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Keywords:	In vitro fertilization, Intracytoplasmic sperm injection, Non-severe male infertility, Assisted reproductive technology

Title

Intracytoplasmic sperm injection (ICSI) versus conventional in vitro fertilization (IVF) in couples with non-severe male infertility (NSMI-ICSI): protocol for a multi-center randomized controlled trial

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Abstracts

Introduction: Intracytoplasmic sperm injection (ICSI), originally introduced as add-on to in vitro fertilization (IVF) for couples with severe male infertility, is in current clinical practice also used in couples with mild male or even unexplained infertility. However, ICSI has involved unresolved concerns regarding the selection and damage to gametes and the health conditions of the offspring, and it is also labour intensive and therefore more expensive than conventional IVF. High quality well powered randomized clinical trials (RCTs) comparing ICSI and IVF are lacking.

Methods and analysis: We propose a multicenter, open-label RCT in six reproductive medical centers across China. We will study couples with non-severe male infertility scheduled for their first or second ICSI or IVF cycle, as low fertility rate after fertilization are more frequent in this population, which could lead to controversy about ICSI or conventional IVF for fertilization. On the day of oocyte retrieval, eligible participants will after informed consent be randomized to undergo either ICSI or conventional IVF in a 1:1 treatment ratio. Other standard assisted reproductive treatments are similar and parallel between two groups. Our primary outcome is ongoing pregnancy leading to live birth after the first cycle with embryo transfer. To demonstrate or refute a difference of 7% between ICSI and conventional IVF, we need to include 2,346 women (1,173 in each intervention arm). In addition, we will follow up neonatal outcomes after delivery to identify the influence of ICSI on offspring.

Ethics and dissemination: Ethical approval was obtained from Peking University Third Hospital medical science research ethics committee. The findings will be disseminated to the public through conference presentations and peer-reviewed scientific journals.

Trial registration number: NCT03298633

Strengths and limitations of this study

- It's the first randomized controlled clinical trial with a large sample size in 6 centers across China.
- Range of sperm parameters in our study is extended based on the fifth edition of WHO manual for the examination and processing of human semen, which will be applicable to couples with non-severe male infertility as many as possible.
- Results in this study will provide evidence on whether conventional ICSI or IVF is the better method for fertilization in terms of live birth for non-severe male infertility.

Keywords: In vitro fertilization; Intracytoplasmic sperm injection; Non-severe male infertility; Assisted reproductive technology.

Introduction

Male infertility is caused by impaired sperm production and function due to different congenital or acquired factors,[1] and has been estimated to be associated with approximately 30% of infertility.[2,3] Assisted reproductive technology (ART) is perceived as a more successful treatment.[4,5] Originally applied in women with tubal damage in 1970s, in-vitro fertilization (IVF) is now acknowledged as an effective treatment for infertility as a major component of ART.[6] However, conventional IVF was much less effective when the semen characteristics were grossly below the standard values according to the WHO fourth edition sperm parameter values and when fertilization rate in previous cycles was low.[7,8]

In 1992,[9] intracytoplasmic sperm injection (ICSI), a technique where a single spermatozoon was injected mechanically into an oocyte in vitro to achieve fertilization, was introduced. While complete fertilization failure was reported up to 50% of the conventional IVF treatments for couples with moderate male infertility (moderate oligozoospermia, asthenozoospermia and teratozoospermia), this occurred in less than 3% of the couples undergoing ICSI.[10-13] Consequently, ICSI has been applied worldwide to treat severe male infertility.[14-16]

The high success rate of ICSI has resulted in its increased use in other populations for whom conventional IVF may be an option, particularly non-male factor infertility. In Europe, in 2012 ICSI was used in 69% of IVF cycles compared to 35% in 1997, while in the Middle East, South-America and South-East Asia, ICSI is performed in 100% of IVF cycles even in some regions.[17,18] In the USA, between 1996 and 2012, the use of ICSI in IVF cycles has increased from 34% to 76%. The greatest increase was documented in non-male factor infertility, where the use of ICSI went from 15% to 67% during this time period.[19]

There are concerns on the increased use of ICSI, as ICSI is time consuming, expensive, and involves unresolved concerns regarding the damage to gametes and the health conditions of the offspring.[20-24] Many studies have indicated the routine use of ICSI in non-male factors infertility was not recommended to improve the clinical outcomes.[25-28] For non-severe male factor infertility, including mild and moderate oligospermia with or without asthenospermia, the fertilization and pregnancy outcome after ICSI compared with conventional IVF is unclear. Studies randomizing sibling oocytes have shown conflicting results. Several studies have documented higher fertilization rates and lower rates of fertilization failure in these couples undergoing ICSI.[29-31] Other studies did not support the

benefit of ICSI in prevention of total fertilization failure as there were no significant differences between ICSI and conventional IVF in embryo quality, implantation, clinical pregnancy, or live birth rates.[32-34] These studies have limitations such as small sample size, non-randomized couples, or no evaluation of live births. In addition, fewer application of ICSI in China may result in low fertility rate for patients with non-severe male infertility, which would give raise to controversy about ICSI or conventional IVF for fertilization during ART in these population.[35]

In view of this situation, we plan an adequately powered multi-center randomized controlled clinical trial to assess whether ICSI is more effective than IVF in couples with non-severe male infertility.

Methods and analysis

Study design

We plan a multi-center, parallel, open-label, randomized controlled clinical trial (1:1 treatment ratio). The flow chart followed SPIRIT checklist showing enrollment, allocation, treatment, and follow-up of participants is presented in Figure 1.[36] In addition, the schedule of enrollment, interventions, and assessments during the study period is shown in Table 1.

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

	Study Period							
	Enrollment	Pre-allocation	Allocation	Post-allocation				Close-out
Content	Screening & Baseline assessment	Controlled ovarian hyperstimulation	Oocyte retrieval & Randomization	Assessment of embryo	Embryo transfer	Evaluation of pregnancy	Follow-up of pregnancy	
Time point	T_0 -3 month	T_1 -1 month	T_2 0 month	T_3 1-3 days	T_4 3 days	T_5 1 month	T_6 3-10 months	T_7 12 months
Enrollment								
Eligibility screen	×	×	×					
Informed consent	×							
Allocation			×					
Interventions								
ICSI			×					
Conventional IVF			×					
Assessments								
Baseline data	×							
Laboratory tests	×	×	×		×	×	×	×
Fertilization				×				
Embryo quality				×				
Pregnancy tests						×		
Pregnancy outcomes							×	×
Fetus information							×	×

Neonate information							×	×
Safety assessment		×	×		×	×	×	×

Study setting

The study will recruit participants from 6 reproductive medical centers across China: Peking University Third Hospital, International Peace Maternity and Child Health Hospital of Shanghai Jiao Tong University, Women's Hospital of Zhejiang University, The Sixth Affiliated Hospital of Sun Yat-Sen University, First Affiliated Hospital of Kunming Medical University, and Haidian Maternal and Child Health Hospital. This trial had been reviewed and approved by the medical science research ethics committee of Peking University Third Hospital: D2017050. The study is registered on ClinicalTrials.gov as NCT03298633. 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.

An independent data and safety monitoring board (DSMB), with members with clinical and statistical expertise, will monitor the trial progress and interim results at regular intervals.

Eligibility criteria

Couples presenting to reproductive medical center of the involved hospitals will be screened for following eligibility to be enrolled in our trial.

Inclusion Criteria:

- Infertile couples scheduled for their first or second IVF/ICSI cycle.
- Male partner has non-severe male infertility, defined as a semen concentrate 5-15$\times 10^6$/ml or sperm with progressive motility (type a+b) 10-32%.
- Women received either gonadotrophin-releasing hormone agonist protocol or gonadotrophin-releasing hormone antagonist protocol as their controlled ovarian hyperstimulation treatment.
- Informed consent obtained.

Exclusion criteria:

- Couple with a contraindication for IVF or ICSI, including poorly controlled type 1 or type 2 diabetes mellitus; undiagnosed 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 eggs.
- Couples undergoing preimplantation genetic testing.

- Sperm concentration with progressive motility used for insemination $<0.1\times10^6$/ml on the day of oocyte retrieval.
- Women with 0 oocyte retrieved.
- Using frozen semen.
- Poor fertilization in previous cycle (≤ 25%).

The sperm parameters defining non-severe male infertility are evaluated according to the WHO fifth edition sperm parameter values and the sperm parameters are subject to the latest sperm analysis.[37] Participants have the right to decline participation during the whole process, and they can withdraw their consent at any time. Their consent or refusal to consent will not affect their conventional clinical treatments.

Recruitment

Infertile couples who come to the outpatient clinic or medical record of infertile couples who have received COH treatment will be screened by a dedicated research team. Eligible couples will then, before oocyte retrieval, explained by a member of the research team the trial details. After this information, couples will be offered time for consideration to decide whether to participate in the trial. Couples who agree to participate will be asked to sign the consent form in their next scheduled visit. An individual record of all non-recruited patients and reasons for exclusion will be obtained and stored. On the day of oocyte retrieval, semen of patients who have signed consent form will be analysed again for the exclusion criteria. Ineligible patients will be further excluded from our trial, continuing their conventional clinical procedures instead.

Randomization

Randomization and allocation of eligible patients to study groups will be performed on the day of oocyte retrieval. This procedure will be performed by administrative staffs in the trial center not involved in the treatment procedure, using an online trial system with a computer-generated randomization list that allocates couples in a 1:1 ratio to ICSI or IVF, with a variable block size of 4 or 6 stratified for center. Stratified permuted block randomization will be centrally controlled.

