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Live birth after letrozole-stimulated cycles versus hormone replacement treatment cycles for the first frozen embryo transfer in women with polycystic ovary syndrome: protocol for a multicentre randomised controlled trial

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ABSTRACT

Introduction Hormone replacement treatment (HRT) is the most commonly used endometrial preparation protocol for frozen embryo transfer (FET) in patients with polycystic ovary syndrome (PCOS). However, studies have found that HRT may increase the risk of hypertensive disorders of pregnancy and some obstetric complications. Letrozole is a new first-line ovulation induction drug for PCOS and can effectively induce spontaneous ovulation by reducing oestrogen levels. However, letrozole is still rarely used in FET and has only been reported in a few studies in Asian populations. High-quality, well-powered randomised controlled trials (RCTs) comparing HRT and letrozole-stimulated protocols are lacking. The aim of this study is to compare the efficacy and safety of two protocols in patients with PCOS.

Methods and analysis This is a multicentre, open-label RCT in four reproductive medical centres in China. In total, 1078 women with PCOS will be randomised (1:1) to the letrozole-stimulated or HRT group in their first FET cycle and their pregnancy and perinatal outcomes during this cycle will be followed up and analysed. The primary outcome is live birth. Secondary outcomes are cycle cancellation rate, biochemical pregnancy, clinical pregnancy, miscarriage, ectopic pregnancy, obstetric and perinatal complications, neonatal complications and birth weight.

Ethics and dissemination Ethical approval was obtained from the Institutional Review Board of Reproductive and Genetic Hospital of CITIC-XIANGYA (LL-SC-2022-001). Written informed consent will be obtained from each participant. The findings will be disseminated through conference presentations and publication in peer-reviewed journals.

Trial registration number NCT05227391.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is a multicentre, randomised, controlled clinical trial with a large sample size comparing letrozole-stimulated protocol and conventional hormone replacement treatment among patients with polycystic ovary syndrome (PCOS).
⇒ Only women with PCOS who undergo the first frozen embryo transfer and have a freeze-all cycle are included and they are randomised in variable block sizes and stratified by study site, which is intended to result in good balance and comparability between the two groups of patients.
⇒ Participants will be followed up to the postpartum period, which will allow the comparison of the effectiveness and safety of the two endometrial preparation protocols to facilitate a more comprehensive evaluation of the pros and cons of the two protocols.
⇒ Neither study participants nor investigators will be blinded, which could potentially introduce bias.
⇒ The sample size was determined on the basis of the primary outcome, and the study has limited power with respect to assessment of the secondary outcomes.

INTRODUCTION

Frozen embryo transfer (FET) has increased dramatically over the past decade owing to improvement of vitrification technique and wide-spread adoption of single-embryo transfer. Compared with fresh embryo transfer, FET can decrease the risk of ovarian hyperstimulation syndrome (OHSS), and increase the live birth rate (LBR), especially in polycystic ovary syndrome (PCOS)/hyper-response populations.[1] Endometrial preparation is the key to the success of FET. There are different protocols for endometrial preparation: natural cycle, hormone replacement treatment (HRT) cycle and stimulation cycle.

Current studies comparing several endometrial preparation protocols have primarily focused on women with regular ovulation and found there is no superiority of any protocol for endometrial preparation over another one in terms of reproductive outcomes.[2] Little interest has been paid to investigate the optimal protocol for women with ovulatory dysfunction, such as PCOS.

Women with PCOS usually have irregular menstruation or oligoanovulation,[3] so the most frequently used cycle regimen for women with PCOS is HRT. In HRT cycles, the endometrium is prepared with programmed oestrogen and progesterone (P), so it is convenient to plan the time of embryo transfer. However, once pregnancy is established, exogenous oestrogen and P cannot be withdrawn until the placenta is formed to substitute for the absent corpus luteum. Recent findings have shown that HRT cycles are associated with a significantly higher risk of hypertensive disorders of pregnancy, pre-eclampsia, postpartum haemorrhage and caesarean section when compared with natural cycle protocols in which a corpus luteum is present.[4] In addition, long-term use of exogenous oestrogen may increase the risk of thromboembolic events.[5]

