Ovulation induction regimens are associated with a higher rate of livebirth after frozen single-blastocyst transfer among women with polycystic ovary syndrome

Yue Niu1,2,3, Dingying Zhao1,2,3, Yuhuan Wang1,2,3, Lu Suo1,2,3, Jialin Zou1,2,3 and Daimin Wei1,2,3*

1Center for Reproductive Medicine, Cheeloo College of Medicine, Shandong University, Jinan, China, 2Key Laboratory of Reproductive Endocrinology of Ministry of Education, Shandong University, Jinan, China, 3Medical Integration and Practice Center, Shandong University, Jinan, China

Background: Hormone replacement therapy (HRT) regimen was suggested to be associated with a decreased rate of livebirth and a higher risk of hypertensive disorders of pregnancy (HDP) after frozen cleavage stage embryo transfer in women with polycystic ovary syndrome (PCOS). With the dramatically increased use of elective single embryo transfer, there is great need to explore the impacts of different endometrial preparation regimens on frozen single-blastocyst transfer in women with PCOS.

Methods: In this study, a total of 3941 women who diagnosed with PCOS and underwent single-blastocyst transfer during their first cycles of frozen embryo transfer (FET) between March 2012 and December 2020 were included. We retrospectively compared the pregnancy and neonatal outcomes after frozen single-blastocyst transfer with endometrial preparation by HRT regimen (n = 3540), ovulation induction by human menopausal gonadotropin (hMG) regimen (n = 226), and ovulation induction by letrozole regimen (n = 175).

Results: After adjustment for confounders with multivariable logistic regression, the hMG regimen group ([58.4% vs. 49.6%; adjusted odds ratio (aOR): 1.43; 95% confidence interval (CI): 1.09-1.89]) and letrozole regimen group (58.9% vs. 49.6%; aOR: 1.42; 95% CI: 1.04-1.93) were associated with a higher rate of livebirth (primary outcome), compared with the group with HRT regimen. As to the secondary outcomes, the rate of pregnancy loss in the hMG regimen group (22.8% vs. 30.3%; aOR: 0.69; 95% CI: 0.48-1.00) and letrozole regimen group (16.9% vs. 30.3%; aOR: 0.48; 95% CI: 0.30-0.78) was also lower than that in the HRT regimen group. The pregnancy outcomes between the hMG regimen group and the letrozole regimen group were similar. We did not observe significant difference in the incidences of maternal and neonatal complications among these three groups.
Conclusion: Ovulation induction regimen with letrozole or hMG for endometrial preparation was associated with a higher livebirth rate and a lower pregnancy loss rate in frozen single-blastocyst transfer cycles among women with PCOS.

KEYWORDS: frozen embryo transfer, hormone replacement therapy, ovulation induction, polycystic ovary syndrome, single-blastocyst transfer

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive age women (1), and is a major cause of anovulatory infertility. In women with PCOS who underwent in vitro fertilization (IVF), our multicenter randomized trial demonstrated freeze-only strategy with deferred frozen embryo transfer (FET) increased the rate of livebirth while decreased the risks of ovarian hyperstimulation syndrome (OHSS) and pregnancy loss compared with fresh embryo transfer (2). FET has been a routine treatment for patients with PCOS in many assisted reproductive technology (ART) centers (3). Furthermore, the use of elective single-blastocyst transfer has been increasingly advocated in FET cycles for an increased livebirth rate and a reduced multiple pregnancy rate (4).

Due to the feature of oligoovulation/anovulation, women with PCOS need hormone replacement therapy (HRT) regimen or ovulation induction regimen for endometrial preparation before FET. For years, HRT regimen was recognized as the first line endometrial preparation protocol before FET in women with PCOS for easily planning (5), decreased rate of cancellation (6), and minimized risk of OHSS. However, HRT regimen was associated with an increased risk of hypertensive disorders of pregnancy (HDP) compared with natural ovulatory cycle, possibly due to its lack of corpus luteum (7). Regarding to the rates of pregnancy and livebirth, in ovulatory women, earlier studies suggested similar efficacy between the natural ovulation regimen and the HRT regimen (8–10). Several studies have focused on the pregnancy outcomes of different endometrial preparation regimens in women with ovulatory dysfunction (11–16). However, most of these studies employed cleavage-stage embryo transfer (11, 12, 14–16), and the efficiency of different ovulation induction regimens was rarely explored. It remains unknown whether ovulation induction regimen is superior to HRT regimens in single-blastocyst transfer cycles, and which medicine is better for ovulation induction regimen is yet to be established.

