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Title

In vitro maturation versus standard *In vitro* fertilisation for women with polycystic ovary syndrome: protocol for a randomised controlled trial

Authors

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Abstract

Introduction： *In vitro* maturation (IVM) of human oocytes is an emerging treatment option in infertile women with PCOS. It is a patient-friendly intervention, avoiding the risk of ovarian hyperstimulation syndrome (OHSS), which is a serious complication of controlled ovarian stimulation (COS). However, there is no randomised controlled trials (RCTs）comparing IVM with the standard IVF. Therefore, we plan a RCT to compare the effectiveness and safety of IVM with the standard IVF with in women with PCOS.

Methods and analysis: This is a single-center, open-label, non-inferiority RCT. Infertile women with PCOS undergoing the first cycle of IVF will be recruited from a large reproductive medicine center in China. Eligible participants will be randomised to receive either IVM or the standard IVF in a 1:1 treatment ratio after informed consent. The primary outcome is ongoing pregnancy leading to live birth within 6 months of the first oocyte retrieval cycle after randomisation. The planned sample size is 350 (175 per group).

Ethics and dissemination: Ethical permission was acquired from the Ethics Committee of Peking University Third Hospital. The results will be issued to publications through scientific journals and conference reports.

Trial registration number: NCT03463772

Strengths and limitations of this study

- It's a randomised controlled trial with adequate sample size to evaluate the IVM treatment in infertile women with PCOS.
- This study will provide evidence on whether the experimental group (IVM) is noninferior to the standard IVF in terms of the live birth.
- To date, there is no recommended standardized IVM procedure. Only single IVM protocol used in this study may limiting the generalizability of the results.

Keywords: *In vitro* fertilisation; polycystic ovary syndrome (PCOS); *In vitro* maturation (IVM); Assisted reproductive technology.

Introduction

Polycystic ovary syndrome (PCOS) is the first common cause of anovulatory infertility and occurs in 5.6% in reproductive-aged women in the Chinese communities[1]. In anovulatory women with PCOS, the first-line treatments are lifestyle interventions and ovulation induction with letrozole or clomiphene citrate[2]. Laparoscopic ovarian drilling or ovarian induction with gonadotrophin (Gn) are considered as the second-line treatment options. *In vitro* fertilisation (IVF) is the third-line treatment and is recommended when previous interventions failed. In standard IVF treatment, women with PCOS are at high risk of ovarian hyperstimulation syndrome (OHSS), which is a serious and common iatrogenic complication of control ovarian stimulation. The incidence of mild OHSS is 20-33% within all IVF cycles[3] and much higher in women PCOS. Severe OHSS is defined by the presence of clinical evidence of ascites with severe abdominal pain and pleural effusion. A large amount of pleural and peritoneal effusion can lead to intravascular blood loss, blood concentration, blood hypercoagulability, hypovolemic shock, severe cardiopulmonary dysfunction, electrolyte imbalance, impaired liver and kidney function, thrombosis, and even life-threatening [4]. Therefore, seeking for an alternative treatment strategy, avoiding the risk of OHSS without compromising pregnancy outcomes is crucial for women with PCOS. *In vitro* maturation (IVM) has been introduced since 1990. Trounson[5] described the first delivery of a healthy baby with IVM technique in a woman with PCOS. From then on, immature oocyte retrieval followed by IVM had been used widely, resulting in the delivery of healthy infants worldwide[6-8]. It is becoming a rising reproductive technology where immature oocytes are collected from antral follicles, typically from unstimulated or minimally stimulated ovaries, then cultured, matured and fertilised *in vitro*[9]. Compared with standard IVF treatment, IVM was performed without ovarian stimulation, and thus preventing the occurrence of OHSS and reducing the costs.

To date, despite the publication of many studies of IVM technique, it is controversial about the effectiveness and safety issue of IVM treatment. Some studies have shown that the live birth rate was significantly lower in the IVM group than in the IVF group[10-12]. Now mature technology, gradually getting more research results indicated

comparable pregnancy outcome between IVM and IVF group[13 14]. So far, all data available have derived from observational studies and non-randomised clinical trials[15]. Given the unique advantages of IVM as safer, simpler and cheaper, we need further evidence from well-designed randomised controlled trials (RCTs) about the live birth rate before we draw the conclusion about the IVM results may be comparable to standard IVF, and clarify the indications and how to optimize treatment between IVM and standard IVF. Therefore, we plan a RCT to determine whether IVM is non-inferior to standard IVF for women with PCOS.

Methods and analysis

Study design

This is a single-center, non-inferiority RCT with 1:1 treatment ratio. The program for enrollment, interventions and evaluation during the study process is shown in Table 1. Figure 1 indicates a flowchart showing the registration, allocation, treatment and follow-up of participants.

Table 1. Schedule of enrollment, interventions, and assessments.

	Study Period							
	Enrollment	Allocation		Post-allocation				Close-out
Content	Screening & Baseline assessment	IVM & IVF Randomisation	Oocyte retrieval	Assessment of embryo	Embryo transfer	Evaluation of pregnancy	Follow-up of pregnancy	
Time point	T_0 -1 month	T_1 o month	T_2 10-14 days	T_3 1-3-5 days after opu	T_4 2-6 month	T_5 3-7 month	T_6 6-10 months	T_7 8-12 months
Enrollment								
Eligibility screen	×	×						
Informed consent	×							
Allocation		×						
Interventions								
IVM		×						
Standard IVF		×						
Assessments								
Baseline data	×							
Laboratory tests	×	×	×		×	×	×	×

Fertilisation				×				
Embryo quality				×				
Pregnancy tests						×		
Pregnancy outcomes							×	×
Fetus information							×	×
Neonate information							×	×
Safety assessment		×	×		×	×	×	×

Study setting

The trial was approved by the Ethics Committee of Peking University Third Hospital (2017sz-066). The study was registered on Clinicaltrials.gov (NCT03463772). Women with PCOS and infertility who underwent their first IVF attempt at Peking University third Hospital (Beijing, China) will be recruited. Potentially eligible women will be given information about the study during their first consultation. All the couples provided written informed consent before participation. The trial progress will be monitored by an independent data and safety monitoring board (DSMB) regularly.

Eligibility criteria

PCOS outpatient women with infertility who come to reproductive center of Peking University Third Hospital will be screened for eligibility by a research team.

Inclusion Criteria:

- Married Chinese women with infertility aged between 20 and 38 years.
- Women with PCOS according to the revised Rotterdam criteria, i.e. two of the three following features are present: 1) Oligo- and/or anovulation; 2) Clinical and/or biochemical signs of hyperandrogenism; 3) Polycystic ovaries. Exclusion of other possible related disorders: ovarian or adrenal androgen-secreting tumors, thyroid disease, hyperprolactinemia, nonclassical adrenal hyperplasia.[16]
- Women undergoing their first IVF/ICSI cycle. Voluntary participation and informed consent obtained.

Exclusion criteria:

- Couples with contraindication for IVF or ICSI.
- Couples receiving donor sperm or donor oocytes.
- Couples with indications or have plan to receive preimplantation genetic testing (PGT).
- Women with a male partner diagnosed with azoospermia.
- Either male partner or female partner with a known abnormal chromosome karyotype (chromosome polymorphisms was not included).
- Women who have undergone unilateral ovariectomy.

Participants can leave the study at any time during the process without any consequences for their clinical treatments.

Recruitment

Potentially eligible patients will be explained about the study during their first consultation. Then the patients will have time for fully consideration. Written informed consent will be acquired in their next

scheduled visit. To initiate the IVM or IVF treatment cycle in anovulatory cases, patients were administrated oral dydrogesterone (Duphaston, Abbott, OLST, Netherlands) 20 mg daily for 10-14 days/ Oral contraceptives Diane-35 (cyproterone acetate 2 mg, ethinylestradiol 35 mg, Bayer, and its generics) for 21 days. After the withdraw bleeding, eligible participants will be assessed again for the exclusion criteria on day 2 or day 3 following the onset of menstrual bleeding.

Randomisation and allocation concealment

Randomisation and allocation of qualified participants will be done on the day 2/3 of the menstrual cycle. Eligible participants will be allocated to IVM or IVF treatment according to a computer-generated randomisation list in a 1:1 ratio, with a variable block size of 4 or 6. Opaque sealed envelopes, with participant's screening order printed outside and randomised assigned group printed inside, will be numbered consecutively. Researchers will enroll the eligible participants after screening, then open each envelope in sequence corresponding with the screening order, and assign the participant into IVM or IVF group. Both investigators and participants will be aware of the allocation of the subsequent treatments.

Interventions

IVM protocol

No gonadotrophins (FSH, HMG or HCG) will be used in the IVM group. After randomisation, participants will visit the clinic on days 6–8 for a transvaginal ultrasound examination to exclude the development of a dominant follicle. Oocyte retrieval will be scheduled once the endometrium thickness reaches at least 6 mm and there is no appearance of dominant follicle (follicle diameter >10 mm). Transvaginal ultrasound–guided oocytes retrieval will be conducted with a single-lumen 19G aspiration needle (K-OPS-7035-REH-ET; Cook, Queensland, Australia) in 90 mmHg suction pressure. Follicular aspirates will be gathered and filtered through a cell strainer (Cell Strainer 352350, 70μm nylon; Falcon, MA,USA). Then the collected aspirates will be washed with pre-warmed Hepe's-HTF (CooperSurgical; Trumbull, CT, USA). The mixture will be examined carefully under a stereomicroscope to search for cumulus-oocyte complexes (COCs). All COCs will be transferred into IVM medium (IVM media kit; Sage, CT, USA) supplemented with 0.075 IU/ml FSH and 0.075 IU/ml LH (Menopur; Ferring, Kiel, Germany) in 5% CO_2 incubator at 37℃. All the COCs will be denuded of cumulus cells after 28-32 h of culture and evaluated the maturation process. The presence of a first polar body indicates that the oocyte enters metaphase II stage. All the metaphase II (MII) oocytes will be inseminated by means of intracytoplasmic sperm injection (ICSI).

IVF protocol

For participants in the IVF group, COS will be performed by a flexible protocol using gonadotrophin-releasing hormone antagonist (GnRH-ant) with recombinant FSH (rFSH) and recombinant human chorionic gonadotrophin (rhCG). Treatment will start on day 2/3 of the menstrual cycle. All participants will be subcutaneous injected rFSH (Gonal-F; Serono, Geneva, Switzerland) with 100-225IU/d initial dosage. Transvaginal ultrasound, serum luteinizing hormone (LH), serum estrogen (E2) and progesterone (P4) will be tested to monitor follicle growth. rFSH doses will be adjusted according to ovarian response. GnRH antagonist-cetrorelix (Cetrotide; Serono, Darmstadt, Germany) 0.25mg daily by subcutaneous injection will begin when at least one follicle has reached a diameter of 12mm which is usually between day 5 to day 8 of ovarian stimulation, until the trigger day (include the trigger day). After 2 or more follicles reach a diameter ≥17 mm, 250ug of rhCG (Ovidrel; Serono, Aubonne, Germany) will be once injected on trigger day. Oocyte retrieval will be performed 36 (±2) hours after rhCG injection. Oocyte collection is conducted via 17G oocyte aspiration needle with use of intravenous sedation. All COCs will be cultured in Human Tubal Fluid medium and incubated in a humidified 37℃ incubator 5% CO2, after oocyte retrieval immediately. The fertilisation method will be selected according to the semen analysis.

Assessment of fertilisation and embryo culture

Fertilisation will be considered normal when two pronuclei are present between 16 and 18h after ICSI or IVF. Normal fertilisation rate will be calculated as the number 2PN over the number of MII in ICSI patients or the number 2PN over the number of COCs for conventional IVF. All zygotes will be cultured in cleavage medium (G-1plus, Vitrolife, USA) for further 48-52h after fertilisation. Cleavage embryonic development will be assessed according to the developmental stage and degree of cytoplasmic fragmentation. All day 3 embryos will be cultured up to 2-3 days (G-2 plus, Vitrolife, USA). From day 5 to day 6, those embryos achieving the blastocyst stage will be evaluated morphologically using a Gardner's grading system.

