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Comparison of endometrial preparation protocols (natural cycle versus hormone replacement cycle) for frozen embryo transfer (COMPETE): a study protocol for a randomised controlled trial

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ABSTRACT

Introduction Natural cycle (NC) and hormone replacement treatment (HT) are frequently used endometrial preparation protocols prior to frozen-thawed embryo transfer in ovulatory women. It is not clear which protocol results in a higher live birth rate. It has been suggested that there is an increased risk in maternal and perinatal morbidity following HT protocol due to the lack of corpus luteum. The objective of this trial is to compare the clinical outcomes of NC and HT protocols in frozen embryo transfer.

Methods and analysis COMPETE is an open-label, single-centre, randomised controlled trial targeting to recruit 888 women, with 444 women each in two arms (1:1 treatment ratio). Women undergoing in vitro fertilisation scheduled for a frozen embryo transfer and have a regular menstrual cycle are eligible. Exclusion criteria include ovulation disorders and intrauterine adhesions. The primary outcome is live birth resulting from the first frozen embryo transfer after randomisation. Secondary outcomes include biochemical pregnancy, clinical pregnancy, multiple pregnancy, ongoing pregnancy, miscarriage, endometrial thickness, cycle cancellation, gestational diabetes mellitus, hypertensive disorders of pregnancy, antepartum haemorrhage, preterm birth, birth weight, large for gestational age, congenital anomaly and perinatal mortality. The data analysis will be following the intention-to-treat principle.

Ethics and dissemination This study has been approved by the Institutional Review Board of Northwest women's and children's hospital (2020008). Written informed consent will be obtained from each participant before randomisation. The results of the trial will be presented via publications.

Trial registration number ChiCTR2000040640.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is the largest randomised controlled trial to date to examine the clinical outcomes between natural cycle and hormone replacement treatment for frozen-thawed embryo transfer.
⇒ This study was performed in a single centre, therefore avoiding different in vitro fertilisation protocols and embryos criteria.
⇒ A limitation is that this trial protocol only targets infertile women with ovulatory.

INTRODUCTION

Frozen-thawed embryo transfer (FET) has been widely used in vitro fertilisation (IVF) because it prevents ovarian hyperstimulation syndrome, facilitates single embryo transfer and improves fertility and pregnancy outcomes.[1] The synchronisation of embryo and endometrium development plays an important role in implantation.[2] Endometrial preparation is of utmost importance for FET to optimise pregnancy rates. At present, the commonly used FET endometrial preparation protocols include the natural cycle (NC) and the hormone replacement treatment (HT) cycle. NC involves a dominant follicle matures and produces estradiol, which leads to endometrium thickening. In contrast, exogenous estradiol and progesterone were administered to prepare endometrium development in HT.

It remains inconclusive regarding which endometrial preparation protocol is more favourable in terms of the live birth rate and maternal and perinatal outcomes. A systematic review and meta-analysis concluded that it is not possible to identify one method of endometrium preparation in FET as being more effective than another based on the current literature.[3] HT requires medication, and in theory, it might be less physiological than a natural ovulatory cycle. In addition, recent studies have suggested that the

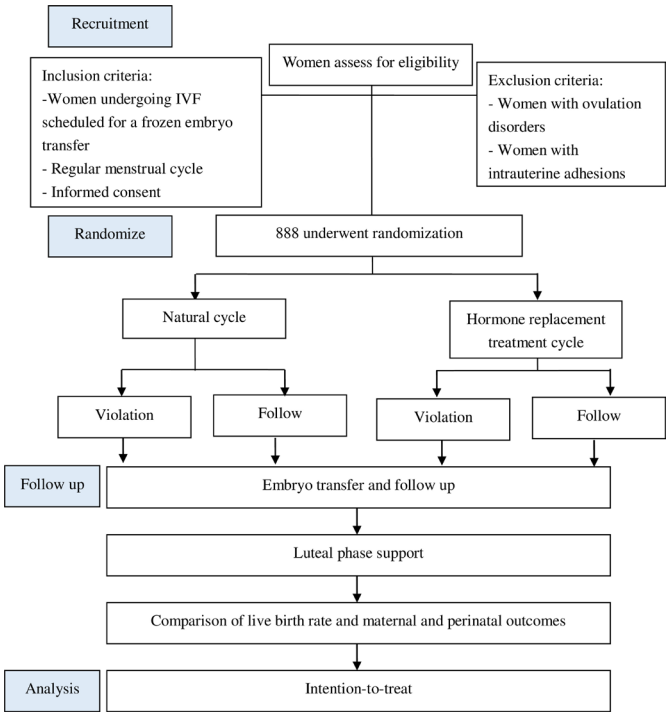

Figure 1 Flow chart of study cohort. IVF, in vitro fertilisation.

