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# BMJ Open

## 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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4 **Full Title:**  
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6 **Comparison of PGS 2.0 versus conventional embryo morphology evaluation for**  
7 **patients with recurrent pregnancy loss: a study protocol for a multicentre**  
8 **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

1  
2  
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4 Ethical approval has been granted by the Ethics Committee of Obstetrics and  
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6 Gynecology Hospital, Fudan University and the participating hospitals. Written  
7  
8 informed consent will be obtained from each couple before any study procedures are  
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10 performed. Data from this study will be stored in the Research Electronic Data  
11  
12 Capture (REDCap). The results of this trial will be presented and published via  
13  
14 peer-reviewed publications and presentations at international conferences.  
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19 **Trial registration number**

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22 NCT03214185; Pre-results.  
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### 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



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4 reasons for frequent aneuploidy cannot be attributed to advanced age alone, and the  
5  
6 mechanisms remain unclear.  
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9 Owing to the high frequency of aneuploidy in RPL patients, pre-implantation  
10 genetic screening (PGS)—now called preimplantation genetic testing-aneuploidy  
11 (PGT-A)—which aims to detect aneuploidy before transfer, is applied to these  
12  
13 patients. In the past two decades, fluorescence in-situ hybridisation (FISH) technology  
14  
15 using limited probes has been applied to detect the five to ten most common  
16  
17 aneuploidies in one or two blastomeres biopsied at day 3 in cleaving embryos.  
18  
19 Although this has been applied to reduce the miscarriage rate and increase the  
20  
21 live-birth rate (LBR) in IVF (PGS 1.0), a few randomised clinical trials have shown a  
22  
23 significant decrease in pregnancy outcomes after PGS 1.0.[10, 11] This disappointing  
24  
25 result might be due to three reasons: first, the cleavage stage biopsy harms the embryo  
26  
27 development potential [12]; second, FISH can detect only a limited number of  
28  
29 aneuploidies; third, mosaicism of the cleaving embryo leads to incorrect assessment  
30  
31 of the embryo. Therefore, a new generation of preimplantation genetic screening  
32  
33 (PGS 2.0) has been introduced to IVF centres; this favors trophectoderm biopsy and  
34  
35 comprehensive chromosome aneuploidy screening.[13, 14] Hence, many reports of  
36  
37 PGS 2.0 have shown increased ongoing pregnancy rates (OPRs) and LBRs.[15-17]  
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39 However, the beneficial effect of PGS 2.0 has not been proven yet in randomised  
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41 controlled trials (RCTs).[18]  
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55 Conventional morphological blastocyst grading systems recommended by  
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57 Gardner and Schoolcraft, which include the degree of blastocoel expansion, inner cell  
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4 mass (ICM), and trophectoderm cells (TE), are used to predict the ploidy status of  
5  
6 blastocysts [6]. More importantly, this grading is completely non-invasive and has no  
7  
8 adverse effects on implantation. Observational studies report a correlation between  
9  
10 good morphology and euploidy embryos,[19, 20] and many researchers propose  
11  
12 embryo morphology as an alternative marker of chromosomal status [21] given the  
13  
14 positive correlation between morphologic grading and the euploid state of the embryo.  
15  
16  
17 However, it has been reported that morphology analysis cannot accurately predict the  
18  
19 genetic status of embryos, because about 50–60% of excellent and good quality  
20  
21 embryos are aneuploid.[22]

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27 In Europe in 2012, the reported mean delivery rates per aspiration for IVF,  
28  
29 intracytoplasmic sperm injection (ICSI), and frozen-thawed transfer (FET) were  
30  
31 21.9%, 20.1%, and 16.0%, respectively.[23] In 2013, the rates were 22.2%, 20.1%,  
32  
33 and 18.0%, respectively.[24] In Europe in 2017, delivery rates after PGS per oocyte  
34  
35 retrieval and per embryo transfer were 13% and 22%, respectively.[25] These data  
36  
37 might be analysed by FISH (PGS1.0). Simon et al. reported LBR per transfer of  
38  
39 64.5% and per retrieval of 45.1% in 1,621 nondonor frozen cycles with PGS in  
40  
41 2018.[26] Lee et al. also reported LBR per initiated cycle of 46.3% in 82 cycles of  
42  
43 RPL couples with PGS in 2019.[27] These data might be analysed by comprehensive  
44  
45 chromosome testing (PGS2.0). We have conducted a retrospective analysis and found  
46  
47 LBR per initiated cycle of 26.6% in RPL couples with PGS, and 15.4% in RPL  
48  
49 couples without PGS (data not yet published).  
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58 For RPL couples who require IVF to help them conceive, we know that PGS  
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4 might increase the LBR per transfer, but whether PGS 2.0 could increase the LBR per  
5  
6 start cycle or the cumulative LBR remains unknown. PGS 2.0 is thought to be a good  
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8 treatment for RPL patients, but whether it should be routinely applied for all couples  
9  
10 with RPL remains controversial. The present protocol describes a multicentre,  
11  
12 prospective, randomised trial assessing PGS 2.0 in the treatment of RPL patients. The  
13  
14 results are very important for clinicians involved in RPL treatment, and for patients  
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16 who experience RPL.  
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## 22 **METHODS AND ANALYSIS**