For all participants, freeze-only blastocyst transfer strategy will be applied. All usable blastocysts (embryos that can grow to expanded or hatching blastocysts earn a score above grade CC) will be cryopreserved by vitrification methods.

Blastocyst vitrification and warm

The expanded blastocysts collapsed after artificial shrinkage will be vitrified and warmed as previously demonstrated[17]. In brief, the blastocysts will be transferred in equilibration medium, which included 7.5% (v/v) dimethyl sulfoxide (DMSO, Sigma Chemical Co., MO, USA) and 7.5% (v/v) ethylene

glycol (EG, Sigma Chemical Co., MO, USA), at 37℃ for 2 min, then placed in vitrification solution for 30 sec that containing 15% DMSO, 15% EG, and 0.65 mol/l sucrose. During this period, shrinkage blastocyst will be loaded on the Cryotop strip (Kitazato, Fuji, Japan), next plunged into liquid nitrogen immediately. For warming, the cryotop will be quickly placed in 0.33 mol/l sucrose at 37℃, and searching for the floated blastocyst under microscope. The blastocyst will be washed several times and placed for 2 min. Then it will be transferred into 0.2mol/l sucrose for 3 min and in a HEPES-buffered medium for 5 min in turn. After 2 hours, the blastocysts will be evaluated the quality. The re-expanded blastocysts will be considered survived and will be transferred to the patients.

Endometrium preparation, blastocyst transfer and luteal support

In vitrified-warmed blastocyst transferred cycles, participants will undergo hormone replacement therapy for their endometrial preparation. All participants once endometrial thickness reaches 6 mm and no ovarian cyst is observed on ultrasound, oral oestradiol valerate (Progynova, Delpharm Lille, Lys-Lez-Lannoy, France) at a dose of 3mg twice daily will be started on day 1–3 of the menstrual cycle. And then ultrasound will be repeated after more than 10 days after administration of HRT, vaginal progesterone gel (Crinone, Merck Serono, Watford, UK) 90 mg per day and oral dydrogesterone (Duphaston, Abbott, OLST, Netherlands) 20 mg twice daily will be added when the endometrial thickness was ≥8 mm. All the ultrasound scans and measurements will be performed with the digital platform Voluson E8 system. Only one blastocyst will be transferred on day 7 after progesterone administration. Luteal phase support was continued until 12 weeks' gestation.

Follow up

All participants will measure the hCG of Urine and blood at the 12 days after blastocyst transfer, in which positive results indicate biochemical pregnancy. On 28 days after transfer, if the gestational sac is observed with ultrasonography, participants will be diagnosed with clinical pregnancy. Ongoing pregnancy is defined as a sac with the fetal heartbeat and occurs after 12 weeks of pregnancy. Ongoing pregnancy leading to live-birth is defined by the live fetus in the uterine after 22 weeks of gestation18. We will collect the following information within 6 weeks after delivery, prenatal testing information (pregnancy complications and fetal information), delivery information (gestational age, mode of delivery, placental condition and birth complications), and newborn information (fetal sex, birth weight and birth defects).

Outcome measures

Primary outcome

Our primary outcome is ongoing pregnancy leading to live birth (gestation age≥22 weeks) within 6 months of the first oocyte retrieval cycle after randomisation[18].

Secondary outcomes

The secondary outcomes in our study include pregnancy outcome, maternal safety and obstetric and perinatal complications. Detailed information is provided in Table 2. Maternal safety is an important part of the secondary outcomes. The incidence of OHSS is the key indicator of maternal safety. Routine evaluation for OHSS will be performed on oocyte retrieval day and on day3 after fertilisation with ultrasound scanning and hormonal result in all participants. It is classified as mild, moderate, or severe according to the RCOG guildline[19].

253

Table 2 Secondary outcomes and related definition

Secondary outcomes	Definition
Pregnancy outcomes	
Implantation	Number of gestational sacs observed per embryo transferred
Clinical pregnancy	One or more observed gestational sac or definitive clinical signs of pregnancy under ultrasonography at 7 weeks of gestation (including clinical documented ectopic pregnancy)
Ongoing pregnancy	Presence of a gestational sac and fetal heartbeat after 12 weeks of gestation
Time to ongoing pregnancy	Time from randomisation to detection of ongoing pregnancy after completion of the transfer
Maternal safety outcomes	
OHSS	Exaggerated systemic response to ovarian stimulation characterized by a wide spectrum of clinical and laboratory manifestations. It is classified as mild, moderate, or severe according to the degree of abdominal distention, ovarian enlargement and respiratory hemodynamic, and metabolic complications. Diagnosed by ultrasound, blood testing and physical examination according the RCOG Guideline[19].
Miscarriage	Spontaneous loss of an intra-uterine pregnancy prior to 28 completed weeks of gestational age
Ectopic pregnancy	A pregnancy outside the uterine cavity, diagnosed by ultrasound, surgical visualization or histopathology
Obstetric and perinatal complications	
Gestational diabetes mellitus	Development of diabetes during pregnancy
Hypertensive disorders of pregnancy	Including pregnancy-induced hypertension, pre-eclampsia and eclampsia
Antepartum haemorrhage	Including placenta previa, placenta accreta and unexplained
Birth weight	Including low birth weight (weight < 2500 gm at birth), very low birth weight (< 1500 gm at birth), high birth weight (>4000 gm at birth) and very high birth weight (>4500 gm at birth)
Large for gestational age	A birth weight greater than the 90th centile of the sex-specific birth weight for a given gestational age reference
Small for gestational age	A birth weight less than the 10th centile for the sex-specific birth weight for a given gestational age reference

Preterm birth	Birth of a fetus delivered after 28 and before 37 completed weeks of gestational age in participants confirmed ongoing pregnancy
Congenital anomaly	Structural or functional disorders that occur during intra-uterine life and can be identified prenatally, at birth or later in life, including trisomy 21 syndrome, neural tube defect, congenital heart disease, cleft lip, excessive numbers of fingers or toes, hydrocephalus.
Perinatal mortality	Fetal or neonatal death occurring during late pregnancy (at 28 completed weeks of gestational age and later), during childbirth, or up to seven completed days after birth)

Statistical analysis

Sample size calculation

The primary hypothesis of this study is that the experimental group (IVM) is noninferior to the control group (IVF-ET) in terms of ongoing pregnancy leading to live birth. On the basis of actual data on the women with PCOS underwent IVM or IVF-ET in our reproductive medicine center, we assume that the proportions of ongoing pregnancy leading to live birth for the PCOS women is 35% per transferred cycle. With a noninferiority margin of 15%, power of 80%, and one-sided α 0.025. We will need to enroll 159 participants in each group (the ratio between groups will be 1:1). Taking consideration of dropout rate as 10% (such as cancellation during trial procedures). Each group will have a minimum of 175 participants for a total of 350 participants.

Statistical analysis

The result will be analyzed according to the intention-to-treat (ITT) principal. Pre-protocol (PP) analysis will also be conducted as a sensitivity analysis. Baseline characteristics will be described by descriptive analysis. For continues variables, the normality distribution will be estimated by using frequency histograms and the Shapiro test initially. If the parameters are normally distributed, they will be presented as means with standard deviations (SDs). If the parameters are non-normally distributed, their medians and inter-quantile ranges (IRQs) will be reported. For categorical variables, we will present the proportion between each group. In addition, the recruitment numbers, those participants lost to follow-up, protocols violations and other relevant data will also be reported.

Comparison between groups will be performed using the independent sample t test, Mann-Whitney U test, Pearson chi-square test or Fisher's exact test as appropriate. The primary outcome, ongoing pregnancy leading to live birth, will be compared using Pearson's chi-square test or Fisher's exact test as appropriate. Categorical secondary outcomes will be compared between two groups using the similar approach as the primary outcome. Student's t test or Wilcoxon test will be used as appropriate for continuous secondary outcomes, such as birthweight and etc. The absolute rate differences (ARD) and their 95% confident interval (CI) between the two groups will be calculated. And the 95% CI of the ARDs will be used to evaluate if the experimental group (IVM) is noninferior to the control group (IVF-ET).

The missing data will be treated as missing at random, and will be imputed using the last observation carry forward (LOCF) method. For the missing values, a sensitivity analysis will be done under the hypothesis of the worst and the best outcomes for each missing individual. Therefore, all secondary outcomes will be considered exploratory.

All statistical analyses will be done using the statistical package SPSS, version 25.0 (SPSS Inc., Chicago, IL). Statistical significance is defined as $p< 0.05$ with two-sided testing except for the non-inferiority test for the primary outcome.

Trial status

The study was designed in 2017. The first recruitment started in March 2018. We completed recruitment in July 2019. The participant follow-up is ongoing. It is expected that data collection will be completed in Oct, 2020.

Ethics and dissemination

The trial had been reviewed and approved by the Science Research Ethics Committee of Peking University Third Hospital (2017sz-066). The study is registered on Clinicaltrials.gov as NCT03463772. Informed consent will be obtained from each participant before randomization. The researchers will permit trial-related monitoring, audits, regulatory inspections, providing direct access to source data and documents. There is no additional data available in this study protocol.

Authors' contributions

JQ and RL conceived the study idea. JQ, RL, RW, BWM, XZ and WG participated in the design of the study, and drafting of the manuscript. XZ, WG, SY and LW participate in recruitment of participants and assessment of clinical outcomes. LZ coordinates data collection, and will perform data analysis. DZ, LZ, RW and BWM will design the statistical analysis plan and oversee statistical analysis. All authors critically reviewed the article and approved the final manuscript.

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Competing interests statement

All authors do not have competing interests to declare. Completed disclosure of interests form available to view online as supporting information.

Reference

1. Li R, Zhang Q, Yang D, et al. Prevalence of polycystic ovary syndrome in women in China: a large community-based study. Hum Reprod 2013;28:2562-9.
2. 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.
3. Mourad S, Brown J, Farquhar C. Interventions for the prevention of OHSS in ART cycles: an overview of Cochrane reviews. Cochrane Database Syst Rev 2017;1:CD012103.
4. Namavar Jahromi BM, Parsanezhad MM, Shomali ZM, et al. Ovarian Hyperstimulation Syndrome: A Narrative Review of Its Pathophysiology, Risk Factors, Prevention, Classification, and Management. Iran J Med Sci 2018;43:248-60.
5. Trounson A, Wood C, Kausche A. In vitro maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. Fertil Steril 1994;62:353-62.
6. Chian RC, Xu CL, Huang JY, et al. Obstetric outcomes and congenital abnormalities in infants conceived with oocytes matured in vitro. Facts Views Vis Obgyn 2014;6:15-8.
7. Mostinckx L, Segers I, Belva F, et al. Obstetric and neonatal outcome of ART in patients with polycystic ovary syndrome: IVM of oocytes versus controlled ovarian stimulation. Hum Reprod 2019;10.1093/humrep/dez086.
8. Yu EJ, Yoon TK, Lee WS, et al. Obstetrical, neonatal, and long-term outcomes of children conceived from in vitro matured oocytes. Fertil Steril 2019;10.1016/j.fertnstert.2019.05.034.
9. Edwards RG. Maturation in vitro of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes. Nature 1965;208:349-51.
10. Das M, Son WY, Buckett W, et al. In-vitro maturation versus IVF with GnRH antagonist for women with polycystic ovary syndrome: treatment outcome and rates of ovarian hyperstimulation syndrome. Reprod Biomed Online 2014;29:545-51.
11. Gremeau AS, Andreadis N, Fatum M, et al. In vitro maturation or in vitro fertilization for women with polycystic ovaries? A case-control study of 194 treatment cycles. Fertil Steril 2012;98:355-60.
12. de Ziegler D, Streuli I, Gayet V, et al. Retrieving oocytes from small non-stimulated follicles in polycystic ovary syndrome (PCOS): in vitro maturation (IVM) is not indicated in the new GnRH antagonist era. Fertil Steril 2012;98:290-3.
13. Walls ML, Hunter T, Ryan JP, et al. In vitro maturation as an alternative to standard in vitro fertilization for patients diagnosed with polycystic ovaries: a comparative analysis of fresh, frozen and cumulative cycle outcomes. Hum Reprod 2015;30:88-96.
14. Ho VNA, Pham TD, Le AH, et al. Live birth rate after human chorionic gonadotropin priming in vitro maturation in women with polycystic ovary syndrome. J Ovarian Res 2018;11:70.
15. Siristatidis CS, Maheshwari A, Vaidakis D, et al. In vitro maturation in subfertile women with polycystic ovarian syndrome undergoing assisted reproduction. Cochrane Database Syst Rev 2018;11:CD006606.
16. Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19:41-7.
17. Chen Y, Zheng XY, Yan J, et al. Neonatal outcomes after the transfer of vitrified blastocysts: closed

versus open vitrification system. Reprod Biol Endocrinol 2013;11.

