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Full Title:

Comparison of PGS 2.0 versus conventional embryo morphology evaluation for patients with recurrent pregnancy loss: a study protocol for a multicentre prospective randomised trial

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ABSTRACT

Introduction

Pregnancy loss (PL) is an adverse life event, and there is no proven effective treatment for recurrent PL (RPL). Preimplantation genetic screening (PGS) can be performed to reduce the risks of PL; however, there is still no solid scientific evidence that PGS improves outcomes for couples experiencing RPL. Comprehensive chromosome screening (PGS 2.0) has become a routine practice in *in vitro* fertilisation (IVF) clinics. Previous studies based on PGS 1.0 with a focus on RPL couples where the female is of advanced maternal age have reported contradictory results. Hence, a multicentre prospective randomised trial is needed to provide evidence for the clinical benefits of PGS 2.0 treatment for RPL couples.

Methods and analysis

A total of 268 RPL couples undergoing IVF cycles will be enrolled. Couples will be randomised according to a unique grouping number generated by a random digital software into (1) PGS 2.0 group and (2) non-PGS (conventional embryo morphology evaluation) group. This study aims to investigate whether the live birth rate (LBR) per initiated cycle after PGS 2.0 is superior to the LBR per initiated cycle after conventional embryo evaluation (non-PGS group). Live birth will be defined as a live baby born after a gestation period of >28 weeks, with a birth weight of more than 1000 g. A multivariate logistic regression model will be used to adjust for confounding factors.

Ethics and dissemination

Ethical approval has been granted by the Ethics Committee of Obstetrics and Gynecology Hospital, Fudan University and the participating hospitals. Written informed consent will be obtained from each couple before any study procedures are performed. Data from this study will be stored in the Research Electronic Data Capture (REDCap). The results of this trial will be presented and published via peer-reviewed publications and presentations at international conferences.

Trial registration number

NCT03214185; Pre-results.

Strengths and limitations of this study

- This will be the first prospective multicentre randomised trial to investigate the effectiveness of PGS 2.0 for the treatment of recurrent pregnancy loss (RPL).
- This is the first trial that seeks to add significantly to the clinical evidence on the positive effects of PGS 2.0 on the live birth rate (LBR) in young RPL couples.
- A multivariable prediction model for future pregnancy outcomes of young RPL couples will be provided based on trial data.
- Bias by adjustment for important confounding factors, including maternal and paternal factors, will be made to investigate the independent effect of PGS 2.0 on RPL.
- Sample size calculation will be based on a 15% difference in the LBR per initiated cycle between the two cohorts, and a smaller difference in the LBR may not be detected.

INTRODUCTION

A pregnancy loss (PL) or miscarriage is defined as the spontaneous demise of a pregnancy before the foetus reaches viability; that is, from the time of conception until 28 weeks of gestation in China [1] or 24 weeks of gestation in European countries[2]; it also includes non-visualised PLs (biochemical PLs or resolved and treated pregnancies of unknown location), and excludes ectopic and molar pregnancies. Recurrent pregnancy loss (RPL) is defined as two or more PLs.[2] Approximately 1–5% of couples trying to conceive experience RPL.[3] Little is known about the cause of RPL; however, this condition is believed to have a multifactorial pathogenesis. Miscarriage specimen examinations have revealed that 50–70% of early PLs are due to chromosomal abnormalities,[4] which can either be of parental origin or arise *de novo* in the embryo from parents with normal karyotypes,[5] often as a random event. Among these, aneuploidy is considered as the main chromosomal abnormality; it is also the main abnormality found in normally developing monospermic embryos during *in vitro* fertilisation (IVF).[6] Recently, a large genetic survey of embryos supported the finding that aneuploidy is the leading chromosomal abnormality in IVF, and it primarily occurs due to errors in maternal meiosis and mitosis.[7] The association between aneuploidy and increasing maternal age has been recognised for a long time;[8] however, the underlying molecular basis has remained elusive. Some studies have provided evidence that the age-related increase in maternal errors is not attributable to one single factor.[9] However, when the female patient in couples with a history of RPL is of relatively young age, the

reasons for frequent aneuploidy cannot be attributed to advanced age alone, and the mechanisms remain unclear.

Owing to the high frequency of aneuploidy in RPL patients, pre-implantation genetic screening (PGS)—now called preimplantation genetic testing-aneuploidy (PGT-A)—which aims to detect aneuploidy before transfer, is applied to these patients. In the past two decades, fluorescence in-situ hybridisation (FISH) technology using limited probes has been applied to detect the five to ten most common aneuploidies in one or two blastomeres biopsied at day 3 in cleaving embryos. Although this has been applied to reduce the miscarriage rate and increase the live-birth rate (LBR) in IVF (PGS 1.0), a few randomised clinical trials have shown a significant decrease in pregnancy outcomes after PGS 1.0.[10, 11] This disappointing result might be due to three reasons: first, the cleavage stage biopsy harms the embryo development potential [12]; second, FISH can detect only a limited number of aneuploidies; third, mosaicism of the cleaving embryo leads to incorrect assessment of the embryo. Therefore, a new generation of preimplantation genetic screening (PGS 2.0) has been introduced to IVF centres; this favors trophectoderm biopsy and comprehensive chromosome aneuploidy screening.[13, 14] Hence, many reports of PGS 2.0 have shown increased ongoing pregnancy rates (OPRs) and LBRs.[15-17] However, the beneficial effect of PGS 2.0 has not been proven yet in randomised controlled trials (RCTs).[18]

Conventional morphological blastocyst grading systems recommended by Gardner and Schoolcraft, which include the degree of blastocoel expansion, inner cell

mass (ICM), and trophectoderm cells (TE), are used to predict the ploidy status of blastocysts [6]. More importantly, this grading is completely non-invasive and has no adverse effects on implantation. Observational studies report a correlation between good morphology and euploidy embryos,[19, 20] and many researchers propose embryo morphology as an alternative marker of chromosomal status [21] given the positive correlation between morphologic grading and the euploid state of the embryo. However, it has been reported that morphology analysis cannot accurately predict the genetic status of embryos, because about 50–60% of excellent and good quality embryos are aneuploid.[22]

In Europe in 2012, the reported mean delivery rates per aspiration for IVF, intracytoplasmic sperm injection (ICSI), and frozen-thawed transfer (FET) were 21.9%, 20.1%, and 16.0%, respectively.[23] In 2013, the rates were 22.2%, 20.1%, and 18.0%, respectively.[24] In Europe in 2017, delivery rates after PGS per oocyte retrieval and per embryo transfer were 13% and 22%, respectively.[25] These data might be analysed by FISH (PGS1.0). Simon et al. reported LBR per transfer of 64.5% and per retrieval of 45.1% in 1,621 nondonor frozen cycles with PGS in 2018.[26] Lee et al. also reported LBR per initiated cycle of 46.3% in 82 cycles of RPL couples with PGS in 2019.[27] These data might be analysed by comprehensive chromosome testing (PGS2.0). We have conducted a retrospective analysis and found LBR per initiated cycle of 26.6% in RPL couples with PGS, and 15.4% in RPL couples without PGS (data not yet published).

For RPL couples who require IVF to help them conceive, we know that PGS

might increase the LBR per transfer, but whether PGS 2.0 could increase the LBR per start cycle or the cumulative LBR remains unknown. PGS 2.0 is thought to be a good treatment for RPL patients, but whether it should be routinely applied for all couples with RPL remains controversial. The present protocol describes a multicentre, prospective, randomised trial assessing PGS 2.0 in the treatment of RPL patients. The results are very important for clinicians involved in RPL treatment, and for patients who experience RPL.

METHODS AND ANALYSIS

Study design

This is a multicentre, prospective, randomised controlled clinical trial which is designed to compare LBR per initiated oocyte retrieval cycle, per patient (cumulative LBR), and per embryo transfer in 268 RPL couples undergoing ICSI. Participants will be enrolled at three hospitals in Shanghai, China. This study has been approved by the ethics committees at the three hospitals. Informed consent will be obtained from the enrolled couples before any study procedures are performed. Reporting of the study results will follow the 2010 revised CONSORT statement [28] and updated guidelines, 2012.[29]

Study population/participants and recruitment

The following inclusion criteria will be applied:

1. Couples who have experienced two or more PLs.
2. Normal karyotypes of both husband and wife (polymorphic chromosomes are considered normal as well).

3. Female aged between ≥20 and <38 years.

The exclusion criteria will include:

1. Females with uterine abnormalities such as uterine malformations (uterus unicorns and duplex uterus), untreated septate uterus, adenomyoma, submucous uterine fibroids, endometrial polyps, or untreated intrauterine adhesions.