### 23 **Study design**

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27 This is a multicentre, prospective, randomised controlled clinical trial which is  
28  
29 designed to compare LBR per initiated oocyte retrieval cycle, per patient (cumulative  
30  
31 LBR), and per embryo transfer in 268 RPL couples undergoing ICSI. Participants will  
32  
33 be enrolled at three hospitals in Shanghai, China. This study has been approved by the  
34  
35 ethics committees at the three hospitals. Informed consent will be obtained from the  
36  
37 enrolled couples before any study procedures are performed. Reporting of the study  
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39 results will follow the 2010 revised CONSORT statement [28] and updated  
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41 guidelines, 2012.[29]  
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### 48 **Study population/participants and recruitment**

49  
50 The following inclusion criteria will be applied:

- 51  
52 1. Couples who have experienced two or more PLs.
- 53  
54 2. Normal karyotypes of both husband and wife (polymorphic chromosomes are  
55  
56 considered normal as well).  
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4 3. Female aged between  $\geq 20$  and  $< 38$  years.  
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6 The exclusion criteria will include:  
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8  
9 1. Females with uterine abnormalities such as uterine malformations (uterus  
10 unicorns and duplex uterus), untreated septate uterus, adenomyoma, submucous  
11 uterine fibroids, endometrial polyps, or untreated intrauterine adhesions.  
12  
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16 2. Females with medical conditions that contraindicates ART or pregnancy such as  
17 deep vein thrombosis, pulmonary embolism, cardiac disease, carcinoma, and severe  
18 anaemia.  
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#### 24 *Interventions*

25  
26 All included couples will be informed of the study procedures and written  
27 informed consent will be signed before controlled ovarian stimulation (COH) is  
28 implemented and any procedures are performed. The included couples will be  
29 randomised 1:1 into either of two groups: group A (PGS 2.0 group) and group B (non  
30 PGS group, conventional embryo morphology evaluation group). Group A will  
31 undergo conventional embryo morphology evaluation and trophectoderm biopsy  
32 before blastocyst cryopreservation, and group B will undergo conventional embryo  
33 morphology evaluation before blastocyst cryopreservation. All patients will undergo a  
34 frozen-thawed embryo transfer once a good quality embryo is chosen.  
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#### 50 **Randomisation**

51  
52 At the start of the study, the grouping results will be generated by random digital  
53 software corresponding to a unique grouping number. The couples will be given a  
54 unique grouping number when they have signed the informed consent form;  
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4 subsequently, they will be randomly divided into group A or group B. Both the  
5  
6 investigators and patients will be aware of the grouping information and interventions.  
7  
8

### 9 **Questionnaire**

10  
11 A questionnaire will be developed for collating the basic characteristics of the  
12  
13 couple; this will include the date of birth of the female, ethnicity, education, annual  
14  
15 income level, occupation, and life-style. The participants will address these questions  
16  
17 on the Research Electronic Data Capture (REDCap) platform. REDCap is a  
18  
19 widely-used secure web interface for ensuring data quality; it checks data accuracy  
20  
21 during data entry.  
22  
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25

### 26 **Patient and Public Involvement**

27  
28 Patients or the public were not involved in the design, or conduct, or reporting, or  
29  
30 dissemination plans of our trial.  
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32  
33