Letrozole, a third-generation aromatase inhibitor, can inhibit aromatase to reduce oestrogen production.[6] The hypo-oestrogenic state releases the hypothalamic–pituitary axis from oestrogenic negative feedback, which, in turn, increases follicle-stimulating hormone secretion and ovarian follicle development. As letrozole does not antagonise oestrogen receptors and maintains normal central feedback, it generally leads to mono-ovulatory cycles, which can reduce the risk of OHSS.[7] Additionally, letrozole has a short half-life of about 48 hours, so oestrogen-targeted tissues (eg, the endometrium and cervix) are potentially spared from adverse effects.[7 8] Legro and Zhang[9] found that compared with clomiphene, letrozole was associated with higher live birth and ovulation rates among infertile women with PCOS. In 2018, the International Evidence-based Guideline recommended that letrozole should be considered a first-line pharmacological treatment for ovulation induction in women with PCOS with anovulatory infertility.[10] Letrozole has shown a good effect in the controlled ovarian hyperstimulation of infertile women with PCOS, but it is still less frequently used in FET. Currently, available literature investigating the use of letrozole in FET for women with PCOS has mainly focused on Asian populations.[11]Only a few retrospective studies and a small-sample randomised controlled trial (RCT) have compared the effects of the letrozole-stimulated protocol and HRT.[11] Limited research suggests that in patients with PCOS undergoing FET, the letrozole-stimulated protocol may be associated with a higher LBR and lower miscarriage rate than HRT.[11 12]

We hypothesised that letrozole would inhibit oestrogen production, resulting in a letrozole-stimulated cycle hypo-oestrogenic and hyperprogestin state,[9] which may improve the LBR of FET in patients with PCOS. However, there is still a lack of sufficient evidence to support this hypothesis. Therefore, we designed a multicentre RCT to compare the effectiveness of the letrozole-stimulated protocol and HRT, with the primary outcome being live birth. In addition, considering that HRT may increase the risk of HDP and individual studies have doubts about the safety of letrozole,[13] we will compare the safety of the two endometrial preparation protocols simultaneously (including maternal complications and congenital malformations).

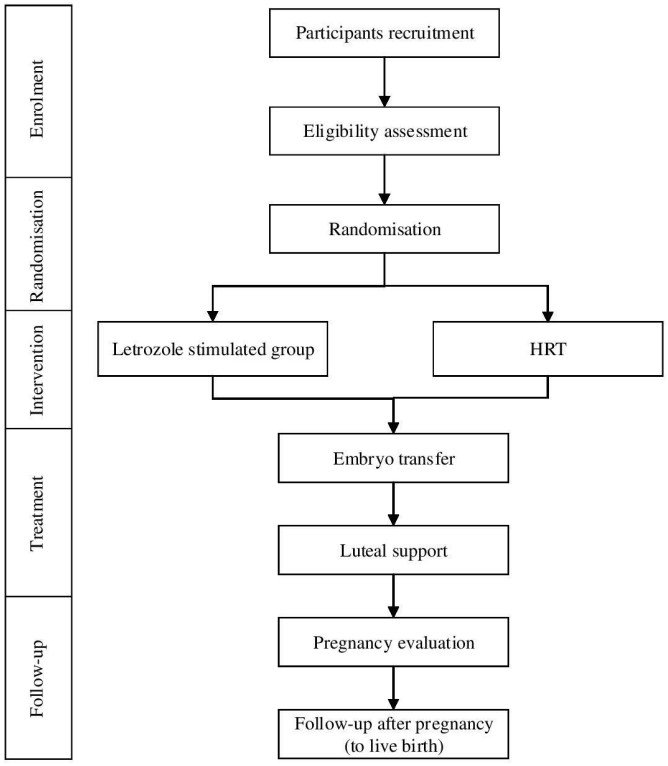

Figure 1 Study flow chart. HRT, hormonal replacement treatment.

METHODS AND ANALYSIS

Study design

This multicentre RCT aims to compare the effectiveness and safety of the letrozole-stimulated protocol versus HRT for the first FET in infertile women with PCOS. Women will be randomised (1:1) to the letrozole-stimulated or HRT group in their first FET cycle, and their pregnancy and perinatal outcomes during this cycle will be followed up and analysed. The flow chart will follow the Standard Protocol Items: Recommendations for Interventional Trials checklist, as shown in figure 1. The schedule of enrolment, randomisation, intervention, treatment and follow-up are shown in online supplemental table 1.