A recent cochrane meta-analysis concluded that for women diagnosed with PCOS who underwent ovulation induction and timed intercourse, letrozole appears to improve live birth and pregnancy rates compared with clomiphene citrate (17). Furthermore, it was demonstrated by another cochrane meta-analysis that in women with clomiphene citrate-resistant PCOS, gonadotrophins resulted in more live births than continued clomiphene citrate (18). The study by Lin et al. showed that the use of letrozole was associated with improved clinical pregnancy outcomes compared with human menopausal gonadotropin (hMG) in ovulatory patients who underwent FET (19). However, the efficiency of letrozole compared with gonadotropin was rarely compared in women diagnosed with PCOS. Several studies revealed that letrozole improved endometrial receptivity as compared with clomiphene in patients with PCOS (20–22). Nonetheless, whether letrozole used for ovulation induction regimen could improve the outcome of frozen single-blastocyst transfer still lacks of evidence and warrants further studies.

In this study, we compared the rates of pregnancy and livebirth as well as the risks of obstetric and neonatal complications among women with PCOS who underwent HRT regimen, hMG ovulation induction regimen, or letrozole ovulation induction regimen for endometrial preparation in frozen single-blastocyst transfer cycles.

Materials and methods

Patients

This was a retrospective study. A total of 3941 infertile women with PCOS were included this study at the Center for Reproductive Medicine of Shandong University from March 2012 to December 2020. Eligible women were aged 20–40 years and diagnosed with PCOS, all of them underwent single-blastocyst transfer at their first attempts of IVF with or without intracytoplasmic sperm injection (ICSI), and the
included cycles were their first FET cycles. To diagnose PCOS, we used Rotterdam consensus (23) as fulfilling at least two of the three criteria and excluding other causes of hyperandrogenism and ovulation dysfunction: oligoovulation or anovulation; clinical or biochemical hyperandrogenism; and polycystic ovarian morphology on ultrasound, as defined by at least one ovary with ≥12 follicles or volume ≥10 cm³. Preimplantation genetic test cycles were excluded from this study. Women with abnormal intrauterine cavity (such as a uterine malformation, adenomyosis, submucous myoma, or intrauterine adhesion), preexisting diabetes and hypertension before pregnancy, or a history of recurrent miscarriage (defined as three or more previous spontaneous pregnancy losses) were also excluded. The study protocol was approved by the Institutional Ethics Committee of the Center for Reproductive Medicine of Shandong University (Ethical Review No.27, 2021).

Embryo culture, evaluation, and selection or transfer

GnRH antagonist protocol or GnRH agonist protocol was used for ovarian stimulation as previously reported (2, 24, 25). Once oocytes retrieval was done after ovarian stimulation, conventional IVF or ICSI was performed approximately 4 to 6 hours later. After 16–18 hours, fertilization was confirmed by the presence of two pronuclei and two polar bodies. On day 3 of embryo culture, embryos were graded by morphological criteria on the basis of the number and size of blastomere and the percentage of fragmentation (26), and the culture medium was changed. The quality of single-blastocyst was assessed according to the Gardner morphological criteria (27). Blastocysts were vitrified on day 5, day 6 or day 7 according to embryo development. For thawing, embryos were transfer into dilution solution in a sequential manner. Blastocyst with score of 4BC or better was transferred. All patients received single-blastocyst transfer.

Endometrial preparation before embryo transfer

The selection of the regimens for endometrial preparation was based on physicians’ experience and patients’ preferences. In the hormone replacement therapy cycle regimen, oral oestradiol valerate (Progynova, Delpharm Lille, Lys-Lez-Lannoy, France) at a dose of 4–6 mg daily was started on cycle day 1–3. Vaginal progesterone gel (Crinone, Merck Serono, Watford, UK) 90 mg per day and oral dydrogesterone (Duphasston, Abbott, OLST, Netherlands) 10 mg twice daily were added when the endometrial thickness reached ≥8 mm. And then the transfer was carried out 5 days after progesterone initiation. If pregnancy was achieved, oral oestradiol valerate was continued until 8 weeks’ gestation, and vaginal progesterone gel and oral dydrogesterone were continued until 12 weeks’ gestation.

In the group underwent hMG stimulated, 75 IU/day of hMG (Le Baode, Livzon, Zhuhai, China) was injected from cycle day 3 for 5 days. Ultrasound monitoring started on days 8 of the menstrual cycle, and the dose of hMG was adjusted according to follicle development. Based on the diameter of the dominant follicle was ≥17 mm or the occurrence of an LH surge, human chorionic gonadotrophin (hCG, Le Baode, Livzon, Zhuhai, China) was administered for triggering ovulation. Oral dydrogesterone 10 mg twice daily was administered for luteal phase support after ovulation, and the transfer of embryos was performed on the 5th days after ovulation. If pregnancy was achieved after frozen blastocyst transfer, luteal phase support was continued until 11 weeks’ gestation.

For the letrozole stimulated group, the patients orally took 2.5–5 mg of letrozole per day (Laiquzuo, Heng-Rui, Jiangshu, China) for 5–7days, from day 3 of menstrual cycle. Ultrasound monitoring started on days 8 of the menstrual cycle. If the dominant follicle reached a diameter of ≥10 mm on cycle day 10, transvaginal ultrasound was repeated every 2 days and no other drug was added until ovulation triggering. If a dominant follicle was <10 mm on day 10, a daily dosage of 75–150 IU hMG was supplemented to stimulate follicle growth. The other procedure was the same as in the hMG group.