18. Zegers-Hochschild F, Adamson GD, Dyer S, et al. The International Glossary on Infertility and Fertility Care, 2017. Hum Reprod 2017;32:1786-801.

19. Royal college of Obstetricians and Gynaecologists. The management of ovarian hyperstimulation syndrome. RCOG Green-top Guildline 2016;5:1-22.

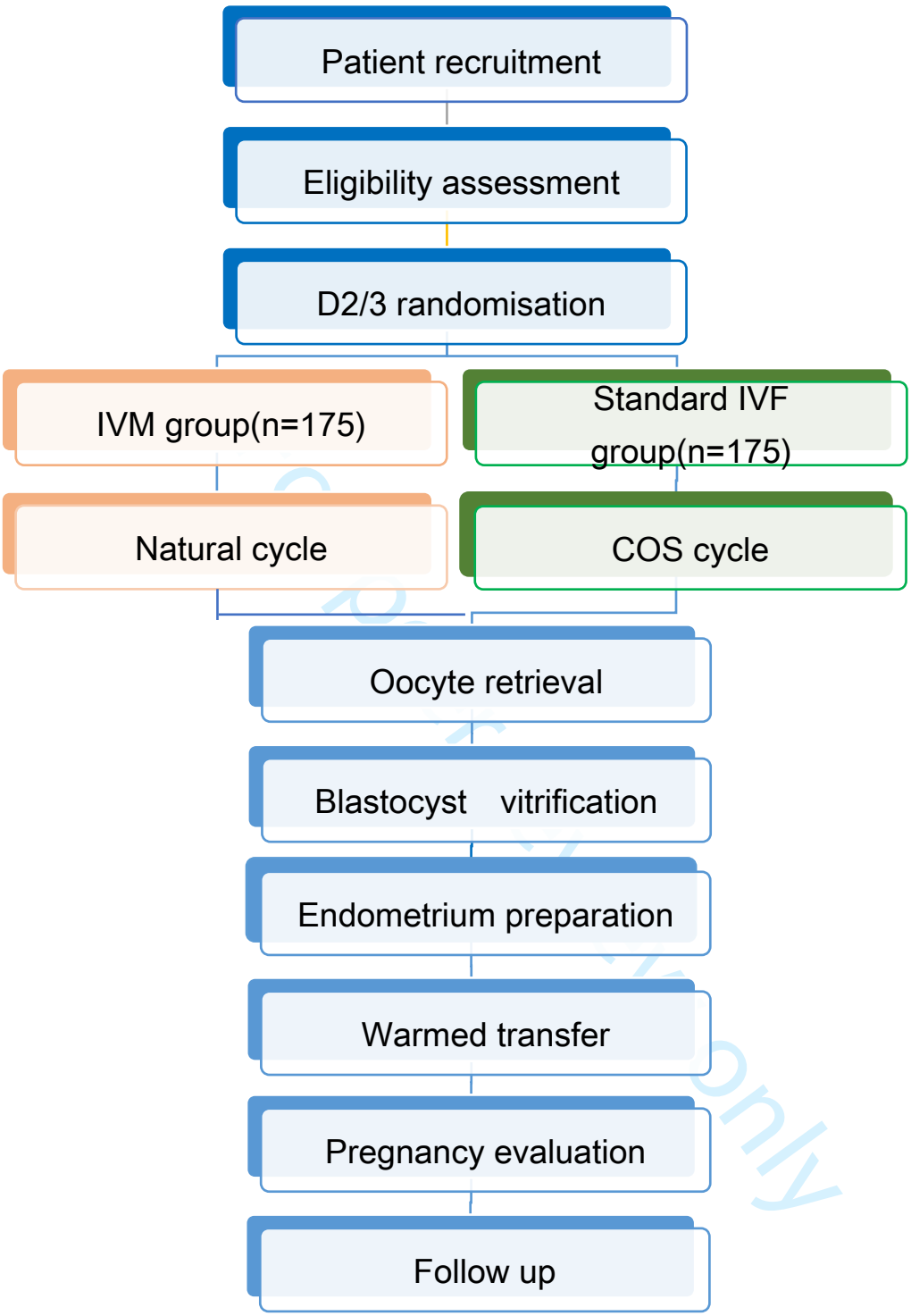

Figure 1. Flow chart of this RCT: IVM vs IVF in women with PCOS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	p1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	P2
	2b	All items from the World Health Organization Trial Registration Data Set	P2
Protocol version	3	Date and version identifier	p1
Funding	4	Sources and types of financial, material, and other support	p15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	p1
	5b	Name and contact information for the trial sponsor	p1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	P2
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	p14

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	P3-4
	6b	Explanation for choice of comparators	P4
Objectives	7	Specific objectives or hypotheses	P4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	P4
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	P7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	P7-8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	P9-11
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	P8
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	P8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	P9-11
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	p12-13
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	P5-6

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	p14
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	P8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	P8
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	P8
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	P8
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	P8-9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	p11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	p11

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	p13-14
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	p14-13
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	p14-13
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	p14-13
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	p13-14
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	p14
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	p14
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	p14
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	p15
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	p15

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	p15
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	p15
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	p15
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	p15
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	p15
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	p15
	31b	Authorship eligibility guidelines and any intended use of professional writers	p15
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	p11
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Live birth after In vitro maturation versus standard In vitro fertilisation for women with polycystic ovary syndrome: protocol for a non-inferiority randomized clinical trial

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Title

Live birth after *In vitro* maturation versus standard *In vitro* fertilisation for women with polycystic ovary syndrome: protocol for a non-inferiority randomized clinical trial

Authors

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Abstract

Introduction: Polycystic ovary syndrome (PCOS) is the first common cause of anovulatory infertility. Currently, *In vitro* fertilisation (IVF) is recommended when conventional attempts have failed. *In vitro* maturation (IVM) of human oocytes is an emerging treatment option in infertile women with PCOS. It is a patient-friendly intervention, avoiding the risk of ovarian hyperstimulation syndrome (OHSS), which is a serious complication of controlled ovarian stimulation (COS) in the standard IVF procedure. We plan a randomised controlled trial (RCT) to evaluate whether IVM is non-inferior to the standard IVF for live birth in women with PCOS.

Methods and analysis: This is a single-center, open-label, non-inferiority RCT performed in a large reproductive medicine center in China. Infertile women with PCOS will be randomised to receive either IVM or standard IVF in a 1:1 treatment ratio after informed consent. IVF procedures used in our study are all standard treatments and other standard assisted reproductive technologies will be similar between the two groups. The primary outcome is ongoing pregnancy leading to live birth within 6 months of the first oocyte retrieval cycle after randomisation. Pregnancy outcome, maternal safety, and obstetric and perinatal complications will be secondary outcomes. The planned sample size is 350 (175 per group).

Ethics and dissemination: Ethical permission was acquired from the Ethics Committee of Peking University Third Hospital. The results will be issued to publications through scientific journals and conference reports.

Trial registration number: NCT03463772

Strengths and limitations of this study:

- This is a randomised controlled trial with an adequate sample size to evaluate IVM treatment in infertile women with PCOS.
- This study will provide evidence on whether the experimental group (IVM) is non-inferior to the standard IVF in terms of live birth.
- Cost-effective analysis is not included in the study, limiting the comprehensiveness of the results.

Keywords: *In vitro* fertilisation; polycystic ovary syndrome (PCOS); *In vitro* maturation (IVM); assisted reproductive technology.

Introduction

Polycystic ovary syndrome (PCOS) is the first common cause of anovulatory infertility and occurs in 5.6% in reproductive-aged women in the Chinese communities[1]. It is also a major metabolic disorder associated with insulin resistance, β -cell dysfunction, and obesity[2]. 50% of women with PCOS present with sub-fertility. In anovulatory women with PCOS, the first-line treatments are lifestyle intervention and ovulation induction with letrozole or clomiphene citrate[3]. Laparoscopic ovarian drilling or ovarian induction with gonadotrophin (Gn) are considered as the second-line treatment options. *In vitro* fertilisation (IVF) is the third-line treatment and is recommended when the above-mentioned treatments have failed.

In standard IVF treatment, women with PCOS are at high risk of ovarian hyperstimulation syndrome (OHSS), which is a serious and common iatrogenic complication of controlled ovarian stimulation. The prevalence of moderate and severe forms of OHSS in women undergoing IVF is 3% to 8% and much higher in women with PCOS[4]. Severe OHSS is defined by the presence of clinical evidence of ascites with severe abdominal pain and pleural effusion. A large amount of pleural and peritoneal effusion can lead to intravascular blood loss, blood concentration, blood hypercoagulability, hypovolemic shock, severe cardiopulmonary dysfunction, electrolyte imbalance, impaired liver and kidney function, thrombosis, and even life-threatening[5]. Therefore, seeking an alternative treatment strategy, avoiding the risk of OHSS without compromising pregnancy outcomes is crucial for women with PCOS.

In vitro maturation (IVM) has been introduced in 1990. Trounson[6] described the first delivery of a healthy baby with the IVM technique in a woman with PCOS. From then on, immature oocyte retrieval followed by IVM had been used widely, resulting in the delivery of thousands healthy infants worldwide[7-9]. In IVM, immature oocytes are collected from antral follicles, typically from unstimulated or minimally stimulated ovaries, then cultured, matured and fertilised in vitro[10]. Compared with standard IVF treatment, IVM is performed without ovarian stimulation, thus preventing the occurrence of OHSS and reducing financial costs.

To date, despite the publication of many studies on IVM, the effectiveness and safety of IVM treatment are still controversial. Some studies showed that the live birth rate was significantly lower in the IVM group than in the IVF group[11-13]. Now mature technology, gradually getting more research results indicated comparable pregnancy

outcomes between IVM and IVF group[14 15]. Until now, all data available have derived from observational studies[16]. Given the unique advantages of IVM as safer, simpler and cheaper[17], we need further evidence from well-designed randomised controlled trials (RCT) on live birth rate before we draw conclusions on the effectiveness and safety of IVM. Therefore, we plan an RCT to determine whether IVM is non-inferior to standard IVF on live birth for women with PCOS.

Methods and analysis

Study design

This is a single-center, non-inferiority RCT with a 1:1 treatment ratio. The program for enrollment, interventions, and evaluation during the study process is shown in Table 1. Figure 1 indicates a flowchart showing the registration, allocation, treatment, and follow-up of participants.

Table 1. Schedule of enrollment, interventions, and assessments

	Study Period							
	Enrollment	Allocation		Post-allocation				Close-out
Content	Screening & Baseline assessment	IVM & IVF Randomisation	Oocyte retrieval	Assessment of embryo	Embryo transfer	Evaluation of pregnancy	Follow-up of pregnancy	
Time point	T_0 -1 month	T_1 o month	T_2 10-14 days	T_3 1-3-5 days after opu	T_4 2-6 month	T_5 1-7 month	T_6 6-10 months	T_7 8-12 months
Enrollment								
Eligibility screen	×	×						
Informed consent	×							
Allocation		×						
Interventions								
IVM		×						
Standard IVF		×						

Assessments								
Baseline data	×							
Laboratory tests	×	×	×		×	×	×	×
Fertilisation				×				
Embryo quality				×				
Pregnancy tests						×		
Pregnancy outcomes							×	×
Fetus information							×	×
Neonate information							×	×
Safety assessment		×	×		×	×	×	×

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Study setting

The trial is approved by the Ethics Committee of Peking University Third Hospital (2017sz-066). The study was registered on Clinicaltrials.gov (NCT03463772). Women with PCOS and infertility who scheduled for their first IVF attempt at Peking University Third Hospital (Beijing, China) will be recruited. Potentially eligible women will be given information about the study during their first consultation. All the couples provided written informed consent before participation. The trial progress will be monitored by an independent data and safety monitoring board (DSMB).

