

# BMJ Open Preimplantation genetic testing for aneuploidy in severe male factor infertility: protocol for a multicenter randomised controlled trial

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## ABSTRACT

**Introduction** Conventional intracytoplasmic sperm injection (ICSI) is a widely used treatment for couples with severe male infertility. However, there are controversies regarding the selection and the damage to gametes during the ICSI procedure. Although preimplantation genetic testing for aneuploidies (PGT-A) can give genetic information about embryos for transfer and improve fertility rate, and it is widely used in women with recurrent spontaneous abortion or advanced age, PGT-A is not only more expensive but also has unclear effectiveness with respect to the improvement of fertility rate among couples with severe male infertility. High-quality, well-powered randomised clinical trials (RCTs) comparing ICSI+PGT-A and ICSI are lacking.

**Methods and analysis** This is a protocol for a multicenter, open-label RCT in four reproductive medical centers qualified for PGT technique in China. We will study couples with severe male infertility scheduled for their fertility treatment. After the blastocyst culture, eligible participants are randomised to the ICSI+PGT-A group or the conventional ICSI group in a 1:1 ratio. Other assisted reproductive procedures are similar and parallel between the two groups. The primary outcome will be live birth rate and cumulative live-birth rate. Secondary outcomes will be embryo implantation rate, biochemical pregnancy rate, clinical pregnancy rate, spontaneous abortion rate, ongoing pregnancy rate, preterm birth rate, fetal chromosomal abnormality rate, birth defect rate and treatment complications. To demonstrate or refute a difference between the two groups, we plan to include 188 participants in each group; taking consideration of 20% of dropout, the total target sample size is 450.

**Ethics and dissemination** Ethical approval was obtained from International Peace Maternity and Child Health Hospital of Shanghai Jiao Tong University Medical Science Research Ethics Committee (GKLW2016-16). Informed consent will be obtained from each participant. The findings will be disseminated to the public through conference presentations and publication in peer-reviewed scientific journals.

**Trial registration number** ClinicalTrials.gov, NCT02941965.

## Strengths and limitation of this study

- ⇒ This study takes place in four infertility medical centers in China, which have obtained the access qualification for preimplantation genetic testing technique.
- ⇒ The primary outcome of this study will be live birth rate and cumulative live-birth rate.
- ⇒ The sample size and power calculation are focused on the primary outcome, with the limited power to detect differences in secondary outcomes.
- ⇒ Due to the type of interventions, blinding of the patients and procedure is not possible.

## INTRODUCTION

Male factor is responsible, at least in part, for about 20%–70% of all the causes of infertility.<sup>1 2</sup> Assisted reproductive technology (ART) has been widely used as an effective way to improve pregnancy rate in infertile couples. Intracytoplasmic sperm injection (ICSI), a technique where a single spermatozoon was injected mechanically into an oocyte in vitro to achieve fertilisation, was introduced in 1992.<sup>3</sup> It has been applied originally to overcome the most severe forms of male factor infertility such as severe oligoasthenoteratozoospermia, which was defined as a semen concentrate less than  $5 \times 10^6$ /mL, and/or with a progressive motility less than 10% based on 2010 WHO reference values.<sup>4</sup> However, there are controversies regarding the selection and the damage to gametes during the ICSI procedure, and, whether the indiscriminate use of ICSI leads to adverse health consequences for the resulting offspring.<sup>5</sup> When technicians inject a sperm into an embryo, it is not only an invasive procedure but also skips the process of sperm capacitation, acrosome reaction, sperm penetration of granular cells and zona

pellucida. After fertilisation through ICSI, the intensity and duration of calcium flow are also different from that of conventional in vitro fertilisation (IVF). ICSI bypasses the natural selection for abnormal sperm during fertilisation, which leads to increasing risk of genetic defection.<sup>6</sup>