2. Females with medical conditions that contraindicates ART or pregnancy such as deep vein thrombosis, pulmonary embolism, cardiac disease, carcinoma, and severe anaemia.

Interventions

All included couples will be informed of the study procedures and written informed consent will be signed before controlled ovarian stimulation (COH) is implemented and any procedures are performed. The included couples will be randomised 1:1 into either of two groups: group A (PGS 2.0 group) and group B (non PGS group, conventional embryo morphology evaluation group). Group A will undergo conventional embryo morphology evaluation and trophectoderm biopsy before blastocyst cryopreservation, and group B will undergo conventional embryo morphology evaluation before blastocyst cryopreservation. All patients will undergo a frozen-thawed embryo transfer once a good quality embryo is chosen.

Randomisation

At the start of the study, the grouping results will be generated by random digital software corresponding to a unique grouping number. The couples will be given a unique grouping number when they have signed the informed consent form;

subsequently, they will be randomly divided into group A or group B. Both the investigators and patients will be aware of the grouping information and interventions.

Questionnaire

A questionnaire will be developed for collating the basic characteristics of the couple; this will include the date of birth of the female, ethnicity, education, annual income level, occupation, and life-style. The participants will address these questions on the Research Electronic Data Capture (REDCap) platform. REDCap is a widely-used secure web interface for ensuring data quality; it checks data accuracy during data entry.

Patient and Public Involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our trial.

COH protocol

1. All patients will undergo three COH cycles unless they become pregnant after the first or second cycle, or they indicate that they wish to stop treatment. If the patient is not pregnant after three COH cycles, she will be automatically withdrawn from the study.

2. A pelvic ultrasound will be performed before the start of COH, and basal hormone levels, including serum follicle stimulating hormone (FSH), luteinising hormone (LH), prolactin (PRL), oestradiol (E2), progesterone (P4), testosterone (T), and anti-Mullerian hormone (AMH), will be examined.

3. A conventional GnRH antagonist COH protocol will be used in all patients either

by using daily recombinant follicle-stimulating hormone (rFSH) or human menopausal gonadotropin (hMG). The gonadotropin stimulation will be performed according to the routine methods used in the clinics of the three hospitals involved in the study. However, this protocol can be changed at any time during the treatment according to the ovarian response. Generally, rFSH or hMG will begin on day 2 or day 3 of the menstrual period; the latter occurring either naturally or induced by exogenous administration of progesterone or oral contraceptive pills. The initiative doses will be 150–300 IU/day according to female age, body mass index (BMI), number of antral follicles, and basal hormone levels. On the sixth day of receiving the rFSH or hMG, transvaginal ultrasound will be performed to examine the diameter of the follicles, and a blood test for serum E2, P, and LH levels will be performed. rFSH or hMG doses will be adjusted according to ovarian response. Subsequently, such monitoring will be performed either every other day or every day. The antagonist regimen are as follows:

Antagonist regimen 1 = rFSH (150–300 IU IM) from day 2 or day 3 followed by rFSH (150–300 IU IM) + Cetrotide (0.25 mg/day SC) from day 8 or day 9.

Antagonist regimen 2 = hMG (150–300 IU IM) from day 2 or day 3 followed by hMG (150–300 IU IM) + Cetrotide (0.25 mg/day SC) from day 8 or day 9.

4. When at least one follicle reaches a mean diameter of 14 mm, or the serum E2 reaches 350 pg/ml, the patient will receive 0.25 mg/day of GnRH antagonist (Cetrotide, Cetrotide, Merck Serono, Shanghai, China) and this will be continued daily until the trigger day.

5. Human chorionic gonadotropin (hCG) trigger for final oocyte maturation: when the mean diameter of at least one follicle is ≥18 mm or two follicles are ≥16 mm, an intramuscular injection of hCG (hCG, HCG, Zhuhai Livzon Pharmaceutical Group, Zhuhai, China) 5000–10000 IU will be administered to the patient. Subsequently, 36 hours after hCG injection, the oocytes will be retrieved under transvaginal ultrasound guidance. On the trigger day, the endometrial thickness and morphology, as well as the number and size of follicles (≥15 mm, 10–15 mm and <10 mm) will be documented.

ICSI and embryo culture

A single sperm will be injected within 4 h after the follicular aspiration. Embryos will be cultured in sequential medium with 5% CO_2 in the atmosphere. The fertilisation state of the embryo will be observed 16–18 hours after ICSI. The observation of blastomere formation (cleavage rate) and scoring of the effective cleavage stage embryos will be performed 72 hours after ICSI; however, the day 3 cleaving embryos will continue to be cultured to blastocysts.

Good quality embryo evaluation

Group A: Blastocysts in group A will first be evaluated according to a widely-used grading system (Gardner and Schoolcraft) as previously described. Subsequently, three to ten trophectoderm cells will be biopsied and immediately transported to the PGD lab for chromosome screening analysis. The day of trophectoderm biopsy will be dependent upon blastocyst development and recorded as day 5 or day 6. Blastocysts will be cryopreserved immediately after the biopsy

procedure is finished. Embryos will be classified as euploid, aneuploid, mosaic, or not classifiable. Consequently, only one euploid and good morphology embryo will be transferred. If no euploid embryo is detected, the transfer cycle will be cancelled.

Group B: Blastocysts in group B will be evaluated according to the Gardner grading system and then cryopreserved. One good quality embryo will be transferred in the next frozen-thawed cycle.

Embryo transfer and luteal phase support

Endometrial preparation will be hormonally induced. Oral E2 valerate (E2V, Progynova, Bayer Schering Pharma, Shanghai, China) will be given to patients at a dose of 4 mg daily from menstrual day 3. The E2V dose will remain unchanged for 10 days and will then be increased to approximately 6–8 mg/day if the endometrial thickness is still less than 8 mm. When the endometrial thickness is ≥8 mm, 60 mg of progesterone (progesterone injection, Xianju pharma, Zhejiang, China) will be injected intramuscularly per day. Six days after the progesterone injections, the blastocyst will be frozen-thawed and transferred. One good quality embryo will be transferred through a catheter guided by transabdominal ultrasound. The patients will lie in bed for half an hour after transfer. The dose of E2V and progesterone will be unchanged until the day on which serum β-hCG levels are measured. If the patient is pregnant, luteal phase support will continue until 11 weeks of gestation and 8% progesterone sustained-release vaginal gel (Crinone, Merck Serono, Shanghai, China; 90 mg per day) will be added.

Pregnancy evaluation

Serum β-hCG will be measured to determine pregnancy 14 days after embryo transfer. If a biochemical pregnancy has been detected, a transvaginal ultrasound scan will be performed 28 days after embryo transfer. If a gestational sac is detected and a heartbeat is seen, a clinical pregnancy is confirmed. The ultrasound scan will be repeated every 2 weeks until 11 weeks. Ongoing pregnancy will be confirmed if the foetal heartbeat is confirmed at 12 weeks of gestation.

Follow-up evaluation

At 12 weeks of gestation, first-trimester pregnancy complications (miscarriage, ectopic pregnancy and gestational trophoblastic neoplasia) will be documented in the case report form (CRF) for the first pregnancy follow-up time point.

At 28 weeks of gestation, the second-trimester pregnancy complications (prenatal diagnosis, abortion, gestational diabetes, preeclampsia, eclampsia, premature rupture of membrane, and placenta abruption) and foetal abnormalities (chromosome abnormalities, foetal malformation, polyhydramnios, oligohydramnios, foetal growth restriction, and foetal distress) will be documented in the CRF for the second pregnancy follow-up time point. If the patient fails to reach 28 weeks of gestation, another frozen-thawed transfer will be arranged and followed up.

At 42 weeks of gestation, delivery information (gestational age, delivery mode, placenta abnormality, and delivery complications), and the new-born information (baby sex, birth weight, Apgar score, and birth defects) will be documented in the CRF for the third pregnancy follow-up time point.

Six weeks after delivery, the postpartum information and neonatal disease

information will be documented in the CRF for the fourth and final pregnancy follow-up time points.

Primary objective

The primary objective of the study is to investigate if the LBR per initiated cycle after PGS is superior compared with the conventional embryo morphology evaluation strategy in the treatment of RPL patients. Live birth will be defined as a live born baby with a gestational period beyond gestational week 28, and birth weight more than 1000 g. Investigation of the cumulative LBR, which is the LBR per patient, and LBR per blastocyst transfer, is also considered a primary aim of the study.