Eligibility criteria

PCOS outpatient women with infertility who come to the reproductive center of Peking University Third Hospital will be screened for eligibility by a research team.

Inclusion Criteria:

- Married Chinese women with infertility aged between 20 and 38 years.
- Women with PCOS according to the revised Rotterdam criteria (i.e. two of the three following features are present: 1) Oligo- and/or anovulation; 2) Clinical and/or biochemical signs of hyperandrogenism; 3) Polycystic ovaries). Exclusion of other possible related disorders: ovarian or adrenal androgen-secreting tumors, thyroid disease, hyperprolactinemia, non-classical adrenal hyperplasia[18].
- Women scheduled for their first IVF/ICSI cycle. Women must have an indication for ART, including ovulation dysfunction and failure to become pregnant after ovulation induction treatment; unilateral or bilateral tubal obstruction, adhesion, salpingectomy or tubal ligation; oligoasthenozoospermia.
- Written Informed consent obtained.

Exclusion criteria:

- Couples with a contraindication for IVF or ICSI (including but not limited to poorly controlled type 1 or type 2 diabetes mellitus; liver disease or dysfunction (based on serum liver enzyme test results); renal disease or abnormal serum renal function; anemia; history of deep venous thrombosis, pulmonary embolus or cerebrovascular accident; uncontrolled hypertension or known symptomatic heart disease; history of (or suspected) cervical carcinoma, endometrial carcinoma or breast carcinoma; and unexplained colporrhagia).
- Couples receiving donor sperm or donor oocytes.
- Couples with indications or have the plan to receive preimplantation genetic testing

(PGT).

- Women with a male partner diagnosed with azoospermia.
- Either a male partner or female partner with a known abnormal chromosome karyotype (chromosome polymorphisms was not included).
- Women who have undergone unilateral ovariectomy.

Participants can leave the study at any time without any consequences for their clinical treatments.

Recruitment

Potentially eligible women will be informed about the trial during their first consultation. If a woman wants to participate, written informed consent will be acquired in their next scheduled visit. To initiate the IVM or IVF treatment cycle in anovulatory cases, patients were administrated oral dydrogesterone (Duphaston, Abbott, OLST, Netherlands) 20 mg daily for 10-14 days/ Oral contraceptives Diane-35 (cyproterone acetate 2 mg, ethinylestradiol 35 mg, Bayer, and its generics) for 21 days. After the withdrawal bleeding, eligible participants will be assessed again for the exclusion criteria on day 2 or day 3 following the onset of menstrual bleeding.

Randomisation and allocation concealment

Randomisation and allocation of qualified participants will be done on day 2/3 of the menstrual cycle. Eligible participants will be allocated to IVM or IVF treatment according to a computer-generated randomisation list in a 1:1 ratio, with a variable block size of 4 or 6. Opaque sealed envelopes, with participant's screening order printed outside and randomised assigned group printed inside, will be numbered consecutively. Researchers will enroll the eligible participants after screening, then open each envelope in a sequence corresponding with the screening order and assign the participant into the IVM or IVF group. Both investigators and participants will be aware of the allocation of the subsequent treatments.

Interventions

IVM protocol

Gonadotrophins (FSH, HMG or HCG) will not be used in the IVM group. After randomisation, participants will visit the clinic on days 6–8 for a transvaginal ultrasound examination to exclude the development of a dominant follicle. Oocyte

retrieval will be scheduled once the endometrium thickness reaches at least 6 mm and there is no appearance of the dominant follicle (follicle diameter >10 mm). Transvaginal ultrasound-guided oocytes retrieval will be conducted with a single-lumen 19G aspiration needle (K-OPS-7035-REH-ET; Cook, Queensland, Australia) in 90 mmHg suction pressure. Follicular aspirates will be gathered and filtered through a cell strainer (Cell Strainer 352350, 70μm nylon; Falcon, MA, USA). Then the collected aspirates will be washed with pre-warmed Hepe's-HTF (CooperSurgical; Trumbull, CT, USA). The mixture will be examined carefully under a stereomicroscope to search for cumulus-oocyte complexes (COCs). All COCs will be transferred into IVM medium (IVM media kit; Sage, CT, USA) supplemented with 0.075 IU/ml FSH and 0.075 IU/ml LH (Menopur; Ferring, Kiel, Germany) in 5% CO2 incubator at 37℃. All the COCs will be denuded of cumulus cells after 28-32 h of culture and evaluated the maturation process. The presence of a first polar body indicates that the oocyte enters the metaphase II stage. All the metaphase II (MII) oocytes will be inseminated by means of intracytoplasmic sperm injection (ICSI).

IVF protocol

For participants in the IVF group, COS will be performed by a flexible protocol using gonadotrophin-releasing hormone antagonist (GnRH-ant) with recombinant FSH (rFSH) and recombinant human chorionic gonadotrophin (rhCG). Treatment will start on day 2/3 of the menstrual cycle. All participants will be subcutaneous injected rFSH (Gonal-F; Serono, Geneva, Switzerland) with 100-225IU/d initial dosage. Transvaginal ultrasound, serum luteinizing hormone (LH), serum estrogen (E2) and progesterone (P4) will be tested to monitor follicle growth. rFSH doses will be adjusted according to ovarian response. GnRH antagonist-cetrorelix (Cetrotide; Serono, Darmstadt, Germany) 0.25mg daily by subcutaneous injection will begin when at least one follicle has reached a diameter of 12mm which is usually between day 5 to day 8 of ovarian stimulation, until the trigger day (include the trigger day). After 2 or more follicles reach a diameter ≥17 mm, 250ug of rhCG (Ovidrel; Serono, Aubonne, Germany) will be once injected on trigger day. Oocyte retrieval will be performed 36 (±2) hours after rhCG injection. Oocyte collection is conducted via 17G oocyte aspiration needle with the use of intravenous sedation. All COCs will be cultured in Human Tubal Fluid medium and incubated in a humidified 37℃ incubator 5% CO2, after oocyte retrieval immediately. The fertilisation method will be selected according to the semen analysis.

Assessment of fertilisation and embryo culture

Fertilisation will be considered normal when two pronuclei are present between 16 and 18h after ICSI or IVF. Normal fertilisation rate will be calculated as the number 2PN over the number of MII in ICSI patients or the number 2PN over the number of COCs for conventional IVF. All zygotes will be cultured in cleavage medium (G-1plus, Vitrolife, USA) for further 48-52h after fertilisation. Cleavage embryonic development will be assessed according to the developmental stage and degree of cytoplasmic fragmentation. All day 3 embryos will be cultured up to 2-3 days (G-2 plus, Vitrolife, USA). From day 5 to day 6, those embryos achieving the blastocyst stage will be evaluated morphologically using a Gardner's grading system.

For all participants, a freeze-only blastocyst transfer strategy will be applied. All usable blastocysts (embryos that can grow to expanded or hatching blastocysts earn a score above grade CC) will be cryopreserved by vitrification methods.

Blastocyst vitrification and warm

The expanded blastocysts collapsed after artificial shrinkage will be vitrified and warmed as previously demonstrated[19]. In brief, the blastocysts will be transferred in equilibration medium, which included 7.5% (v/v) dimethyl sulfoxide (DMSO, Sigma Chemical Co., MO, USA) and 7.5% (v/v) ethylene glycol (EG, Sigma Chemical Co., MO, USA), at 37℃ for 2 min, then placed in vitrification solution for 30 sec that containing 15% DMSO, 15% EG, and 0.65 mol/l sucrose. During this period, shrinkage blastocyst will be loaded on the Cryotop strip (Kitazato, Fuji, Japan), next plunged into liquid nitrogen immediately. For warming, the cryotop will be quickly placed in 0.33 mol/l sucrose at 37℃, and searching for the floated blastocyst under microscope. The blastocyst will be washed several times and placed for 2 min. Then it will be transferred into 0.2mol/l sucrose for 3 min and in a HEPES-buffered medium for 5 min in turn. After 2 hours, the blastocysts will be evaluated the quality. The re-expanded blastocysts will be considered survived and will be transferred to the patients.

Endometrium preparation, blastocyst transfer, and luteal support

In vitrified-warmed blastocyst transferred cycles, participants will undergo hormone replacement therapy for their endometrial preparation. All participants' once endometrial thickness reaches 6 mm and no ovarian cyst is observed on ultrasound, oral

oestradiol valerate (Progynova, Delpharm Lille, Lys-Lez-Lannoy, France) at a dose of 3mg twice daily will be started on day 1–3 of the menstrual cycle. And then an ultrasound will be repeated after more than 10 days after administration of HRT, vaginal progesterone gel (Crinone, Merck Serono, Watford, UK) 90 mg per day and oral dydrogesterone (Duphaston, Abbott, OLST, Netherlands) 20 mg twice daily will be added when the endometrial thickness was ≥8 mm. All the ultrasound scans and measurements will be performed with the digital platform Voluson E8 system. Only one blastocyst will be transferred on day 7 after progesterone administration. Luteal phase support was continued until 12 weeks' gestation.

Follow up

All participants will measure the hCG of Urine and blood at the 12 days after blastocyst transfer, in which positive results indicate biochemical pregnancy. On 28 days after transfer, if the gestational sac is observed with ultrasonography, participants will be diagnosed with clinical pregnancy. Ongoing pregnancy is defined as a sac with the fetal heartbeat and occurs after 12 weeks of pregnancy. Ongoing pregnancy leading to live-birth is defined by the live fetus in the uterine after 22 weeks of gestation. We will collect the following information within 6 weeks after delivery, prenatal testing information (pregnancy complications and fetal information), delivery information (gestational age, mode of delivery, placental condition and birth complications), and newborn information (fetal sex, birth weight, and birth defects).

Outcome measures

Primary outcome

Our primary study endpoint is ongoing pregnancy leading to live birth (gestation age≥22 weeks) within 6 months of the first oocyte retrieval cycle after randomisation[20].

Secondary outcomes

Secondary outcomes of our trial include pregnancy outcomes, maternal safety, and obstetric and perinatal complications. Detailed information is provided in Table 2. Maternal safety is an important part of the secondary outcomes. The incidence of OHSS is the key indicator of maternal safety. Routine evaluation for OHSS will be performed on oocyte retrieval day and on day3 after fertilisation with ultrasound scanning and hormonal result in all participants. It is classified as mild, moderate, or severe according

to the RCOG guildline[21].

Safety Reporting

Adverse events (AE) are defined as any undesirable experience occurring to a subject during the trial. A serious adverse event (SAE) is any untoward medical events, that results in death; is life-threatening (at the time of the event); requires hospitalization or prolongation of existing inpatients' hospitalization; results in persistent or significant disability or incapacity; is a congenital anomaly or birth defect; is a new event of the trial likely to affect the safety of the subjects, such as an unexpected outcome of an adverse reaction.

SAE in this study includes: moderate/severe OHSS; intraperitoneal hemorrhage or ovarian torsion after oocyte retrieval; ectopic pregnancy; severe preeclampsia; pregnancy complications leading to hospitalization; stillbirth; birth defects; other serious medical events judged by researchers to meet the criteria of SAE.

All SAEs will be reported to the DSMB and accredited Medical Education Technology Committee (METC) that approved the protocol, according to the requirements of that METC.