Researches about the safety of ICSI are inconsistent. Some have reported that the birth defect rate through ICSI is higher than that of the IVF and natural pregnancy.<sup>7,8</sup> Other studies have shown that ICSI does not increase the occurrence rate of chromosomal abnormalities and congenital abnormalities in the offspring. The increasing risk is mainly due to the defects of the sperm itself, rather than the ICSI procedure.<sup>9</sup> The guideline of the European Society of Urology emphasises that the infertile sperm has a potential risk of passing on genetic diseases to the next generation.<sup>10</sup> Risks of chromosomal abnormalities have been observed to be greater in infants born as a result of ICSI than in naturally conceived children.<sup>11</sup> Therefore, though ICSI greatly improves the pregnancy rate for couples with severe male infertility, it also transmits chromosomal abnormalities, especially sex chromosomal abnormalities to the next generation.<sup>12</sup>

Preimplantation genetic testing for aneuploidies (PGT-A), formerly known as preimplantation genetic screening, has been developing rapidly these years. It is a procedure that determines the chromosomal status of embryos prior to transfer. As is known to us, the chromosomal abnormalities are the main causes of embryonic development defection. The indications of PGT-A can increase implantation rate, pregnancy rate and live birth rate, while reducing the risk of miscarriage and aneuploidy.<sup>13</sup> Researches show that PGT-A can increase the possibility of pregnancy per transfer, reduce the risk of miscarriage in recurrent abortion patients from 33.5% to 6.9%,<sup>14</sup> and increase the clinical pregnancy rate from 45.8% to 70.9%.<sup>15</sup> Keltz *et al* reported that PGT-A can significantly improve implantation rate, clinical pregnancy rate and sustained pregnancy rate, and reduce miscarriage rate and multiple birth rate.<sup>16</sup> A meta-analysis that includes four randomised clinical trials (RCTs) and seven cohort studies compared the effects of PGT-A and traditional embryo morphological assessment on IVF/ICSI pregnancy outcomes. The result shows that embryos diagnosed as euploid with PGT-A are more likely to achieve live birth.<sup>17</sup>

Male infertility often accompanies decrease in sperm number, motility rate and increase in proportion of abnormal sperm. In severe male infertility patients, these three conditions often exist simultaneously. Genetic factors, environmental toxins and mental stress are the common causes of sperm abnormalities. In 1995, Lancet reported an increased risk in miscarriage and birth defects of severe male infertility.<sup>12</sup> Subsequent studies have shown that the male gonadal dysfunction (high serum FSH level) is related to sperm chromosome aneuploidy.<sup>18</sup> And oligospermia is significantly related to blastocyst chromosome aneuploidy, especially sex chromosome.<sup>9</sup> These studies also show that there is no difference in the incidence of embryo aneuploidy with normal motility sperm

in IVF or ICSI procedure.<sup>9</sup> These results strongly suggest the necessity of early chromosomal screening for patients with severe male infertility. Therefore, PGT-A after ICSI seems to be an ideal solution to avoid embryos with chromosome abnormalities being transplanted in severe male infertility treatment. However, PGT-A costs much more than conventional ICSI. And whether PGT-A can improve the pregnancy rate in couples with severe male infertility still lacks strong evidence such as RCT study.<sup>19</sup> In this situation, we planned a multicenter randomised controlled clinical trial to compare the efficacy between ICSI+PGT-A and conventional ICSI in severe male infertility couples.

## METHODS AND ANALYSIS

### Objectives

ICSI is a widely used treatment for couples with severe male infertility. However, there are controversies regarding the selection and the damage to gametes during the ICSI procedure. Although PGT-A can give genetic information about embryos to help to select the embryo for transfer and improve fertility rate, whether it can improve fertility rate of couples with severe male infertility remains unclear. Therefore, the present study aims to compare the ICSI procedure with ICSI and PGT-A for the improvement of live birth rate and cumulative live birth rate among couples with severe male infertility.

### Trial design

We plan a multicenter, open-label, parallel, randomised controlled, clinical trial (1:1 treatment ratio). The flowchart followed Standard Protocol Items: Recommendations for Interventional Trials checklist showing enrollment, allocation, treatment and follow-up of participants is presented in [figure 1](#). In addition, the schedule of enrollment, interventions and assessments during the study period is shown in [table 1](#).