Study outcomes

The primary outcome was livebirth after the frozen single-blastocyst transfer. Secondary outcomes were endometrial thickness, biochemical pregnancy, clinical pregnancy, ongoing pregnancy, pregnancy loss, and birth weight. Safety outcomes included ectopic pregnancy and other obstetrical and perinatal complications [i.e., preterm delivery, gestational diabetes mellitus (GDM), HDP, small-for-gestation-age (SGA) neonates, large-for-gestation-age (LGA) neonates, congenital anomalies]. Livebirth was defined as the delivery of any viable neonate who was 28 weeks of gestation or older. Biochemical pregnancy was defined as a serum hCG level >10 IU/L at 12–14 days after FET. Clinical pregnancy was defined as the presence of at least one gestational sac in the uterine cavity on ultrasound at 5 weeks after FET. Ongoing pregnancy was defined as available pregnancy after 12 weeks’ gestation. Miscarriage was defined as pregnancy loss before the 28th gestational week. Preterm birth was defined as delivery before 37 weeks of gestation. GDM was defined as one or more of the 75 g OGTT glucose level indexes above the following cut-off values screened between 24–28 weeks of gestation after excluding pregestational diabetes mellitus: fasting plasma glucose ≥5.1mmol/l; 1 h plasma glucose ≥10.0 mmol/l; 2 h plasma glucose ≥8.5 mmol/l (28). HDP was defined
as sustained blood pressure ≥140/90 mmHg after 20 weeks, including gestational hypertension, pre-eclampsia, eclampsia and without a prior history of hypertension (29). SGA was defined as birthweight lower than the 10th percentile of referential birthweight. LGA was defined as birthweight higher than the 90th percentile of referential birthweight. Low birth weight was defined as birth weight smaller than 2500 g, and macrosomia was defined as birth weight greater than 4000 g.

Statistical analysis

For continuous variables, the normality was tested by the graphical use of histograms, Q–Q plots and the Shapiro–Wilk test. If data are normally distributed, then they were described as mean ± standard deviation (SD); otherwise, they were presented as median (25th percentile to 75th percentile). Categorical variables were expressed as number of cases (n) and percentage of occurrence (%). Continuous data were compared with the use of one way ANOVA test or Kruskal-Wallis test. Categorical variables were assessed by χ² analysis and Fisher’s exact test. Pairwise comparisons among groups were performed with the Bonferroni method to modify the significance levels. Multivariable logistic regression analysis was used to evaluate the possible relationship between the type of endometrial preparation and outcomes after adjusting for confounding factors, including age, body mass index, duration of infertility, gravidity, year of treatment, and number of oocytes retrieved. All statistical analyses were performed by Statistical Package for Social Sciences (SPSS) version 26.0. A P value <0.05 was considered to be statistically significant.

Results

Baseline characteristics

As is illustrated in Table 1, the three groups exhibited similar baseline characteristics, including age, body mass index, gravidity, parity, infertility causes, fertilization method, donor sperm, ovarian stimulation protocols, days of ovarian stimulation, total gonadotropin dose, and number of oocytes retrieved. The HRT group had a longer duration of infertility compared to the hMG group [4.0 (2.5-5.5) vs. 3.0 (2.0-5.0), P=0.021]. There was a significant difference in the year of treatment among the HRT regimen, hMG regimen and letrozole regimen groups (P=0.273 for HRT regimen group vs. hMG regimen group, P<0.001 for HRT regimen group vs. letrozole regimen group, P=0.057 for hMG regimen group vs. letrozole regimen group). In addition, the letrozole regimen achieved thicker endometrium [10.0 (8.5-11.0) vs. 9.0 (8.5-10.0), P=0.001] compared with the HRT group.

Reproductive outcomes

The comparison of the reproductive and neonatal outcomes between the three groups is presented in Table 2. The rates of livebirth (58.4% vs. 49.6%, P=0.030) and ongoing pregnancy (60.2% vs. 51.5%, P=0.033) were higher in the hMG regimen group compared with the HRT regimen group. The rate of livebirth was of borderline statistical difference between the letrozole regimen group and the HRT regimen group (58.9% vs. 49.6%, P=0.051). The letrozole regimen group also had a lower rate of total pregnancy loss among conception (16.9% vs. 30.3%, P=0.003) and a lower rate of biochemical miscarriage (4.8% vs. 12.3%, P=0.036) compared with the HRT regimen group. Stratified analyses of reproductive outcomes by ovarian stimulation protocols are showed in Supplemental Table 1. For patients who underwent agonist protocol, the letrozole regimen group was associated with higher rates of livebirth (61.9% vs. 49.1%, P=0.042) and ongoing pregnancy (63.9% vs. 50.7%, P=0.033) than the HRT regimen group, the hMG regimen group had a tendency of increased livebirth rate (56.8% vs. 49.1%) compared with the HRT regimen group. For patients who underwent antagonist protocol, the HRT regimen group also had the lowest rate of livebirth (50.6%, 59.4%, and 55.3%, in HRT group, hMG group, and letrozole group, respectively), although no significant difference.