Table 2. Secondary outcomes and related definition

Secondary outcomes	Definition
Pregnancy outcomes	
Implantation	Number of gestational sacs observed per embryo transferred
Clinical pregnancy	One or more observed gestational sac or definitive clinical signs of pregnancy under ultrasonography at 7 weeks of gestation (including clinical documented ectopic pregnancy)
Ongoing pregnancy	Presence of a gestational sac and fetal heartbeat after 12 weeks of gestation
Time to ongoing pregnancy leading to live birth*	Time from randomisation to detection of ongoing pregnancy after completion of the transfer
Maternal safety outcomes	
OHSS	Exaggerated systemic response to ovarian stimulation characterized by a wide spectrum of clinical and laboratory manifestations. It is classified as mild, moderate, or severe according to the degree of abdominal distention, ovarian enlargement and respiratory hemodynamic, and metabolic complications. Diagnosed by ultrasound, blood testing and physical examination according the RCOG Guideline[21].
Miscarriage	Spontaneous loss of an intra-uterine pregnancy prior to 28 completed weeks of gestational age
Ectopic pregnancy	A pregnancy outside the uterine cavity, diagnosed by ultrasound, surgical visualization or histopathology

Obstetric and perinatal complications	
Gestational diabetes mellitus	Development of diabetes during pregnancy
Hypertensive disorders of pregnancy	Including pregnancy-induced hypertension, pre-eclampsia and eclampsia
Antepartum haemorrhage	Including placenta previa, placenta accreta and unexplained
Birth weight	Including low birth weight (weight < 2500 gm at birth), very low birth weight (< 1500 gm at birth), high birth weight (>4000 gm at birth) and very high birth weight (>4500 gm at birth)
Large for gestational age	A birth weight greater than the 90th centile of the sex-specific birth weight for a given gestational age reference
Small for gestational age	A birth weight less than the 10th centile for the sex-specific birth weight for a given gestational age reference
Preterm birth	Birth of a fetus delivered after 28 and before 37 completed weeks of gestational age in participants confirmed ongoing pregnancy
Congenital anomaly	Structural or functional disorders that occur during intra-uterine life and can be identified prenatally, at birth or later in life, including trisomy 21 syndrome, neural tube defect, congenital heart disease, cleft lip, excessive numbers of fingers or toes, hydrocephalus.
Perinatal mortality	Fetal or neonatal death occurring during late pregnancy (at 28 completed weeks of gestational age and later), during childbirth, or up to seven completed days after birth)

297 * only ongoing pregnancy leading to live birth within 6 months of the first oocyte retrieval cycle after randomisation will be counted.

Statistical analysis

Sample size calculation

The primary hypothesis of this study is that the experimental group (IVM) is non-inferior to the control group (IVF) in terms of ongoing pregnancy leading to live birth. On the basis of actual data on the women with PCOS who underwent IVM or IVF-ET in our reproductive medicine center, we assume that after IVF the proportions of ongoing pregnancy leading to live birth for the PCOS women are 35% per transferred cycle[22 23]. With a non-inferiority margin of 15%, power of 80%, and one-sided α 0.025. We will need to enroll 159 participants in each group (the ratio between groups will be 1:1). Taking consideration of dropout rate as 10% (such as cancellation during trial procedures). Each group will include 175 participants (a total of 350 participants).

Statistical analysis

The result will be analyzed according to the intention-to-treat (ITT) principle. Pre-protocol (PP) analysis will also be conducted as a sensitivity analysis. Baseline characteristics will be described by descriptive analysis, and the balance among groups or subgroups will be assessed by analysis for different kinds of data. For continuous variables, the normality distribution will be estimated by using frequency histograms and the Kolmogorov-Smirnov test initially. If the continuous variables are normally distributed, they will be presented as means with standard deviations (SDs). If the continuous variables are non-normally distributed, their medians and inter-quantile ranges (IQRs) will be reported. For categorical variables, we will present the proportion between each group. In addition, the recruitment numbers, those participants lost to follow-up, protocols violations and other relevant data will also be reported. A comparison between groups will be performed using the independent sample t-test, Mann-Whitney U test for continuous variables, or Pearson chi-square test/Fisher's exact test for categorical variables as appropriate. The primary outcome, ongoing pregnancy leading to live birth, will be compared using Pearson's chi-square test or Fisher's exact test as appropriate. Categorical secondary outcomes will be compared between two groups using a similar approach as the primary outcome. Student's t-test or Wilcoxon test will be used as appropriate for continuous secondary outcomes, such as birth weight and etc. The relative risks (RR) and absolute rate differences (ARD) and their 95% confidence interval (CI) between the two groups will be calculated. And the 95% CI of the ARDs will be used to evaluate if the experimental group (IVM) is

non-inferior to the control group (IVF-ET). For the time-to-event outcome, the Kaplan-Meier curve will be used.

Missing data will be treated as missing at random and will be imputed using the last observation carried forward (LOCF) method. For the missing values, a sensitivity analysis will be done under the hypothesis of the worst and the best outcomes for each missing individual. Therefore, all secondary outcomes will be considered exploratory. All statistical analyses will be done using the statistical package SPSS, version 25.0 (SPSS Inc., Chicago, IL). Statistical significance is defined as $p< 0.05$ with two-sided testing except for the non-inferiority test for the primary outcome.

Trial status

The study was designed in 2017. The first participant was recruited in March 2018 and the last participant was recruited in July 2019. The follow-up is ongoing. It is expected that data collection will be completed in Oct 2020.

Patient and Public Involvement

This research was done without patient or public involvement. Neither patients nor the public was involved in the development of the research question, study design or implementation of this trial. Patients will not be invited to develop patient-relevant outcomes or interpret the results, as well as the writing or editing of the final manuscript for readability or accuracy. As interventions in our study are both routine procedures during clinical work, the burden of the intervention is assessed by patients themselves.

Ethics and dissemination

The trial had been reviewed and approved by the Science Research Ethics Committee of Peking University Third Hospital (2017sz-066). The study is registered on Clinicaltrials.gov as NCT03463772. Informed consent will be obtained from each participant before randomization. The researchers will permit trial-related monitoring, audits, regulatory inspections, providing direct access to source data and documents. There are no additional data available in this study protocol.

Authors' contributions

JQ and RL conceived the study idea. JQ, RL, RW, BWM, XZ, WG, and DZ participated in the design of the study and drafting of the manuscript. XZ, WG, SY, and LW

participate in the recruitment of participants and the assessment of clinical outcomes. LZ and DZ coordinates data collection and will perform data analysis. All authors critically reviewed the article and approved the final manuscript.

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Competing interests statement

All authors do not have competing interests to declare.

Figure 1. Flow chart of this RCT: IVM vs IVF in women with PCOS

Reference

1. Li R, Zhang Q, Yang D, et al. Prevalence of polycystic ovary syndrome in women in China: a large community-based study. *Hum Reprod* 2013;**28**:2562-9.
2. Messinis IE, Messini CI, Anifandis G, et al. Polycystic ovaries and obesity. *Best Pract Res Clin Obstet Gynaecol* 2015;**29**:479-88.
3. 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.
4. Mourad S, Brown J, Farquhar C. Interventions for the prevention of OHSS in ART cycles: an overview of Cochrane reviews. *Cochrane Database Syst Rev* 2017;**1**:CD012103.
5. Namavar Jahromi BM, Parsanezhad MM, Shomali ZM, et al. Ovarian Hyperstimulation Syndrome: A Narrative Review of Its Pathophysiology, Risk Factors, Prevention, Classification, and Management. *Iran J Med Sci* 2018;**43**:248-60.
6. Trounson A, Wood C, Kausche A. In vitro maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. *Fertil Steril* 1994;**62**:353-62.
7. Chian RC, Xu CL, Huang JY, et al. Obstetric outcomes and congenital abnormalities in infants conceived with oocytes matured in vitro. *Facts Views Vis Obgyn* 2014;**6**:15-8.
8. Mostinckx L, Segers I, Belva F, et al. Obstetric and neonatal outcome of ART in patients with polycystic ovary syndrome: IVM of oocytes versus controlled ovarian stimulation. *Hum Reprod* 2019;10.1093/humrep/dez086.
9. Yu EJ, Yoon TK, Lee WS, et al. Obstetrical, neonatal, and long-term outcomes of children conceived from in vitro matured oocytes. *Fertil Steril* 2019;10.1016/j.fertnstert.2019.05.034.
10. Edwards RG. Maturation in vitro of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes. *Nature* 1965;**208**:349-51.
11. Das M, Son WY, Buckett W, et al. In-vitro maturation versus IVF with GnRH antagonist for women with polycystic ovary syndrome: treatment outcome and rates of ovarian hyperstimulation syndrome. *Reprod Biomed Online* 2014;**29**:545-51.
12. Gremeau AS, Andreadis N, Fatum M, et al. In vitro maturation or in vitro fertilization for women with polycystic ovaries? A case-control study of 194 treatment cycles. *Fertil Steril* 2012;**98**:355-60.
13. de Ziegler D, Streuli I, Gayet V, et al. Retrieving oocytes from small non-stimulated follicles in polycystic ovary syndrome (PCOS): in vitro maturation (IVM) is not indicated in the new GnRH antagonist era. *Fertil Steril* 2012;**98**:290-3.
14. Walls ML, Hunter T, Ryan JP, et al. In vitro maturation as an alternative to standard in vitro fertilization for patients diagnosed with polycystic ovaries: a comparative analysis of fresh, frozen and cumulative cycle outcomes. *Hum Reprod* 2015;**30**:88-96.
15. Ho VNA, Pham TD, Le AH, et al. Live birth rate after human chorionic gonadotropin priming in vitro maturation in women with polycystic ovary syndrome. *J Ovarian Res* 2018;**11**:70.
16. Siristatidis CS, Maheshwari A, Vaidakis D, et al. In vitro maturation in subfertile women with polycystic ovarian syndrome undergoing assisted reproduction. *Cochrane Database Syst Rev* 2018;**11**:CD006606.
17. Ellenbogen A, Shavit T, Shalom-Paz E. IVM results are comparable and may have advantages over standard IVF. *Facts Views Vis Obgyn* 2014;**6**:77-80.
18. Rotterdam EA-SPcwg. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 2004;**19**:41-7.
19. Chen Y, Zheng XY, Yan J, et al. Neonatal outcomes after the transfer of vitrified blastocysts: closed versus open vitrification system. *Reprod Biol Endocrinol* 2013;**11**.
20. Zegers-Hochschild F, Adamson GD, Dyer S, et al. The International Glossary on Infertility and Fertility Care, 2017. *Fertility and sterility* 2017;**108**:393-406.
21. Royal college of Obstetricians and Gynaecologists. The management of ovarian hyperstimulation syndrome. *RCOG Green-top Guildline* 2016;**5**:1-22.
22. Yang R,Luo L, Wang Y,Li R, Liu P,Qiao J. clinical choice of ovarian stimulation protocol in the first IVF/ICSI cycle of polycystic ovary syndrome (PCOS) patienYangts. *Reproduction & contraception* 2015;**35**:241-46.
23. Chen ZJ, Shi Y, Sun Y, et al. Fresh versus Frozen Embryos for Infertility in the Polycystic Ovary

Syndrome. *The New England journal of medicine* 2016;**375**:523-33.