### Study setting

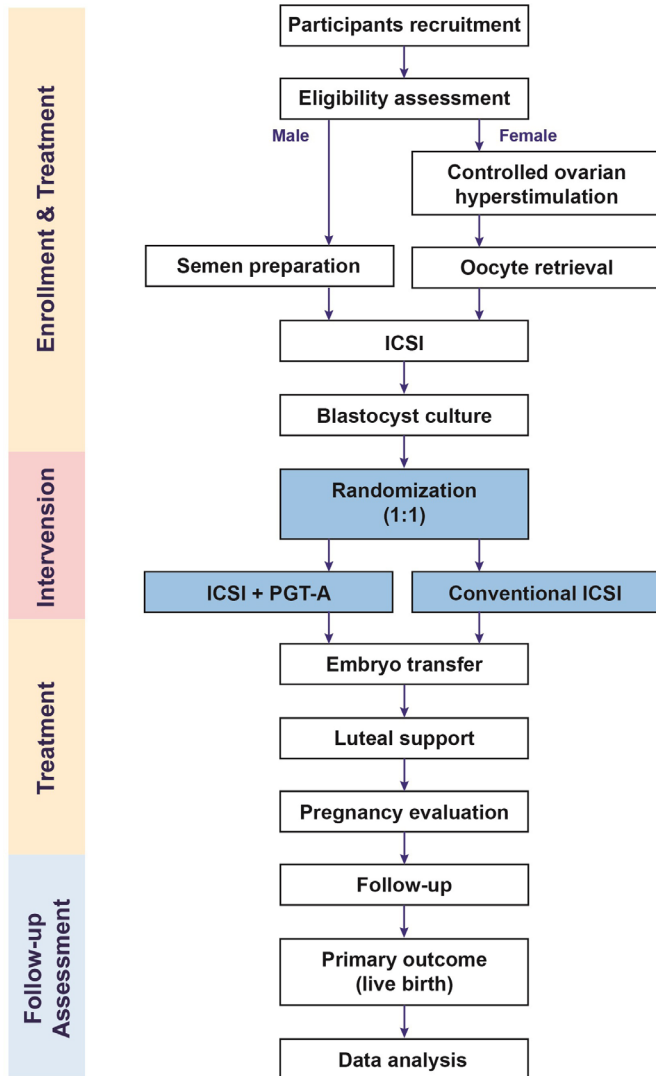
This study will recruit participants from four reproductive medical centers in China, which have obtained the access qualification for PGT technique: International Peace Maternity and Child Health Hospital of Shanghai Jiao Tong University, Renji Hospital of Shanghai Jiao Tong University, Obstetrics and Gynecology Hospital of Fudan University, Sir Run Run Shaw Hospital of Zhejiang University. An independent data and safety monitoring board, with members with clinical and statistical expertise, will monitor the trial progress and interim results at regular intervals.

### Eligibility criteria

The involved hospitals will screen the couples presenting to the reproductive medical centers for following eligibility in order to be enrolled in our trial.

### Inclusion criteria

1. Male partner age of 20–55 years old, Chinese.



**Figure 1** Flowchart for enrollment, allocation, treatment and follow-up of study participants. ICSI, intracytoplasmic sperm injection; PGT-A, preimplantation genetic testing for aneuploidies.

2. Male partner has severe male infertility (defined as a semen concentrate less than  $5 \times 10^6$ /mL, and/or with a progressive motility less than 10%).
3. Proposed ICSI to assist pregnancy.
4. Fully explain the nature of the research and obtain the informed consent of the participants before carrying out any procedure in the research protocol. If a participant is not capable of expressing opinions, the legal representative of the participant can sign the informed consent on behalf of the participants.

### Exclusion criteria

Any one of the following criteria should be excluded from this study:

1. Male partner had been diagnosed with obstructive azoospermia, sexual dysfunction and immune infertility.
2. Female partner at 38 years of age and older.
3. Female partner has uterine abnormalities such as uterine malformations (unicornuate uterus, mediastinal

uterus, double uterus, double horn uterus, etc), adenomyosis, submucosal fibroids or intrauterine adhesions.