absence of the corpus luteum in HT is responsible for the increased risk of maternal complications such as hypertensive disorders in pregnancy.[4]

In our retrospective cohort study comparing NC and HT in young women with regular menstrual cycles, we found that NC has a higher live birth rate and lower miscarriage rate than HT.[5] There are few randomised controlled studies comparing these two protocols in women with regular menstrual cycles. One open-label, single-centre, randomised controlled trial (RCT) compared natural and downregulated HT, and found women with ovulatory cycles had similar live birth rates between the two groups.[6] However, this RCT only included 159 patients and lacks power to draw firm conclusions. Another RCT randomised women into four groups of endometrial preparation: NC with or without human chorionic gonadotropin (hCG), HT with or without pretreatment using gonadotropin releasing hormone agonist, and no significant differences were found for pregnancy, miscarriage and live birth between the four groups.[7] This study was also underpowered and suffered from incomplete reporting of the study design and method of randomisation.

Therefore, we have developed this large randomised controlled trial with adequate power to investigate if NC leads to a higher live birth rate than HT as endometrial preparation protocol in FET.

METHODS AND ANALYSIS

Study setting

This study protocol describes the design of a single-centre, parallel- group RCT (1:1 ratio) conducted at Northwest women's and children's hospital in China. The RCT will conform to the Consolidated Standards of Reporting Trials statement for reporting RCTs.[8] The assisted reproduction centre, Northwest women's and children's hospital (China) will recruit participants for the study. The study is registered at www.chictr.org.cn (ChiCTR2000040640) in December 2020. Participant enrolment started in December 2020 and is expected to be completed in December 2022. This study has been approved by the Institutional Review Board of Northwest women's and children's hospital (2020008). Informed consent will be obtained from each patient before any study procedure. The study flow chart is shown in figure 1.

Eligibility criteria

Eligible participants will need to fulfil the following inclusion criteria and none of the exclusion criteria.

Inclusion criteria

- Women undergoing IVF scheduled for a frozen embryo transfer.
- Regular menstrual cycle.
- Informed consent.

Exclusion criteria:

- Women with ovulation disorders.
- Women with intrauterine adhesions.

Sample size estimation

According to our retrospective cohort study,[5] live birth rates in the HT group were 55.1% in our centre. Based on studies within fertility care as well as the discussion by gynaecologist and epidemiologists, we assumed that the minimal clinical important difference to make NC preferable over HT for FET would be 10%. Therefore, we need to include 370 women in each group with two-sided test, 5.0% alpha error and 80% statistical power. Assuming a 20% drop-out rate, this requires 444 participants in each group. The ratio between intervention and control groups will be 1:1.

Randomisation and blinding

Women fulfilling the eligibility criteria and willing to participate will be randomly allocated to one of the two arms (NC or HT) in a ratio of 1:1 on menstrual cycle day 5. Computer-generated random numbers are prepared by an independent statistician. Simple randomisation is centrally controlled by using a web-based electronic data capture (ResMan). Both the investigators and patients will be aware of the allocation. Blinding of intervention is impossible for women and doctors administering the intervention. To minimise bias, embryologists and doctors involved in the embryo transfer are blinded to the group assignments of the participants in the trial.

Endometrial preparation protocols

Natural cycle

Women in the NC group will undergo transvaginal ultrasound from day 5 of the menstrual cycle. Follicular growth will be monitored through transvaginal ultrasound and serum luteinising hormone (LH). When LH >20 IU/L, transvaginal ultrasound will be performed daily until

ovulation. If the leading follicle reaches a mean diameter of >17 mm while LH <20 IU/L, 10 000 IU of hCG will be administered to trigger oocytes' ovulation. When the ovulation is confirmed by transvaginal ultrasound, 200 mg of vaginal micronised progesterone will be initiated three times a day and continued for 3 days for cleavage stage embryos and 5 days for blastocyst stage embryos. Thereafter, progesterone support will continue and oral dydrogesterone 10 mg three times a day and continued until 10 weeks of gestation.