Figure 1. Flow chart of this RCT: IVM vs IVF in women with PCOS

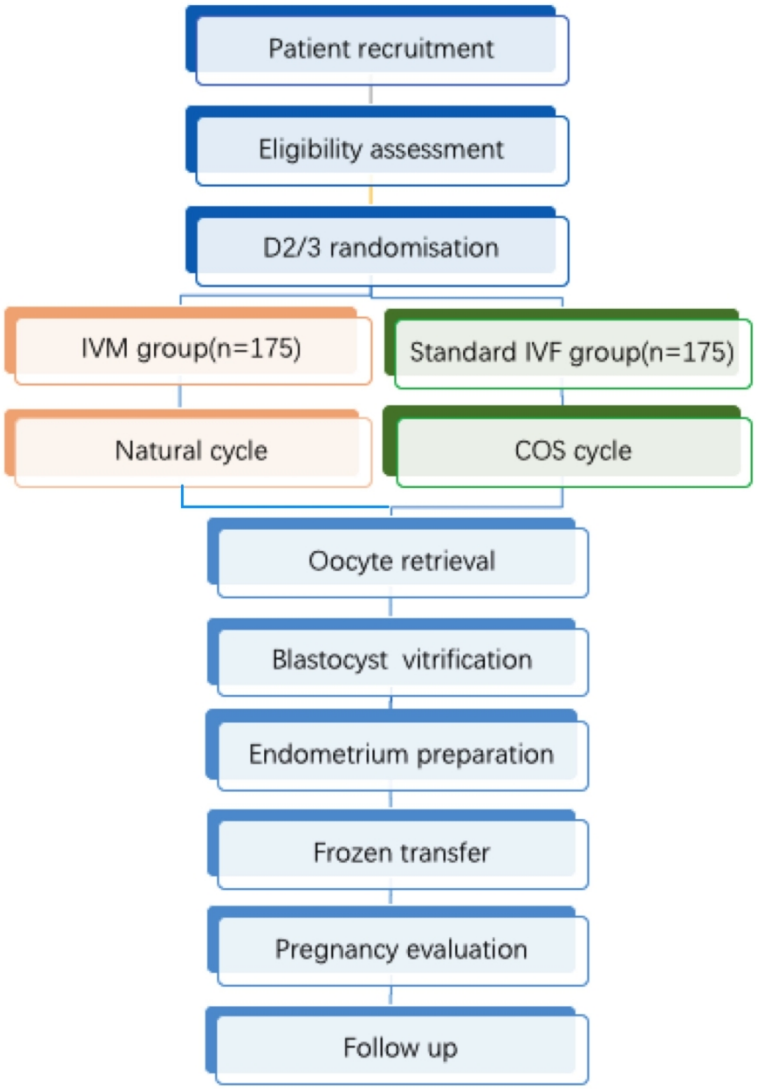

Figure 1. Flow chart of this RCT: IVM vs IVF in women with PCOS

172x239mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	p1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	P2
	2b	All items from the World Health Organization Trial Registration Data Set	P2
Protocol version	3	Date and version identifier	p1
Funding	4	Sources and types of financial, material, and other support	p15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	p1
	5b	Name and contact information for the trial sponsor	p1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	P2
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	p14

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	P3-4
	6b	Explanation for choice of comparators	P4
Objectives	7	Specific objectives or hypotheses	P4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	P4
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	P7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	P7-8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	P9-11
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	P8
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	P8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	P9-11
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	p12-13
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	P5-6

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	p14
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	P8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	P8
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	P8
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	P8
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	P8-9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	p11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	p11

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	p13-14
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	p14-13
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	p14-13
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	p14-13
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	p13-14
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	p14
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	p14
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	p14
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	p15
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	p15

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	p15
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	p15
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	p15
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	p15
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	p15
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	p15
	31b	Authorship eligibility guidelines and any intended use of professional writers	p15
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	p11
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Live birth after In vitro maturation versus standard In vitro fertilisation for women with polycystic ovary syndrome: protocol for a non-inferiority randomized clinical trial

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Title

Live birth after *In vitro* maturation versus standard *In vitro* fertilisation for women with polycystic ovary syndrome: protocol for a non-inferiority randomized clinical trial

Authors

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Abstract

Introduction: Polycystic ovary syndrome (PCOS) is the first common cause of anovulatory infertility. Currently, *In vitro* fertilisation (IVF) is recommended when conventional attempts have failed. *In vitro* maturation (IVM) of human oocytes is an emerging treatment option in infertile women with PCOS. It is a patient-friendly intervention, avoiding the risk of ovarian hyperstimulation syndrome (OHSS), which is a serious complication of controlled ovarian stimulation (COS) in the standard IVF procedure. We plan a randomised controlled trial (RCT) to evaluate whether IVM is non-inferior to the standard IVF for live birth in women with PCOS.

Methods and analysis: This is a single-center, open-label, non-inferiority RCT performed in a large reproductive medicine center in China. Infertile women with PCOS will be randomised to receive either IVM or standard IVF in a 1:1 treatment ratio after informed consent. IVF procedures used in our study are all standard treatments and other standard assisted reproductive technologies will be similar between the two groups. The primary outcome is ongoing pregnancy leading to live birth within 6 months of the first oocyte retrieval cycle after randomisation. Pregnancy outcome, maternal safety, and obstetric and perinatal complications will be secondary outcomes. The planned sample size is 350 (175 per group).

Ethics and dissemination: Ethical permission was acquired from the Ethics Committee of Peking University Third Hospital. The results will be issued to publications through scientific journals and conference reports.

Trial registration number: NCT03463772

Strengths and limitations of this study:

- This is a randomised controlled trial with an adequate sample size to evaluate IVM treatment in infertile women with PCOS.
- This study will provide evidence on whether the experimental group (IVM) is non-inferior to the standard IVF in terms of live birth.
- The cost-effective analysis is not included in the study, limiting the comprehensiveness of the results.

Keywords: *In vitro* fertilisation; polycystic ovary syndrome (PCOS); *In vitro* maturation (IVM); assisted reproductive technology.

Introduction

Polycystic ovary syndrome (PCOS) is the first common cause of anovulatory infertility and occurs in 5.6% in reproductive-aged women in the Chinese communities[1]. It is also a major metabolic disorder associated with insulin resistance, β -cell dysfunction, and obesity[2]. 50% of women with PCOS present with sub-fertility. In anovulatory women with PCOS, the first-line treatments are lifestyle intervention and ovulation induction with letrozole or clomiphene citrate[3]. Laparoscopic ovarian drilling or ovarian induction with gonadotrophin (Gn) are considered as the second-line treatment options. *In vitro* fertilisation (IVF) is the third-line treatment and is recommended when the above-mentioned treatments have failed.

In standard IVF treatment, women with PCOS are at high risk of ovarian hyperstimulation syndrome (OHSS), which is a serious and common iatrogenic complication of controlled ovarian stimulation. The prevalence of moderate and severe forms of OHSS in women undergoing IVF is 3% to 8% and much higher in women with PCOS[4]. Severe OHSS is defined by the presence of clinical evidence of ascites with severe abdominal pain and pleural effusion. A large amount of pleural and peritoneal effusion can lead to intravascular blood loss, blood concentration, blood hypercoagulability, hypovolemic shock, severe cardiopulmonary dysfunction, electrolyte imbalance, impaired liver and kidney function, thrombosis, and even life-threatening[5]. Therefore, seeking an alternative treatment strategy, avoiding the risk of OHSS without compromising pregnancy outcomes is crucial for women with PCOS.

In vitro maturation (IVM) has been introduced in 1990. Trounson[6] described the first delivery of a healthy baby with the IVM technique in a woman with PCOS. From then on, immature oocyte retrieval followed by IVM had been used widely, resulting in the delivery of thousands healthy infants worldwide[7-9]. In IVM, immature oocytes are collected from antral follicles, typically from unstimulated or minimally stimulated ovaries, then cultured, matured and fertilised in vitro[10]. Compared with standard IVF treatment, IVM is performed without ovarian stimulation, thus preventing the occurrence of OHSS and reducing financial costs.

To date, despite the publication of many studies on IVM, the effectiveness and safety of IVM treatment are still controversial. Some studies showed that the live birth rate was significantly lower in the IVM group than in the IVF group[11-13]. Now mature technology, gradually getting more research results indicated comparable pregnancy

outcomes between IVM and IVF group[14 15]. Until now, all data available have derived from observational studies[16]. Given the unique advantages of IVM as safer, simpler and cheaper[17], we need further evidence from well-designed randomised controlled trials (RCT) on the live birth rate before we draw conclusions on the effectiveness and safety of IVM. Therefore, we plan an RCT to determine whether IVM is non-inferior to standard IVF on live birth for women with PCOS.

Methods and analysis

Study design

This is a single-center, non-inferiority RCT with a 1:1 treatment ratio. The program for enrollment, interventions, and evaluation during the study process is shown in Table 1. Figure 1 indicates a flowchart showing the registration, allocation, treatment, and follow-up of participants.

Table 1. Schedule of enrollment, interventions, and assessments

	Study Period							
	Enrollment	Allocation		Post-allocation				Close-out
Content	Screening & Baseline assessment	IVM & IVF Randomisation	Oocyte retrieval	Assessment of embryo	Embryo transfer	Evaluation of pregnancy	Follow-up of pregnancy	
Time point	T_0 -1 month	T_1 o month	T_2 10-14 days	T_3 1-3-5 days after opu	T_4 2-6 month	T_5 1-7 month	T_6 6-10 months	T_7 8-12 months
Enrollment								
Eligibility screen	×	×						
Informed consent	×							
Allocation		×						
Interventions								
IVM		×						
Standard IVF		×						

Assessments								
Baseline data	×							
Laboratory tests	×	×	×		×	×	×	×
Fertilisation				×				
Embryo quality				×				
Pregnancy tests						×		
Pregnancy outcomes							×	×
Fetus information							×	×
Neonate information							×	×
Safety assessment		×	×		×	×	×	×

Study setting

The trial is approved by the Ethics Committee of Peking University Third Hospital (2017sz-066). The study was registered on Clinicaltrials.gov (NCT03463772). Women with PCOS and infertility who scheduled for their first IVF attempt at Peking University Third Hospital (Beijing, China) will be recruited. Potentially eligible women will be given information about the study during their first consultation. All the couples provided written informed consent before participation. The trial progress will be monitored by an independent data and safety monitoring board (DSMB).

Eligibility criteria

PCOS outpatient women with infertility who come to the reproductive center of Peking University Third Hospital will be screened for eligibility by a research team.

Inclusion Criteria:

- Married Chinese women with infertility aged between 20 and 38 years.
- Women with PCOS according to the revised Rotterdam criteria (i.e. two of the three following features are present: 1) Oligo- and/or anovulation; 2) Clinical and/or biochemical signs of hyperandrogenism; 3) Polycystic ovaries). Exclusion of other possible related disorders: ovarian or adrenal androgen-secreting tumors, thyroid disease, hyperprolactinemia, non-classical adrenal hyperplasia[18].
- Women scheduled for their first IVF/ICSI cycle. Women must have an indication for ART, including ovulation dysfunction and failure to become pregnant after ovulation induction treatment; unilateral or bilateral tubal obstruction, adhesion, salpingectomy or tubal ligation; oligoasthenozoospermia.
- Written Informed consent obtained.

Exclusion criteria:

- Couples with a contraindication for IVF or ICSI (including but not limited to poorly controlled type 1 or type 2 diabetes mellitus; liver disease or dysfunction (based on serum liver enzyme test results); renal disease or abnormal serum renal function; anemia; history of deep venous thrombosis, pulmonary embolus or cerebrovascular accident; uncontrolled hypertension or known symptomatic heart disease; history of (or suspected) cervical carcinoma, endometrial carcinoma or breast carcinoma; and unexplained colporrhagia).
- Couples receiving donor sperm or donor oocytes.
- Couples with indications or have the plan to receive preimplantation genetic testing

(PGT).

- Women with a male partner diagnosed with azoospermia.
- Either a male partner or female partner with a known abnormal chromosome karyotype (chromosome polymorphisms was not included).
- Women who have undergone unilateral ovariectomy.

Participants can leave the study at any time without any consequences for their clinical treatments.

Recruitment

Potentially eligible women will be informed about the trial during their first consultation. If a woman wants to participate, written informed consent will be acquired in their next scheduled visit. To initiate the IVM or IVF treatment cycle in anovulatory cases, patients were administrated oral dydrogesterone (Duphaston, Abbott, OLST, Netherlands) 20 mg daily for 10-14 days/ Oral contraceptives Diane-35 (cyproterone acetate 2 mg, ethinylestradiol 35 mg, Bayer, and its generics) for 21 days. After the withdrawal bleeding, eligible participants will be assessed again for the exclusion criteria on day 2 or day 3 following the onset of menstrual bleeding.