4. Female partner has a history of recurrent abortion, including biochemical pregnancy ( $\geq 3$  time miscarriages).
5. One of the couples has abnormal chromosomal karyotypes, excluding chromosomal polymorphisms.
6. Female partner has contraindications for assisted reproduction, such as poorly controlled type 1 or type 2 diabetes mellitus; undiagnosed liver disease or abnormal liver function (abnormal serum liver enzymes); kidney disease or abnormal kidney function; severe anaemia; history of deep venous thrombosis; history of pulmonary embolism; history of cerebrovascular accident; poorly controlled hypertension or diagnosed heart disease; history of cervical cancer, endometrial cancer or breast cancer; unexplained vaginal bleeding.
7. Male partner has contraindications for assisted reproduction, such as poorly controlled type 1 or type 2 diabetes mellitus; undiagnosed liver disease or abnormal liver function (abnormal serum liver enzymes); kidney disease or abnormal kidney function; severe anaemia; history of deep venous thrombosis; history of pulmonary embolism; history of cerebrovascular accident; poorly controlled hypertension or diagnosed heart disease.
8. One of the couple refuses to cooperate with the study.
9. Patients who have been included in the experimental group or control group of this study.

### Withdrawal criteria

The research treatment and evaluation of participants can suspend at any time to include the following:

1. The participant wishes to quit the study. The participant is free to withdraw from the study at any time without affecting further treatment.
2. Failed blastocyst culture, no blastocyst for transfer.
3. Incorrectly selected participants who do not fit the study.

### Recruitment procedure

Infertile couples who come to the outpatient clinic will be screened by our dedicated research team and process necessary tests. Then a member of the research team will explain the trial details to the eligible couples who have been proposed ICSI for their treatment. The couples will be asked to sign the consent form (online supplemental material) in their next visit if they agree to participate in the trial. On the day of oocyte retrieval, semen sample from patients who agreed to participate in the study will be analysed again for the inclusion criteria. Ineligible patients will be further excluded from the trial. In addition, an individual record of non-recruited patients and the reasons for exclusion will be obtained and stored.

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

Content	Enrollment		Pre allocation		Allocation		Post allocation		Close-out	
	Screening and baseline assessment	T0 3 months	Controlled ovarian hyperstimulation	T1 1 month	Oocyte retrieval and semen preparation	ICSI and Assessment of embryo	Blastocyst culture and randomization	PGT-A and/or Embryo frozen	Embryo transfer	Evaluation of pregnancy
Time point	T0 3 months	T1 1 month	T2 0 months	T3 1–2 days	T4 5–6 days	T5 1–2 months	T6 4–5 days	T7 1 month	T8 3–10 months	T9 3 months
<b>Enrollment</b>										
Eligibility screen	√	√	√	√	√					
Informed consent	√									
Allocation					√					
<b>Interventions</b>										
Conventional ICSI						√				
ICSI+PGT-A						√				
<b>Assessment</b>										
Baseline data	√									
Laboratory tests	√	√	√	√	√		√	√	√	√
Fertilisation				√						
Embryo quality				√	√					
Pregnancy tests								√		
Pregnancy outcomes									√	√
Fetus information									√	√
Safety assessment		√	√	√	√		√	√	√	√
ICSI, intracytoplasmic sperm injection; PGT-A, preimplantation genetic testing for aneuploidies.										

## Randomisation

After oocyte and sperm retrieval, standard ICSI procedure will be proceeded by qualified technician and zygotes will be cultured to day 5–6 (blastocyst). Randomisation and allocation of eligible patients will be performed on the patients with at least four good-quality embryos on day 3 or 1 blastocyst on day 5–6. This procedure will be done by staffs who are not involved in the treatment procedure, using an online trial system with a computer-generated randomisation list that allocates couples in a 1:1 ratio to ICSI+PGT-A or ICSI.

## Open-label design

The trial was originally designed to be performed as a double-blind trial, in which everybody should be blinded until the primary outcome occurred except embryologists and geneticists who perform ICSI, PGT-A and analyse genetic results. However, due to the type of intervention, neither the participants nor the treatment providers can be blinded to treatment allocation. The participants want to know about their allocation of fertilisation method and need to bear the cost of PGT-A, the design is then changed to an open-label study. After the ICSI procedure and fertilised eggs have been cultured into embryos or blastocysts, administrative staff in the IVF laboratory will log into the trial system to do randomisation and to allocate participants to the conventional ICSI group or ICSI+PGT-A group. All the embryos obtained during one treatment cycle are advised to culture to blastocyst and all the blastocysts (ICSI+PGT-A group after the blastocyst trophectoderm biopsy) will go into the freezing strategy waiting for the upcoming procedure.