HT cycle

Endometrial preparation will be initiated with oral estradiol valerate (Progynova; Bayer Schering Pharma AG, Berlin, Germany) at a daily dose of 6 mg from day 5 of menstrual cycle. A transvaginal ultrasound and serum progesterone level will be performed 10–12 days later. Provided the endometrial thickness reaches 7 mm or more and p<1.5 ng/mL, 200 mg of vaginal micronised progesterone will be initiated three times a day and continued for 4 days for cleavage stage embryos and 5 days for blastocyst stage embryos. Thereafter, progesterone support will continue and oral dydrogesterone 10 mg three times a day and continued until 10 weeks of gestation. We use dydrogesterone because it has better affinity for the progesterone receptors, and oral dydrogesterone 30 mg daily is the most commonly used dose.[9] Progesterone production will shift from the corpora lutea towards the placenta between 7 and 9 weeks of gestation to maintain pregnancy.[10]

Embryo transfer

Embryo transfer could be performed either on day 3 (cleavage stage) or day 5/6 (blastocyst stage). The decision to transfer day 3 or day 5/6 embryos will be evaluated by the embryologists according to the Gardner's score of the embryos.[11] For couples with sufficient good quality embryos (more than 4) on day 3, blastocyst culture and transfer will be performed. To reduce the risk of high-order multiple pregnancies, the number of embryos replaced will be mostly limited to one or two best-quality embryos. In both groups, the best embryos will be transferred first. The embryologists are not aware of the allocation of the study.

Pregnancy confirmation

Serum ß-hCG levels are measured 14 days after embryo transfer. If serum ß-hCG is positive, luteal support is continued and an ultrasound is carried out 4–5 weeks later.

Outcome measurements

Primary outcome

The primary outcome will be live birth resulting from the first frozen embryo transfer after randomisation. Live birth is defined as a delivery of one or more living infants ≥28 week's gestation.

Secondary outcomes

To assess the effectiveness of the treatment, we will record these secondary outcomes in terms of effectiveness (from the first transfer after randomisation):

- Biochemical pregnancy: defined as serum level of ß-hCG>50 mIU/mL.
- Clinical pregnancy: defined as one or more observed gestational sac or definitive clinical signs of pregnancy under ultrasonography at 7 weeks after embryo transfer (including clinically documented ectopic pregnancy).
- Multiple pregnancy: defined as a pregnancy with two or more gestational sacs or positive heart beats at 7 weeks of gestation.
- Ongoing pregnancy: defined as the presence of a gestational sac and fetal heartbeat after 12 weeks of gestation.
- Miscarriage (pregnancy loss at <28 weeks).
- Endometrial thickness.
- Cycle cancellation: defined as cancellation of the cycle prior to embryo transfer.

In case of ongoing pregnancy, we will also collect the following obstetric and perinatal complications:

- Gestational diabetes mellitus .
- Hypertensive disorders of pregnancy (comprising pregnancy-induced hypertension; pre-eclampsia (PET, a hypertensive disorder of pregnancy predominantly occuring after 20-week gestation with either proteinuria or evidence of systemic involvement) and eclampsia).
- Antepartum haemorrhage, including placenta previa, placenta accreta and unexplained.
- Preterm birth: defined as birth of a fetus delivered after 28 and before 37 completed weeks of gestational age in participants confirmed ongoing pregnancy. We will also collect causes of preterm birth; that is, spontaneous and iatrogenic delivery.
- Birth weight, including low birth weight (defined as weight <2500 g at birth), very low birth weight (defined as <1500 g at birth), high birth weight (defined as >4000 g at birth) and very high birth weight (defined as >4500 g at birth).
- Large for gestational age (defined as birth weight >90th centile for gestation, based on standardised ethnicity-based charts) and small for gestational age (defined as less than 10th centile for gestational age at delivery based on standardised ethnicity-based charts); birthweight percentage.
- Congenital anomaly (any congenital anomaly will be included).
- Perinatal mortality: defined as fetal or neonatal death occurring during late pregnancy (at 24 completed weeks of gestational age and later), during childbirth or up to seven completed days after birth.