The comparison of maternal and neonatal complications between the three groups is presented in Table 3. No significant difference in maternal and neonatal complications (including preterm delivery, GDM, HDP, SGA, LGA, low birth weight, macrosomia, and congenital anomalies) was observed among the three groups. There were still no significant differences in these complications among the three groups after stratified analyses by ovarian stimulation protocols (Supplemental Table 2). After adjustment for the above-mentioned confounding factors (Table 4), the hMG regimen group [adjusted odds ratio (aOR): 1.43; 95% confidence interval (CI): 1.09-1.89] and the letrozole regimen group (aOR: 1.42; 95% CI: 1.04-1.93) had a higher rate of livebirth, compared with the group with HRT regimen. The rate of ongoing pregnancy was also higher in the hMG regimen group (aOR: 1.44; 95% CI: 1.09-1.89) and the letrozole regimen group (aOR: 1.38; 95% CI: 1.01-1.89) than the HRT group. Both the hMG regimen group (aOR: 0.69; 95% CI: 0.48-1.00) and the letrozole regimen group (aOR: 0.48; 95% CI: 0.30-0.78) were associated with a lower risk of pregnancy loss. The risks of biochemical miscarriage (aOR: 0.39; 95% CI: 0.17-0.89) and clinical miscarriage (aOR: 0.50; 95% CI: 0.28-0.90) were
TABLE 1 Basic characteristics of the participants at cycle level by the different protocols.

| Characteristic                       | HRT regimen (n = 3540) | hMG regimen (n = 226) | Letrozole regimen (n = 175) | P Value |
|--------------------------------------|------------------------|-----------------------|-----------------------------|---------|
| Age (years)                          | 29.0 (27.0-31.0)       | 28.0 (26.0-31.0)      | 28.0 (26.0-30.0)             | 0.155   |
| Body mass index (kg/m²)              | 24.6 (21.9-27.4)       | 24.2 (21.6-27.2)      | 24.6 (22.4-27.3)             | 0.419   |
| Duration of infertility (years)      | 4.0 (2.5-5.5)a         | 3.0 (2.0-5.0)         | 3.5 (2.0-5.0)                | 0.013   |
| Gravidity (%)                        |                        |                       |                             | 0.110   |
| 0                                    | 2380 (67.2%)           | 136 (60.2%)           | 110 (62.9%)                 |         |
| 1                                    | 780 (22.0%)            | 56 (24.8%)            | 46 (26.3%)                  |         |
| ≥2                                   | 380 (10.7%)            | 34 (15.0%)            | 19 (10.9%)                  |         |
| Parity (%)                           |                        |                       |                             | 0.389   |
| 0                                    | 3232 (91.3%)           | 201 (88.9%)           | 157 (89.7%)                 |         |
| ≥1                                   | 308 (8.7%)             | 25 (11.1%)            | 18 (10.3%)                  |         |
| Infertility causes (%)               |                        |                       |                             | 0.373   |
| PCOS only                            | 265 (7.5%)             | 13 (5.8%)             | 15 (8.6%)                   |         |
| PCOS + tubal factor                  | 2112 (59.7%)           | 145 (64.2%)           | 102 (58.3%)                 |         |
| PCOS + male factor                   | 265 (7.5%)             | 15 (6.6%)             | 17 (9.7%)                   |         |
| PCOS + other factors                 | 75 (2.1%)              | 9 (4.0%)              | 5 (2.9%)                    |         |
| PCOS + mixed factors                 | 823 (23.2%)            | 44 (19.5%)            | 36 (20.6%)                  |         |
| Fertilization method (%)             |                        |                       |                             | 0.483   |
| IVF                                  | 2824 (79.8%)           | 185 (81.9%)           | 140 (80.0%)                 |         |
| ICSI                                 | 572 (16.2%)            | 28 (12.4%)            | 28 (16.0%)                  |         |
| Half IVF + half ICSI                 | 144 (4.1%)             | 13 (5.8%)             | 7 (4.0%)                    |         |
| Donor sperm (%)                      | 175 (4.9%)             | 12 (5.3%)             | 5 (2.9%)                    |         |
| Year of treatment (%)                |                        |                       |                             | <0.001  |
| 2012-2014                            | 499 (93.3%)b           | 29 (5.4%)             | 7 (1.3%)                    |         |
| 2015-2016                            | 1122 (87.0%)ab         | 90 (7.0%)             | 77 (6.0%)                   |         |
| 2017-2018                            | 954 (90.3%)            | 53 (5.0%)             | 50 (4.7%)                   |         |
| 2019-2020                            | 965 (91.0%)            | 54 (5.1%)             | 41 (3.9%)                   |         |
| Ovarian stimulation protocols (%)    |                        |                       |                             | 0.663   |
| Long agonist protocol                | 1807 (51.0%)           | 108 (47.8%)           | 94 (53.7%)                  |         |
| Short agonist protocol               | 86 (2.4%)              | 10 (4.4%)             | 3 (1.7%)                    |         |
| GnRH antagonist protocol             | 1610 (45.5%)           | 106 (46.9%)           | 76 (43.4%)                  |         |
| Other protocols                      | 37 (1.0%)              | 2 (0.9%)              | 2 (1.1%)                    |         |
| Days of ovarian stimulation (days)   | 110 (9.0-13.0)         | 110 (9.0-13.0)        | 110 (9.0-13.0)              | 0.755   |
| Total gonadotropin dose (IU)         | 1525.0                 | 1500.0                | 1481.3                      | 0.769   |
| (1200.0-2250.0)                      | (1200.0-2250.0)        | (1125.0-2250.0)       |                             |         |
| Number of oocytes retrieved (n)      | 17.0 (12.0-21.0)       | 16.0 (12.0-20.0)      | 17.0 (13.0-22.0)            | 0.134   |
| Endometrium thickness (mm)           | 9.0 (8.5-10.0)b        | 9.0 (8.0-10.0)        | 10.0 (8.5-11.0)             | 0.001   |