Secondary objectives

The secondary objectives are as follows:

1. To analyse clinical pregnancy rate per transfer, per initiative and cumulative pregnancy rate in the two groups. Clinical pregnancy will be defined as the presence of an intrauterine gestation sac 4 weeks after embryo transfer.

2. To measure time-to-pregnancy from the date of starting COH to the date of the first ongoing pregnancy in the two groups (the longest follow-up time will be 2 years; hence, failure will be defined as no pregnancy over the 2-year period from the start of COH).

3. To measure the miscarriage rate in the two groups. Miscarriage will be defined as the termination of the pregnancy at <28 weeks of gestation with a miscarried foetal weight less than 1000 g.

Sample size calculation

The three study centres had an average 15% LBR per initiated retrieval cycle and an average 30% LBR per initiated cycle following PGS and frozen-thawed transfer strategy for the last 3 years. For the sample size calculations, we aim to detect an increase of 15% of LBR following PGS strategy with an alpha error level of 0.05 and a beta error level of 0.2. The number will be set to 1:1 in each group, and the minimum sample size will be 242 participants for each group. Considering a dropout rate of 10%, we expect to have a total of 268 participants, with 134 participants in each group.

Outcome measurements (primary and secondary)

Four investigators from the three centres have composed a Data Monitoring Group (DMG), that is responsible for data integrity and accuracy. All the data will be stored in the REDCap, and this interface will automatically ensure accuracy during data entry. We included data obtained from participants completing the self-administered basic characteristics survey questionnaire. We included outcome data from the whole COH cycle and follow-up evaluations. We will use the full analysis set (FAS), an intent-to-treat (ITT) approach, to examine differences in the LBR per initiated cycle in the two treatment arms in the primary analysis using a Pearson $\chi 2$ test. Clinical pregnancy rate and other rates will be analysed using the Pearson $\chi 2$ test and logistic regression. Cox proportional hazards models and the Kaplan-Meier method will be used to compare differences of time to pregnancy and cumulative LBR. The DMG will audit the data quarterly.

Ethics and dissemination

RPL is unexplained in about 50% of young couples, and the effectiveness of treatments, such as anticoagulation,[30] corticosteroids,[31] and other such treatments, is controversial. In current practice, RPL is considered an issue derived mostly from embryo causes. However, it is questionable whether this embryo-centred approach is correct.

In this trial, we hypothesise that euploid embryos will increase the LBR for young RPL couples. Many observational studies have shown that PGS can increase the LBR per transfer, but may decrease the LBR per initiated cycle in women of advanced age.[10, 22] To the best of our knowledge, this trial is the first RCT to analyse LBR in young RPL couples.

The limitations of this RCT are that the sample size calculation is based on a difference in the LBR per initiated cycle of 15% between the two cohorts; hence, it a may not be able to detect smaller differences in LBR. Larger effect sizes may be achieved in more controlled settings; however, this is a trade-off for studying the complex, heterogeneous RPL population who might receive other individualised and complex treatment. Additionally, the centres included in this RCT are all in Shanghai, although included couples may come from all over the country. Therefore, the generalisability of the results may be limited and the inclusion of sites and patient populations from around the country may have provided a more diverse and larger sample size. We will try to minimise this by using randomisation and by choosing young couples who have travelled from other parts of China for treatment.

Counselling of young couples confronted with unexplained RPL regarding its

aetiology and prognosis is an essential part of the treatment process, and the advice will allow them to choose their treatment modalities and decide for or against future attempts. This study may prove that PGS is a quick and safe future treatment option.

Ethical approval has been granted by the Ethics Committees of Obstetrics and Gynecology Hospital, Fudan University (2017-85), the Shanghai JiAi Genetics & IVF Institute (JIAI E2017-15), the coordinated centres of Renji Hospital, Shanghai Jiao Tong University School of Medicine (2017072101), and The International Peace Maternity & Child Health Hospital of China welfare institute, Shanghai Jiao Tong University School of Medicine (GKLW2017-13)(supplementary files). Written informed consent will be obtained from each couple before any study procedure is performed. Data from this study are/will be stored in the Research Electronic Data Capture (REDCap). To improve adherence to intervention protocols, the investigators will keep the proper scientific research attitude, and be able to answer the participants' various questions to increase participants' compliance. There will be no interim analysis during the study period. The results of this trial will be presented and published via peer-reviewed publications and presentations at international conferences.

Trial status

The first participant was randomised in March 22, 2018. We aim to complete the recruitment by March 31, 2020.

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AUTHORS' CONTRIBUTIONS

Contributors LCX, SYL, and SXX from the sponsor hospital have designed the whole study. SXX is responsible for the whole project. LCX, SXX, SY, and JL will be responsible for patient recruitment and randomisation. LCX, LY, XJ, and YJF will form the data management team responsible for collecting and analysing all data. LCX, YJF, SXX, SY, and JL will supervise the data. The manuscript will be drafted by LCX. All the authors will participate in reviewing, curating, and approval of the final manuscript.

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Competing interests: The authors declare that they have no competing interests.

REFERENCES

1. Shi Y, Wei D, Liang X, et al. Live birth after fresh embryo transfer vs elective embryo cryopreservation/frozen embryo transfer in women with polycystic ovary syndrome undergoing IVF (FreFro-PCOS): study protocol for a multicenter, prospective, randomized controlled clinical trial. *Trials* 2014;15:154. doi:

10.1186/1745-6215-15-154.

2. Group EEPGD. Recurrent Pregnancy Loss Guideline of the European Society of Human Reproduction and Embryology 2017. www.eshre.eu/guidelines, Last update: NOVEMBER 2017.

3. El Hachem H, Crepaux V, May-Panloup P, et al. Recurrent pregnancy loss: current perspectives. *Int J Womens Health* 2017;9:331–45. doi: 10.2147/IJWH.S100817.

4. Wang Y, Cheng Q, Meng L, et al. Clinical application of SNP array analysis in first-trimester pregnancy loss: a prospective study. *Clin Genet* 2017;91:849–58. doi: 10.1111/cge.12926.

5. Robinson WP, McFadden DE, Stephenson MD. The origin of abnormalities in recurrent aneuploidy/polyploidy. *Am J Hum Genet* 2001;69:1245–54. doi: 10.1086/324468.

6. Babariya D, Fragouli E, Alfarawati S, et al. The incidence and origin of segmental aneuploidy in human oocytes and preimplantation embryos. *Hum Reprod* 2017;32:2549–60. doi: 10.1093/humrep/dex324.

7. McCoy RC, Demko ZP, Ryan A, et al. Evidence of selection against complex mitotic-origin aneuploidy during preimplantation development. *Plos Genet* 2015;11:e1005601. doi: 10.1371/journal.pgen.1005601.

8. Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. *Nat Rev Genet* 2001;2:280–91. doi: 10.1038/35066065.

9. Nagaoka SI, Hassold TJ, Hunt PA. Human aneuploidy: mechanisms and new insights into an age-old problem. *Nat Rev Genet* 2012;13:493–504. doi:

10.1038/nrg3245.

10. Staessen C, Platteau P, Van Assche E, et al. Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. *Hum Reprod* 2004;19:2849–58. doi: 10.1093/humrep/deh536.

11. Mastenbroek S, Twisk M, van Echten-Arends J, et al. In vitro fertilization with preimplantation genetic screening. *N Engl J Med* 2007;357:9–17. doi: 10.1056/NEJMoa067744.

12. Scott RT Jr., Upham KM, Forman EJ, et al. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. *Fertil Steril* 2013;100:624–30. doi: 10.1016/j.fertnstert.2013.04.039.

13. Geraedts J, Sermon K. Preimplantation genetic screening 2.0: the theory. *Mol Hum Reprod* 2016;22:839–44. doi: 10.1093/molehr/gaw033

14. Meldrum DR, Su HI, Katz-Jaffe MG, et al. Preimplantation genetic screening 2.0: an evolving and promising technique. *Fertil Steril* 2016;106:64–5. doi: 10.1016/j.fertnstert.2016.05.002.

15. Keltz MD, Vega M, Sirota I, et al. Preimplantation genetic screening (PGS) with Comparative genomic hybridization (CGH) following day 3 single cell blastomere biopsy markedly improves IVF outcomes while lowering multiple pregnancies and miscarriages. *J Assist Reprod Genet* 2013;30:1333–9. doi: 10.1007/s10815-013-0070-6.

16. Dahdouh EM, Balayla J, Garcia-Velasco JA. Comprehensive chromosome screening improves embryo selection: a meta-analysis. *Fertil Steril* 2015;104:1503–12. doi: 10.1016/j.fertnstert.2015.08.038.