### 34 **COH protocol**

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37 1. All patients will undergo three COH cycles unless they become pregnant after the  
38  
39 first or second cycle, or they indicate that they wish to stop treatment. If the patient is  
40  
41 not pregnant after three COH cycles, she will be automatically withdrawn from the  
42  
43 study.  
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48 2. A pelvic ultrasound will be performed before the start of COH, and basal hormone  
49  
50 levels, including serum follicle stimulating hormone (FSH), luteinising hormone  
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52 (LH), prolactin (PRL), oestradiol (E2), progesterone (P4), testosterone (T), and  
53  
54 anti-Mullerian hormone (AMH), will be examined.  
55  
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57  
58 3. A conventional GnRH antagonist COH protocol will be used in all patients either  
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4 by using daily recombinant follicle-stimulating hormone (rFSH) or human  
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6 menopausal gonadotropin (hMG). The gonadotropin stimulation will be performed  
7  
8 according to the routine methods used in the clinics of the three hospitals involved in  
9  
10 the study. However, this protocol can be changed at any time during the treatment  
11  
12 according to the ovarian response. Generally, rFSH or hMG will begin on day 2 or  
13  
14 day 3 of the menstrual period; the latter occurring either naturally or induced by  
15  
16 exogenous administration of progesterone or oral contraceptive pills. The initiative  
17  
18 doses will be 150–300 IU/day according to female age, body mass index (BMI),  
19  
20 number of antral follicles, and basal hormone levels. On the sixth day of receiving the  
21  
22 rFSH or hMG, transvaginal ultrasound will be performed to examine the diameter of  
23  
24 the follicles, and a blood test for serum E2, P, and LH levels will be performed. rFSH  
25  
26 or hMG doses will be adjusted according to ovarian response. Subsequently, such  
27  
28 monitoring will be performed either every other day or every day. The antagonist  
29  
30 regimen are as follows:

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32 Antagonist regimen 1 = rFSH (150–300 IU IM) from day 2 or day 3 followed by  
33  
34 rFSH (150–300 IU IM) + Cetrotide (0.25 mg/day SC) from day 8 or day 9.

35  
36 Antagonist regimen 2 = hMG (150–300 IU IM) from day 2 or day 3 followed by  
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38 hMG (150–300 IU IM) + Cetrotide (0.25 mg/day SC) from day 8 or day 9.

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51 4. When at least one follicle reaches a mean diameter of 14 mm, or the serum E2  
52  
53 reaches 350 pg/ml, the patient will receive 0.25 mg/day of GnRH antagonist  
54  
55 (Cetrotide, Cetrotide, Merck Serono, Shanghai, China) and this will be continued  
56  
57 daily until the trigger day.  
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4 5. Human chorionic gonadotropin (hCG) trigger for final oocyte maturation: when the  
5  
6 mean diameter of at least one follicle is  $\geq 18$  mm or two follicles are  $\geq 16$  mm, an  
7  
8 intramuscular injection of hCG (hCG, HCG, Zhuhai Livzon Pharmaceutical Group,  
9  
10 Zhuhai, China) 5000–10000 IU will be administered to the patient. Subsequently, 36  
11  
12 hours after hCG injection, the oocytes will be retrieved under transvaginal ultrasound  
13  
14 guidance. On the trigger day, the endometrial thickness and morphology, as well as  
15  
16 the number and size of follicles ( $\geq 15$  mm, 10–15 mm and  $< 10$  mm) will be  
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18 documented.  
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### 24 **ICSI and embryo culture**

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27 A single sperm will be injected within 4 h after the follicular aspiration. Embryos  
28  
29 will be cultured in sequential medium with 5% CO<sub>2</sub> in the atmosphere. The  
30  
31 fertilisation state of the embryo will be observed 16–18 hours after ICSI. The  
32  
33 observation of blastomere formation (cleavage rate) and scoring of the effective  
34  
35 cleavage stage embryos will be performed 72 hours after ICSI; however, the day 3  
36  
37 cleaving embryos will continue to be cultured to blastocysts.  
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### 43 **Good quality embryo evaluation**

44  
45 Group A: Blastocysts in group A will first be evaluated according to a  
46  
47 widely-used grading system (Gardner and Schoolcraft) as previously described.  
48  
49 Subsequently, three to ten trophectoderm cells will be biopsied and immediately  
50  
51 transported to the PGD lab for chromosome screening analysis. The day of  
52  
53 trophectoderm biopsy will be dependent upon blastocyst development and recorded as  
54  
55 day 5 or day 6. Blastocysts will be cryopreserved immediately after the biopsy  
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4 procedure is finished. Embryos will be classified as euploid, aneuploid, mosaic, or not  
5  
6 classifiable. Consequently, only one euploid and good morphology embryo will be  
7  
8 transferred. If no euploid embryo is detected, the transfer cycle will be cancelled.  
9  
10

