)	0.826
Embryos Number	0.91 (0.71, 1.16)	0.441
High Quality Embryo (Yes vs. No)	0.69 (0.40, 1.19)	0.185
Treatment (GnRHa + HRT vs. NC)	0.69 (0.40, 1.21)	0.200
Treatment (HRT vs. NC)	0.77 (0.44, 1.35)	0.361
Treatment (Ovulation Induction vs. NC)	0.62 (0.32, 1.18)	0.145
Endometrial Morphology (A vs. C)	1.02 (0.74, 1.38)	0.923
Endometrial Morphology (B vs. C)	1.11 (0.78, 1.58)	0.555
Endometrial Thickness (mm)	1.02 (0.96, 1.08)	0.484

OI ovulation induction, NC natural cycle, GnRHa+HRT GnRHa pretreatment, HRT hormone replacement Treatment. BMI body mass index

Multivariate analysis

Multiple logistic regression was performed to determine the association between positive hCG and population characteristics. After adjusting for confounding variables, we found that the number of embryos (OR = 1.37; 95% CI, 1.17–1.61, $p < 0.001$) and high-quality embryos (OR = 1.62; 95% CI, 1.10–2.37, $p < 0.014$) increased the positive hCG rate, while age reduced it (OR = 0.97; 95% CI, 0.96–0.99, $p < 0.007$). Other population characteristics showed no significant differences in positive hCG rates (Table 4). Additionally, none of these factors affected the chemical pregnancy rate (Table 5).

Table 6 Multiple logistic regression of clinical pregnancy by Treatment Group

Factors	OR (95% CI)	*P*
BMI	1.01 (0.99, 1.03)	0.370
Progesterone Day (E2)	1.00 (1.00, 1.00)	0.237
Progesterone Day (P)	0.94 (0.85, 1.04)	0.231
Age (Years)	0.98 (0.96, 1.00)	0.015*
Embryos Number	1.37 (1.18, 1.60)	< 0.001*
High Quality Embryo (Yes vs. No)	1.82 (1.25, 2.66)	0.002*
Treatment (GnRHa + HRT vs. NC)	1.45 (0.99, 2.11)	0.055
Treatment (HRT vs. NC)	1.21 (0.82, 1.77)	0.332
Treatment (Ovulation Induction vs. NC)	1.09 (0.72, 1.66)	0.679
Endometrial Morphology (A vs. C)	0.86 (0.71, 1.03)	0.108
Endometrial Morphology (B vs. C)	0.85 (0.68, 1.06)	0.148
Endometrial Thickness (mm)	1.02 (0.98, 1.06)	0.296

OI ovulation induction, NC natural cycle, GnRHa+HRT GnRHa pretreatment, HRT hormone replacement Treatment. BMI body mass index. (*P<0.05)

After adjusting for confounding variables, we found that several factors influenced clinical pregnancy (Table 6). The number of embryos (OR = 1.37; 95% CI, 1.17–1.61, $p < 0.001$) and high-quality embryos (OR = 1.82; 95% CI, 1.25–2.66, $p = 0.002$) increased clinical pregnancy rates, while age reduced it (OR = 0.98; 95% CI, 0.96-1, $p = 0.015$). BMI (OR = 1.06; 95% CI, 1.02–1.09, $p = 0.002$) was associated with an increased abortion rate, while age reduced clinical pregnancy rates (OR = 0.98; 95% CI, 0.96-1, $p = 0.015$). (Table 7).

Finally, the number of embryos (OR = 1.33; 95% CI, 1.15–1.55, $p < 0.001$), high-quality embryos (OR = 1.70; 95% CI, 1.15–2.52, $p = 0.008$), and endometrial thickness (OR = 1.04; 95% CI, 1-1.08, $p = 0.028$) increased live birth rates. In contrast, age (OR = 0.97; 95% CI, 0.95–0.99, $p < 0.001$) and endometrial morphology type B (OR = 0.80; 95% CI, 0.64–0.99, $p = 0.043$) reduced live birth rates. Moreover, live birth rates were significantly higher in the GnRHa + HRT group compared to other groups (OR = 1.56; 95% CI, 1.06–2.30, $p = 0.024$). (Table 8).