17. Kushnir VA, Darmon SK, Albertini DF, et al. Effectiveness of in vitro fertilization with preimplantation genetic screening: a reanalysis of United States assisted reproductive technology data 2011-2012. *Fertil Steril* 2016;106:75–9. doi: 10.1016/j.fertnstert.2016.02.026.

18. Orvieto R. Preimplantation genetic screening- the required RCT that has not yet been carried out. *Reprod Biol Endocrinol* 2016;14:35. doi: 10.1186/s12958-016-0171-z.

19. Capalbo A, Rienzi L, Cimadomo D, et al. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts. *Hum Reprod* 2014;29:1173–81. doi: 10.1093/humrep/deu033.

20. Kim MK, Park JK, Jeon Y, et al. Correlation between morphologic grading and euploidy rates of blastocysts, and clinical outcomes in in vitro fertilization preimplantation genetic screening. *J Korean Med Sci* 2019;34:e27. doi: 10.3346/jkms.2019.34.e27.

21. Alfarawati S, Fragouli E, Colls P, et al. The relationship between blastocyst morphology, chromosomal abnormality, and embryo gender. *Fertil Steril* 2011;95:520–4. doi: 10.1016/j.fertnstert.2010.04.003.

22. Vinals Gonzalez X, Odia R, Naja R, et al. Euploid blastocysts implant irrespective

of their morphology after NGS-(PGT-A) testing in advanced maternal age patients. *J Assist Reprod Genet* 2019;36:1623–29. doi: 10.1007/s10815-019-01496-9.

23. European IVF-Monitoring Consortium (EIM) for the European Society of Human Reproduction and Embryology (ESHRE), Calhaz-Jorge C, de Geyter C, et al. Assisted reproductive technology in Europe, 2012: results generated from European registers by ESHRE. *Hum Reprod* 2016;31:1638–52. doi: 10.1093/humrep/dew151.

24. European IVF-Monitoring Consortium, European Society of Human Reproduction and Embryology, Calhaz-Jorge C, et al. Assisted reproductive technology in Europe, 2013: results generated from European registers by ESHRE. *Hum Reprod* 2017;32:1957–73. doi: 10.1093/humrep/dex264.

25. De Rycke M, Goossens V, Kokkali G, et al. ESHRE PGD Consortium data collection XIV-XV: cycles from January 2011 to December 2012 with pregnancy follow-up to October 2013. *Hum Reprod* 2017;32:1974–94. doi: 10.1093/humrep/dex265.

26. Simon AL, Kiehl M, Fischer E, et al. Pregnancy outcomes from more than 1,800 in vitro fertilization cycles with the use of 24-chromosome single-nucleotide polymorphism-based preimplantation genetic testing for aneuploidy. *Fertil Steril* 2018;110:113–21. doi: 10.1016/j.fertnstert.2018.03.026.

27. Lee CI, Wu CH, Pai YP, et al. Performance of preimplantation genetic testing for aneuploidy in IVF cycles for patients with advanced maternal age, repeat implantation failure, and idiopathic recurrent miscarriage. *Taiwan J Obstet Gynecol* 2019;58:239–43. doi: 10.1016/j.tjog.2019.01.013.

28. Moher D, Hopewell S, Schulz KF, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *BMJ* 2010;340:c869. doi: 10.1136/bmj.c869.

29. Moher D, Hopewell S, Schulz KF, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *Int J Surg* 2012;10:28–55. doi: 10.1016/j.ijsu.2011.10.001.

30. Rottenstreich A, Amsalem H, Kleinstern G, et al. Outcomes of threatened abortions after anticoagulation treatment to prevent recurrent pregnancy loss. *Reprod Biomed Online* 2017;35:461–67. doi: 10.1016/j.rbmo.2017.06.018.

31. Laskin CA, Bombardier C, Hannah ME, et al. Prednisone and aspirin in women with autoantibodies and unexplained recurrent fetal loss. *N Engl J Med* 1997;337:148–53. doi: 10.1056/NEJM199707173370302.

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

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	3
Funding	4	Sources and types of financial, material, and other support	3，18
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	18

	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	19
	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)	19
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	5-6
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	14-15
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)	8
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	8
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)	8-9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-12
	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)	10
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	18-19
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A

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	15-16
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)	17
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	15
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	15
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	9

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	9
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	18
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
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	10
	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	13-14

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	10
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	15-16
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A
	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)	N/A
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	15-16

	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	17
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	N/A
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	17
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	19
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)	N/A
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	8

	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
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	15
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	20
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	19
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	N/A
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	17
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
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	N/A

*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.

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Comparison of PGS2.0 versus conventional embryo morphology evaluation for patients with recurrent pregnancy loss: a study protocol for a multicentre randomised trial

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Full Title:

Comparison of PGS2.0 versus conventional embryo morphology evaluation for patients with recurrent pregnancy loss: a study protocol for a multicentre randomised trial

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ABSTRACT

Introduction

Pregnancy loss (PL) is an adverse life event, and there is no proven effective treatment for recurrent PL (RPL). Preimplantation genetic screening (PGS) can be performed to reduce the risks of PL; however, there is still no solid scientific evidence that PGS improves outcomes for couples experiencing RPL. Comprehensive chromosome screening (PGS2.0) has become a routine practice in *in vitro* fertilisation (IVF) clinics. Previous studies based on PGS1.0 with a focus on RPL couples where the female is of advanced maternal age have reported contradictory results. Hence, a multicentre, randomised trial is needed to provide evidence for the clinical benefits of PGS2.0 treatment for RPL couples.

Methods and analysis

Overall, 268 RPL couples undergoing IVF cycles will be enrolled. Couples will be randomised according to a unique grouping number generated by a random digital software into (1) PGS2.0 group and (2) non-PGS (conventional embryo morphology evaluation) group. This study aims to investigate whether the live birth rate (LBR) per initiated cycle after PGS2.0 is superior to the LBR per initiated cycle after conventional embryo evaluation (non-PGS group). Live birth will be defined as a live baby born after a gestation period of >28 weeks, with a birth weight of more than 1000 g. A multivariate logistic regression model will be used to adjust for confounding factors.

Ethics and dissemination

Ethical approval has been granted by the Ethics Committee of Obstetrics and

Gynecology Hospital, Fudan University and the participating hospitals. Written informed consent will be obtained from each couple before any study procedure is performed. Data from this study will be stored in the Research Electronic Data Capture (REDCap). The results of this trial will be presented and published via peer-reviewed publications and presentations at international conferences.

Trial registration number

NCT03214185; Pre-results.

Strengths and limitations of this study

- This will be the first multicentre randomised trial to investigate the effectiveness of PGS2.0 for the treatment of recurrent pregnancy loss (RPL).
- This is the first trial that seeks to add significantly to the clinical evidence on the positive effects of PGS2.0 on the live birth rate (LBR) in young RPL couples.
- A multivariable prediction model for future pregnancy outcomes of young RPL couples will be provided based on trial data.
- Bias by adjustment for important confounding factors, including maternal and paternal factors, will be made to investigate the independent effect of PGS2.0 on RPL.
- Sample size calculation will be based on a difference of 15%-points in the LBR per initiated cycle between the two cohorts, and a smaller difference in the LBR may not be detected.

INTRODUCTION

A pregnancy loss (PL) or miscarriage is defined as the spontaneous demise of a pregnancy before the foetus reaches viability; that is, from the time of conception until 28 weeks of gestation in China,[1 2] 24 weeks of gestation in European countries,[3] or 22 weeks gestation according to the international glossary on infertility and fertility care.[4] It also includes non-visualised PLs (biochemical PLs or resolved and treated pregnancies of unknown location), and excludes ectopic and molar pregnancies.[3] Recurrent pregnancy loss (RPL) is defined as two or more PLs.[3 5] Approximately 1–5% of couples trying to conceive experience RPL.[6] Little is known about the cause of RPL; however, this condition is believed to have a multifactorial pathogenesis. Miscarriage specimen examinations have revealed that 50–70% of early PLs are due to chromosomal abnormalities,[7] which can either be of parental origin or arise *de novo* in the embryo from parents with normal karyotypes,[8] often as a random event. Among these, aneuploidy is considered as the main chromosomal abnormality; it is also the main abnormality found in normally developing monospermic embryos during *in vitro* fertilisation (IVF).[9] Recently, a large genetic survey of embryos supported the finding that aneuploidy is the leading chromosomal abnormality in IVF, and it primarily occurs due to errors in maternal meiosis and mitosis.[10] The association between aneuploidy and increasing maternal age has been recognised for a long time,[11] however, the underlying molecular basis has remained elusive. Some studies have provided evidence that the age-related increase in maternal errors is not attributable to one single factor. [12] However, when the female patient in couples with a history of RPL is of relatively

young age, the reasons for frequent aneuploidy cannot be attributed to advanced age alone, and the mechanisms remain unclear.