HRT, hormone replacement therapy; hMG, human menopausal gonadotropin; PCOS, polycystic ovary syndrome; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection. a There were significant differences between the HRT group and the hMG group. b There were significant differences between the HRT group and the Letrozole group.

Reduced in letrozole regimen group in comparison to the HRT regimen group. Stratified logistic regression of pregnancy outcomes by ovarian stimulation protocols is showed in Supplemental Table 3. For patients who underwent agonist protocol, the letrozole regimen group also had higher rates of livebirth rate (aOR: 1.70; 95% CI: 1.11-2.59), clinical pregnancy (aOR: 1.71; 95% CI: 1.08-2.71), and ongoing pregnancy (aOR: 1.76; 95% CI: 1.15-2.72), and a lower rate of total pregnancy loss among conception (aOR: 0.54; 95% CI: 0.30-0.97) compared with the HRT regimen group. For patients who underwent antagonist protocol, the letrozole regimen group was associated with a lower rate of total pregnancy loss among conception (aOR: 0.35; 95% CI: 0.15-0.83) compared with the HRT regimen group.

Discussion

In this study, we found that both the hMG regimen group and the letrozole regimen group were associated with a higher rate of livebirth and a lower rate of pregnancy loss compared with the group with HRT regimen. When stratified by ovarian stimulation protocols, the letrozole regimen was associated with
a higher rate of live birth rate in patients who underwent agonist protocol, and a lower rate of pregnancy loss in patients who underwent antagonist protocol, as compared with the HRT regimen group. We did not observe significant difference in the incidences of maternal and neonatal complications among these three groups. Thus, ovulation induction regimen may be a better choice for endometrial preparation for frozen single-blastocyst transfer in women with PCOS.

Our results were consistent with several previous studies conducted in patients with ovulation disorders (13, 15, 30, 31). Man et al. reported a higher rate of live birth in hMG regimen compared with HRT regimen in patients with PCOS who underwent frozen single-blastocyst transfer (13). The study by Hu et al. showed that that in 120 patients with PCOS, letrozole ovulation induction regimen was associated with higher rates of implantation, clinical pregnancy and ongoing pregnancy, when compared with HRT regimen (30). Zhang and coworkers observed that in patients with PCOS, most of whom underwent cleavage stage embryo transfer, the letrozole regimen group was associated with a higher rate of livebirth and a lower rate of pregnancy loss than the HRT regimen group (15). The study by Li et al. also demonstrated higher implantation rate, clinical pregnancy rate, and livebirth rate but a lower abortion rate with letrozole ovulation induction regimen cycles compared with HRT cycles in patients with ovulation disorders (31).