Randomisation and allocation concealment

Randomisation and allocation of qualified participants will be done on day 2/3 of the menstrual cycle. Eligible participants will be allocated to IVM or IVF treatment according to a computer-generated randomisation list in a 1:1 ratio, with a variable block size of 4 or 6. Opaque sealed envelopes, with participant's screening order printed outside and randomised assigned group printed inside, will be numbered consecutively. Researchers will enroll the eligible participants after the screening, then open each envelope in a sequence corresponding with the screening order and assign the participant into the IVM or IVF group. Both investigators and participants will be aware of the allocation of the subsequent treatments.

Interventions

IVM protocol

Gonadotrophins (FSH, HMG or HCG) will not be used in the IVM group. After randomisation, participants will visit the clinic on days 6–8 for a transvaginal ultrasound examination to exclude the development of a dominant follicle. Oocyte

retrieval will be scheduled once the endometrium thickness reaches at least 6 mm and there is no appearance of the dominant follicle (follicle diameter >10 mm). Transvaginal ultrasound-guided oocytes retrieval will be conducted with a single-lumen 19G aspiration needle (K-OPS-7035-REH-ET; Cook, Queensland, Australia) in 90 mmHg suction pressure. Follicular aspirates will be gathered and filtered through a cell strainer (Cell Strainer 352350, 70μm nylon; Falcon, MA, USA). Then the collected aspirates will be washed with pre-warmed Hepe's-HTF (CooperSurgical; Trumbull, CT, USA). The mixture will be examined carefully under a stereomicroscope to search for cumulus-oocyte complexes (COCs). All COCs will be transferred into IVM medium (IVM media kit; Sage, CT, USA) supplemented with 0.075 IU/ml FSH and 0.075 IU/ml LH (Menopur; Ferring, Kiel, Germany) in 5% CO2 incubator at 37℃. All the COCs will be denuded of cumulus cells after 28-32 h of culture and evaluated the maturation process. The presence of a first polar body indicates that the oocyte enters the metaphase II stage. All the metaphase II (MII) oocytes will be inseminated by means of intracytoplasmic sperm injection (ICSI).

IVF protocol

For participants in the IVF group, COS will be performed by a flexible protocol using gonadotrophin-releasing hormone antagonist (GnRH-ant) with recombinant FSH (rFSH) and recombinant human chorionic gonadotrophin (rhCG). Treatment will start on day 2/3 of the menstrual cycle. All participants will be subcutaneous injected rFSH (Gonal-F; Serono, Geneva, Switzerland) with 100-225IU/d initial dosage. Transvaginal ultrasound, serum luteinizing hormone (LH), serum estrogen (E2) and progesterone (P4) will be tested to monitor follicle growth. rFSH doses will be adjusted according to ovarian response. GnRH antagonist-cetrorelix (Cetrotide; Serono, Darmstadt, Germany) 0.25mg daily by subcutaneous injection will begin when at least one follicle has reached a diameter of 12mm which is usually between day 5 to day 8 of ovarian stimulation, until the trigger day (include the trigger day). After 2 or more follicles reach a diameter ≥17 mm, 250ug of rhCG (Ovidrel; Serono, Aubonne, Germany) will be once injected on trigger day. Oocyte retrieval will be performed 36 (±2) hours after rhCG injection. Oocyte collection is conducted via 17G oocyte aspiration needle with the use of intravenous sedation. All COCs will be cultured in Human Tubal Fluid medium and incubated in a humidified 37℃ incubator 5% CO2, after oocyte retrieval immediately. The fertilisation method will be selected according to the semen analysis.

Assessment of fertilisation and embryo culture

Fertilisation will be considered normal when two pronuclei are present between 16 and 18h after ICSI or IVF. Normal fertilisation rate will be calculated as the number 2PN over the number of MII in ICSI patients or the number 2PN over the number of COCs for conventional IVF. All zygotes will be cultured in cleavage medium (G-1plus, Vitrolife, USA) for further 48-52h after fertilisation. Cleavage embryonic development will be assessed according to the developmental stage and degree of cytoplasmic fragmentation. All day 3 embryos will be cultured up to 2-3 days (G-2 plus, Vitrolife, USA). From day 5 to day 6, those embryos achieving the blastocyst stage will be evaluated morphologically using Gardner's grading system.

For all participants, a freeze-only blastocyst transfer strategy will be applied. All usable blastocysts (embryos that can grow to expanded or hatching blastocysts earn a score above grade CC) will be cryopreserved by vitrification methods.

Blastocyst vitrification and warm

The expanded blastocysts collapsed after artificial shrinkage will be vitrified and warmed as previously demonstrated[19]. In brief, the blastocysts will be transferred in equilibration medium, which included 7.5% (v/v) dimethyl sulfoxide (DMSO, Sigma Chemical Co., MO, USA) and 7.5% (v/v) ethylene glycol (EG, Sigma Chemical Co., MO, USA), at 37℃ for 2 min, then placed in vitrification solution for 30 sec that containing 15% DMSO, 15% EG, and 0.65 mol/l sucrose. During this period, shrinkage blastocyst will be loaded on the Cryotop strip (Kitazato, Fuji, Japan), next plunged into liquid nitrogen immediately. For warming, the cryotop will be quickly placed in 0.33 mol/l sucrose at 37℃ and searching for the floated blastocyst under the microscope. The blastocyst will be washed several times and placed for 2 min. Then it will be transferred into 0.2mol/l sucrose for 3 min and in a HEPES-buffered medium for 5 min in turn. After 2 hours, the blastocysts will be evaluated the quality. The re-expanded blastocysts will be considered to survive and will be transferred to the patients.

Endometrium preparation, blastocyst transfer, and luteal support

In vitrified-warmed blastocyst transferred cycles, participants will undergo hormone replacement therapy for their endometrial preparation. All participants' once endometrial thickness reaches 6 mm and no ovarian cyst is observed on ultrasound, oral

oestradiol valerate (Progynova, Delpharm Lille, Lys-Lez-Lannoy, France) at a dose of 3mg twice daily will be started on day 1–3 of the menstrual cycle. And then an ultrasound will be repeated after more than 10 days after administration of HRT, vaginal progesterone gel (Crinone, Merck Serono, Watford, UK) 90 mg per day and oral dydrogesterone (Duphaston, Abbott, OLST, Netherlands) 20 mg twice daily will be added when the endometrial thickness was ≥8 mm. All the ultrasound scans and measurements will be performed with the digital platform Voluson E8 system. Only one blastocyst will be transferred on day 7 after progesterone administration. Luteal phase support was continued until 12 weeks' gestation.

Follow up

All participants will measure the hCG of Urine and blood at the 12 days after blastocyst transfer, in which positive results indicate biochemical pregnancy. On 28 days after transfer, if the gestational sac is observed with ultrasonography, participants will be diagnosed with clinical pregnancy. Ongoing pregnancy is defined as a sac with the fetal heartbeat and occurs after 12 weeks of pregnancy. Ongoing pregnancy leading to live-birth is defined by the live fetus in the uterine after 22 weeks of gestation. We will collect the following information within 6 weeks after delivery, prenatal testing information (pregnancy complications and fetal information), delivery information (gestational age, mode of delivery, placental condition and birth complications), and newborn information (fetal sex, birth weight, and birth defects).

Outcome measures

Primary outcome

Our primary study endpoint is ongoing pregnancy leading to live birth (gestation age≥22 weeks) within 6 months of the first oocyte retrieval cycle after randomisation[20].

Secondary outcomes

Secondary outcomes of our trial include pregnancy outcomes, maternal safety, and obstetric and perinatal complications. Detailed information is provided in Table 2. Maternal safety is an important part of the secondary outcomes. The incidence of OHSS is the key indicator of maternal safety. Routine evaluation for OHSS will be performed on oocyte retrieval day and on day3 after fertilisation with ultrasound scanning and hormonal result in all participants. It is classified as mild, moderate, or severe according

to the RCOG guildline[21].

Safety Reporting

Adverse events (AE) are defined as any undesirable experience occurring to a subject during the trial. A serious adverse event (SAE) is any untoward medical events, that results in death; is life-threatening (at the time of the event); requires hospitalization or prolongation of existing inpatients' hospitalization; results in persistent or significant disability or incapacity; is a congenital anomaly or birth defect; is a new event of the trial likely to affect the safety of the subjects, such as an unexpected outcome of an adverse reaction.

SAE in this study includes: moderate/severe OHSS; intraperitoneal hemorrhage or ovarian torsion after oocyte retrieval; ectopic pregnancy; severe preeclampsia; pregnancy complications leading to hospitalization; stillbirth; birth defects; other serious medical events judged by researchers to meet the criteria of SAE.

All SAEs will be reported to the DSMB and accredited Medical Education Technology Committee (METC) that approved the protocol, according to the requirements of that METC.

Table 2. Secondary outcomes and related definition

Secondary outcomes	Definition
Pregnancy outcomes	
Implantation	Number of gestational sacs observed per embryo transferred
Clinical pregnancy	One or more observed gestational sac or definitive clinical signs of pregnancy under ultrasonography at 7 weeks of gestation (including clinical documented ectopic pregnancy)
Ongoing pregnancy	Presence of a gestational sac and fetal heartbeat after 12 weeks of gestation
Time to ongoing pregnancy leading to live birth*	Time from randomisation to detection of ongoing pregnancy after completion of the transfer
Maternal safety outcomes	
OHSS	Exaggerated systemic response to ovarian stimulation characterized by a wide spectrum of clinical and laboratory manifestations. It is classified as mild, moderate, or severe according to the degree of abdominal distention, ovarian enlargement and respiratory hemodynamic, and metabolic complications. Diagnosed by ultrasound, blood testing and physical examination according the RCOG Guideline[21].
Miscarriage	Spontaneous loss of an intra-uterine pregnancy prior to 28 completed weeks of gestational age
Ectopic pregnancy	A pregnancy outside the uterine cavity, diagnosed by ultrasound, surgical visualization or histopathology

Obstetric and perinatal complications	
Gestational diabetes mellitus	Development of diabetes during pregnancy
Hypertensive disorders of pregnancy	Including pregnancy-induced hypertension, pre-eclampsia and eclampsia
Antepartum haemorrhage	Including placenta previa, placenta accreta and unexplained
Birth weight	Including low birth weight (weight < 2500 gm at birth), very low birth weight (< 1500 gm at birth), high birth weight (>4000 gm at birth) and very high birth weight (>4500 gm at birth)
Large for gestational age	A birth weight greater than the 90th centile of the sex-specific birth weight for a given gestational age reference
Small for gestational age	A birth weight less than the 10th centile for the sex-specific birth weight for a given gestational age reference
Preterm birth	Birth of a fetus delivered after 28 and before 37 completed weeks of gestational age in participants confirmed ongoing pregnancy
Congenital anomaly	Structural or functional disorders that occur during intra-uterine life and can be identified prenatally, at birth or later in life, including trisomy 21 syndrome, neural tube defect, congenital heart disease, cleft lip, excessive numbers of fingers or toes, hydrocephalus.
Perinatal mortality	Fetal or neonatal death occurring during late pregnancy (at 28 completed weeks of gestational age and later), during childbirth, or up to seven completed days after birth)

297 * only ongoing pregnancy leading to live birth within 6 months of the first oocyte retrieval cycle after randomisation will be counted.

Statistical analysis

Sample size calculation

The primary hypothesis of this study is that the experimental group (IVM) is non-inferior to the control group (IVF) in terms of ongoing pregnancy leading to live birth. On the basis of actual data on the women with PCOS who underwent IVM or IVF-ET in our reproductive medicine center, we assume that after IVF the proportions of ongoing pregnancy leading to live birth for the PCOS women are 35% per transferred cycle[22 23]. With a non-inferiority margin of 15%, power of 80%, and one-sided α 0.025. We will need to enroll 159 participants in each group (the ratio between groups will be 1:1). Taking consideration of dropout rate as 10% (such as cancellation during trial procedures). Each group will include 175 participants (a total of 350 participants).