Owing to the high frequency of aneuploidy in RPL patients, pre-implantation genetic screening (PGS)—now called preimplantation genetic testing-aneuploidy (PGT-A)—which aims to detect aneuploidy before transfer, is applied to these patients. In the past two decades, fluorescence in-situ hybridisation (FISH) technology using limited probes has been applied to detect the five to ten most common aneuploidies in one or two blastomeres biopsied at day 3 in cleaving embryos. Although this has been applied to reduce the miscarriage rate and increase the live-birth rate (LBR) in IVF (PGS1.0), a few randomised clinical trials have shown a significant decrease in pregnancy outcomes after PGS1.0.[13 14] This disappointing result might be due to three reasons: first, the cleavage stage biopsy harms the embryo development potential;[15] second, FISH can detect only a limited number of aneuploidies; third, mosaicism of the cleaving embryo leads to incorrect assessment of the embryo. Therefore, a new generation of preimplantation genetic screening (PGS2.0) has been introduced to IVF centres; this favours trophectoderm biopsy and comprehensive chromosome aneuploidy screening,[16 17] Hence, many reports of PGS2.0 have shown increased ongoing pregnancy rates (OPRs) and LBRs.[18-20] However, the beneficial effect of PGS2.0 has not been proven yet in randomised controlled trials (RCTs).[21]

Conventional morphological blastocyst grading systems recommended by Gardner and Schoolcraft, which include the degree of blastocoel expansion, inner cell mass (ICM), and trophectoderm cells (TE), are used to predict the ploidy status of blastocysts,

[9] More importantly, this grading is completely non-invasive and has no adverse effects on implantation. Observational studies report a correlation between good morphology and euploidy embryos,[22 23] and many researchers propose embryo morphology as an alternative marker of chromosomal status,[24] given the positive correlation between morphologic grading and the euploid state of the embryo. However, it has been reported that morphology analysis cannot accurately predict the genetic status of embryos, because about 50–60% of excellent and good quality embryos are aneuploid.[25]

In Europe in 2012, the reported mean delivery rates per aspiration for IVF, intracytoplasmic sperm injection (ICSI), and frozen-thawed transfer (FET) were 21.9%, 20.1%, and 16.0%, respectively.[26] In 2013, the rates were 22.2%, 20.1%, and 18.0%, respectively.[27] In Europe in 2017, delivery rates after PGS per oocyte retrieval and per embryo transfer were 13% and 22%, respectively.[28] These data might be analysed by FISH (PGS1.0). Simon et al. reported LBR per transfer of 64.5% and per retrieval of 45.1% in 1,621 nondonor frozen cycles with PGS in 2018.[29] Lee et al. also reported LBR per initiated cycle of 46.3% in 82 cycles of RPL couples with PGS in 2019.[30] These data might be analysed by comprehensive chromosome testing (PGS2.0). We have conducted a retrospective analysis and found LBR per initiated cycle of 26.6% in RPL couples with PGS, and 15.4% in RPL couples without PGS.[31]

For RPL couples who require IVF to help them conceive, we know that PGS might increase the LBR per transfer, but whether PGS2.0 could increase the LBR per start cycle or the cumulative LBR remains unknown. PGS2.0 is thought to be a good treatment for RPL patients, but whether it should be routinely applied for all couples

with RPL remains controversial. The present protocol describes a multicentre, randomised trial assessing PGS2.0 in the treatment of RPL patients. The results are very important for clinicians involved in RPL treatment, and for patients who experience RPL.

METHODS AND ANALYSIS

Study design

This is a multicentre, randomised controlled clinical trial which is designed to compare LBR per initiated oocyte retrieval cycle, per patient (cumulative LBR), and per embryo transfer in 268 RPL couples undergoing ICSI. Participants will be enrolled at three hospitals in Shanghai, China. This study has been approved by the ethics committees at the three hospitals. Informed consent will be obtained from the enrolled couples before any study procedures are performed. Reporting of the study results will follow the 2010 revised CONSORT statement[32] and updated guidelines, 2012.[33]

Study population/participants and recruitment

The following inclusion criteria will be applied:

1. Couples who have experienced two or more PLs.
2. Normal karyotypes of both husband and wife (polymorphic chromosomes are considered normal as well).
3. Female aged between 20 and 38 years (≥20 and <38 years).

The exclusion criteria will include:

1. Females with uterine abnormalities such as uterine malformations (uterus unicorns and duplex uterus), untreated septate uterus, adenomyoma, submucous uterine

fibroids, endometrial polyps, or untreated intrauterine adhesions.

2. Females with medical conditions that contraindicate ART or pregnancy such as deep vein thrombosis, pulmonary embolism, cardiac disease, carcinoma, and severe anaemia.

In order to achieve adequate participant enrolment to reach the target sample size, we will use the following strategies:

1. at the waiting rooms of the three IVF centers, posters will be put to let more people know this study.

2. the doctors at the three IVF centers will be encouraged to introduce the study to their patients to let more people know this study.

3. a study contact will be designated for any person who want to know details of this study.

Interventions

Randomisation will take place during the couple's first visit to the clinic or on the first day of stimulation. All included couples will be informed of the study procedures and written informed consent will be signed before controlled ovarian stimulation (COH) is implemented and any procedures are performed. The included couples will be randomised 1:1 into either of two groups: group A (PGS2.0 group) and group B (non-PGS group, conventional embryo morphology evaluation group). Group A will undergo conventional embryo morphology evaluation and trophectoderm biopsy before blastocyst cryopreservation, and group B will undergo conventional embryo morphology evaluation before blastocyst cryopreservation. All patients will undergo a

frozen-thawed embryo transfer once a good quality embryo or an euploid embryo after PGS2.0 is chosen. Evaluation of blastocyst stage embryos are based on three aspects: the expansion of the blastocoele cavity (EH stage), the number and cohesiveness of the inner cell mass (ICM grade) and trophectodermal cells (TE grade) according to the Gardner and Schoolcraft grading system[34-36]. The EH stage is assessed as one of the following: (1) an early blastocyst with the volume of the blastocoele is less than half of that of an embryo; (2) a blastocyst with the volume of the blastocoele is at least half that of the embryo; (3) a full blastocyst with a completely filling blastocoele of the embryo; (4) an expanded, thinning zona blastocyst with the volume of the blastocoele larger than that of the full blastocyst; (5) a hatching blastocyst with the TE starting to herniate through the zona; and (6) a hatched blastocyst completely escaped from the zona. ICM and TE grade are evaluated after EH stage is assessed. The ICM is assessed as one of the following: (A) tightly packed, many cells; (B) loosely grouped, several cells; and (C) very few cells. The TE is assessed as one of the following: (A) many cells forming a cohesive epithelium; (B) few cells forming a loose epithelium; and (C) very few, large cells.

Randomisation

At the start of the study, the grouping results will be generated by random digital software corresponding to a unique grouping number. The couples will be given a unique grouping number when they have signed the informed consent form; subsequently, they will be randomly divided into group A or group B. Both the investigators and patients will be aware of the grouping information and interventions.

There will be no blinding of the treatment allocation to the doctors and participants in the study. The embryologist performing the embryo quality evaluation will be blinded to the allocated treatment.

Questionnaire

A questionnaire will be developed for collating the basic characteristics of the couple; this will include the date of birth of the couple, ethnicity, education, annual income level, occupation, and lifestyle. The participants will address these questions on the Research Electronic Data Capture (REDCap) platform. REDCap is a widely used secure web interface for ensuring data quality; it checks data accuracy during data entry.

Patient and Public Involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our trial.

COH protocol

1. All patients will undergo up to three COH cycles unless they indicate that they wish to stop treatment. If the patient is not pregnant after three COH cycles and has no surplus embryos for transfer, she will be automatically withdrawn from the study.

2. A 2D ultrasound pelvic ultrasound will be performed before the start of COH, and basal hormone levels, including serum follicle stimulating hormone (FSH), luteinising hormone (LH), prolactin (PRL), oestradiol (E2), progesterone (P4), testosterone (T), and anti-Mullerian hormone (AMH), will be examined.