TABLE 2 Reproductive and neonatal outcomes after FET according to the type of endometrial preparation treatments.

| Characteristic                  | HRT regimen (n = 3540) | hMG regimen (n = 226) | Letrozole regimen (n = 175) | P Value |
|--------------------------------|------------------------|-----------------------|-----------------------------|---------|
| Livebirth (%)                  | 1756 (49.6%)           | 132 (58.4%)           | 103 (58.9%)                 | 0.003   |
| Singleton livebirth (%)        | 1737 (49.1%)           | 126 (55.8%)           | 99 (56.6%)                  | 0.028   |
| Twin livebirth (%)             | 19 (0.5%)              | 6 (2.7%)              | 4 (2.3%)                    | 0.001   |
| Sex of neonates                |                        |                       |                             | 0.767   |
| Male (%)                       | 979/1775 (55.2%)       | 71/138 (51.4%)        | 64/107 (59.8%)              |         |
| Female (%)                     | 796/1775 (44.8%)       | 67/126 (48.6%)        | 43/107 (40.2%)              |         |
| Birthweight (g)*               | 3500.0 (3200.0-3800.0) | 3475.0 (3100.0-3780.0)| 3350.0 (3030.0-3750.0)      | 0.309   |
| Biochemical pregnancy (%)      | 2521 (71.2%)           | 171 (75.7%)           | 124 (70.9%)                 | 0.351   |
| Clinical pregnancy (%)         | 2195 (62.0%)           | 156 (69.0%)           | 117 (66.9%)                 | 0.053   |
| Ongoing pregnancy (%)          | 1822 (51.5%)           | 136 (60.2%)           | 105 (60.0%)                 | 0.005   |
| Total pregnancy loss among conception (%)| 765/2521 (30.3%)| 39/171 (22.8%) | 21/124 (16.9%) | 0.001   |
| Biochemical miscarriage (%)    | 310/2521 (12.3%)       | 13/171 (7.6%)         | 6/124 (4.8%)                | 0.010   |
| Clinical miscarriage (%)       | 431/2195 (19.6%)       | 24/156 (15.4%)        | 13/117 (11.1%)              | 0.036   |
| First trimester miscarriage (%)| 373/2195 (17.0%)       | 20/156 (12.8%)        | 12/117 (10.3%)              | 0.073   |
| Second trimester miscarriage (%)| 58/2195 (2.6%)    | 4/156 (2.6%)          | 1/117 (0.9%)                | 0.602   |
| Ectopic pregnancy (%)          | 16/2195 (0.6%)         | 2/171 (1.2%)          | 1/124 (0.8%)                | 0.371   |
| Induced delivery (%)           | 8/2195 (0.4%)          | 0                     | 1/117 (0.9%)                | 0.429   |

HRT, hormone replacement therapy; hMG, human menopausal gonadotropin. *There were significant differences between the HRT group and the hMG group. **There were significant differences between the HRT group and the Letrozole group. *Birthweight of 3 newborn babies in the HRT group and 2 newborn babies in the hMG group was missing.

TABLE 3 Maternal and neonatal complications.

| Characteristic                  | HRT regimen (n = 3540) | hMG regimen (n = 226) | Letrozole regimen (n = 175) | P-Value |
|--------------------------------|------------------------|-----------------------|-----------------------------|---------|
| Maternal complications         |                        |                       |                             |         |
| Preterm delivery (%)           | 163/2195 (7.4%)        | 14/156 (9.0%)         | 10/117 (8.5%)               | 0.718   |
| GDM (%)                        | 132/2195 (6.0%)        | 14/156 (9.0%)         | 7/117 (6.0%)                | 0.332   |
| HDP (%)                        | 145/2195 (6.6%)        | 5/156 (3.2%)          | 9/117 (7.7%)                | 0.211   |
| Neonatal complications         |                        |                       |                             |         |
| SGA (%)*                       | 47/1772 (2.7%)         | 4/136 (2.9%)          | 7/107 (6.5%)                | 0.065   |
| LGA (%)*                       | 469/1772 (26.5%)       | 31/136 (22.8%)        | 23/107 (21.5%)              | 0.355   |
| Low birth weight (%)*          | 96/1772 (5.4%)         | 8/136 (5.9%)          | 7/107 (6.5%)                | 0.868   |
| Macrosomia (%)*                | 228/1772 (12.9%)       | 16/136 (11.8%)        | 14/107 (13.1%)              | 0.929   |
| Congenital anomalies (%)       | 20/1775 (1.1%)         | 4/138 (2.9%)          | 1/107(0.9%)                 | 0.185   |

HRT, hormone replacement therapy; hMG, human menopausal gonadotropin; GDM, gestational diabetes mellitus; HDP, hypertensive disorders of pregnancy; SGA, small-for-gestation-age; LGA, large-for-gestation-age. *Birthweight of 3 newborn babies in the HRT group and 2 newborn babies in the hMG group was missing.
The study by Conrad et al. discovered that the absence of a corpus luteum has potential influence on angiogenesis and immunology (33). The study by Conrad et al. discovered that the absence of a corpus luteum plays a role. Besides production of steroid hormone, corpus luteum has potential influence on angiogenesis and immunology (33). The study by Conrad et al. discovered that the absence of a corpus luteum in IVF cycles was related to maternal cardiovascular dysregulation in early age of pregnancy (34). The suboptimal maternal cardiovascular function may contribute to compromised pregnancy outcomes. In addition, the possibility that inadequate or excessive dosage or imprecise timing of estrogen and progesterone administration in HRT cycles may also adversely affect endometrial function and subsequently lead to adverse pregnancy outcomes (35). It was demonstrated that estrogen administration exceeded 28 days or high serum estradiol levels (≥400 pg/ml) prior to progesterone administration was associated with a decreased live birth rate during frozen blastocyst transfer cycles (36, 37).