Discussion

The choice of FET transfer protocols has been extensively researched by reproductive physicians. This study retrospectively analyzed 3,030 cycles in a general population

Table 7 Multiple logistic regression of abortion by treatment group

Variable	OR (95% CI)	*P*
BMI	1.06 (1.02, 1.09)	0.002*
Progesterone Day (E2)	1.00 (1.00, 1.00)	0.391
Progesterone Day (P)	0.97 (0.81, 1.16)	0.714
Age (Years)	1.04 (1.01, 1.07)	0.017*
Embryos Number	1.04 (0.82, 1.33)	0.733
High Quality Embryo (Yes vs. No)	1.20 (0.63, 2.27)	0.579
Treatment (GnRHa + HRT vs. NC)	0.83 (0.46, 1.49)	0.527
Treatment (HRT vs. NC)	0.87 (0.48, 1.58)	0.655
Treatment (Ovulation Induction vs. NC)	0.68 (0.35, 1.35)	0.271
Endometrial Morphology (A vs. C)	0.77 (0.55, 1.07)	0.119
Endometrial Morphology (B vs. C)	1.20 (0.86, 1.68)	0.288
Endometrial Thickness (mm)	0.95 (0.89, 1.01)	0.072

OI ovulation induction, NC natural cycle, GnRHa+HRT GnRHa pretreatment, HRT hormone replacement Treatment. BMI body mass index. (*$P<0.05$)

Table 8 Multiple logistic regression of live birth by treatment group

Variable	OR (95% CI)	*P*
BMI	0.99 (0.97, 1.01)	0.338
Progesterone Day (E2)	1.00 (1.00, 1.00)	0.142
Progesterone Day (P)	0.95 (0.86, 1.05)	0.326
Age (Years)	0.97 (0.95, 0.99)	< 0.001*
Embryos Number	1.33 (1.15, 1.55)	< 0.001*
High Quality Embryo (Yes vs. No)	1.70 (1.15, 2.52)	0.008*
Treatment (GnRHa + HRT vs. NC)	1.56 (1.06, 2.30)	0.024*
Treatment (HRT vs. NC)	1.31 (0.88, 1.94)	0.178
Treatment (Ovulation Induction vs. NC)	1.29 (0.84, 1.98)	0.243
Endometrial Morphology (A vs. C)	0.91 (0.75, 1.10)	0.326
Endometrial Morphology (B vs. C)	0.80 (0.64, 0.99)	0.043*
Endometrial Thickness (mm)	1.04 (1.00, 1.08)	0.028*

OI ovulation induction, NC natural cycle, GnRHa+HRT GnRHa pretreatment, HRT hormone replacement Treatment. BMI body mass index. (*$P<0.05$)

aged less than 35 years, with a BMI less than 24, and no significant differences in embryo quality or the number of embryos transferred. Four endometrial preparation protocols were used: ovulation induction, NC, artificial cycle, and artificial cycle after down-regulation. We found significant differences in hCG positivity, clinical pregnancy rate, and birth rate among the four endometrial preparation protocols. However, there were no significant differences in biochemical pregnancy rate and miscarriage rate.

When using the ovulation induction protocol as a control group, hCG positivity and clinical pregnancy rates were higher than those of the GnRHa + HRT protocol. However, there were no differences in hCG positivity, biochemical pregnancy rate, clinical pregnancy rate, miscarriage rate, and live birth rate between the ovulation induction protocol and the NC or artificial cycle protocols.

Using the NC protocol for endometrial preparation as a control group, we found that the hCG positivity rate, clinical pregnancy rate, and live birth rate were higher compared to the artificial cycle after down-regulation. However, there were no significant differences in miscarriage rate and biochemical pregnancy rate between these groups. Notably, a study by Xiao et al. [16] compared the NC protocol with the artificial cycle protocol for endometrial preparation when all embryos were 8-cell cleavage embryos. They found that the implantation rate, biochemical pregnancy rate, clinical pregnancy rate, and live birth rate were significantly higher in the NC protocol. In contrast, Alur-Gupta et al. [17] found no significant differences in pregnancy outcomes, including biochemical pregnancy rate, miscarriage rate, clinical pregnancy rate, ectopic pregnancy rate, and live birth, when comparing the NC with the artificial cycle protocol after adjusting for factors such as embryo quality, BMI, and age.

Our findings suggest that, in the general population, a NC protocol for endometrial preparation is more advantageous in the absence of other risk factors. However, while NC preparation avoids exogenous hormone therapy and is considered a simple and cost-effective transplantation option, the need to synchronize endogenous LH with endometrial conditions results in a higher cycle cancellation rate, making artificial cycles a preferred option for fertility clinicians. When comparing artificial cycles with downregulated artificial cycles, we found that the downregulated artificial cycle regimen had higher clinical pregnancy and live birth rates, with no other significant differences. GnRHa has been shown to improve endometrial receptivity and promote markers such as LIF, MEIS1 and HOXA10, in addition to endometrial cytosol synapses [18, 19]. Yu et al. [20], showed that the live birth rates were significantly higher and miscarriage rates significantly lower in the GnRHa + HRT group compared to the HRT group, while the biochemical pregnancy, clinical pregnancy, multiparity, and full-term birth rate were higher in the GnRHa + HRT group compare to those of the control group. Retrospective studies in polycystic ovary syndrome (PCOS) [12], endometriosis [21] and adenomyosis populations [10], have shown that GnRHa pretreatment significantly improved outcomes.