11       Group B: Blastocysts in group B will be evaluated according to the Gardner  
12  
13 grading system and then cryopreserved. One good quality embryo will be transferred  
14  
15 in the next frozen-thawed cycle.  
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17

### 18 19 **Embryo transfer and luteal phase support**

20  
21       Endometrial preparation will be hormonally induced. Oral E2 valerate (E2V,  
22  
23 Progynova, Bayer Schering Pharma, Shanghai, China) will be given to patients at a  
24  
25 dose of 4 mg daily from menstrual day 3. The E2V dose will remain unchanged for 10  
26  
27 days and will then be increased to approximately 6–8 mg/day if the endometrial  
28  
29 thickness is still less than 8 mm. When the endometrial thickness is  $\geq 8$  mm, 60 mg of  
30  
31 progesterone (progesterone injection, Xianju pharma, Zhejiang, China) will be  
32  
33 injected intramuscularly per day. Six days after the progesterone injections, the  
34  
35 blastocyst will be frozen-thawed and transferred. One good quality embryo will be  
36  
37 transferred through a catheter guided by transabdominal ultrasound. The patients will  
38  
39 lie in bed for half an hour after transfer. The dose of E2V and progesterone will be  
40  
41 unchanged until the day on which serum  $\beta$ -hCG levels are measured. If the patient is  
42  
43 pregnant, luteal phase support will continue until 11 weeks of gestation and 8%  
44  
45 progesterone sustained-release vaginal gel (Crinone, Merck Serono, Shanghai, China;  
46  
47 90 mg per day) will be added.  
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### 58 **Pregnancy evaluation**



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4 Serum  $\beta$ -hCG will be measured to determine pregnancy 14 days after embryo  
5  
6 transfer. If a biochemical pregnancy has been detected, a transvaginal ultrasound scan  
7  
8 will be performed 28 days after embryo transfer. If a gestational sac is detected and a  
9  
10 heartbeat is seen, a clinical pregnancy is confirmed. The ultrasound scan will be  
11  
12 repeated every 2 weeks until 11 weeks. Ongoing pregnancy will be confirmed if the  
13  
14 foetal heartbeat is confirmed at 12 weeks of gestation.  
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18

### 19 **Follow-up evaluation**

20  
21  
22 At 12 weeks of gestation, first-trimester pregnancy complications (miscarriage,  
23  
24 ectopic pregnancy and gestational trophoblastic neoplasia) will be documented in the  
25  
26 case report form (CRF) for the first pregnancy follow-up time point.  
27  
28  
29

30 At 28 weeks of gestation, the second-trimester pregnancy complications (prenatal  
31  
32 diagnosis, abortion, gestational diabetes, preeclampsia, eclampsia, premature rupture  
33  
34 of membrane, and placenta abruption) and foetal abnormalities (chromosome  
35  
36 abnormalities, foetal malformation, polyhydramnios, oligohydramnios, foetal growth  
37  
38 restriction, and foetal distress) will be documented in the CRF for the second  
39  
40 pregnancy follow-up time point. If the patient fails to reach 28 weeks of gestation,  
41  
42 another frozen-thawed transfer will be arranged and followed up.  
43  
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48 At 42 weeks of gestation, delivery information (gestational age, delivery mode,  
49  
50 placenta abnormality, and delivery complications), and the new-born information  
51  
52 (baby sex, birth weight, Apgar score, and birth defects) will be documented in the  
53  
54 CRF for the third pregnancy follow-up time point.  
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58 Six weeks after delivery, the postpartum information and neonatal disease  
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4 information will be documented in the CRF for the fourth and final pregnancy  
5  
6 follow-up time points.  
7

### 8 9 **Primary objective**

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

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29 The secondary objectives are as follows:  
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- 31  
32 1. To analyse clinical pregnancy rate per transfer, per initiative and cumulative  
33 pregnancy rate in the two groups. Clinical pregnancy will be defined as the presence  
34 of an intrauterine gestation sac 4 weeks after embryo transfer.  
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36  
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- 39  
40 2. To measure time-to-pregnancy from the date of starting COH to the date of the first  
41 ongoing pregnancy in the two groups (the longest follow-up time will be 2 years;  
42 hence, failure will be defined as no pregnancy over the 2-year period from the start of  
43 COH).  
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- 49  
50 3. To measure the miscarriage rate in the two groups. Miscarriage will be defined as  
51 the termination of the pregnancy at <28 weeks of gestation with a miscarried foetal  
52 weight less than 1000 g.  
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59 Sample size calculation  
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4 The three study centres had an average 15% LBR per initiated retrieval cycle and  
5  
6 an average 30% LBR per initiated cycle following PGS and frozen-thawed transfer  
7  
8 strategy for the last 3 years. For the sample size calculations, we aim to detect an  
9  
10 increase of 15% of LBR following PGS strategy with an alpha error level of 0.05 and  
11  
12 a beta error level of 0.2. The number will be set to 1:1 in each group, and the  
13  
14 minimum sample size will be 242 participants for each group. Considering a dropout  
15  
16 rate of 10%, we expect to have a total of 268 participants, with 134 participants in  
17  
18 each group.  
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23