## Interventions

### Controlled ovarian hyperstimulation (COH)

All participants will receive standard COH treatment in each center selected by physicians depending on their circumstances, including either using gonadotrophin releasing hormone agonist (GnRH-a) on the basis of a short GnRH-a long protocol or GnRH-a prolonged protocol or gonadotrophin releasing hormone antagonist (GnRH-ant) protocol. Menstrual cycle of participants includes spontaneous menstrual cycle or the cycle use of oral contraceptives or progesterone. Before gonadotropin (Gn) treatment, baseline transvaginal ultrasound and basic serum hormones (such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone, oestrogen) will be measured to confirm the follicle status. In short GnRH-a long protocol, GnRH-a will be used in the midluteal phase of the previous menstrual cycle, then Gn treatment starts 14 days later along with confirmation of pituitary downregulation. In GnRH-a prolonged protocol, GnRH-a is injected on day 2 of the menstrual cycle, then ovarian stimulation start 28–35 days later along with confirmation of pituitary downregulation. In GnRH-a short protocol, participants will receive GnRH-a on day 2 or 3 of the menstrual cycle and then Gn treatment will be applied 1 day later. The initial

dosage of Gn is 150–300 mg/day and will be adjusted according to the individual response. Gn treatment will be continued to the trigger day. After two or more follicles reach a diameter  $\geq 18$  mm, human chorionic gonadotropin (hCG) will be once injected on trigger day. In the GnRH-ant protocol, participants will be injected Gn daily from day 3 of menstrual cycle until at least one follicle reaches a diameter of 12 mm or on day 6 of ovarian stimulation, GnRH-ant will then be applied until the trigger day.

### Oocyte retrieval and preparation

Oocyte retrieval is performed 36 hours ( $\pm 2$ ) after hCG injection. Routine oocyte pick-up is done under transvaginal ultrasound guidance via 17–18G oocyte aspiration needle with use of intravenous sedation. The retrieved cumulus oocyte complexes will be placed immediately in culture medium covered by lightweight paraffin oil and incubated in a humidified 37°C, 5%/6% CO<sub>2</sub> incubator, and incubated for 2–6 hours before injection.

### Semen preparation and ICSI

Semen samples will be obtained on the day of oocyte retrieval by masturbation after 3–7 days of abstention from sexual intercourse. Sperm sample are assessed by computer-assisted semen analysis according to the fifth edition of WHO laboratory standards of human semen and sperm.<sup>20</sup> All semen samples are prepared by discontinuous density gradient centrifugation or swim-up protocol. Microscopes (200–400 times) will be used to observe whether there are serious abnormalities in sperm morphology. When the sperm sample fits our eligibility criteria, a standard ICSI procedure will be performed as previously described<sup>21</sup> by a certificated technician. Only the oocytes that extrude the first polar body (metaphase-II oocytes) will be microinjected.

### Assessment of fertilisation and embryo quality

Assessment of fertilisation is carried out about 16–18 hours (day 1) after ICSI. Normal fertilisation was determined by the presence of two pronuclei and a second polar body. Morphological embryo evaluation was performed on day 3 after retrieval (68 hours after insemination or ICSI).<sup>22</sup> The embryos were classified into four morphological grades according to the conventional method on the basis of blastomere size and the amount of anucleate fragmentation<sup>23</sup>: grade 1, blastomere uniform in size and shape and little or no fragmentation; grade 2, blastomeres uneven in size and shape and/or fragmentation  $< 10\%$  of the embryonic surface; grade 3, fragmentation of  $10\% \pm 30\%$  of the embryonic surface; and grade 4, fragments  $> 30\%$  of the embryonic surface. Embryos evaluated as grade 1 or grade 2 were defined as good-quality embryos. All the zygotes were cultured in cleavage medium to day 5 or 6 (blastocyst). The blastocyst quality will be scored using Gardner blastocyst grading system,<sup>24</sup> and only the blastocyst of medium-quality (B3BC) or better will further be frozen and/or for biopsy.