Although accumulated evidences showed HRT regimen for endometrial preparation was associated with an increased risk of HDP compared with natural regimen where the corpus luteum exists in ovulatory women (7), there was no difference in the risk of HDP in our study when the corpus luteum status varied. Women with PCOS are at higher risk of adverse pregnancy and birth outcomes (38), in that condition, the negative effects caused by lack of corpus luteum may be aggravated, the establishment and maintaining of pregnancy was compromised, and subsequently lead to increased pregnancy loss and decreased incidence for acquiring livebirth, while the difference of the risk of HDP became unsignificant. The study by Zhang et al. showed that HRT regimen for endometrial preparation was associated with an increased risk of HDP compared with ovulation regimen among women with PCOS (16). However, most patients in their study underwent double cleavage stage embryos transfer, and above one third patients achieved twin delivery.

In ovulation regimens, the receptivity of endometrium to embryo implantation relies on endogenous estrogen, progesterone, and other factors produced during the maturity of a dominant follicle, ovulation, and subsequent formulation of a corpus luteum. Accordant with the recent meta-analysis (39), our study showed that both hMG regimen and letrozole regimen result in a higher livebirth rate compared with HRT regimen, while hMG regimen and letrozole regimen achieved comparable rate of livebirth. However, when stratified by ovarian stimulation protocols, the difference in terms of livebirth between hMG regimen and HRT regimen was attenuated. Up to now, which medicine is superior for ovulation induction has yet to be determined. However, infertile people with PCOS might benefit from the use of letrozole (40).

Our study indicated the endometrial thickness before embryo transfer was thicker in the letrozole group compared with the HRT group. Letrozole may induce a molecular response in the endometrium of PCOS patients in the Wnt/B-catenin pathway, which plays a pivotal role in cell proliferation, differentiation, motility, survival, embryo implantation, and placental development (41, 42). Secondly, letrozole can decrease the production of estrogen, as a result, intraovarian and serum estrogen levels were reduced (43). Low estrogen levels left a large number of estrogen receptors unoccupied, in order to increase sensitivity to subsequent estrogen rise (44). Furthermore, letrozole have a positive effect on the expression

### Table 4 Logistic regression of pregnancy outcomes.

| Characteristic                             | hMG regimen vs. HRT regimen | Letrozole regimen vs. HRT regimen |
|--------------------------------------------|------------------------------|----------------------------------|
|                                            | aOR (95% CI) | P Value | aOR (95% CI) | P Value |
| Livebirth                                  | 1.43 (1.09-1.89) | 0.011    | 1.42 (1.04-1.93) | 0.029 |
| Singleton livebirth                         | 1.30 (0.99-1.71) | 0.059    | 1.31 (0.97-1.79) | 0.083 |
| Twin livebirth                              | 5.29 (2.06-13.58) | 0.001    | 5.40 (1.77-16.53) | 0.003 |
| Biochemical pregnancy                       | 1.31 (0.95-1.79) | 0.099    | 0.99 (0.71-1.38) | 0.947 |
| Clinical pregnancy                          | 1.39 (1.04-1.86) | 0.029    | 1.21 (0.87-1.67) | 0.252 |
| Ongoing pregnancy                           | 1.44 (1.09-1.91) | 0.010    | 1.38 (1.01-1.89) | 0.042 |
| Total pregnancy loss among conception       | 0.69 (0.48-1.00) | 0.048    | 0.48 (0.30-0.78) | 0.003 |
| Biochemical miscarriage                     | 0.58 (0.37-1.04) | 0.069    | 0.39 (0.17-0.89) | 0.025 |
| Clinical pregnancy loss                     | 0.76 (0.49-1.20) | 0.234    | 0.50 (0.28-0.90) | 0.020 |

aOR, adjusted odds ratio; CI, confidence interval. Analyses were adjusted for age, body mass index, duration of infertility, gravidity, year of treatment, and number of oocytes retrieved.

During HRT cycles, high doses of exogenous estrogen and progesterone are needed for luteal phase support in the first trimester. Although it has been proposed that the use of HRT regimen should be abandoned in ovulatory women (32), HRT regimen still is the first choice for women who have irregular menstrual cycles. Our study provides additional evidence for the previous findings that the HRT regimen was associated with a lower rate of livebirth and a higher rate of pregnancy loss compared with the ovulation induction regimens for women with PCOS, even after single-blastocyst transfer. The underlying mechanism is unclear. However, the absence of a corpus luteum may plays a role.