### 24 **Outcome measurements (primary and secondary)**

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27 Four investigators from the three centres have composed a Data Monitoring  
28  
29 Group (DMG), that is responsible for data integrity and accuracy. All the data will be  
30  
31 stored in the REDCap, and this interface will automatically ensure accuracy during  
32  
33 data entry. We included data obtained from participants completing the  
34  
35 self-administered basic characteristics survey questionnaire. We included outcome  
36  
37 data from the whole COH cycle and follow-up evaluations. We will use the full  
38  
39 analysis set (FAS), an intent-to-treat (ITT) approach, to examine differences in the  
40  
41 LBR per initiated cycle in the two treatment arms in the primary analysis using a  
42  
43 Pearson  $\chi^2$  test. Clinical pregnancy rate and other rates will be analysed using the  
44  
45 Pearson  $\chi^2$  test and logistic regression. Cox proportional hazards models and the  
46  
47 Kaplan-Meier method will be used to compare differences of time to pregnancy and  
48  
49 cumulative LBR. The DMG will audit the data quarterly.  
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### 58 **Ethics and dissemination**

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4 RPL is unexplained in about 50% of young couples, and the effectiveness of  
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6 treatments, such as anticoagulation,[30] corticosteroids,[31] and other such  
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8 treatments, is controversial. In current practice, RPL is considered an issue derived  
9  
10 mostly from embryo causes. However, it is questionable whether this embryo-centred  
11  
12 approach is correct.  
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16  
17 In this trial, we hypothesise that euploid embryos will increase the LBR for  
18  
19 young RPL couples. Many observational studies have shown that PGS can increase  
20  
21 the LBR per transfer, but may decrease the LBR per initiated cycle in women of  
22  
23 advanced age.[10, 22] To the best of our knowledge, this trial is the first RCT to  
24  
25 analyse LBR in young RPL couples.  
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30 The limitations of this RCT are that the sample size calculation is based on a  
31  
32 difference in the LBR per initiated cycle of 15% between the two cohorts; hence, it a  
33  
34 may not be able to detect smaller differences in LBR. Larger effect sizes may be  
35  
36 achieved in more controlled settings; however, this is a trade-off for studying the  
37  
38 complex, heterogeneous RPL population who might receive other individualised and  
39  
40 complex treatment. Additionally, the centres included in this RCT are all in Shanghai,  
41  
42 although included couples may come from all over the country. Therefore, the  
43  
44 generalisability of the results may be limited and the inclusion of sites and patient  
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46 populations from around the country may have provided a more diverse and larger  
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48 sample size. We will try to minimise this by using randomisation and by choosing  
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50 young couples who have travelled from other parts of China for treatment.  
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58 Counselling of young couples confronted with unexplained RPL regarding its  
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4 aetiology and prognosis is an essential part of the treatment process, and the advice  
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6 will allow them to choose their treatment modalities and decide for or against future  
7  
8 attempts. This study may prove that PGS is a quick and safe future treatment option.  
9

10  
11 Ethical approval has been granted by the Ethics Committees of Obstetrics and  
12  
13 Gynecology Hospital, Fudan University (2017-85), the Shanghai JiAi Genetics & IVF  
14  
15 Institute (JIAI E2017-15), the coordinated centres of Renji Hospital, Shanghai Jiao  
16  
17 Tong University School of Medicine (2017072101), and The International Peace  
18  
19 Maternity & Child Health Hospital of China welfare institute, Shanghai Jiao Tong  
20  
21 University School of Medicine (GKLW2017-13)(supplementary files). Written  
22  
23 informed consent will be obtained from each couple before any study procedure is  
24  
25 performed. Data from this study are/will be stored in the Research Electronic Data  
26  
27 Capture (REDCap). To improve adherence to intervention protocols, the investigators  
28  
29 will keep the proper scientific research attitude, and be able to answer the participants'  
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31 various questions to increase participants' compliance. There will be no interim  
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33 analysis during the study period. The results of this trial will be presented and  
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35 published via peer-reviewed publications and presentations at international  
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37 conferences.  
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#### 48 **Trial status**