### Preimplantation genetic testing for aneuploidies

After randomisation, the embryos from conventional ICSI group, which undergo embryo frozen procedure will wait for further transplantation. While the embryos from ICSI+PGT-A group will proceed with a biopsy procedure before the embryo are frozen. The biopsy will be carried out by certificated technicians, usually removes 3–10 cells from the trophectoderm, which is the outer layer of cells that will become the placenta as the embryo develops. The biopsy does not remove any cells from the inner cell mass, which will develop into the fetus. The removed cells are then sent to our cytogenetic laboratories to be analysed using the next-generation sequencing platform. The result will be interpreted by our geneticists.

### Embryo transfer and luteal support

The time of embryo transfer will be decided by physicians according to conditions of participants. According to the national ART specification, only one embryo can be transferred in PGT group. Hence, the number of embryos replaced will be limited to one best-quality embryos in all study centers. Luteal support is performed by standard routines at each study center: for patients in natural ovulation cycle is started from the day of ovulation with oral dydrogesterone (Duphaston) at a dose of 10 mg two times per day and is continued until the day of serum hCG testing. While for patients using an artificial regimen, they will use vaginal progesterone gel (Crinone at a dose of 90 mg daily or Utrogestan at a dose of 600 mg daily) or progesterone injection at a dose of 60 mg daily with oral dydrogesterone (Duphaston) at a dose of 10 mg two times per day and continue until the day of serum hCG testing.

### Follow-Up

According to the outcomes we track, serum hCG will be measured after 14 days of embryo transfer, and positive results indicate biochemical pregnancy. If the gestational sac is observed with ultrasonography in the uterus after 28 days of embryo transfer, clinical pregnancy will be confirmed. Ongoing pregnancy is defined by intrauterine live fetus after 20 weeks of gestation. After delivery, the information of pregnancy, delivery and infant characteristics will all be collected in a form designed for this trail.

### Outcome measures

Baseline characteristics such as age, height, weight, body mass index, education, duration of infertility, gravidity, parity and basal hormone levels will be collected.

### Primary outcome

Our primary outcome will be live birth rate and cumulative live-birth rate. Live birth rate is defined as delivery of any viable infant at 28 weeks or more of gestation after initial embryo transfer to the number of transplant cycles. And cumulative live-birth rate is defined as the proportion of deliveries with at least one live birth after transfers of all euploid embryos in the PGT-A group or up to three blastocysts in conventional ICSI group within 1 year

after randomization to the total number of participants assigned to the group.

### Secondary outcomes

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

1. Embryo implantation rate: defined as the ratio of the number of gestational sac with fetal heartbeat in the uterus to the number of embryo transferred.
2. Biochemical pregnancy rate: defined as the ratio of biochemical pregnancy (serum hCG evaluated >5 mIU/L after 14 days of embryo transfer) cycle to the number of transplant cycle.
3. Clinical pregnancy rate: defined as the ratio of clinical pregnancy (one or more observed gestational sac with fetal heartbeat in the uterus under ultrasonography at 28 days after embryo transfer) cycle to the number of transplant cycle.
4. Spontaneous abortion rate: defined as the ratio of spontaneous abortion (spontaneous pregnancy loss before 20 weeks of gestation) cycle to the number of transplant cycle.
5. Ongoing pregnancy rate: defined as the ratio of ongoing pregnancy (intrauterine live fetus under ultrasonography after 20 weeks of gestation) cycle to the number of transplant cycle.
6. Preterm birth rate: defined as the ratio of preterm birth (delivery  $\geq 28$  and  $< 37$  weeks of gestation) cycle to the number of transplant cycle.
7. Fetal chromosomal abnormality rate: defined as the ratio of fetal chromosomal abnormality cycle to the number of transplant cycle. The test of fetal chromosomes is done by collecting abortion fetal tissues, or amniotic fluid puncture/umbilical blood puncture during the second trimester of pregnancy, or by collecting cord blood/oral mucosa DNA of newborns.
8. Birth defect rate: defined as the ratio of birth defect (fetus body structure, function or metabolism congenital abnormalities) cycle to the number of transplant cycle.