3. Conventional GnRH antagonist COH protocols will be used in all patients either by using daily recombinant follicle-stimulating hormone (rFSH) or human menopausal

gonadotropin (hMG).[37] The gonadotropin stimulation will be performed according to the routine methods used in the clinics of the three hospitals involved in the study. Generally, rFSH or hMG will begin on day 2 or day 3 of the menstrual period; the latter occurring either naturally or induced by exogenous administration of progesterone or oral contraceptive pills. The initial doses will be 150–300 IU/day according to female age, body mass index (BMI), number of antral follicles, and basal hormone levels.[38] On the sixth day of receiving the rFSH or hMG, transvaginal ultrasound will be performed to examine the diameter of the follicles, and a blood test for serum E2, P, and LH levels will be performed. rFSH or hMG doses will be adjusted according to ovarian response. Subsequently, such monitoring will be performed either every other day or every day. The antagonist regimen is as follows:

Antagonist regimen 1 = rFSH (150–300 IU IM) from day 2 or day 3 followed by rFSH (150–300 IU IM) + Cetrotide (0.25 mg/day SC) from day 8 or day 9.

Antagonist regimen 2 = hMG (150–300 IU IM) from day 2 or day 3 followed by hMG (150–300 IU IM) + Cetrotide (0.25 mg/day SC) from day 8 or day 9.

4. When at least one follicle reaches a mean diameter of 14 mm, or the serum E2 reaches 1000 pg/ml, the patient will receive 0.25 mg/day of GnRH antagonist (Cetrotide, Merck Serono, Shanghai, China) and this will be continued daily until the trigger day.

5. Human chorionic gonadotropin (hCG) trigger or a GnRH agonist for final oocyte maturation: when the mean diameter of at least one follicle is ≥18 mm or two follicles are ≥16 mm, an intramuscular injection of hCG (hCG, HCG, Zhuhai Livzon Pharmaceutical Group, Zhuhai, China) 5000–10000 IU or Triptorelin (Triptorelin

Pamoate, Ferring, Switzerland) 0.1 mg will be administered to the patient. Subsequently, 36 hours after hCG or Triptorelin injection, the oocytes will be retrieved under transvaginal ultrasound guidance. On the trigger day, the endometrial thickness and morphology, as well as the number and size of follicles (≥15 mm, 10–15 mm and <10 mm) will be documented.

ICSI and embryo culture

A single sperm will be injected within 4 h after the follicular aspiration. Embryos will be cultured in sequential medium with 5% CO_2 in the atmosphere. The fertilisation state of the embryo will be observed 16–18 hours after ICSI. The observation of blastomere formation (cleavage rate) and scoring of the effective cleavage stage embryos will be performed 72 hours after ICSI; however, the day 3 cleaving embryos will continue to be cultured to blastocysts.

Good quality embryo evaluation

Group A: Blastocysts in group A will first be evaluated according to a widely used grading system (Gardner and Schoolcraft) as previously described.[35 39] Subsequently, three to ten trophectoderm cells will be biopsied and immediately transported to the PGD lab for chromosome screening analysis. The day of trophectoderm biopsy will be dependent upon blastocyst development and recorded as day 5 or day 6. The amplified products will be preserved according to the requirements of the genetic laboratory. Blastocysts will be cryopreserved immediately after the biopsy procedure is finished. Embryos will be classified as euploid, aneuploid, mosaic, or not classifiable. Consequently, only one euploid and good morphology embryo will be transferred. If

no euploid embryo is detected, the transfer cycle will be cancelled.

Group B: Blastocysts in group B will be evaluated according to the Gardner grading system as described above and then cryopreserved. One good quality embryo will be transferred in the next frozen-thawed cycle.

The freeze-all strategy used here is to reduce the potential risk of ovarian hyper-stimulation syndrome which could happen on some of these patients. If that was happened, we will record these adverse events and give appropriate and timely treatment.

Embryo transfer and luteal phase support

Endometrial preparation will be hormonally induced. Oral E2 valerate (E2V, Progynova, Bayer Schering Pharma, Shanghai, China) will be given to patients at a dose of 4 mg daily from menstrual day 3. The E2V dose will remain unchanged for 10 days and will then be increased to approximately 6–8 mg/day if the endometrial thickness is still less than 8 mm. When the endometrial thickness is ≥8 mm, 60 mg of progesterone (progesterone injection, Xianju pharma, Zhejiang, China) will be injected intramuscularly per day. Six days after the progesterone injections, the blastocyst will be frozen-thawed and transferred. One good quality embryo will be transferred through a catheter guided by transabdominal ultrasound. The patients will lie in bed for half an hour or be free to walk around after transfer. The dose of E2V and progesterone will be unchanged until the day on which serum β-hCG levels are measured. If the patient is pregnant, luteal phase support will continue until 11 weeks of gestation and 8% progesterone sustained-release vaginal gel (Crinone, Merck Serono, Shanghai, China;

90 mg per day) will be added.

Pregnancy evaluation

Serum β-hCG will be measured to determine pregnancy 14 days after embryo transfer. If a biochemical pregnancy has been detected, a transvaginal ultrasound scan will be performed 28 days after embryo transfer. If a gestational sac is detected and a heartbeat is seen, a clinical pregnancy is confirmed. The ultrasound scan will be repeated every 2 weeks until 11 weeks. Ongoing pregnancy will be confirmed if the foetal heartbeat is confirmed at 12 weeks of gestation.

Follow-up evaluation

At 12 weeks of gestation, first-trimester pregnancy complications (miscarriage, ectopic pregnancy and gestational trophoblastic neoplasia) will be documented in the case report form (CRF) for the first pregnancy follow-up time point. Antenatal care will be referred for these women when the ongoing pregnancy is beyond 12 weeks.

At 28 weeks of gestation, the situation of mothers and foetuses will be documented in the CRF at the second pregnancy follow-up time point. If the patient fails to have a live birth, another frozen-thawed transfer will be arranged and followed up. Perinatal care will be introduced to these mothers when the pregnancy is beyond 28 weeks.

At 42 weeks of gestation, delivery information (gestational age, delivery mode, placenta abnormality, and delivery complications), and the newborn information (baby sex, birth weight, Apgar score, and birth defects) will be documented in the CRF for the third pregnancy follow-up time point. Postpartum care will be introduced to these mothers to help with postpartum recovery.

Six weeks after delivery, the postpartum information and neonatal disease information will be documented in the CRF for the fourth and final pregnancy follow-up time points.

Primary objective

The primary objective of the study is to investigate if the LBR per initiated cycle after PGS is superior compared with the conventional embryo morphology evaluation strategy in the treatment of RPL patients. Live birth will be defined as a live-born baby with a gestational period beyond gestational week 28, and birth weight more than 1000 g. Investigation of the cumulative LBR, which is the LBR per patient, and LBR per blastocyst transfer, is also considered a primary aim of the study.

Secondary objectives

The secondary objectives are as follows:

1. To analyse clinical pregnancy rate per transfer, per initiative and cumulative pregnancy rate in the two groups. Clinical pregnancy will be defined as the presence of an intrauterine gestation sac 4 weeks after embryo transfer.

2. To measure time-to-pregnancy from the date of starting COH to the date of the first ongoing pregnancy in the two groups (the longest follow-up time will be 2 years; hence, failure will be defined as no pregnancy over the 2-year period from the start of COH).

3. To measure the miscarriage rate in the two groups. Miscarriage will be defined as the termination of the pregnancy at <28 weeks of gestation with a miscarried foetal weight less than 1000 g.

Sample size calculation

The three study centres had an average 15% LBR per initiated retrieval cycle and an average 30% LBR per initiated cycle following PGS and frozen-thawed transfer strategy for the last 3 years. For the sample size calculations, we aim to detect an increase of 15% of LBR following PGS strategy with an alpha error level of 0.05 and a beta error level of 0.2. The number will be set to 1:1 in each group, and the minimum sample size will be 242 participants. Considering a dropout rate of 10%, we expect to have a total of 268 participants, with 134 participants in each group.

Outcome measurements (primary and secondary)

Four investigators from the three centres have composed a Data Monitoring Group (DMG), that is responsible for data integrity and accuracy. All the data will be stored in the REDCap, and this interface will automatically ensure accuracy during data entry. We included data obtained from participants completing the self-administered basic characteristics survey questionnaire. We included outcome data from the whole COH cycle and follow-up evaluations. We will use the full analysis set (FAS), an intent-to-treat (ITT) approach, to examine differences in the LBR per initiated cycle in the two treatment arms in the primary analysis using a Pearson $\chi 2$ test. Clinical pregnancy rate and other rates will be analysed using the Pearson $\chi 2$ test and logistic regression. Cox proportional hazards models and the Kaplan-Meier method will be used to compare differences of time to pregnancy and cumulative LBR. Multiple imputation will be conducted for analysis of missing data. The DMG will audit the data quarterly.