Study setting

Participants will be enrolled at four hospitals located in four provinces of China: the Reproductive and Genetic Hospital of CITIC-Xiangya, Henan Provincial People's Hospital, The First People's Hospital of Yunnan Province

and Guizhou Provincial People's Hospital. An independent data and safety monitoring board (DSMB), including members with clinical, statistical and ethical expertise, will monitor the trial progress and interim results at regular intervals.

Eligibility criteria

Eligible patients will need to meet all of the following inclusion criteria:

1. Women diagnosed with PCOS according to the modified Rotterdam criteria.[14]
2. Women undergoing their first controlled ovarian hyperstimulation cycle of in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI).
3. Women who received either the gonadotropin-releasing hormone antagonist protocol or long agonist protocol as their controlled ovarian hyperstimulation treatment.
4. Women with a freeze-all cycle.
5. Women aged 20–38 years.
6. Women with a body mass index of 18–30 kg/m^2.
7. Women with at least one good-quality embryo suitable for transfer, including day 3 cleavage stage embryos with grade 7CI/8CI or day 5/6 blastocysts of grade 4BB or higher.

The modified Rotterdam criteria[14] are menstrual abnormalities (irregular uterine bleeding, oligomenorrhoea or amenorrhoea) with hyperandrogenism and/or polycystic ovaries. Hyperandrogenism will be diagnosed as hirsutism and/or hyperandrogenaemia. Polycystic ovaries will be defined as the presence of either an ovary containing ≥12 antral follicles measuring 2–9 mm in diameter and/or increased ovarian volume (>10 cm^3)[15] on transvaginal ultrasonographic scanning. Other causes of hyperandrogenism and ovulation dysfunction, including tumours, congenital adrenal hyperplasia, hyperprolactinaemia and thyroid dysfunction, will be excluded before PCOS diagnosis.

Women will be excluded if they meet any of the following exclusion criteria:

1. Women with a history of recurrent spontaneous abortion.
2. Women with unilateral/bilateral oophorectomy.
3. Women with untreated hydrosalpinx.
4. Women with a uterine cavity abnormality, for example, a uterine congenital malformation, untreated uterine septum (except partial septum uterus), adenomyosis, submucosal myoma or moderate-to-severe intrauterine adhesions.
5. Women with uncontrolled diabetes mellitus, thyroid disease and hypertension.
6. Women undergoing preimplantation genetic test.

Recruitment

All infertile women with PCOS will be screened after they decide to freeze all embryos. If eligible, the investigator will explain the details of the study to the patients and allow them sufficient time to consider whether or not to participate in the trial. Couples/women who agree to participate will be asked to sign an informed consent form. Those who do not participate in this trial will undergo assisted reproductive treatment according to the routine clinical procedures, and the reasons for refusal or exclusion will be recorded separately. After completing the relevant examinations and prior to endometrial preparation treatment (or randomisation), the investigators will evaluate the inclusion and exclusion criteria for patients who have signed the informed consent form. Ineligible patients will be further excluded, and the reasons for exclusion will be registered.

Randomisation

On days 3–5 of the FET cycle, when women will have been confirmed as eligible to participate in the trial and endometrial preparation can be started, authorised investigators will log into the central randomisation system (an interactive web response system (IWRS)) using user unique identifications and passwords to allocate groups for the eligible patients. An independent statistician will prepare the computer-generated randomisation scheme in a 1:1 ratio between the two arms (letrozole-stimulated and HRT groups). Permuted block sizes of 2, 4 and 6 will be used for randomisation. The randomisation scheme will be stratified by the study site. The allocation table will be uploaded to the IWRS by an independent statistician and computer professionals, and concealed from the clinical staff performing the randomisation.

Blinding

This study is an open-label trial. Participants, investigators, date collectors and analysts will not be blinded to the group assignment.