Blinding

The trial was originally designed and performed as a double-blind trial, in which participants and clinicians/nurses who performed embryo transfer or follow-up, as well as the investigators and assessors will be blinded until the primary outcome occurred, while embryologists who performed IVF and ICSI were not blinded. Recruitment was slow due to the double-blind design, as participants

wanted to know about their allocation of fertilization method as soon as possible. Therefore, after recruitment of 115 participants, the design has been changed to an open-label study, in which researchers and clinicians are informed about the randomized allocation on the day of embryo transfer for participants with fresh embryo transfers and the day of embryo freezing for couples with freeze-all strategies. Prior to these dates, participants and clinicians will still be unaware of randomization allocation.

Interventions

Controlled ovarian hyperstimulation

All couples will receive controlled ovarian hyperstimulation (COH) treatment, which is performed by standard routine according to each study center. The COH treatment includes either gonadotrophin-releasing hormone agonist (GnRH-a) protocol or gonadotrophin-releasing hormone antagonist (GnRH-ant) protocol, and the selection of protocol will be done by physicians. In the GnRH-ant protocol, participants will be injected Gonadotropin (Gonal-F® or Pouliquen® or HMG®) daily from cycle day 2 or 3. When at least one follicle has reached a diameter of 12mm or on day 6 of ovarian stimulation, GnRH-ant (Cetrotide® or Ganirelix®) will be administered subcutaneously until the trigger day (include the trigger day). For super long GnRH-a protocol, GnRH-a (Alarelin® or Triptorelin®) will be used in previous menstrual cycle, gonadotropin treatment starts after 28-35 days on GnRH agonist downregulation. For long GnRH-a protocol, pituitary down-regulation will be initiated 7-10 days before the menstrual cycle with GnRH-a (Alarelin® or Triptorelin®). After 10-14 days or on day 2 of menstrual cycle, gonadotropin treatment will start. For short GnRH-a protocol, participants will receive Alarelin® or Triptorelin® for the pituitary down-regulation on day 2/3 of menstrual cycle. Gonadotropin will be used on the same time. For above treatments, menstrual cycle of patient includes spontaneous menstrual cycle, and irregular menstrual cycle by the use of oral contraceptives (OC) or progestins. Before gonadotrophin treatment, baseline pelvic ultrasound, as well as basic serum hormones (such as FSH, luteinizing hormone (LH), progesterone (P) and β-hCG) will be measured to confirm the follicle status. The initial dosage gonadotrophin (Gonal-F® or Pouliquen® or HMG®) is 150-300mg/d and the subsequent dose will be adjusted according to the individual response. Gonadotrophin treatment will be continued to the trigger day. After two or more follicles reach a diameter ≥18 mm, 250ug of hCG (Ovitrelle, 250 μg s.c.) will be once injected on trigger day.

Oocyte retrieval and preparation

Oocyte retrieval is scheduled for 36h (±2) after hCG injection. Routine oocyte pick-up is performed under transvaginal ultrasound guidance via 17G oocyte aspiration needle with use of intravenous sedation.

Semen preparation

Fresh ejaculate semen samples will be obtained by masturbation after 2-7 days' abstention from sexual intercourse on the day of oocyte retrieval. Sperm concentration and progressive motility are assessed by computer-assisted semen analysis according to the fifth edition of World Health Organization (WHO) laboratory standards of human semen and sperm.[37] All semen samples are prepared by discontinue density gradient centrifugation or swim-up protocol according to local routines.

ICSI Group

Oocytes in couples allocated to ICSI, will undergo ICSI as been previously described.[38] In short, as the enzymatic removal was done in oocyte preparation, the denuded oocytes are examined to assess integrity and maturity. Only those oocytes that have extruded the first polar body (metaphase-Ⅱ oocytes) will be microinjected.

IVF Group

All the oocytes in couples allocated to IVF will be treated by conventional IVF, in which every oocyte will be inseminated by sperm with progressive motility concentrate $0.1\text{-}0.2\times10^6$ approximately 39-42h after hCG injection.

Assessment of fertilization and embryo quality

Apart from the fertilization procedure, assisted reproductive treatments will be similar for the two groups. Assessment of fertilization is carried out about 16-18h (day 1) after fertilization. After the evaluation on day 1, zygotes are left in culture for a further 48 hours, and the cleavage embryo quality will be observed at 67-69 (day 3) hours after fertilization. The embryos are scored according to the quality, numbers, size of the blastomeres and the amount of anucleate fragmentation.

Embryo transfer and luteal support

Fresh or frozen-thawed embryo transfer will be decided and performed by physicians in three or five days following the day of oocytes collection for participants receive fresh embryo transfer, and 4-6 day after progesterone initiation for participants undergo frozen thawed embryo transfer (within 6

months after oocyte aspiration). To reduce the risk of high-order multiple pregnancies, the number of embryos replaced will be mostly limited to two best-quality embryos. Luteal support, as well as embryo freezing and thawing is performed by standard routines at each study center, as we assume that the different protocols will be equally distributed in the interventional and control groups.

Follow-up

Urine and blood hCG will be measured 14 days after embryo transfer, and positive results indicate biochemical pregnancy. If the gestational sac is observed with ultrasonography on 7 weeks after transfer, clinical pregnancy will be confirmed. Ongoing pregnancy is defined by the presence of a gestational sac with fetal heartbeat after 12 weeks of gestation. Women with confirmed as ongoing pregnancy will be asked to notify researchers the time of delivery. In 2 weeks after delivery, the information of pregnancy (pregnancy complications, and fetus information), delivery information (gestational age, delivery mode, placenta abnormality and/or delivery complications), infant information (such as sex, birth weight, birth defect) will be collected by completing forms designed for this visit.

Outcome measures

Primary outcome

Our primary outcome will be ongoing pregnancy leading to live birth after the first cycle with embryo transfer. Live birth will be defined as a delivery of one or more living infants (≥22 week's gestation or birth weight more than 1,000g).[17]

Secondary outcomes

For the effectiveness of the treatment, we will record these secondary outcomes in terms of effectiveness:

- Fertilization: defined as number of zygotes with 2PN (per oocyte retrieved and per oocyte inseminated/injected).
- Total fertilization failure: defined as no oocyte formed 2PN in this given cycle.
- Available embryo: defined as number of embryos ≥4 cells and ≤30% fragmentation (except embryos developed from ≥3PN zygotes) on day 3 observation.
- Good quality embryo: defined as number of embryos with ≥6 cells and ≤10% fragmentation developed from 2PN zygotes on day 3 observation.
- Implantation: defined as the number of gestational sacs observed per embryo transferred.

- Clinical pregnancy: defined as one or more observed gestational sac or definitive clinical signs of pregnancy under ultrasonography at 7 weeks after embryo transfer (including clinically documented ectopic pregnancy).
- Multiple pregnancy: defined as a pregnancy with two or more gestational sacs or positive heart beats at 7 weeks of gestation.
- Ongoing pregnancy: defined as the presence of a gestational sac and fetal heartbeat after 12 weeks of gestation.

For the safety of the treatment, we will record the following treatment complications as secondary outcomes:

- Moderate/severe ovarian hyperstimulation syndrome (OHSS): defined as 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.
- Miscarriage: defined as the spontaneous loss of an intra-uterine pregnancy prior to 22 completed weeks of gestational age.
- Ectopic pregnancy: defined as the implantation takes place outside the uterine cavity, confirmed by sonography or laparoscopy.

We will also collect the following obstetric and perinatal complications:

- Gestational diabetes mellitus (GDM).
- Hypertensive disorders of pregnancy (comprising pregnancy induced hypertension (PIH); pre-eclampsia (PET) and eclampsia).
- Antepartum haemorrhage, including placenta previa, placenta accreta and unexplained.
- Preterm birth: defined as birth of a fetus delivered after 28 and before 37 completed weeks of gestational age in participants confirmed ongoing pregnancy.
- Birth weight, including low birth weight (defined as weight < 2500 gm at birth), very low birth weight (defined as < 1500 gm at birth), high birth weight (defined as >4000 gm at birth) and very high birth weight (defined as >4500 gm at birth).
- Large for gestational age (defined as birth weight >90th centile for gestation, based on standardized ethnicity based charts) and small for gestational age (defined as less than 10th centile for gestational age at delivery based on standardized ethnicity based charts).
- Congenital anomaly (any congenital anomaly will be included).

- Perinatal mortality: defined as 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.

Data management

The data collected for the trial will be a mixture of routinely clinical data and information from follow-up, which are verifiable from the medical record or telephone interview. To guarantee the authentic study results, all of our researchers and clinicians are required to master all details about this study. All the characteristics in our study are collected at baseline and follow-up through a standard clinical electronic data collection system (EDC). All participant-identifiable data, such as consent forms, screening and identification logs will be stored in the investigator site files, accessible only to delegated members of the study team.

Safety reporting will be in accordance with plan and all adverse events will be recorded and informed DSMB. The DSMB will perform an interim analysis three months after the first 600 randomized participants have completed embryo transfer. They will do so using the endpoint ongoing pregnancy, as data on live birth will not be available. Also, the DSMB will oversee the SAE's that have occurred.