In addition to the endometrial preparation protocol, factors such as the patient's age, BMI, embryo quality, number of embryos transferred, endometrial thickness on the day of transfer, and endometrial receptivity affect FET pregnancy outcomes. Our logistic analysis revealed that, in women of advanced age, ovarian function decreases, and oocyte quality deteriorates, negatively impacting pregnancy outcomes. Notably, in FET, embryo non-integrity increases with age, and the uterine cavity is susceptible to environmental influences, reducing

endometrial tolerance [22]. Therefore, it is not surprising that we found that in women under 35 years, age was a risk factor for hCG positivity rate, clinical pregnancy rate, live birth rate, and a protective factor for miscarriage rate, with no effect on biochemical pregnancy rate. This aligns with previous studies concluding that older age correlates with poorer pregnancy outcomes.

Additionally, increased BMI impacts assisted reproduction technique (ART) outcomes, with obese patients showing significantly higher incidences of insulin resistance and hyperandrogenemia, affecting ovarian responsiveness and pregnancy outcomes post-embryo transfer [23]. Another study by Kelton et al. [24] indicated that fetal aneuploidy rates were higher in miscarriages among obese women, resulting in significantly lower clinical pregnancy and live birth rates, and significantly higher miscarriage rates. However, other studies shown that obesity is not associated with fetal aneuploidy, yet it remains a risk factor for miscarriage even with aneuploid embryos [25, 26]. Our study also found BMI to be a risk factor for miscarriage, which increased with higher BMI. Notably, there was no significant difference in hCG positivity rate, biochemical pregnancy rate, clinical pregnancy rate, and live birth rate, likely because our study population had normal BMIs, with no overweight or obese participants.

Embryo quality and the number of embryos transferred are crucial for successful pregnancy in assisted reproduction. Several studies have concluded that the higher numbers of embryos transferred correlate with higher clinical pregnancy rates, but also higher multiple pregnancy rates. For example, Guerif et al. [27] showed that the implantation and clinical pregnancy rates were significantly higher in the group transferred with one eugenic embryo compared to no eugenic embryos. However, there was no significant difference in clinical pregnancy rates among groups with one, two, or three eugenic embryos in ET. Another study by Vergouw et al. [28] also reported no significant difference in pregnancy rates between groups with one and two eugenic embryos, though the latter had significantly higher multiple birth rates.

Several studies have also shown that transferring two or more embryos significantly increases the live birth rate in frozen-thawed embryo transfer cycles. The quality of the embryos is also crucial, with studies indicating significantly higher pregnancy rates when two good quality blastocysts are transferred compared to one or no good quality embryos [2]. Our study further supports that the number and quality of embryos positively influences the hCG positivity rate, clinical pregnancy rate, and live birth rate.

In conclusion, our study suggests that the NC endometrial preparation protocol yields better pregnancy outcomes than other protocols in the general population, with downregulated artificial cycle protocols being more favorable than regular artificial cycle protocols. However, this study did not exclude the effect of embryo quality on pregnancy outcomes and had a limited sample size. Therefore, further research with larger sample sizes and controls for embryo quality is needed to better understand the impact of endometrial preparation protocols on pregnancy outcomes.

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Author contributions
ML and NNL supervised the study, JM and YL carried out statistics collection, JM wrote the manuscript. All authors read and approved the final version. All Authors reviewed the manuscript.

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Data availability
This study was approval by The Reproductive Hospital of Jiangxi University of Traditional Chinese Medicine（2023101）and adhere to tenets of Declaration of Helsinki. All the patients signed informed consent before starting IVF treatment at The Reproductive Hospital, which included consent for the use of summary statistic and not published at the individual level. Date not contain any identifying information。.

Declarations

Consent for publication
Not application.

Competing interests
The authors declare no competing interests.

Human ethics and consent to participate
All methods were carried out in accordance with relevant guidelines and regulations. Study involving human date, have were performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all subjects. The study was approval by The Reproductive Hospital of Jiangxi University of Traditional Chinese Medicine (2023101).

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