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

1. Any discomfort or deterioration of existing discomfort: during the whole treatment and follow-up procedure, any discomfort or deterioration of existing discomfort in the patient, regardless of whether it is related to research interventions. Symptoms such as abdominal pain, bloating, chest tightness etc, or physical signs such as positive shifting dullness, adnexa mass, etc, or abnormal medical auxiliary examination results.
2. Ectopic pregnancy: defined as implantation that takes place outside the uterine cavity.
3. Moderate/severe ovarian hyperstimulation syndrome (OHSS): defined as an exaggerated systemic response to ovarian stimulation characterised. According to the degree of abdominal distention, ovarian enlargement, and respiratory, haemodynamic, and metabolic complications, it is classified as mild, moderate or severe.

4. Internal bleeding or infection: defined as the complications caused by medical treatment such as egg retrieval, embryo transplant, etc.

### Data management

The informed consent will be in compliance with laws relevant to privacy protection. And according to this consent, participants will authorise researchers to collect, use and publish their data for research purposes. All the researchers and clinicians who participate in this trial are required to master all details about this trial so that to guarantee the authentic study results. The characteristics and medical records of each participant are collected through a standard electronic data collection system from baseline to follow-up procedure. Inspectors will continuously conduct data verification and quality inspections at each research center, and work with researchers to guarantee the accuracy of the data. Adding or correcting any data of the participant must be carried out by a qualified researcher with initials and dates.

All the adverse events will be recorded and inform data and safety monitoring board and safety report will be in accordance with plan. After the first 300 randomised participants have completed their embryo transfer, the data and safety monitoring board will perform an interim analysis using the endpoint ongoing pregnancy. The data and safety monitoring board will also oversee the severe adverse events that have occurred.

### Sample size

According to our previous research and the average live birth rate calculated over all study sites, the live birth rate of conventional IVF with non-male factor infertility is about 35% per cycle. And the live birth rate of ICSI with male factor infertility is about 25% per cycle. If we estimated the live birth rate of our control group (conventional ICSI group) is 25%, and ICSI+PGT-A can improve the live birth rate of severe male factor infertility to about 35%. Set  $\alpha=0.05$  and  $\beta=0.20$ , according to the sample size calculation formula, we need 188 participants in each group. Plus taking consideration a dropout of 20%, the total participant recruitment number is 450 (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 0.05 for the final analysis.<sup>16</sup>

### Statistical analysis

All tests will be two tailed, and differences with  $p$  value  $<0.05$  for final analysis are considered statistically significant. For continues variables, parameters normally distributed will be expressed as mean with SD and compared using Student's t-test. If the parameters are non-normally distributed, their medians and IQRs will be reported and compared using the Wilcoxon rank sum test. Categorical variables will be reported as frequencies and percentages and compared by means of the  $\chi^2$  test. No scenarios will be used to impute missing values. All statistical analyses will

be performed with the SAS software package V.9.3. The statistical analysis will be done by an independent statistician, overseen by International Peace Maternity and Child Health Hospital of Shanghai Jiao Tong University.

### Patient and public involvement

Neither patients nor the public was involved in the study design, development of the research question or implementation of this study.

### ETHICS AND DISSEMINATION

Ethical approval was obtained from International Peace Maternity and Child Health Hospital of Shanghai Jiao Tong University Medical Science Research Ethics Committee (GKLW2016-16). Informed consent will be obtained from each participant before randomisation. The findings will be disseminated to the public through conference presentations and peer-reviewed scientific journals. All data and documents will be available on request.

### Trial status

The recruitment in each study center started in July 2017. The estimated end date of the last recruitment for this study is August 2022.

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**Contributors** H-FH, YS and Y-TW conceived the study idea and supervised trial processing in each study center. X-HL, M-XG and D-DW participated in the study design, participant recruitment, data analysis and drafting the manuscript. Y-TW, X-XS, S-YZ, YL, LJ, J-LZ and C-MX supervised recruitment and diagnosis in each center, and coordinates of the data collection. C-LZ and S-CC participate in the proposal development. CZ will perform data analysis. LW oversees laboratory work. All authors critically reviewed the article and approved the final manuscript.

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