Sample size

Among couples with non-severe male infertility, the average live birth rate after IVF during 2014-2015 calculated over all study sites was 40% per cycle. Based on other studies within fertility care as well as the discussion by gynaecologist and methodologists, we assumed that the minimal clinical important difference to make ICSI preferable over IVF would be 7%. To demonstrate this difference with two-sided test, 5% alpha-error, 90% statistical power, and taking consideration a dropout of 10%, we will need to enroll 1,173 participants in each group, i.e. a total of 2,346 participants (the ratio between groups will be 1:1). For the interim analysis, we will use the Haybittle–Peto boundary. The significance level for the interim analysis will be 0.001 and for the final analysis 0.05.[39]

Statistical analysis

For continues variables, parameters normally distributed will be expressed as mean with standard deviation (SD) and compared using student t-test. If the parameters are non-normally distributed, their medians and inter-quantile ranges (IRQs) will be reported, the Mann-Whitney-U test will be utilized to test the distribution of these variables as well. For categorical variables, we will present the proportion between each group and distributions will be compared using Pearson's chi-square test and Fisher's exact test when appropriate. Data analysis of this trial will follow intention-to-treatment, in

which all randomized women will be considered in the primary comparison between treatment groups. Per-protocol analysis will be conducted as a secondary analysis in participants who complied with protocol and per-treatment analysis will be used according to actual treatments that participants received. For missing values, a range of clinically plausible scenarios will be used to impute missing values in order to test the robustness of the findings. For losses to follow-up and protocol violations, we will attempt sensitive analyses to explore the effect of these factors on the trial findings. All tests will be two-tailed, and differences with p value <0.001 for interim analysis or p value <0.05 for final analysis are considered statistically significant. All statistical analyses will be performed with the SAS software package V.9.4. The statistical analysis will be done by an independent statistician, overseen by Clinical Epidemiology Research Center of Peking University Third Hospital. The analysis will be described in detail in a statistical analysis plan.

Ethics and dissemination

This trial had been reviewed and approved by the medical science research ethics committee of Peking University Third Hospital: D2017050. The study is registered on ClinicalTrials.gov as NCT03298633. Informed consent will be obtained from each participant before randomisation. The researchers will permit trial-related monitoring, audits, regulatory inspections, providing direct access to source data and documents.

Trial status

The recruitment in each study center started in April 2018. The estimated end date of the last recruitment for this study is April 2020.

Authors' contributions

JQ, HH, RL, RW and BWM conceived the study idea. JQ, HH, RL, RY, YL, DZ, participated in the design of the study, recruitment of participants, and drafting of the manuscript. DZ and RY participate in recruitment of participants and assessment of clinical outcomes. HH, YZ, XL, LT, and HW supervised patient diagnosis and recruitment in each study center. DZ and LZ coordinates of the data collection. LZ oversees data collection will performed data analysis. DZ, LZ, RW and BWM will design the statistical analysis plan and oversee statistical analysis. YL oversees laboratory work among 6 centers. All authors critically reviewed the article and approved the final manuscript.

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Competing interests statement

None declared. Completed disclosure of interests form available to view online as supporting information.

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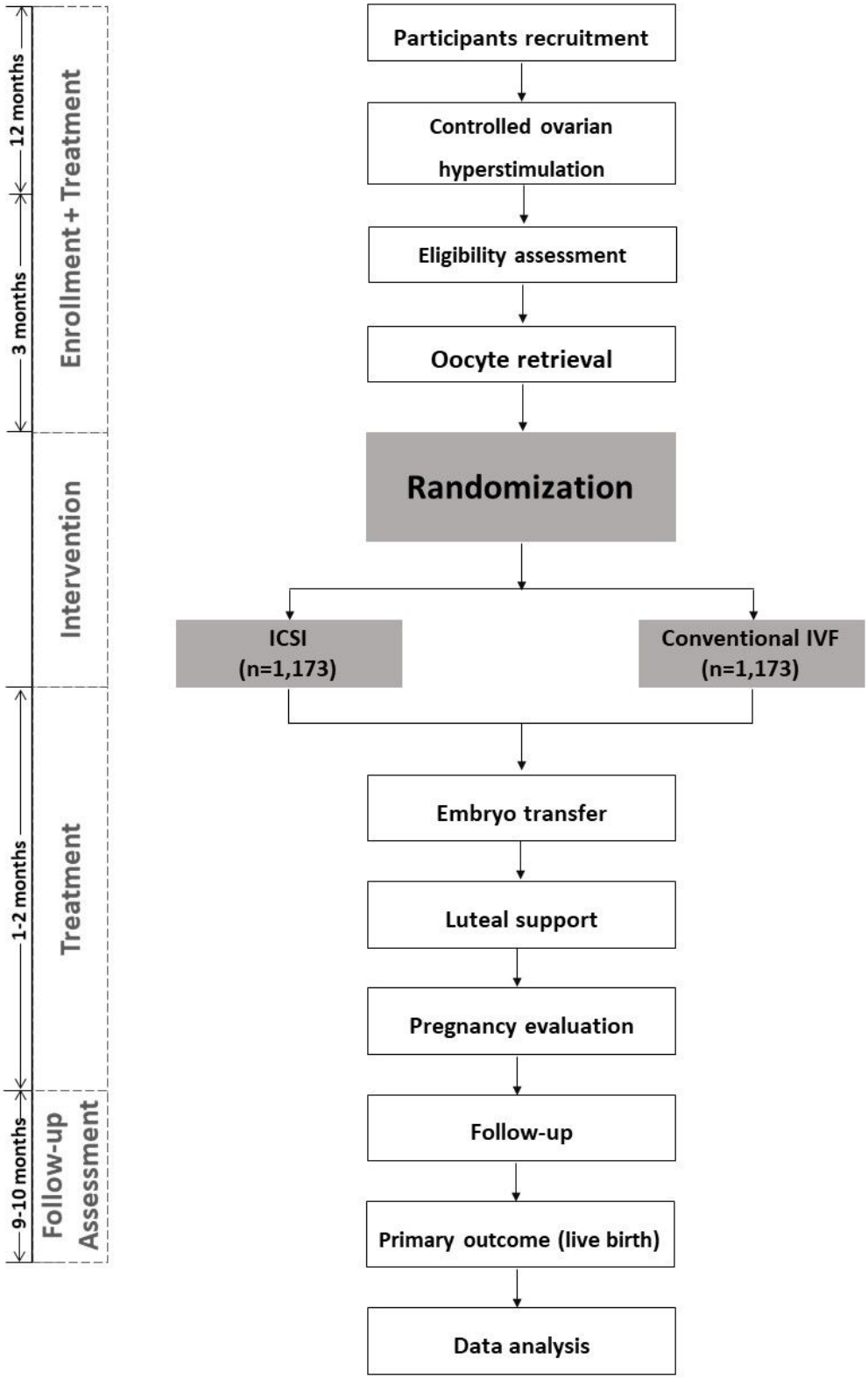

Figure 1. Flow chart followed SPIRIT checklist showing participants enrollment, allocation, treatment, and follow-up of participants.

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SPIRIT 2013 Statement: Defining Standard Protocol Items for Clinical Trials

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Abstract

The protocol of a clinical trial serves as the foundation for study planning, conduct, reporting, and appraisal. However, trial protocols and existing protocol guidelines vary greatly in content and quality. This article describes the systematic development and scope of SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) 2013, a guideline for the minimum content of a clinical trial protocol.

The 33-item SPIRIT checklist applies to protocols for all clinical trials and focuses on content rather than format. The checklist recommends a full description of what is planned; it does not prescribe how to design or conduct a trial. By providing guidance for key content, the SPIRIT recommendations aim to facilitate the drafting of high-quality protocols. Adherence to SPIRIT would also enhance the transparency and completeness of trial protocols for the benefit of investigators, trial participants, patients, sponsors, funders, research ethics committees or institutional review boards, peer reviewers, journals, trial registries, policymakers, regulators, and other key stakeholders.

The protocol of a clinical trial plays a key role in study planning, conduct, interpretation, oversight, and external review by detailing the plans from ethics approval to dissemination

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of results. A well-written protocol facilitates an appropriate assessment of scientific, ethical, and safety issues before a trial begins; consistency and rigor of trial conduct; and full appraisal of the conduct and results after trial completion. The importance of protocols has been emphasized by journal editors (1–6), peer reviewers (7–10), researchers (11–15), and public advocates (16).

Despite the central role of protocols, a systematic review revealed that existing guidelines for protocol content vary greatly in their scope and recommendations, seldom describe how the guidelines were developed, and rarely cite broad stakeholder involvement or empirical evidence to support their recommendations (17). These limitations may partly explain why an opportunity exists to improve the quality of protocols. Many protocols for randomized trials do not adequately describe the primary outcomes (inadequate for 25% of trials) (18, 19), treatment allocation methods (inadequate for 54% to 79%) (20, 21), use of blinding (inadequate for 9% to 34%) (21, 22), methods for reporting adverse events (inadequate for 41%) (23), components of sample size calculations (inadequate for 4% to 40%) (21, 24), data analysis plans (inadequate for 20% to 77%) (21, 24–26), publication policies (inadequate for 7%) (27), and roles of sponsors and investigators in study design or data access (inadequate for 89% to 100%) (28, 29). The problems that underlie these protocol deficiencies may in turn lead to avoidable protocol amendments, poor trial conduct, and inadequate reporting in trial publications (15, 30).

In response to these gaps in protocol content and guidance, we launched the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) initiative in 2007. This international project aims to improve the completeness of trial protocols by producing evidence-based recommendations for a minimum set of items to be addressed in protocols. The SPIRIT 2013 Statement includes a 33-item checklist (Table 1) and diagram (Figure). An associated explanatory paper (SPIRIT 2013 Explanation and Elaboration) (31) details the rationale and supporting evidence for each checklist item, along with guidance and model examples from actual protocols.