Ethics and dissemination

RPL is unexplained in about 50% of young couples, and the effectiveness of

treatments, such as anticoagulation,[40] corticosteroids,[41] and other such treatments, is controversial. In current practice, RPL is considered an issue derived mostly from embryo causes. However, it is questionable whether this embryo-centred approach is correct.

In this trial, we hypothesise that euploid embryos will increase the LBR for young RPL couples. Many observational studies have shown that PGS can increase the LBR per transfer, but may decrease the LBR per initiated cycle in women of advanced age.[13] [25] To the best of our knowledge, this trial is the first RCT to analyse LBR in young RPL couples.

The limitations of this RCT are that the sample size calculation is based on a difference in the LBR per initiated cycle of 15% between the two cohorts; hence, it a may not be able to detect smaller differences in LBR. Larger effect sizes may be achieved in more controlled settings; however, this is a trade-off for studying the complex, heterogeneous RPL population who might receive other individualised and complex treatment. Additionally, the centres included in this RCT are all in Shanghai, although included couples may come from all over the country. Therefore, the generalisability of the results may be limited and the inclusion of sites and patient populations from around the country may have provided a more diverse and larger sample size. We will try to minimise this by using randomisation and by choosing young couples who have travelled from other parts of China for treatment.

No blinding of the treatment allocation to the doctors in the study might cause the doctors to choose a higher stimulation dose in the PGS2.0 group in order to get more

oocytes for selection. However, the dose of the Gonadotropins and euploidy rate is controversial.[38 42] The initiative doses will be 150–300 IU/day according to female age, BMI, number of antral follicles, and basal hormone levels. To choose PGS or not is not considered when choosing the initiative stimulation dose, and the adjustment of dose will be based on the women's ovarian response. We use the randomized trial to reduce confounders.

Counselling of young couples confronted with unexplained RPL regarding its aetiology and prognosis is an essential part of the treatment process, and the advice will allow them to choose their treatment modalities and decide for or against future attempts. This study may prove that PGS is a quick and safe future treatment option.

Amendments to the protocol will be agreed on by the ethics committee, data and safety monitoring committee and will be approved by the ethics committee prior to implementation.

Ethical approval has been granted by the Ethics Committees of Obstetrics and Gynecology Hospital, Fudan University (2017-85), the Shanghai JiAi Genetics & IVF Institute (JIAI E2017-15), the coordinated centres of Renji Hospital, Shanghai Jiao Tong University School of Medicine (2017072101), and The International Peace Maternity & Child Health Hospital of China Welfare Institute, Shanghai Jiao Tong University School of Medicine (GKLW2017-13). Written informed consent will be obtained from each couple before any study procedure is performed. Data from this study are/will be stored in the Research Electronic Data Capture (REDCap). To improve adherence to intervention protocols, the investigators will keep the proper

scientific research attitude, and be able to answer the participants' various questions to increase participants' compliance. The personal information of the enrolled participants will be removed during collecting, sharing, and maintaining in order to protect confidentiality of the participants, and all COH cycles assigned to the participant will be identified by a consistent patient identification. There will be no interim analysis during the study period. The results of this trial will be presented and published via peer-reviewed publications and presentations at international conferences.

Trial status

The study was designed in July 2017, and the first participant was randomised on March 22, 2018. At the time of the manuscript preparation, we have recruited 100 couples and the recruitment is ongoing. Trial registration number: NCT03214185 and stage: Pre-results. We aim to complete the recruitment by March 31, 2021.

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AUTHORS' CONTRIBUTIONS

Contributors LCX, SYL, and SXX from the sponsor hospital have designed the whole study. SXX is responsible for the whole project. LCX, SXX, SY, and JL will be responsible for patient recruitment and randomisation. LCX, LY, XJ, and YJF will form the data management team responsible for collecting and analysing all data. LCX, YJF,

SXX, SY, and JL will supervise the data. The manuscript will be drafted by LCX. All authors will participate in reviewing, curating, and the approval of the final manuscript.

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Competing interests: The authors declare that they have no competing interests.

REFERENCES

1. Shi Y, Wei D, Liang X, et al. Live birth after fresh embryo transfer vs elective embryo cryopreservation/frozen embryo transfer in women with polycystic ovary syndrome undergoing IVF (FreFro-PCOS): study protocol for a multicenter, prospective, randomized controlled clinical trial. *Trials* 2014;15:154. doi: 10.1186/1745-6215-15-154 [published Online First: 2014/06/03]

2. Shi Y, Sun Y, Hao C, et al. Transfer of Fresh versus Frozen Embryos in Ovulatory Women. *The New England journal of medicine* 2018;378(2):126-36. doi: 10.1056/NEJMoa1705334 [published Online First: 2018/01/11]

3. Group EEPGD. RECURRENT PREGNANCY LOSS Guideline of the European Society of Human Reproduction and Embryology,www.eshre.eu/guidelines, Last update: NOVEMBER 2017.2017.

4. Zegers-Hochschild F, Adamson GD, Dyer S, et al. The International Glossary on Infertility

and Fertility Care, 2017. *Human reproduction* 2017;32(9):1786-801. doi: 10.1093/humrep/dex234 [published Online First: 2017/11/09]

5. Practice Committee of American Society for Reproductive M. Definitions of infertility and recurrent pregnancy loss: a committee opinion. *Fertility and sterility* 2013;99(1):63. doi: 10.1016/j.fertnstert.2012.09.023 [published Online First: 2012/10/26]

6. El Hachem H, Crepaux V, May-Panloup P, et al. Recurrent pregnancy loss: current perspectives. *Int J Womens Health* 2017;9:331-45. doi: 10.2147/IJWH.S100817 [published Online First: 2017/05/30]

7. Wang Y, Cheng Q, Meng L, et al. Clinical application of SNP array analysis in first-trimester pregnancy loss: a prospective study. *Clin Genet* 2017;91(6):849-58. doi: 10.1111/cge.12926 [published Online First: 2016/11/25]

8. Robinson WP, McFadden DE, Stephenson MD. The origin of abnormalities in recurrent aneuploidy/polyploidy. *American journal of human genetics* 2001;69(6):1245-54. doi: 10.1086/324468 [published Online First: 2001/10/25]

9. Babariya D, Fragouli E, Alfarawati S, et al. The incidence and origin of segmental aneuploidy in human oocytes and preimplantation embryos. *Human reproduction* 2017;32(12):2549-60. doi: 10.1093/humrep/dex324 [published Online First: 2017/11/11]

10. McCoy RC, Demko ZP, Ryan A, et al. Evidence of Selection against Complex Mitotic-Origin Aneuploidy during Preimplantation Development. *Plos Genet* 2015;11(10):e1005601. doi: 10.1371/journal.pgen.1005601 [published Online First: 2015/10/23]

11. Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. *Nature*

reviews Genetics 2001;2(4):280-91. doi: 10.1038/35066065 [published Online First: 2001/04/03]

12. Nagaoka SI, Hassold TJ, Hunt PA. Human aneuploidy: mechanisms and new insights into an age-old problem. *Nature reviews Genetics* 2012;13(7):493-504. doi: 10.1038/nrg3245 [published Online First: 2012/06/19]

13. Staessen C, Platteau P, Van Assche E, et al. Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. *Human reproduction* 2004;19(12):2849-58. doi: 10.1093/humrep/deh536 [published Online First: 2004/10/09]

14. Mastenbroek S, Twisk M, van Echten-Arends J, et al. In vitro fertilization with preimplantation genetic screening. *The New England journal of medicine* 2007;357(1):9-17. doi: 10.1056/NEJMoa067744 [published Online First: 2007/07/06]

15. Scott RT, Jr., Upham KM, Forman EJ, et al. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. *Fertility and sterility* 2013;100(3):624-30. doi: 10.1016/j.fertnstert.2013.04.039 [published Online First: 2013/06/19]

16. Geraedts J, Sermon K. Preimplantation genetic screening 2.0: the theory. *Molecular human reproduction* 2016;22(8):839-44. doi: 10.1093/molehr/gaw033 [published Online First: 2016/06/04]

17. Meldrum DR, Su HI, Katz-Jaffe MG, et al. Preimplantation genetic screening 2.0: an evolving and promising technique. *Fertility and sterility* 2016;106(1):64-65. doi:

10.1016/j.fertnstert.2016.05.002 [published Online First: 2016/05/29]