Patient and public involvement

Patients and the public are not involved in the process of the study. The participants will be informed of the

study results via peer-reviewed journals and conference presentations.

Withdrawal of individual participants

Participants have the right to withdraw from the trial at any time during the process. The decision to withdraw will neither affect their conventional clinical treatments nor their relationship with clinicians.

Statistical analysis

Baseline characteristics will be described by descriptive analysis, and the balance between the two arms will be assessed. For continuous variables, the normality test will be initially estimated using frequency histograms and the Shapiro test. If the parameters are non-normally distributed, their medians and IQRs will be reported. For categorical variables, we will present the proportions of the two arms. In addition, we will also report the numbers of recruitment, participants lost to follow-up, protocols violation and other relevant descriptive data.

Primary outcome

Data analysis will follow the intention-to-treat principle. We will include all randomised women in the primary comparison between the two arms. Per-protocol analysis may be conducted as a secondary analysis. The primary outcome, live birth rate, will be compared between the two arms using Pearson's χ^2 test or Fisher's exact test for unadjusted analysis. We will also compute unadjusted risk ratio (RR) and its 95% CI. We will perform multivariable Poisson regression or log-bnomial model to compute adjusted RR and its 95% CI in the event of prominent imbalance of potential confounders between the two arms.

Secondary outcomes

Secondary outcomes will be compared between the two arms using the similar approach described for the primary outcome.

Subgroup analysis

Primary and secondary outcomes will be compared between the two arms within several clinically important subgroups including female age groups (<35/≥35 years), whether adopted freeze-all policy in fresh cycles, and embryo stages (cleavage/blastocyst) in which the effects on outcomes might be modified. Due to the concern over multiplicity of subgroup analysis, we will place limited importance on subgroup findings.

DISCUSSION

Endometrial preparation is one of the most important steps of FET. Synchronised development of the embryos and endometrium, and cross-talk between them are essential to embryo implantation.[12 13] The 'window of implantation' is the time when the endometrium is most able to support trophoblast-endometrial interactions, which is maintained for a limited period.[14] Previous studies suggested that high serum estradiol level could damage the endometrium and shorten the window of implantation and inhibit embryo implantation.[15] In addition, high progesterone level in the early third trimester is shown to be at high risk of later development of pre-eclampsia.[16]

NC without exposing to the risk of exogenous hormones is considered to be a safer and more natural way of endometrial preparation. The timing of embryo transfer is determined by LH surge or triggering ovulation with hCG.[3] However, women with NC require frequent monitoring for ovulation, while the risk of cycle cancellation is high. The HT protocol offers conveniences for women and doctors. However, it becomes evident that corpus luteum plays an important role in maternal cardiovascular adaptation to pregnancy.[17] Corpus luteum secrets not only estradiol and progesterone, but also relaxin, oxytocin, renin, aldosterone, vascular endothelial growth factor and other vasoactive compounds.[18] The excessive supplementation of estradiol and progesterone, together with absence of corpus luteum in the HT protocol, might contribute to an increased risk of adverse maternal and perinatal outcomes.

Despite the various experiences and doctors' preferences with endometrial preparation, there is a lack of evidence to support the superiority of one method over the other.[19] A Cochrane database included 18 RCTs and did not find sufficient evidence to support the use of one cycle regimen in preference to another.[20] A secondary analysis of a multicentre RCT demonstrated that the NC protocol yielded a higher implantation rate than the HT cycle.[21] However, the imbalance of the number of women between the two groups and restricted inclusion criteria (age <35 years) make the results hard to be extrapolated to the general population. One register-based cohort study demonstrated that obstetric and perinatal outcomes are adversely affected in HT.[22] However, this study did not adjust for some covariates due to missing data. Therefore, a properly sized RCT of FET in NC versus HT cycle is warranted.

The COMPETE randomised trial attempts to establish the optimum endometrial preparation protocol for ovulatory women. Our findings may contribute to developing clinical guidelines for good clinical practice of FET protocols.

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Contributors XL designed the study and write the manuscript. WW, TaoW, TS, TingW, NZ, DP, HC, JX, XLiu, ZS, RW, XLi, NL, RP, LT, BM, HB, HZ recruited patients, PQ, DZ, BWM and WL contributed to the design, revised the manuscript and edited language. JS conceived and designed the study.

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Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

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