Development of the SPIRIT 2013 Statement

The SPIRIT 2013 Statement was developed in broad consultation with 115 key stakeholders, including trial investigators (n = 30); health care professionals (n = 31); methodologists (n = 34); statisticians (n = 16); trial coordinators (n = 14); journal editors (n = 15); and representatives from the research ethics community (n = 17), industry and nonindustry funders (n = 7), and regulatory agencies (n = 3), whose roles are not mutually exclusive. As detailed later, the SPIRIT guideline was developed through 2 systematic reviews, a formal Delphi consensus process, 2 face-to-face consensus meetings, and pilot-testing (32).

The SPIRIT checklist evolved through several iterations. The process began with a preliminary checklist of 59 items derived from a systematic review of existing protocol guidelines (17). In 2007, 96 expert panelists from 17 low(n = 1), middle- (n = 6), and high-income (n = 10) countries refined this initial checklist over 3 iterative Del-phi consensus survey rounds by e-mail (33). Panelists rated each item on a scale of 1 (not important) to 10 (very important), suggested new items, and provided comments that were circulated in

subsequent rounds. Items with a median score of 8 or higher in the final round were included, whereas those rated 5 or lower were excluded. Items rated between 5 and 8 were retained for further discussion at the consensus meetings.

After the Delphi survey, 16 members of the SPIRIT Group (named as authors of this paper) met in December 2007 in Ottawa, Ontario, Canada, and 14 members met in September 2009 in Toronto, Ontario, Canada, to review the survey results, discuss controversial items, and refine the draft checklist. After each meeting, the revised checklist was recirculated to the SPIRIT Group for additional feedback.

A second systematic review identified empirical evidence about the relevance of specific protocol items to trial conduct or risk of bias. The results of this review informed the decision to include or exclude items on the SPIRIT checklist. This review also provided the evidence base of studies cited in the SPIRIT 2013 Explanation and Elaboration paper (31). Some items had little or no identified empirical evidence (for example, the title) and are included in the checklist on the basis of a strong pragmatic or ethical rationale.

Finally, we pilot-tested the draft checklist in 2010 and 2011 with University of Toronto graduate students who used the document to develop trial protocols as part of a master's-level course on clinical trial methods. Their feedback on the content, format, and usefulness of the checklist was obtained through an anonymous survey and incorporated into the final SPIRIT checklist.

Definition of a Clinical Trial Protocol

Although every study requires a protocol, the precise definition of a protocol varies among individual investigators, sponsors, and other stakeholders. For the SPIRIT initiative, the protocol is defined as a document that provides sufficient detail to enable understanding of the background, rationale, objectives, study population, interventions, methods, statistical analyses, ethical considerations, dissemination plans, and administration of the trial; replication of key aspects of trial methods and conduct; and appraisal of the trial's scientific and ethical rigor from ethics approval to dissemination of results.

The protocol is more than a list of items. It should be a cohesive document that provides appropriate context and narrative to fully understand the elements of the trial. For example, the description of a complex intervention may need to include training materials and figures to enable replication by persons with appropriate expertise.

The full protocol must be submitted for approval by an institutional review board (IRB) or research ethics committee (34). It is recommended that trial investigators or sponsors address the SPIRIT checklist items in the protocol before submission. If the details for certain items have not yet been finalized, then this should be stated in the protocol and the items updated as they evolve.

The protocol is a "living" document that is often modified during the trial. A transparent audit trail with dates of important changes in trial design and conduct is an essential part of the scientific record. Trial investigators and sponsors are expected to adhere to the protocol

as approved by the IRB and to document amendments made in the most recent protocol version. Important protocol amendments should be reported to IRBs and trial registries as they occur and subsequently be described in trial reports.

Scope of the SPIRIT 2013 Statement

The SPIRIT 2013 Statement applies to the content of a clinical trial protocol, including its appendices. A clinical trial is a prospective study in which 1 or more interventions are assigned to human participants to assess the effects on health-related outcomes. The primary scope of SPIRIT 2013 relates to randomized trials, but the same considerations substantially apply to all types of clinical trials, regardless of study design, intervention, or topic.

The SPIRIT 2013 Statement provides guidance for minimum protocol content. Certain circumstances may warrant additional protocol items. For example, a factorial study design may require specific justification; crossover trials have unique statistical considerations, such as carryover effects; and industry-sponsored trials may have additional regulatory requirements.

The protocol and its appendices are often the sole repository of detailed information relevant to every SPIRIT checklist item. Using existing trial protocols, we have been able to identify model examples of every item (31), which illustrates the feasibility of addressing all checklist items in a single protocol document. For some trials, relevant details may appear in related documents, such as statistical analysis plans, case record forms, operations manuals, or investigator contracts (35, 36). In these instances, the protocol should outline the key principles and refer to the separate documents so that their existence is known.

The SPIRIT 2013 Statement primarily relates to the content of the protocol rather than its format, which is often subject to local regulations, traditions, or standard operating procedures. Nevertheless, adherence to certain formatting conventions, such as a table of contents; section headings; glossary; list of abbreviations; list of references; and a schematic schedule of enrollment, interventions, and assessments, will facilitate protocol review (Figure).

Finally, the intent of SPIRIT 2013 is to promote transparency and a full description of what is planned—not to prescribe how a trial should be designed or conducted. The checklist should not be used to judge trial quality, because the protocol of a poorly designed trial may address all checklist items by fully describing its inadequate design features. Nevertheless, the use of SPIRIT 2013 may improve the validity and success of trials by reminding investigators about important issues to consider during the planning stages.

Relation to Existing Clinical Trial Guidance

With its systematic development process, consultation with international stakeholders, and explanatory paper citing relevant empirical evidence (31), SPIRIT 2013 builds on other international guidance applicable to clinical trial protocols. It adheres to the ethical principles mandated by the 2008 Declaration of Helsinki, particularly the requirement that the protocol address specific ethical considerations, such as competing interests (34).

In addition, SPIRIT 2013 encompasses the protocol items recommended by the International Conference on Harmonisation Good Clinical Practice E6 guidance, written in 1996 for clinical trials whose data are intended for submission to regulatory authorities (37). The SPIRIT Statement builds on the Good Clinical Practice guidance by providing additional recommendations on specific key protocol items (for example, allocation concealment, trial registration, and consent processes). In contrast to SPIRIT, the Good Clinical Practice guidance used informal consensus methods, has unclear contributorship, and lacks citation of supporting empirical evidence (38).

The SPIRIT 2013 Statement also supports trial registration requirements from the World Health Organization (39), the International Committee of Medical Journal Editors (40), legislation pertaining to ClinicalTrials.gov (41), the European Commission (42), and others. For example, item 2b of the SPIRIT checklist recommends that the protocol list the World Health Organization Trial Registration Data Set (Appendix Table, available at www.annals.org), which is the minimum amount of information that the International Committee of Medical Journal Editors mandates for trial registries. Having this data set in its own protocol section is intended not only to serve as a form of trial summary but also to help improve the quality of information in registry entries. Registration-specific data could be easily identified in the protocol section and copied into the registry fields. In addition, protocol amendments applicable to this section could prompt investigators to update their registry data.

The SPIRIT 2013 Statement mirrors applicable items from CONSORT 2010 (Consolidated Standards of Reporting Trials) (43). Consistent wording and structure used for items common to both checklists will facilitate the transition from a SPIRIT-based protocol to a final report based on CONSORT. The SPIRIT Group has also engaged leaders of other initiatives relevant to protocol standards, such as trial registries, the Clinical Data Interchange Standards Consortium Protocol Representation Group, and Pragmatic Randomized Controlled Trials in Health-Care, to align international efforts in promoting transparency and high-quality protocol content.

Potential Effect

An extensive range of stakeholders could benefit from widespread use of the SPIRIT 2013 Statement and its explanatory paper (Table 2). Pilot-testing and informal feedback have shown that it is particularly valuable for trial investigators when they draft their protocols. It can also serve as an informational resource for new investigators, peer reviewers, and IRB members.

There is also potential benefit for trial implementation. The excessive delay from the time of protocol development to ethics approval and the start of participant recruitment remains a major concern for clinical trials (44). Improved completeness of protocols could help increase the efficiency of protocol review by reducing avoidable queries to investigators about incomplete or unclear information. With full documentation of key information and increased awareness of important considerations before the trial begins, the use of SPIRIT may also help to reduce the number and burden of subsequent protocol amendments—many

of which can be avoided with careful protocol drafting and development (15). Widespread adoption of SPIRIT 2013 as a single standard by IRBs, funding agencies, regulatory agencies, and journals could simplify the work of trial investigators and sponsors, who could fulfill the common application requirements of multiple stakeholders with a single SPIRIT-based protocol. Better protocols would also help trial personnel to implement the study as the protocol authors intended.

Furthermore, adherence to SPIRIT 2013 could help ensure that protocols contain the requisite information for critical appraisal and trial interpretation. High-quality protocols can provide important information about trial methods and conduct that is not available from journals or trial registries (45–47). As a transparent record of the researchers' original intent, comparisons of protocols with final trial reports can help to identify selective reporting of results and undisclosed amendments (48), such as changes to primary outcomes (19, 49). However, clinical trial protocols are not generally accessible to the public (45). The SPIRIT 2013 Statement will have a greater effect when protocols are publicly available to facilitate full evaluation of trial validity and applicability (11, 12, 14, 50).