Interventions

Preparation of the endometrium

On days 3–5 of a natural or P-induced menstrual cycle,[16] ultrasonography and E_2 and P measurements will be performed. Endometrial preparation can be started if it is confirmed that there are no follicles >10 mm, the endometrium thickness is ≤5 mm, E_2 level is <80 pg/mL and P level is <1 ng/mL.

In the letrozole-stimulated group, letrozole (2.5 mg/day, Zhejiang Hisun Pharmaceutical, Zhejiang, China) will be administered for five consecutive days from days 3 to 5 of a natural or P-induced menstrual cycle. Ultrasound monitoring and serum hormone measurements will be performed from cycle days 12–13 onwards.

1. If the leading follicle reaches a diameter of ≥12 mm on cycle days 12–13, transvaginal ultrasonography will be repeated every 2 days. When the dominant follicle reaches a diameter of ≥16 mm, ultrasonography will be performed daily until ovulation. When the diameter of the dominant follicle is ≥18–20 mm without ovulation and the endometrium thickness is ≥8 mm, the serum E2, luteinising hormone (LH) and P levels will be measured, and if the P level is <1 ng/mL, 5000–10 000 IU

human chorionic gonadotropin (HCG) ampules will be injected intramuscularly for the final oocyte triggering. Luteal support (10 mg, two times per day; Duphaston, Abbott Biologicals B.V., Abbott Park, Illinois, USA) will be initiated on the day of ovulation and continued for 55 days after embryo transfer if pregnancy occurs. For luteinised unruptured follicles, the day when the P level is ≥1.5 ng/mL after 48 hours of injecting HCG or the day when the follicular diameter is ≥18–20 mm, urine LH level is +/±, and P level is ≥1.5 ng/mL will be assumed to be the ovulation day. Oral dedrogesterone tablets (10 mg two times per day; Duphaston, Abbott Biologicals B.V.) will be taken from ovulation day until 55 days after embryo transfer.

2. If the diameter of the dominant follicle is <12 mm on days 12–13, the serum E_2, LH and P levels will be measured, and if the P level is <1 ng/mL, a daily dose of 75 IU human menopausal gonadotropin (HMG) will be supplemented to stimulate follicle growth. Transvaginal ultrasonography will be performed after using HMG for 3–5 days. If a dominant follicle is present, HMG will be used continuously until the day of HCG; if there is still no dominant follicle but the thickness of the endometrium reaches ≥8 mm and the P level is <1 ng/mL, HMG will be replaced with HRT to transform the endometrium.

In the HRT group, oral E_2 valerate tablets (3 mg two times per day, Progynova; Bayer Schering Pharma, Berlin, Germany) will be administered on days 3–5 of the natural or P-induced menstrual cycle. Ten days later, ultrasonography will be conducted to measure the endometrial thickness and ensure that no dominant follicle has emerged. Additionally, serum E_2, LH and P measurements will be performed. When the endometrial thickness reaches ≥8 mm with a P level <1 ng/mL, P vaginal suppositories (200 mg, three times daily; Utrogestan, Besins Healthcare, Paris, France) and oral dydrogesterone tablets (10 mg two times per day; Duphaston, Abbott Biologicals B.V.) will be initiated. Clinical pregnancy will be detected approximately 28 days after embryo transfer. If clinical pregnancy is confirmed, the E_2 valerate dose will be reduced to 4 mg/day, and reduced again to 2 mg/day at 45 days after embryo transfer until the drug is discontinued at 55 days after embryo transfer. The combination of Utrogestan and Duphaston will be used until 45 days after embryo transfer, and Duphaston alone will be used until 55 days after embryo transfer.

Embryo vitrification, thawing and transfer

All embryos will be graded before freezing. On day 3, embryos will be scored using the Puissant criterion, and blastocysts will be graded according to the Gardner and Schoolcraft system on days 5/6/7. All embryos will be vitrified and thawed using a Kitazato vitrification kit (Kitazato Biopharma, Shizuoka, Japan) in combination with a closed high-security vitrification straw (Cryo Bio System, L'Aigle, France). The embryos will be thawed on the day of transfer. Thawed embryos will be prioritised based on their best quality before freezing. The thawed embryos will be transferred to G 2.5 medium and cultured for 2–6 hours. Embryos will be considered to have survived and be suitable for transfer when half or more of the blastomeres are recovered or the blastocyst re-expanded.