Statistical analysis

The result will be analyzed according to the intention-to-treat (ITT) principle. Pre-protocol (PP) analysis will also be conducted as a sensitivity analysis. Baseline characteristics will be described by descriptive analysis, and the balance among groups or subgroups will be assessed by analysis for different kinds of data. For continuous variables, the normality distribution will be estimated by using frequency histograms and the Kolmogorov-Smirnov test initially. If the continuous variables are normally distributed, they will be presented as means with standard deviations (SDs). If the continuous variables are non-normally distributed, their medians and inter-quantile ranges (IQRs) will be reported. For categorical variables, we will present the proportion between each group. In addition, the recruitment numbers, those participants lost to follow-up, protocols violations and other relevant data will also be reported. A comparison between groups will be performed using the independent sample t-test, Mann-Whitney U test for continuous variables, or Pearson chi-square test/Fisher's exact test for categorical variables as appropriate. The primary outcome, ongoing pregnancy leading to live birth, will be compared using Pearson's chi-square test or Fisher's exact test as appropriate. Categorical secondary outcomes will be compared between two groups using a similar approach as the primary outcome. Student's t-test or Wilcoxon test will be used as appropriate for continuous secondary outcomes, such as birth weight and etc. The relative risks (RR) and absolute rate differences (ARD) and their 95% confidence interval (CI) between the two groups will be calculated. And the 95% CI of the ARDs will be used to evaluate if the experimental group (IVM) is

non-inferior to the control group (IVF-ET). For the time-to-event outcome, the Kaplan-Meier curve will be used. Multiple variable logistic regression models will be used to assess the treatment effect adjusting for other potential confounding variables that are unbalanced in the baseline.

Missing data will be treated as missing at random and will be imputed using the last observation carried forward (LOCF) method. For the missing values, a sensitivity analysis will be done under the hypothesis of the worst and the best outcomes for each missing individual. Therefore, all secondary outcomes will be considered exploratory. All statistical analyses will be done using the statistical package SPSS, version 25.0 (SPSS Inc., Chicago, IL). Statistical significance is defined as $p< 0.05$ with two-sided testing except for the non-inferiority test for the primary outcome.

Trial status

The study was designed in 2017. The first participant was recruited in March 2018 and the last participant was recruited in July 2019. The follow-up is ongoing. It is expected that data collection will be completed in Oct 2020.

Patient and Public Involvement

This research was done without patient or public involvement. Neither patients nor the public was involved in the development of the research question, study design or implementation of this trial. Patients will not be invited to develop patient-relevant outcomes or interpret the results, as well as the writing or editing of the final manuscript for readability or accuracy. As interventions in our study are both routine procedures during clinical work, the burden of the intervention is assessed by patients themselves.

Ethics and dissemination

The trial had been reviewed and approved by the Science Research Ethics Committee of Peking University Third Hospital (2017sz-066). The study is registered on Clinicaltrials.gov as NCT03463772. Informed consent will be obtained from each participant before randomization. The researchers will permit trial-related monitoring, audits, regulatory inspections, providing direct access to source data and documents. There are no additional data available in this study protocol.

Authors' contributions

JQ and RL conceived the study idea. JQ, RL, RW, BWM, XZ, and WG participated in the design of the study and drafting of the manuscript. XZ, WG, SY, and LW participate in the recruitment of participants and the assessment of clinical outcomes. LZ and DZ coordinate data collection and will perform data analysis. All authors critically reviewed the article and approved the final manuscript.

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Competing interests statement

All authors do not have competing interests to declare.

Figure 1. Flow chart of this RCT: IVM vs IVF in women with PCOS

Reference

1. Li R, Zhang Q, Yang D, et al. Prevalence of polycystic ovary syndrome in women in China: a large community-based study. *Hum Reprod* 2013;**28**:2562-9.
2. Messinis IE, Messini CI, Anifandis G, et al. Polycystic ovaries and obesity. *Best Pract Res Clin Obstet Gynaecol* 2015;**29**:479-88.
3. 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.
4. Mourad S, Brown J, Farquhar C. Interventions for the prevention of OHSS in ART cycles: an overview of Cochrane reviews. *Cochrane Database Syst Rev* 2017;**1**:CD012103.
5. Namavar Jahromi BM, Parsanezhad MM, Shomali ZM, et al. Ovarian Hyperstimulation Syndrome: A Narrative Review of Its Pathophysiology, Risk Factors, Prevention, Classification, and Management. *Iran J Med Sci* 2018;**43**:248-60.
6. Trounson A, Wood C, Kausche A. In vitro maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. *Fertil Steril* 1994;**62**:353-62.
7. Chian RC, Xu CL, Huang JY, et al. Obstetric outcomes and congenital abnormalities in infants conceived with oocytes matured in vitro. *Facts Views Vis Obgyn* 2014;**6**:15-8.
8. Mostinckx L, Segers I, Belva F, et al. Obstetric and neonatal outcome of ART in patients with polycystic ovary syndrome: IVM of oocytes versus controlled ovarian stimulation. *Hum Reprod* 2019;10.1093/humrep/dez086.
9. Yu EJ, Yoon TK, Lee WS, et al. Obstetrical, neonatal, and long-term outcomes of children conceived from in vitro matured oocytes. *Fertil Steril* 2019;10.1016/j.fertnstert.2019.05.034.
10. Edwards RG. Maturation in vitro of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes. *Nature* 1965;**208**:349-51.
11. Das M, Son WY, Buckett W, et al. In-vitro maturation versus IVF with GnRH antagonist for women with polycystic ovary syndrome: treatment outcome and rates of ovarian hyperstimulation syndrome. *Reprod Biomed Online* 2014;**29**:545-51.
12. Gremeau AS, Andreadis N, Fatum M, et al. In vitro maturation or in vitro fertilization for women with polycystic ovaries? A case-control study of 194 treatment cycles. *Fertil Steril* 2012;**98**:355-60.
13. de Ziegler D, Streuli I, Gayet V, et al. Retrieving oocytes from small non-stimulated follicles in polycystic ovary syndrome (PCOS): in vitro maturation (IVM) is not indicated in the new GnRH antagonist era. *Fertil Steril* 2012;**98**:290-3.
14. Walls ML, Hunter T, Ryan JP, et al. In vitro maturation as an alternative to standard in vitro fertilization for patients diagnosed with polycystic ovaries: a comparative analysis of fresh, frozen and cumulative cycle outcomes. *Hum Reprod* 2015;**30**:88-96.
15. Ho VNA, Pham TD, Le AH, et al. Live birth rate after human chorionic gonadotropin priming in vitro maturation in women with polycystic ovary syndrome. *J Ovarian Res* 2018;**11**:70.
16. Siristatidis CS, Maheshwari A, Vaidakis D, et al. In vitro maturation in subfertile women with polycystic ovarian syndrome undergoing assisted reproduction. *Cochrane Database Syst Rev* 2018;**11**:CD006606.
17. Ellenbogen A, Shavit T, Shalom-Paz E. IVM results are comparable and may have advantages over standard IVF. *Facts Views Vis Obgyn* 2014;**6**:77-80.
18. Rotterdam EA-SPcwg. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 2004;**19**:41-7.
19. Chen Y, Zheng XY, Yan J, et al. Neonatal outcomes after the transfer of vitrified blastocysts: closed versus open vitrification system. *Reprod Biol Endocrinol* 2013;**11**.
20. Zegers-Hochschild F, Adamson GD, Dyer S, et al. The International Glossary on Infertility and Fertility Care, 2017. *Fertility and sterility* 2017;**108**:393-406.
21. Royal college of Obstetricians and Gynaecologists. The management of ovarian hyperstimulation syndrome. *RCOG Green-top Guildline* 2016;**5**:1-22.
22. Yang R,Luo L, Wang Y,Li R, Liu P,Qiao J. clinical choice of ovarian stimulation protocol in the first IVF/ICSI cycle of polycystic ovary syndrome (PCOS) patienYangts. *Reproduction & contraception* 2015;**35**:241-46.
23. Chen ZJ, Shi Y, Sun Y, et al. Fresh versus Frozen Embryos for Infertility in the Polycystic Ovary

Syndrome. *The New England journal of medicine* 2016;**375**:523-33.

Figure 1. Flow chart of this RCT: IVM vs IVF in women with PCOS

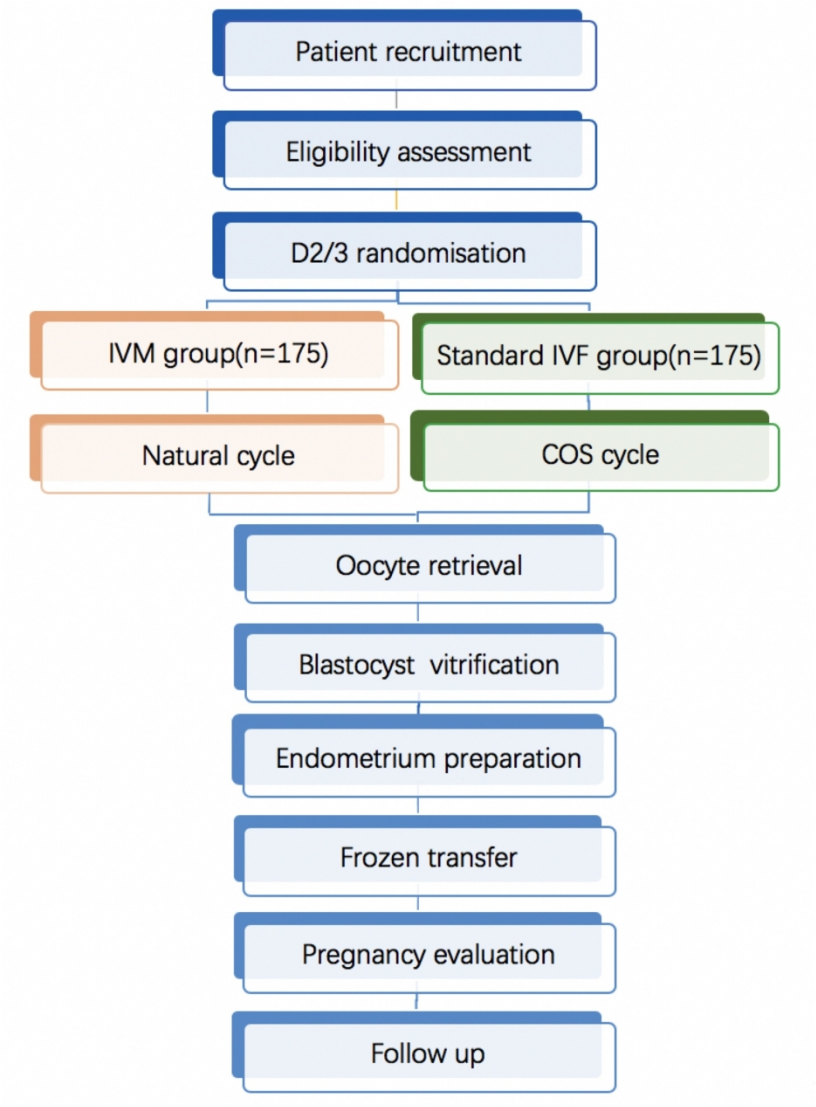

Figure 1. Flow chart of this RCT: IVM vs IVF in women with PCOS

175x249mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	p1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	P2
	2b	All items from the World Health Organization Trial Registration Data Set	P2
Protocol version	3	Date and version identifier	p1
Funding	4	Sources and types of financial, material, and other support	p15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	p1
	5b	Name and contact information for the trial sponsor	p1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	P2
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	p14

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	P3-4
	6b	Explanation for choice of comparators	P4
Objectives	7	Specific objectives or hypotheses	P4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	P4
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	P7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	P7-8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	P9-11
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	P8
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	P8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	P9-11
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	p12-13
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	P5-6

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	p14
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	P8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	P8
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	P8
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	P8
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	P8-9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	p11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	p11

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	p13-14
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	p14-13
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	p14-13
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	p14-13
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	p13-14
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	p14
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	p14
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	p14
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	p15
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	p15

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	p15
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	p15
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	p15
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	p15
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	p15
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	p15
	31b	Authorship eligibility guidelines and any intended use of professional writers	p15
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	p11
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.