The SPIRIT 2013 guideline needs the support of key stakeholders to achieve its greatest impact (Table 2), as seen with widely adopted reporting guidelines, such as CONSORT (51). We will post the names of organizations that have endorsed SPIRIT 2013 on the SPIRIT Web site (www.spirit-statement.org) and provide resources to facilitate implementation. Widespread adoption of the SPIRIT recommendations can help improve protocol drafting, content, and implementation; facilitate registration, efficiency, and appraisal of trials; and ultimately enhance transparency for the benefit of patient care.

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	Study Period							
	Enrollment	Allocation	Postallocation					Closeout
Time point*	$-t_1$	0	t_1	t_2	t_3	t_4	etc.	t_x
Enrollment:								
Eligibility screen	X							
Informed consent	X							
[List other procedures]	X							
Allocation		X						
Interventions:								
[Intervention A]			◆	—	◆			
[Intervention B]			X		X			
[List other study groups]			◆	—	—	◆		
Assessments:								
[List baseline variables]	X	X						
[List outcome variables]				X		X	etc.	X
[List other data variables]			X	X	X	X	etc.	X

Recommended content can be displayed using various schematic formats. See SPIRIT 2013 Explanation and Elaboration (31) for examples. This template is copyrighted by the SPIRIT Group and is reproduced with permission. SPIRIT = Standard Protocol Items: Recommendations for Interventional Trials.
* List specific time points in this row.

Figure.
Example template of recommended content for the schedule of enrollment, interventions, and assessments.

Table 1

SPIRIT 2013 Checklist: Recommended Items to Address in a Clinical Trial Protocol and Related Documents*

Section/Item	Item Number	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry.
	2b	All items from the World Health Organization Trial Registration Data Set (Appendix Table, available at www.annals.org)
Protocol version	3	Date and version identifier
Funding	4	Sources and types of financial, material, and other support
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors
	5b	Name and contact information for the trial sponsor
	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
	5d	Composition, roles, and responsibilities of the coordinating center, steering committee, end point adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see item 21a for DMC)
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
	6b	Explanation for choice of comparators
Objectives	7	Specific objectives or hypotheses
Trial design	8	Description of trial design, including type of trial (e.g., parallel group, crossover, factorial, single group), allocation ratio, and framework (e.g., superiority, equivalence, noninferiority, exploratory)
Methods		
Participants, interventions, and outcomes		
Study setting	9	Description of study settings (e.g., community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centers and individuals who will perform the interventions (e.g., surgeons, psychotherapists)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (e.g., drug dose change in response to harms, participant request, or improving/worsening disease)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (e.g., drug tablet return, laboratory tests)

Section/Item	Item Number	Description
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (e.g., systolic blood pressure), analysis metric (e.g., change from baseline, final value, time to event), method of aggregation (e.g., median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline	13	Time schedule of enrollment, interventions (including any runins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (Figure).
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
Recruitment	15	Strategies for achieving adequate participant enrollment to reach target sample size
Assignment of interventions (for controlled trials)		
Allocation Sequence generation	16a	Method of generating the allocation sequence (e.g., computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (e.g., blocking) should be provided in a separate document that is unavailable to those who enroll participants or assign interventions.
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (e.g., central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	16c	Who will generate the allocation sequence, who will enroll participants, and who will assign participants to interventions
Blinding (masking)	17a	Who will be blinded after assignment to interventions (e.g., trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
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 (e.g., duplicate measurements, training of assessors) and a description of study instruments (e.g., 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.
	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
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (e.g., double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol.
Statistical methods	20a	Statistical methods for analyzing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol.
	20b	Methods for any additional analyses (e.g., subgroup and adjusted analyses)
	20c	Definition of analysis population relating to protocol nonadherence (e.g., as-randomized analysis), and any statistical methods to handle missing data (e.g., multiple imputation)

Section/Item	Item Number	Description
Monitoring		
Data monitoring	21a	Composition of 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.
	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
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
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor
Ethics and dissemination		
Research ethics approval	24	Plans for seeking REC/IRB approval
Protocol amendments	25	Plans for communicating important protocol modifications (e.g., changes to eligibility criteria, outcomes, analyses) to relevant parties (e.g., investigators, RECs/IRBs, trial participants, trial registries, journals, regulators)
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorized surrogates, and how (see item 32)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
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
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site
Access to data	29	Statement of who will have access to the final trial data set, and disclosure of contractual agreements that limit such access for investigators
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
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, health care professionals, the public, and other relevant groups (e.g., via publication, reporting in results databases, or other data-sharing arrangements), including any publication restrictions
	31b	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level data set, and statistical code
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorized surrogates
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

DMC = data monitoring committee; IRB = institutional review board; REC = research ethics committee; SPIRIT = Standard Protocol Items: Recommendations for Interventional Trials.

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation and Elaboration (31) for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group and is reproduced with permission.

Table 2

Potential Benefits and Proposed Stakeholder Actions for Supporting Adherence to SPIRIT 2013

Stakeholder	Proposed Actions	Potential Benefits
Clinical trial groups, investigators, sponsors	Adopt SPIRIT as standard guidance Use as tool for writing protocols	Improved quality, completeness, and consistency of protocol content Enhanced understanding of rationale and issues to consider for key protocol items Increased efficiency of protocol review
Research ethics committees/ institutional review boards, funding agencies, regulatory agencies	Mandate or encourage adherence to SPIRIT for submitted protocols Use as training tool	Improved quality, completeness, and consistency of protocol submissions Increased efficiency of review and reduction in queries about protocol requirements
Educators	Use SPIRIT checklist and explanatory paper as a training tool	Enhanced understanding of the rationale and issues to consider for key protocol items
Patients, trial participants, policymakers	Advocate use of SPIRIT by trial investigators and sponsors	Improved protocol content relevant to transparency, accountability, critical appraisal, and oversight
Trial registries	Encourage SPIRIT-based protocols Register full protocols to accompany results disclosure	Improved quality of registry records Prompt for trialists to update registry record when SPIRIT checklist item 2b (Registration Data Set) is updated Improved quality, completeness, and consistency of protocol content for registries that house full protocols and results
Journal editors and publishers	Endorse SPIRIT as standard guidance for published and unpublished protocols Include reference to SPIRIT in instructions for authors Ask that protocols be submitted with manuscripts, circulate them to peer reviewers, and encourage authors to make them available as Web appendices	Improved quality, completeness, and consistency of protocol content Enhanced peer review of trial manuscripts through improved protocol content, which can be used to assess protocol adherence and selective reporting Improved transparency and interpretation of trials by readers

SPIRIT = Standard Protocol Items: Recommendations for Interventional Trials.

Appendix Table

World Health Organization Trial Registration Data Set*

Item	Description
1. Primary registry and trial-identifying number	Name of primary registry and the unique identifier assigned by the primary registry
2. Date of registration in primary registry	Date when the trial was officially registered in the primary registry
3. Secondary identifying numbers	Other identifiers, if any Universal Trial Number Identifiers assigned by the sponsor Other trial registration numbers issued by other registries Identifiers issued by funding bodies, collaborative research groups, regulatory authorities, ethics committees/institutional review boards, etc.
4. Sources of monetary or material support	Major sources of monetary or material support for the trial (e.g., funding agency, foundation, company, institution)
5. Primary sponsor	Person, organization, group, or other legal entity that takes responsibility for initiating and managing a study
6. Secondary sponsor(s)	Additional persons, organizations, or other legal persons, if any, who have agreed with the primary sponsor to take on responsibilities of sponsorship
7. Contact for public queries	E-mail address, telephone number, and postal address of the contact who will respond to general queries, including information about current recruitment status
8. Contact for scientific queries	Name and title, e-mail address, telephone number, postal address, and affiliation of the principal investigator and e-mail address, telephone number, postal address, and affiliation of the contact for scientific queries about the trial (if applicable)
9. Public title	Title intended for the lay public in easily understood language
10. Scientific title	Scientific title of the study as it appears in the protocol submitted for funding and ethical review; include trial acronym, if available
11. Countries of recruitment	Countries from which participants will be recruited
12. Health condition(s) or problem(s) studied	Primary health condition(s) or problem(s) studied (e.g., depression, breast cancer, medication error)
13. Intervention(s)	For each group of the trial, record a brief intervention name plus an intervention description Intervention name: For drugs, use the generic name; for other types of interventions, provide a brief descriptive name Intervention description: Must be sufficiently detailed for it to be possible to distinguish between the groups of a study; for example, interventions involving drugs may include dosage form, dosage, frequency, and duration
14. Key inclusion and exclusion criteria	Inclusion and exclusion criteria for participant selection, including age and sex
15. Study type	Method of allocation (randomized/nonrandomized) Blinding/masking (identify who is blinded) Assignment (e.g., single group, parallel, crossover, factorial) Purpose Phase (if applicable) For randomized trials: Method of sequence generation and allocation concealment
16. Date of first enrollment	Anticipated or actual date of enrollment of the first participant
17. Target sample size	Total number of participants to enroll
18. Recruitment status	Pending: Participants are not yet being recruited or enrolled at any site Recruiting Suspended: Temporary halt in recruitment and enrollment Complete: Participants are no longer being recruited or enrolled Other

Item	Description
19. Primary outcome(s)	The primary outcome should be the outcome used in sample size calculations or the main outcome used to determine the effects of the intervention For each primary outcome provide: Name of the outcome (do not use abbreviations) Metric or method of measurement used (be as specific as possible) Time point of primary interest
20. Key secondary outcome(s)	As for primary outcomes, for each secondary outcome provide: Name of the outcome (do not use abbreviations) Metric or method of measurement used (be as specific as possible) Time point of interest

* Adapted from www.who.int/ictrp/network/trds/en/index.html.