In the letrozole-stimulated and HRT groups, two cleavage-stage embryos (at least one embryo graded as 8CI/7CI) or one top-quality blastocyst (≥4BB) will be recommended for transfer. In the letrozole-stimulated group, the day of ovulation will be day 0, cleavage-stage embryos will be transferred on day 3, and blastocysts will be transferred on day 5. In the HRT group, the day of P supplementation will be considered as p+0, cleavage-stage embryos will be transferred on p+3 and blastocysts will be transferred on p+5.[17]

Criteria for cycle cancellation

A letrozole-stimulated cycle will be cancelled if any of the following criteria are met.

1. More than three dominant follicles (follicle diameter ≥14 mm).
2. Endometrial thickness <8 mm on the day before transfer.

An HRT cycle will be cancelled if any of the following criteria are met.

1. P level >1 ng/mL on the day of luteal support.
2. Endometrial thickness <8 mm on the day before transfer.

Pregnancy evaluation

Approximately 14 days after embryo transfer, serum β-hCG levels will be measured to determine biochemical pregnancy. If a biochemical pregnancy has been achieved, a transvaginal ultrasonography will be performed to evaluate the clinical pregnancy approximately 28 days after embryo transfer. Approximately 70 days after embryo transfer, ultrasonography will be repeated to confirm ongoing pregnancy.

During each visit, the women will also be asked whether they have experienced any adverse reactions after embryo transfer, such as vaginal bleeding, lower abdominal pain or gastrointestinal discomfort, as well as if the women are taking drugs.

Follow-up after pregnancy

At 12–14 weeks gestation, the first-trimester pregnancy complications (miscarriage and ectopic pregnancy) will be collected via medical records or follow-up telephone calls for participants examined at other hospitals.

At 28–30 weeks gestation, second-trimester pregnancy complications (abortion, prenatal diagnosis test results, gestational diabetes, gestational hypertension/pre-eclampsia/eclampsia, placenta previa and intrauterine growth retardation) will be followed up by telephone.

At 42–44 weeks gestation, the final follow-up visit will be conducted by telephone to collect the third-trimester pregnancy complications (preterm labour, placental abruption, placenta accreta, placenta previa, gestational

diabetes, gestational hypertension/pre-eclampsia/eclampsia, intrauterine growth retardation, premature rupture of membrane and/or abnormality of amniotic fluid), delivery information (gestational age, the number of live births, delivery mode and/or delivery complications) and infant information (sex, birth weight, birth defect, etc). If necessary, the woman's medical records will be requested to be sent online to check the information.

All follow-up records will be recorded in the electronic data capture (EDC) system.

Outcomes

Primary outcome

The primary outcome is live birth, which will be defined as the delivery of any viable infant at 28 weeks or longer.

Secondary outcomes

Secondary effectiveness outcomes are cycle cancellation rate, biochemical pregnancy, clinical pregnancy and neonatal birth weight. Cycle cancellation rate refers to the number of women who initiated endometrial preparation without embryo transfer divided by the number of women randomised to the specific group. Biochemical pregnancy will be defined as a serum β-hCG level ≥10 IU/L measured approximately 14 days after embryo transfer. Clinical pregnancy will be defined as the presence of at least one gestational sac in the uterine cavity on ultrasonography at approximately 28 days after embryo transfer. Birth weight will refer to the weight of the newborn at birth.

Safety outcomes are miscarriage, ectopic pregnancy, obstetric and perinatal complications, and neonatal complications. Miscarriage will be defined as the spontaneous loss of an intrauterine pregnancy prior to 22 weeks of gestational age. Ectopic pregnancy will be defined as implantation outside the uterine cavity, as confirmed by sonography or laparoscopy. Important complications will be defined as follows: HDP will be defined as the development of blood pressure >140/90 mm Hg after pregnancy with or without proteinuria or other signs of pre-eclampsia, including pre-eclampsia and gestational hypertension and excludes chronic hypertension; gestational diabetes mellitus will be defined as carbohydrate intolerance of variable severity with onset or first recognition during pregnancy as determined from the diagnosis in the obstetrical medical record; postpartum haemorrhage will be defined as the loss of 500 mL of blood or more after completion of the third stage of labour; preterm birth will be defined as delivery of a fetus at less than 37 and more than 28 weeks' gestational age; stillbirth will be defined as death of a child born at a gestational age of ≥20 weeks or weighing ≥500 g; small gestational age will refer to birth weight below the 10th percentile for gestational age, and large gestational age will refer to birth weight above the 90th percentile for gestational age, the reference of birth weight for gestational age and neonatal gender will be based on the Chinese population.[18 19]