Accordant with the recent meta-analysis (39), the absence of a corpus luteum may play a role. Besides production of steroid hormone, corpus luteum has potential influence on angiogenesis and immunology (33). The suboptimal maternal cardiovascular function may contribute to compromised pregnancy outcomes. In addition, the possibility that inadequate or excessive dosage or imprecise timing of estrogen and progesterone administration in HRT cycles may also adversely affect endometrial function and subsequently lead to adverse pregnancy outcomes (35). It was demonstrated that estrogen administration exceeded 28 days or high serum estradiol levels (≥400 pg/ml) prior to progesterone administration was associated with a decreased live birth rate during frozen blastocyst transfer cycles (36, 37).
of molecules related to endometrial receptivity (19). Promoted integrin expression and improved pregnancy and implantation rates was founded after co-treatment with letrozole in patient with defected endometrial receptivity (45). Thus, the treatment with letrozole for endometrial preparation in patients with PCOS may offer greater potential.

A strength of our study was its large sample size and data integrity. Additionally, we only included patients who underwent frozen single-blastocyst transfer, which has been increasingly advocated. We acknowledge that this study has limitations. First, it was a retrospective observational study, although we only enrolled women who underwent their first FET cycles at the first attempts of IVF, and adjusted confounders with multivariable logistic regression, the bias and confounding factors may still influence the results of our study. Furthermore, due to the great sample difference among the three groups, we were at risks for both a Type 1 and a Type 2 error. Additionally, as a result of small sample, when it comes to maternal and neonatal complications, there may lack of statistics validity. Large sample and multicenter randomized controlled trial are needed to assess the efficacy of ovulation induction regimen as a method of endometrial preparation for FET in patients with PCOS.

Conclusion

We found that for patients with PCOS who underwent frozen single-blastocyst transfer, both hMG ovulation induction regimen and letrozole ovulation induction regimen for endometrial preparation were associated with better pregnancy outcomes compared with HRT regimen, such as a higher livebirth rate and a lower pregnancy loss rate. Further studies are warranted to explore which ovulation induction regimen is superior for endometrial preparation before FET.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by The Institutional Ethics Committee of the Center for Reproductive Medicine of Shandong University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

DW supervised the entire study and revised the manuscript; YN collected data, analyzed the data, and drafted the manuscript; DZ, YW, LS, and JZ collected data and analyzed the data. All authors have been involved in interpreting the data and have approved the final version.

Funding

This work was supported by grants from the National Natural Science Foundation of China (82071718).

Acknowledgments

The authors thank the patients and staff of the Center for Reproductive Medicine of Shandong University for their cooperation and support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.987813/full#supplementary-material
38. Roos N, Kiefer H, Sahlin L, Ekman-Ordeberg G, Falconer H, Stephansson O. Risk of adverse pregnancy outcomes in women with polycystic ovary syndrome: Population based cohort study. *Bmj* (2011) 343:d6309. doi: 10.1136/bmj.d6309

39. Zhang Y, Wu L, Li TC, Wang CC, Zhang T, Chung J. Systematic review update and meta-analysis of randomized and non-randomized controlled trials of ovarian stimulation versus artificial cycle for endometrial preparation prior to frozen embryo transfer in women with polycystic ovary syndrome. *Reprod Biomed Online* (2022) 20(1):62. doi: 10.1186/s12958-022-00931-4

40. Palomba S. Aromatase inhibitors for ovulation induction. *J Clin Endocrinol Metab* (2015) 100(5):1742–7. doi: 10.1210/jc.2014-4235

41. Mehdinejadani S, Amidi F, Mehdizadeh M, Barati M, Pazhoohan A, Alyasin A, et al. Effects of letrozole and clomiphene citrate on Wnt signaling pathway in endometrium of polycystic ovarian syndrome and healthy women. *Biol Reprod* (2019) 100(3):641–8. doi: 10.1093/biolre/ioy187

42. Mehdinejadani S, Amidi F, Mehdizadeh M, Barati M, Safdarian L, Aflatoonian R, et al. The effects of letrozole and clomiphene citrate on ligands expression of Wnt3, Wnt7a, and Wnt8b in proliferative endometrium of women with polycystic ovarian syndrome. *Gynecol Endocrinol* (2018) 34(9):775–80. doi: 10.1080/09513590.2018.1446934

43. Garcia-Velasco JA. The use of aromatase inhibitors in in vitro fertilization. *Fertil Steril* (2012) 98(6):1356–8. doi: 10.1016/j.fertnstert.2012.09.042

44. Tatsumi T, Jwa SC, Kuwahara A, Irahara M, Kubota T, Saito H. Pregnancy and neonatal outcomes following letrozole use in frozen-thawed single embryo transfer cycles. *Hum Reprod* (2017) 32(6):1244–8. doi: 10.1093/humrep/dex066

45. Miller PB, Parnell BA, Bushnell G, Tallman N, Forstein DA, Higdon HL3rd, et al. Endometrial receptivity defects during IVF cycles with and without letrozole. *Hum Reprod* (2012) 27(5):881–8. doi: 10.1093/humrep/der452