BMJ Open

Intracytoplasmic sperm injection (ICSI) versus conventional in vitro fertilization (IVF) in couples with non-severe male infertility (NSMI-ICSI): protocol for a multi-center randomized controlled trial

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Secondary Subject Heading:	Reproductive medicine
Keywords:	In vitro fertilization, Intracytoplasmic sperm injection, Non-severe male infertility, Assisted reproductive technology

Title

Intracytoplasmic sperm injection (ICSI) versus conventional in vitro fertilization (IVF) in couples with non-severe male infertility (NSMI-ICSI): protocol for a multi-center randomized controlled trial

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Abstracts

Introduction: Intracytoplasmic sperm injection (ICSI), originally introduced as add-on to in vitro fertilization (IVF) for couples with severe male infertility, is in current clinical practice also used in couples with mild male or even unexplained infertility. However, ICSI has involved unresolved concerns regarding the selection and damage to gametes and the health conditions of the offspring, and it is also labour intensive and therefore more expensive than conventional IVF. High quality well powered randomized clinical trials (RCTs) comparing ICSI and IVF are lacking.

Methods and analysis: We propose a multicenter, open-label RCT in ten reproductive medical centers across China. We will study couples with non-severe male infertility (defined as a semen concentrate 5-15×10^6/ml or sperm with a progressive motility 10-32%) scheduled for their first or second ICSI or IVF cycle, as low fertility rate after fertilization are more frequent in this population, which could lead to controversy about ICSI or conventional IVF for fertilization. On the day of oocyte retrieval, eligible participants are after informed consent be randomized to undergo either ICSI or conventional IVF in a 1:1 treatment ratio. Other standard assisted reproductive treatments are similar and parallel between two groups. Our primary outcome is ongoing pregnancy leading to live birth after the first cycle with embryo transfer. To demonstrate or refute a difference of 7% between ICSI and conventional IVF, we need to include 2,346 women (1,173 in each intervention arm). In addition, we will follow up neonatal outcomes after delivery to identify the influence of ICSI on offspring.

Ethics and dissemination: Ethical approval was obtained from Peking University Third Hospital medical science research ethics committee. The findings will be disseminated to the public through conference presentations and peer-reviewed scientific journals.

Trial registration number: NCT03298633

Strengths and limitations of this study

- It's the first randomized controlled clinical trial with a large sample size comparing ICSI and conventional IVF among patients with non-severe male infertility in 10 centers across China.
- This study will provide evidence on whether ICSI or conventional IVF is the better method for fertilization in terms of live birth for non-severe male infertility.
- Range of sperm parameters (semen concentrate 5-15×10^6/ml or sperm with progressive motility 10-32%) in our study based on the fifth edition of WHO manual will be applicable to couples with non-severe male infertility as many as possible.

- The sample size and power calculation were focused on the primary outcome of this study, with the limited power to detect other secondary outcomes.

Keywords: In vitro fertilization; Intracytoplasmic sperm injection; Non-severe male infertility; Assisted reproductive technology.

Introduction

Male infertility is caused by impaired sperm production and function due to different congenital or acquired factors,[1] and has been estimated to be associated with approximately 30% of infertility.[2,3] Assisted reproductive technology (ART) is perceived as a more successful treatment.[4,5] Originally applied in women with tubal damage in 1970s, in-vitro fertilization (IVF) is now acknowledged as an effective treatment for infertility as a major component of ART.[6] However, conventional IVF was much less effective when the semen characteristics were grossly below the standard values according to the WHO fourth edition sperm parameter values and when fertilization rate in previous cycles was low.[7,8]

In 1992,[9] intracytoplasmic sperm injection (ICSI), a technique where a single spermatozoon was injected mechanically into an oocyte in vitro to achieve fertilization, was introduced. While complete fertilization failure was reported up to 50% of the conventional IVF treatments for couples with moderate male infertility (moderate oligozoospermia, asthenozoospermia and teratozoospermia), this occurred in less than 3% of the couples undergoing ICSI.[10-13] Consequently, ICSI has been applied worldwide to treat severe male infertility.[14-16]

The high success rate of ICSI has resulted in its increased use in other populations for whom conventional IVF may be an option, particularly non-male factor infertility. In Europe, in 2012 ICSI was used in 69% of IVF cycles compared to 35% in 1997, while in the Middle East, South-America and South-East Asia, ICSI is performed in 100% of IVF cycles even in some regions.[17,18] In the USA, between 1996 and 2012, the use of ICSI in IVF cycles has increased from 34% to 76%. The greatest increase was documented in non-male factor infertility, where the use of ICSI went from 15% to 67% during this time period.[19]

There are concerns on the increased use of ICSI, as ICSI is time consuming, expensive, and involves unresolved concerns regarding the damage to gametes and the health conditions of the offspring.[20-24] Many studies have indicated the routine use of ICSI in non-male factors infertility was not recommended to improve the clinical outcomes.[25-28] For non-severe male factor infertility, including mild and moderate oligospermia with or without asthenospermia, the fertilization and pregnancy outcome after ICSI compared with conventional IVF is unclear. Studies randomizing sibling oocytes have shown conflicting results. Several studies have documented higher fertilization rates and lower rates of fertilization failure in these couples undergoing ICSI.[29-31] Other studies did not support the

benefit of ICSI in prevention of total fertilization failure as there were no significant differences between ICSI and conventional IVF in embryo quality, implantation, clinical pregnancy, or live birth rates.[32-34] These studies have limitations such as small sample size, non-randomized couples, or no evaluation of live births. In addition, fewer application of ICSI in China may result in low fertility rate for patients with non-severe male infertility, which would give raise to controversy about ICSI or conventional IVF for fertilization during ART in these population.[35]

In view of this situation, we plan an adequately powered multi-center randomized controlled clinical trial to assess whether ICSI is more effective than IVF in couples with non-severe male infertility.

Methods and analysis

Study design

We plan a multi-center, parallel, open-label, randomized controlled clinical trial (1:1 treatment ratio). The flow chart followed SPIRIT checklist showing enrollment, allocation, treatment, and follow-up of participants is presented in Figure 1.[36] In addition, the schedule of enrollment, interventions, and assessments during the study period is shown in Table 1.

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

	Study Period							
	Enrollment	Pre-allocation	Allocation	Post-allocation				Close-out
Content	Screening & Baseline assessment	Controlled ovarian hyperstimulation	Oocyte retrieval & Randomization	Assessment of embryo	Embryo transfer	Evaluation of pregnancy	Follow-up of pregnancy	
Time point	T_0 -3 month	T_1 -1 month	T_2 0 month	T_3 1-3 days	T_4 3 days	T_5 1 month	T_6 3-10 months	T_7 12 months
Enrollment								
Eligibility screen	×	×	×					
Informed consent	×							
Allocation			×					
Interventions								
ICSI			×					
Conventional IVF			×					
Assessments								
Baseline data	×							
Laboratory tests	×	×	×		×	×	×	×
Fertilization				×				
Embryo quality				×				
Pregnancy tests						×		
Pregnancy outcomes							×	×
Fetus information							×	×

Neonate information							×	×
Safety assessment		×	×		×	×	×	×

Study setting

The study will recruit participants from 10 reproductive medical centers across China: Peking University Third Hospital, International Peace Maternity and Child Health Hospital of Shanghai Jiao Tong University, Women's Hospital of Zhejiang University, The Sixth Affiliated Hospital of Sun Yat-Sen University, First Affiliated Hospital of Kunming Medical University, Haidian Maternal and Child Health Hospital, First Affiliated Hospital of Anhui Medical University, The Second Hospital of Hebei Medical University, The Third Affiliated Hospital of Guangzhou Medical University, and General Hospital of Ningxia Medical University. This trial had been reviewed and approved by the medical science research ethics committee of Peking University Third Hospital: D2017050. The study is registered on ClinicalTrials.gov as NCT03298633. 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.

An independent data and safety monitoring board (DSMB), with members with clinical and statistical expertise, will monitor the trial progress and interim results at regular intervals.

Eligibility criteria

Couples presenting to reproductive medical center of the involved hospitals will be screened for following eligibility to be enrolled in our trial.

Inclusion Criteria:

- Infertile couples scheduled for their first or second IVF/ICSI cycle.
- Male partner has non-severe male infertility, defined as a semen concentrate 5-15$\times 10^6$/ml or sperm with progressive motility (type a+b) 10-32%.
- Women received either gonadotrophin-releasing hormone agonist protocol or gonadotrophin-releasing hormone antagonist protocol as their controlled ovarian hyperstimulation treatment.
- Informed consent obtained.

Exclusion criteria:

- Couple with a contraindication for IVF or ICSI, including poorly controlled type 1 or type 2 diabetes mellitus; undiagnosed 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 eggs.

- Couples undergoing preimplantation genetic testing.
- Sperm concentration with progressive motility used for insemination $<0.1\times10^6$/ml on the day of oocyte retrieval.
- Women with 0 oocyte retrieved.
- Using frozen semen.
- Poor fertilization in previous cycle ($\leq$ 25%).