Statistical considerations

Sample size

To date, the largest study comparing the endometrial preparation protocols in patients with PCOS is the retrospective cohort study conducted by Zhang *et al*,[20] which analysed 2664 FET cycles of PCOS and found that the LBRs of the first FET were 50.7% in HRT cycles and 54.4% in letrozole-stimulated cycles. The LBR was significantly higher in letrozole-stimulated cycles than in HRT cycles (adjusted OR 1.33, 95% CI 1.09 to 1.61) after adjusting for possible confounding factors. Additionally, we summarised the data of CITIC-Xiangya from August 2019 to March 2020, including 493 HRT cycles and 91 letrozole-stimulated cycles, and the LBRs of the two groups were 50% and 64%, respectively, after propensity score matching.

Based on the aforementioned results, we assumed that the HRT cycles would achieve an LBR of 50%. To detect an absolute difference in LBRs of 10% (anticipated LBR of 50% in the HRT group vs 60% in the letrozole-stimulated group), with a power of 80% and an alpha error of 0.05, 916 women will need to be included. The ratio between the groups will be 1:1. Considering a drop-out rate of 15% between randomisation and follow-up, we plan to include 1078 women.

Statistical analysis

All statistical analyses will be performed according to the intention-to-treat principle. Categorical data will be described as frequency and percentage; between-group differences will be assessed by χ^2 analysis, with use of the Fisher's exact test for expected frequencies <5. Continuous data will be presented as mean (±SD), and differences between the study groups will be analysed using the Student's t-test for normally distributed data or Wilcoxon rank-sum test for non-normally distributed data. Pregnancy outcomes will be compared by calculating the relative risks with the corresponding 95% CIs. A per-protocol analysis will be conducted as a secondary analysis in participants who comply with the protocol (if necessary). For issues such as lost to follow-up, missing data and protocol violations, we will perform sensitivity analyses to explore the effect of these factors on the findings. The Haybittle-Peto boundary will be used for the interim analysis.

All tests will be two tailed, and differences with $p<0.05$ for final analysis are considered statistically significant. Statistical analyses will be performed using SPSS Statistics for Windows (V.24.0; IBM).

Data collection and management

An EDC will be used to record and deposit the study data. The data managers will take responsibility for managing the user's accounts of each subcentre and assign jurisdiction to the users. Data entry personnel will be responsible for the entry and correction of study data, which will mainly be derived from medical records and postpregnancy telephone follow-up records. To ensure the accuracy of the input data, a three-level data quality control

will be conducted. The first level will be real-time logical and range checking built into the EDC. The second will be remote data monitoring and validation conducted by the EDC data manager and programmer. Comprehensive data checks can identify more complicated and less common errors. The third level of quality control will be site visits conducted by the clinical research associate, where data in the EDC will be compared against data from source documents. Identified errors will be flagged, and data entry personnel or investigators will be notified to verify and correct errors. All participant-identifiable data, such as consent forms, screening and identification records, will be stored in site files and accessible only to delegated members of the study team.

All adverse events will be recorded and reported to the DSMB. The DSMB will perform an interim analysis 3 months after the first 400 randomised participants have completed embryo transfer. The DSMB will do so by using ongoing pregnancy as an endpoint, as data on live births will not be available. In addition, the DSMB will oversee all severe adverse events that have occurred.

Patient and public involvement

None.

ETHICS AND DISSEMINATION

This study has been approved by the Institutional Review Board of Reproductive and Genetic Hospital of CITIC-XIANGYA (approval number LL-SC-2022-001). Written informed consent will be obtained from each patient before any study procedure is performed (see online supplemental file for the informed consent form). The trial is registered with ClinicalTrial.gov (NCT05227391).