18. Keltz MD, Vega M, Sirota I, et al. Preimplantation genetic screening (PGS) with Comparative genomic hybridization (CGH) following day 3 single cell blastomere biopsy markedly improves IVF outcomes while lowering multiple pregnancies and miscarriages. *Journal of assisted reproduction and genetics* 2013;30(10):1333-9. doi: 10.1007/s10815-013-0070-6 [published Online First: 2013/08/21]
19. Dahdouh EM, Balayla J, Garcia-Velasco JA. Comprehensive chromosome screening improves embryo selection: a meta-analysis. *Fertility and sterility* 2015;104(6):1503-12. doi: 10.1016/j.fertnstert.2015.08.038 [published Online First: 2015/09/20]
20. Kushnir VA, Darmon SK, Albertini DF, et al. Effectiveness of in vitro fertilization with preimplantation genetic screening: a reanalysis of United States assisted reproductive technology data 2011-2012. *Fertility and sterility* 2016;106(1):75-79. doi: 10.1016/j.fertnstert.2016.02.026 [published Online First: 2016/03/10]
21. Orvieto R. Preimplantation genetic screening- the required RCT that has not yet been carried out. *Reproductive biology and endocrinology : RB&E* 2016;14(1):35. doi: 10.1186/s12958-016-0171-z [published Online First: 2016/06/28]
22. Capalbo A, Rienzi L, Cimadomo D, et al. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts. *Human reproduction* 2014;29(6):1173-81. doi: 10.1093/humrep/deu033 [published Online First: 2014/03/01]
23. Kim MK, Park JK, Jeon Y, et al. Correlation between Morphologic Grading and Euploidy Rates of Blastocysts, and Clinical Outcomes in In Vitro Fertilization Preimplantation

Genetic Screening. *J Korean Med Sci* 2019;34(4):e27. doi: 10.3346/jkms.2019.34.e27 [published Online First: 2019/01/29]

24. Alfarawati S, Fragouli E, Colls P, et al. The relationship between blastocyst morphology, chromosomal abnormality, and embryo gender. *Fertility and sterility* 2011;95(2):520-4. doi: 10.1016/j.fertnstert.2010.04.003 [published Online First: 2010/06/12]

25. Vinals Gonzalez X, Odia R, Naja R, et al. Euploid blastocysts implant irrespective of their morphology after NGS-(PGT-A) testing in advanced maternal age patients. *Journal of assisted reproduction and genetics* 2019;36(8):1623-29. doi: 10.1007/s10815-019-01496-9 [published Online First: 2019/06/06]

26. European IVFMCftESoHR, Embryology, Calhaz-Jorge C, et al. Assisted reproductive technology in Europe, 2012: results generated from European registers by ESHRE. *Human reproduction* 2016;31(8):1638-52. doi: 10.1093/humrep/dew151 [published Online First: 2016/08/09]

27. European IVFmC, European Society of Human R, Embryology, et al. Assisted reproductive technology in Europe, 2013: results generated from European registers by ESHRE. *Human reproduction* 2017;32(10):1957-73. doi: 10.1093/humrep/dex264 [published Online First: 2017/11/09]

28. De Rycke M, Goossens V, Kokkali G, et al. ESHRE PGD Consortium data collection XIV-XV: cycles from January 2011 to December 2012 with pregnancy follow-up to October 2013. *Human reproduction* 2017;32(10):1974-94. doi: 10.1093/humrep/dex265 [published Online First: 2017/11/09]

29. Simon AL, Kiehl M, Fischer E, et al. Pregnancy outcomes from more than 1,800 in vitro

fertilization cycles with the use of 24-chromosome single-nucleotide polymorphism-based preimplantation genetic testing for aneuploidy. *Fertility and sterility* 2018;110(1):113-21. doi: 10.1016/j.fertnstert.2018.03.026 [published Online First: 2018/06/18]

30. Lee CI, Wu CH, Pai YP, et al. Performance of preimplantation genetic testing for aneuploidy in IVF cycles for patients with advanced maternal age, repeat implantation failure, and idiopathic recurrent miscarriage. *Taiwanese journal of obstetrics & gynecology* 2019;58(2):239-43. doi: 10.1016/j.tjog.2019.01.013 [published Online First: 2019/03/27]
31. Lei C-X, Ye J-F, Sui Y-L, et al. Retrospective cohort study of preimplantation genetic testing for aneuploidy with comprehensive chromosome screening versus nonpreimplantation genetic testing in normal karyotype, secondary infertility patients with recurrent pregnancy loss. *Reproductive and Developmental Medicine* 2019;3(4):205-12. doi: 10.4103/2096-2924.274544 [published Online First: 2019/09]
32. Moher D, Hopewell S, Schulz KF, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *Bmj* 2010;340:c869. doi: 10.1136/bmj.c869 [published Online First: 2010/03/25]
33. Moher D, Hopewell S, Schulz KF, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *Int J Surg* 2012;10(1):28-55. doi: 10.1016/j.ijsu.2011.10.001 [published Online First: 2011/11/01]
34. Practice Committees of American Society for Reproductive M, Society for Assisted Reproductive T. Mature oocyte cryopreservation: a guideline. *Fertility and sterility* 2013;99(1):37-43. doi: 10.1016/j.fertnstert.2012.09.028 [published Online First:

2012/10/23]

35. Li X, Xu Y, Fu J, et al. Non-invasive metabolomic profiling of embryo culture media and morphology grading to predict implantation outcome in frozen-thawed embryo transfer cycles. *Journal of assisted reproduction and genetics* 2015;32(11):1597-605. doi: 10.1007/s10815-015-0578-z [published Online First: 2015/10/16]

36. Gu R, Feng Y, Guo S, et al. Improved cryotolerance and developmental competence of human oocytes matured in vitro by transient hydrostatic pressure treatment prior to vitrification. *Cryobiology* 2017;75:144-50. doi: 10.1016/j.cryobiol.2016.12.009 [published Online First: 2017/01/07]

37. Bar Hava I, Blueshtein M, Ganer Herman H, et al. Gonadotropin-releasing hormone analogue as sole luteal support in antagonist-based assisted reproductive technology cycles. *Fertility and sterility* 2017;107(1):130-35 e1. doi: 10.1016/j.fertnstert.2016.10.011 [published Online First: 2017/02/24]

38. Wu Q, Li H, Zhu Y, et al. Dosage of exogenous gonadotropins is not associated with blastocyst aneuploidy or live-birth rates in PGS cycles in Chinese women. *Human reproduction* 2018;33(10):1875-82. doi: 10.1093/humrep/dey270 [published Online First: 2018/08/24]

39. Fu J, Shao J, Li X, et al. Non-invasive metabolomic profiling of day 3 embryo culture media using near-infrared spectroscopy to assess the development potential of embryos. *Acta Biochim Biophys Sin (Shanghai)* 2013;45(12):1074-8. doi: 10.1093/abbs/gmt115 [published Online First: 2013/10/22]

40. Rottenstreich A, Amsalem H, Kleinstern G, et al. Outcomes of threatened abortions after

anticoagulation treatment to prevent recurrent pregnancy loss. *Reproductive biomedicine online* 2017;35(4):461-67. doi: 10.1016/j.rbmo.2017.06.018 [published Online First: 2017/08/02]

41. Laskin CA, Bombardier C, Hannah ME, et al. Prednisone and aspirin in women with autoantibodies and unexplained recurrent fetal loss. *The New England journal of medicine* 1997;337(3):148-53. doi: 10.1056/NEJM199707173370302 [published Online First: 1997/07/17]
42. McCulloh DH, Alikani M, Norian J, et al. Controlled ovarian hyperstimulation (COH) parameters associated with euploidy rates in donor oocytes. *Eur J Med Genet* 2019;62(8):103707. doi: 10.1016/j.ejmg.2019.103707 [published Online First: 2019/06/30]

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

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	8-19
Protocol version	3	Date and version identifier	3
Funding	4	Sources and types of financial, material, and other support	20
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	20

	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	20
	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)	19-20
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	5-6
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	15-16
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)	8
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	8
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)	8-9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-12
	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)	12
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	15
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	11-12, 15

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	15-16
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)	17
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	16
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9
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	10

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	10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	20
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
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	11
	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	15-16

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	11
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	17-18
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A
	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)	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	17

	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	20
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	14
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	18
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	19
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)	19
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	19-20

	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
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	20
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	21
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	21
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	15-16
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	17
	31b	Authorship eligibility guidelines and any intended use of professional writers	19
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	11

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	19
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	13

*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.