In this study, couples with various female indications for IVF will be included. The sperm parameters defining non-severe male infertility are evaluated according to the WHO fifth edition sperm parameter values and the sperm parameters are subject to the latest sperm analysis.[37] Participants have the right to decline participation during the whole process, and they can withdraw their consent at any time. Their consent or refusal to consent will not affect their conventional clinical treatments.

Recruitment

Infertile couples who come to the outpatient clinic or medical record of infertile couples who have received COH treatment will be screened by a dedicated research team. Eligible couples will then, before oocyte retrieval, explained by a member of the research team the trial details. After this information, couples will be offered time for consideration to decide whether to participate in the trial. Couples who agree to participate will be asked to sign the consent form in their next scheduled visit. An individual record of all non-recruited patients and reasons for exclusion will be obtained and stored. On the day of oocyte retrieval, semen of patients who have signed consent form will be analysed again for the exclusion criteria. Ineligible patients will be further excluded from our trial, continuing their conventional clinical procedures instead.

Randomization

Randomization and allocation of eligible patients to study groups will be performed on the day of oocyte retrieval. This procedure will be performed by administrative staffs in the trial center not involved in the treatment procedure, using an online trial system with a computer-generated randomization list that allocates couples in a 1:1 ratio to ICSI or IVF, with a variable block size of 4 or 6 stratified for center. Stratified permuted block randomization will be centrally controlled.

Blinding

The trial was originally designed and performed as a double-blind trial, in which participants and clinicians/nurses who performed embryo transfer or follow-up, as well as the investigators and assessors will be blinded until the primary outcome occurred. While embryologists who performed

IVF and ICSI were not blinded. Recruitment was slow due to the double-blind design, as participants wanted to know about their allocation of fertilization method as soon as possible. Therefore, after recruitment of 115 participants, the design was changed to an open-label study: On the day of oocyte retrieval, administrative staff in the IVF laboratory will log into the trial system to randomize and allocate participants to receive either ICSI or IVF. Initially, only embryologists will know the allocation. Participants and clinicians will be informed about the randomized allocation on the day of embryo transfer for participants with fresh embryo transfers and the day of embryo freezing for couples with freeze-all strategies. Prior to these dates, participants and clinicians will still be unaware of randomization allocation.

Interventions

Controlled ovarian hyperstimulation

All couples will receive controlled ovarian hyperstimulation (COH) treatment, which is performed by standard routine according to each study center. The COH treatment includes either gonadotrophin-releasing hormone agonist (GnRH-a) protocol or gonadotrophin-releasing hormone antagonist (GnRH-ant) protocol, and the selection of protocol will be done by physicians. In the GnRH-ant protocol, participants will be injected Gonadotropin (Gonal-F® or Pouliquen® or HMG®) daily from cycle day 2 or 3. When at least one follicle has reached a diameter of 12mm or on day 6 of ovarian stimulation, GnRH-ant (Cetrotide® or Ganirelix®) will be administered subcutaneously until the trigger day (include the trigger day). For super long GnRH-a protocol, GnRH-a (Alarelin® or Triptorelin®) will be used in previous menstrual cycle, gonadotropin treatment starts after 28-35 days on GnRH agonist downregulation. For long GnRH-a protocol, pituitary down-regulation will be initiated 7-10 days before the menstrual cycle with GnRH-a (Alarelin® or Triptorelin®). After 10-14 days or on day 2 of menstrual cycle, gonadotropin treatment will start. For short GnRH-a protocol, participants will receive Alarelin® or Triptorelin® for the pituitary down-regulation on day 2/3 of menstrual cycle. Gonadotropin will be used on the same time. For above treatments, menstrual cycle of patient includes spontaneous menstrual cycle, and irregular menstrual cycle by the use of oral contraceptives (OC) or progestins. Before gonadotrophin treatment, baseline pelvic ultrasound, as well as basic serum hormones (such as FSH, luteinizing hormone (LH), progesterone (P) and β-hCG) will be measured to confirm the follicle status. The initial dosage gonadotrophin (Gonal-F® or Pouliquen® or HMG®) is 150-300mg/d and the subsequent dose will be adjusted according to the individual response. Gonadotrophin treatment will be continued to the trigger day. After two or more follicles reach a diameter ≥18 mm, 250ug of hCG (Ovitrelle, 250 μg s.c.) will be once injected on trigger day.

Oocyte retrieval and preparation

Oocyte retrieval is scheduled for 36h (±2) after hCG injection. Routine oocyte pick-up is performed under transvaginal ultrasound guidance via 17-18G oocyte aspiration needle with use of intravenous sedation. The retrieved cumulus oocyte complexes (COC) will be placed in culture medium covered by lightweight paraffin oil and incubated in a humidified 37℃, 5%/6% CO_2 incubator after oocyte retrieval immediately. Besides, the COCs are incubated for 2-6h before insemination or injection.

Semen preparation

Fresh ejaculate semen samples will be obtained by masturbation after 2-7 days' abstention from sexual intercourse on the day of oocyte retrieval. Sperm concentration and progressive motility are assessed by computer-assisted semen analysis according to the fifth edition of World Health Organization (WHO) laboratory standards of human semen and sperm.[37] All semen samples are prepared by discontinue density gradient centrifugation or swim-up protocol according to local routines. Microscopes (200-400 times) will be used to observe whether there are serious abnormalities in sperm morphology that could lead to fertilization failure, such as globozoospermia.

ICSI Group

Oocytes in couples allocated to ICSI, will undergo ICSI which has been previously described.[38] In short, as the enzymatic removal was done in oocyte preparation, the denuded oocytes are examined to assess integrity and maturity. Only those oocytes that have extruded the first polar body (metaphase-Ⅱ oocytes) will be microinjected.

IVF Group

All the oocytes in couples allocated to IVF will be treated by conventional IVF which is adhered to every study, in which every oocyte will be inseminated by sperm with progressive motility concentrate $0.1\text{-}0.2\times10^6$ approximately 39-42h after hCG injection.

Assessment of fertilization and embryo quality

Apart from the fertilization procedure, assisted reproductive treatments will be similar for the two groups. Assessment of fertilization is carried out about 16-18h (day 1) after fertilization. Normal fertilization was assessed by the presence of two pronuclei and a second polar body. The zygotes were cultured in cleavage medium to day 3, and the cleavage embryo quality will be observed at 67-69 (day

3) hours after fertilization. The embryos are scored according to the quality, numbers, size of the blastomeres and the amount of anucleate fragmentation.

Embryo transfer and luteal support

Fresh or frozen-thawed embryo transfer will be decided by physicians according to conditions of patients. Transfer of fresh embryos is the usual practice when fresh embryos are available in all our study centres. In some cases, all embryos may be cryopreserved without a fresh-embryo transfer, most commonly to prevent the ovarian hyperstimulation syndrome. In addition, a freeze-all strategy will be used in the following scenarios: hydrosalpinx, elevated progesterone in hGC day, endometrial factors (endometrial polyps, endometrial cavity fluid and thin endometrium), systematic diseases (stomach-ache, fever or cold), and sudden accident of patients.

Fresh or frozen-thawed embryo transfer will be performed by physicians in three or five days following the day of oocytes collection for participants receive fresh embryo transfer, and 4-6 day after progesterone initiation for participants undergo frozen thawed embryo transfer (within 6 months after oocyte aspiration). To reduce the risk of high-order multiple pregnancies, the number of embryos replaced will be limited up to two best-quality embryos in all study centres (one embryo would be transferred if there is uterine malformation, history of uterine surgery or cesarean section). Luteal support, as well as embryo freezing and thawing is performed by standard routines at each study center, as we assume that the different protocols will be equally distributed in the interventional and control groups.

Follow-up

Urine and blood hCG will be measured 14 days after embryo transfer, and positive results indicate biochemical pregnancy. If the gestational sac is observed with ultrasonography on 7 weeks after transfer, clinical pregnancy will be confirmed. Ongoing pregnancy is defined by the presence of a gestational sac with fetal heartbeat after 12 weeks of gestation. In 6 weeks after delivery, the information of pregnancy (pregnancy complications, and fetus information), delivery information (gestational age, delivery mode, placenta abnormality and/or delivery complications), infant information (such as sex, birth weight, birth defect) will be collected by completing forms designed for this visit.

Outcome measures

Primary outcome

Our primary outcome will be ongoing pregnancy leading to live birth after the first embryo transfer. Live birth will be defined as a delivery of one or more living infants (≥22 week's gestation or birth weight more than 1,000g).[17]

Secondary outcomes

For the effectiveness of the treatment, we will record these secondary outcomes in terms of effectiveness:

- Fertilization: defined as number of zygotes with 2PN (per woman randomized and per oocyte retrieved).
- Total fertilization failure: defined as no oocyte formed 2PN in this given cycle.
- Available embryo: defined as number of embryos ≥4 cells and ≤30% fragmentation (except embryos developed from ≥3PN zygotes) on day 3 observation.
- Good quality embryo: defined as number of embryos with ≥6 cells and ≤10% fragmentation developed from 2PN zygotes on day 3 observation.
- Implantation: defined as the number of gestational sacs observed per embryo transferred.
- Clinical pregnancy: defined as one or more observed gestational sac or definitive clinical signs of pregnancy under ultrasonography at 7 weeks after embryo transfer (including clinically documented ectopic pregnancy).
- Multiple pregnancy: defined as a pregnancy with two or more gestational sacs or positive heart beats at 7 weeks of gestation.
- Ongoing pregnancy: defined as the presence of a gestational sac and fetal heartbeat after 12 weeks of gestation.