The researchers will permit trial-related monitoring, audits, regulatory inspections and direct access to the source data and documents. The findings of this study will be presented at national and international conferences and published in peer-reviewed scientific journals.

Trial status

Recruitment to the first study centre started in March 2022. The estimated end date of the last recruitment in this study is June 2024.

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Contributors FG, GL, YL and XW contributed to the study conception and design, as well as supervised the study. FG, YL, CZ, YRF, BD, SZ, YM and YW participate in recruitment of participants and assessment of clinical outcomes. XW and YL coordinates of the data collection and performed data analysis. The draft of the manuscript was written by XW and YL. All authors read and approved the manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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REFERENCES

1 Roque M, Haahr T, Geber S, *et al*. Fresh versus elective frozen embryo transfer in IVF/ICSI cycles: a systematic review and meta-analysis of reproductive outcomes. *Hum Reprod Update* 2019;25:2–14.
2 Glujovsky D, Pesce R, Sueldo C, *et al*. Endometrial preparation for women undergoing embryo transfer with frozen embryos or embryos derived from donor oocytes. *Cochrane Database Syst Rev* 2020;10:CD006359.
3 Escobar-Morreale HF. Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. *Nat Rev Endocrinol* 2018;14:270–84.
4 Busnelli A, Schirripa I, Fedele F, *et al*. Obstetric and perinatal outcomes following programmed compared to natural frozen-thawed embryo transfer cycles: a systematic review and meta-analysis. *Hum Reprod* 2022;37:1619–41.
5 Vinogradova Y, Coupland C, Hippisley-Cox J. Use of hormone replacement therapy and risk of venous thromboembolism: nested case-control studies using the QResearch and CPRD databases. *BMJ* 2019;364:k4810.
6 Cole PA, Robinson CH. Mechanism and inhibition of cytochrome P-450 Aromatase. *J Med Chem* 1990;33:2933–42.
7 Palomba S. Aromatase inhibitors for ovulation induction. *J Clin Endocrinol Metab* 2015;100:1742–7.
8 Jirge PR, Patil RS. Comparison of endocrine and ultrasound profiles during ovulation induction with clomiphene citrate and letrozole in ovulatory volunteer women. *Fertil Steril* 2010;93:174–83.
9 Legro RS, Zhang H, Eunice Kennedy Shriver NICHD Reproductive Medicine Network. Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. *N Engl J Med* 2014;371:1463–4.
10 Teede HJ, Misso ML, Costello MF, *et al*. Recommendations from the International evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Hum Reprod* 2018;33:1602–18.
11 Zhang Y, Wu L, Li TC, *et al*. 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 Biol Endocrinol* 2022;20:62.
12 Zhang J, Liu H, Wang Y, *et al*. Letrozole use during frozen embryo transfer cycles in women with polycystic ovary syndrome. *Fertil Steril* 2019;112:371–7.
13 Biljan MM, Hemmings R, Brassard N. The outcome of 150 babies following the treatment with Letrozole or Letrozole and Gonadotropins. *Fertility and Sterility* 2005;84:S95.
14 Consensus on diagnostic criteria and longterm health risks related to polycystic ovary syndrome. *Fertility and Sterility* 2004;81:19–25.
15 Balen AH, Laven JSE, Tan S-L, *et al*. Ultrasound assessment of the polycystic ovary: International consensus definitions. *Hum Reprod Update* 2003;9:505–14.
16 ACOG committee opinion no. 738: Aromatase inhibitors in gynecologic practice. *Obstet Gynecol* 2018;131:1.
17 Mackens S, Santos-Ribeiro S, van de Vijver A, *et al*. Frozen embryo transfer: a review on the optimal endometrial preparation and timing. *Hum Reprod* 2017;32:2234–42.
18 Dai L, Deng C, Li Y, *et al*. Population-based birth weight reference percentiles for Chinese twins. *Ann Med* 2017;49:470–8.
19 Dai L, Deng C, Li Y, *et al*. Birth weight reference percentiles for Chinese. *PLoS One* 2014;9:e104779.
20 Zhang J, Liu H, Wang Y, *et al*. Letrozole use during frozen embryo transfer cycles in women with polycystic ovary syndrome. *Fertility and Sterility* 2019;112:371–7.