For the safety of the treatment, we will record the following treatment complications as secondary outcomes:

- Moderate/severe ovarian hyperstimulation syndrome (OHSS): defined as 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.
- Miscarriage: defined as the spontaneous loss of an intra-uterine pregnancy prior to 22 completed weeks of gestational age.
- Ectopic pregnancy: defined as the implantation takes place outside the uterine cavity, confirmed by sonography or laparoscopy.

We will also collect the following obstetric and perinatal complications:

- Gestational diabetes mellitus (GDM).
- Hypertensive disorders of pregnancy (comprising pregnancy induced hypertension (PIH); pre-eclampsia (PET) and eclampsia).
- Antepartum haemorrhage, including placenta previa, placenta accreta and unexplained.
- Preterm birth: defined as birth of a fetus delivered after 28 and before 37 completed weeks of gestational age in participants confirmed ongoing pregnancy.
- Birth weight, including low birth weight (defined as weight < 2500 gm at birth), very low birth weight (defined as < 1500 gm at birth), high birth weight (defined as >4000 gm at birth) and very high birth weight (defined as >4500 gm at birth).
- Large for gestational age (defined as birth weight >90th centile for gestation, based on standardized ethnicity according the charts) and small for gestational age (defined as less than 10th centile for gestational age at delivery based on standardized ethnicity according the charts).
- Congenital anomaly (defined as structural or functional disorders that occur during intra-uterine life and can be identified prenatally, at birth or later in life), including trisomy 13, 18, 21, neural tube defect, congenital heart disease, cleft lip, excessive numbers of fingers or toes, hydrocephalus. Clinical diagnosis of congenital anomaly is defined according to the International Classification of Diseases, revision 10 (ICD-10) criteria.[39]
- Perinatal mortality (defined as 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)
- Neonatal mortality (defined as death of a live born baby within 28 days of birth).

Data management

The data collected for the trial will be a mixture of routinely clinical data and information from follow-up, which are verifiable from the medical record. To guarantee the authentic study results, all of our researchers and clinicians are required to master all details about this study. All the characteristics in our study are collected at baseline and follow-up through a standard clinical electronic data collection system (EDC). All participant-identifiable data, such as consent forms, screening and identification logs will be stored in the investigator site files, accessible only to delegated members of the study team. Safety reporting will be in accordance with plan and all adverse events will be recorded and informed DSMB. The DSMB will perform an interim analysis three months after the first 600 randomized

participants have completed embryo transfer. They will do so using the endpoint ongoing pregnancy, as data on live birth will not be available. Also, the DSMB will oversee the SAE's that have occurred.

Sample size

Among couples with non-severe male infertility, the average live birth rate after IVF during 2014-2015 calculated over all study sites was 40% per cycle. Based on other studies within fertility care as well as the discussion by gynaecologist and methodologists, we assumed that the minimal clinical important difference to make ICSI preferable over IVF would be 7%. To demonstrate this difference with two-sided test, 5% alpha-error, 90% statistical power, and taking consideration a dropout of 10%, we will need to enroll 1,173 participants in each group, i.e. a total of 2,346 participants (the ratio between groups will be 1:1). For the interim analysis, we will use the Haybittle–Peto boundary. The significance level for the interim analysis will be 0.001 and for the final analysis 0.05.[40]

Statistical analysis

For continues variables, parameters normally distributed will be expressed as mean with standard deviation (SD) and compared using student t-test. If the parameters are non-normally distributed, their medians and inter-quantile ranges (IRQs) will be reported, the Mann-Whitney-U test will be utilized to test the distribution of these variables as well. For categorical variables, we will present the proportion between each group and distributions will be compared using Pearson's chi-square test and Fisher's exact test when appropriate. Data analysis of this trial will follow intention-to-treatment, in which all randomized women will be considered in the primary comparison between treatment groups. Per-protocol analysis will be conducted as a secondary analysis in participants who complied with protocol.

For missing values, a range of clinically plausible scenarios will be used to impute missing values in order to test the robustness of the findings. For losses to follow-up and protocol violations, we will attempt sensitive analyses to explore the effect of these factors on the trial findings. All tests will be two-tailed, and differences with p value <0.001 for interim analysis or p value <0.05 for final analysis are considered statistically significant. All statistical analyses will be performed with the SAS software package V.9.4. The statistical analysis will be done by an independent statistician, overseen by Clinical Epidemiology Research Center of Peking University Third Hospital. The analysis will be described in detail in a statistical analysis plan.

Patient and Public Involvement

This research was done without patient or public involvement. Neither patients nor the public were 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 final manuscript for readability or accuracy. As interventions in our study are both routine procedures during clinical work, burden of the intervention is assessed by patients themselves.

Ethics and dissemination

This trial had been reviewed and approved by the medical science research ethics committee of Peking University Third Hospital: D2017050. The study is registered on ClinicalTrials.gov as NCT03298633. 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.

Trial status

The recruitment in each study center started in April 2018. The estimated end date of the last recruitment for this study is April 2020.

Authors' contributions

JQ, HH, RL, RW and BWM conceived the study idea. JQ, HH, RL, RY, YL, DZ, participated in the design of the study, recruitment of participants, and drafting of the manuscript. DZ and RY participate in recruitment of participants and assessment of clinical outcomes. JQ, HH, YZ, XL, LT, HW, YC, GH, JL and JZ supervised patient diagnosis and recruitment in each study center. DZ and LZ coordinates of the data collection. LZ oversees data collection will performed data analysis. DZ, LZ, RW and BWM will design the statistical analysis plan and oversee statistical analysis. YL oversees laboratory work among 10 centers. All authors critically reviewed the article and approved the final manuscript.

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Competing interests statement

None declared. Completed disclosure of interests form available to view online as supporting information.

Figure 1. Flow chart followed SPIRIT checklist showing patient enrollment, allocation, treatment, and follow-up of participants.

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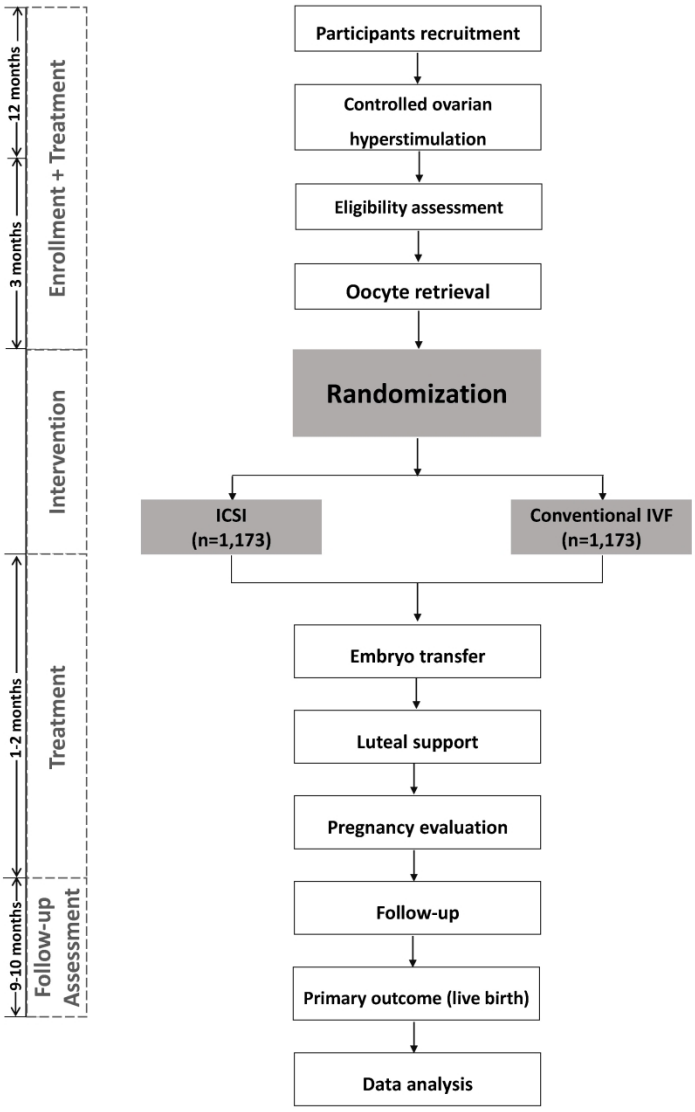

Figure 1. Flow chart followed SPIRIT checklist showing patient enrollment, allocation, treatment, and follow-up of participants.

215x350mm (300 x 300 DPI)

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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	p3
	2b	All items from the World Health Organization Trial Registration Data Set	P3
Protocol version	3	Date and version identifier	p1
Funding	4	Sources and types of financial, material, and other support	p18
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	p3
	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)	p16

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	p5-6
	6b	Explanation for choice of comparators	p6
Objectives	7	Specific objectives or hypotheses	p6
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)	p6
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	p9
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)	p9-10
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	p11-13
	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)	p10
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	p10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	p11-13
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	p14-15
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)	P7-8

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	p16
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	p10
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	p10
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	p10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	p10
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	p10-11
	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	p13
	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	p13

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	p15-16
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	p16-17
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	p16-17
	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)	p16-17
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	p15-16
	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	p16
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	p16
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	p16
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	p17
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)	p17

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	p17
	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	p17
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	p18
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	p17
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	p17
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	p17
	31b	Authorship eligibility guidelines and any intended use of professional writers	p17
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	p13
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.