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51 The first participant was randomised in March 22, 2018. We aim to complete the  
52  
53 recruitment by March 31, 2020.  
54

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57  
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59  
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5  
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7  
8

## 9 **AUTHORS' CONTRIBUTIONS**

10  
11 Contributors LCX, SYL, and SXX from the sponsor hospital have designed the whole  
12  
13 study. SXX is responsible for the whole project. LCX, SXX, SY, and JL will be  
14  
15 responsible for patient recruitment and randomisation. LCX, LY, XJ, and YJF will  
16  
17 form the data management team responsible for collecting and analysing all data.  
18  
19 LCX, YJF, SXX, SY, and JL will supervise the data. The manuscript will be drafted  
20  
21 by LCX. All the authors will participate in reviewing, curating, and approval of the  
22  
23 final manuscript.  
24  
25  
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27  
28

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34  
35 study was sponsored by Obstetrics and Gynecology Hospital of Fudan University.  
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39  
40 Competing interests: The authors declare that they have no competing interests.  
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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description	Page
<b>Administrative information</b>			
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

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	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**

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4	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
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9	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
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15	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-12
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20		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
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25		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	18-19
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30		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
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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
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4	Allocation	16b	Mechanism of implementing the allocation sequence	9
5	concealment		(eg, central telephone; sequentially numbered,	
6	mechanism		opaque, sealed envelopes), describing any steps to	
7			conceal the sequence until interventions are assigned	
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9	Implementation	16c	Who will generate the allocation sequence, who will	18
10			enrol participants, and who will assign participants to	
11			interventions	
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14	Blinding (masking)	17a	Who will be blinded after assignment to interventions	9
15			(eg, trial participants, care providers, outcome	
16			assessors, data analysts), and how	
17				
18		17b	If blinded, circumstances under which unblinding is	N/A
19			permissible, and procedure for revealing a participant's	
20			allocated intervention during the trial	
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23	<b>Methods: Data collection, management, and analysis</b>			
24				
25	Data collection	18a	Plans for assessment and collection of outcome,	10
26	methods		baseline, and other trial data, including any related	
27			processes to promote data quality (eg, duplicate	
28			measurements, training of assessors) and a	
29			description of study instruments (eg, questionnaires,	
30			laboratory tests) along with their reliability and validity,	
31			if known. Reference to where data collection forms can	
32			be found, if not in the protocol	
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35		18b	Plans to promote participant retention and complete	13-14
36			follow-up, including list of any outcome data to be	
37			collected for participants who discontinue or deviate	
38			from intervention protocols	
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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
<b>Methods: Monitoring</b>			
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

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4		21b	Description of any interim analyses and stopping
5			guidelines, including who will have access to these
6			interim results and make the final decision to terminate
7			the trial
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9	Harms	22	Plans for collecting, assessing, reporting, and
10			managing solicited and spontaneously reported
11			adverse events and other unintended effects of trial
12			interventions or trial conduct
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15	Auditing	23	Frequency and procedures for auditing trial conduct, if
16			any, and whether the process will be independent from
17			investigators and the sponsor
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19			
20	<b>Ethics and dissemination</b>		
21			
22	Research ethics	24	Plans for seeking research ethics
23	approval		committee/institutional review board (REC/IRB)
24			approval
25			
26	Protocol	25	Plans for communicating important protocol
27	amendments		modifications (eg, changes to eligibility criteria,
28			outcomes, analyses) to relevant parties (eg,
29			investigators, REC/IRBs, trial participants, trial
30			registries, journals, regulators)
31			
32			
33	Consent or assent	26a	Who will obtain informed consent or assent from
34			potential trial participants or authorised surrogates, and
35			how (see Item 32)
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	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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.

# BMJ Open

## 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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<b>Primary Subject Heading</b>:	Obstetrics and gynaecology
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4 **Full Title:**  
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6 **Comparison of PGS2.0 versus conventional embryo morphology evaluation for**  
7 **patients with recurrent pregnancy loss: a study protocol for a multicentre**  
8 **randomised trial**  
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14 Caixia Lei<sup>1</sup>, Yilun Sui<sup>1</sup>, Jiangfeng Ye<sup>3</sup>, Yao Lu<sup>4</sup>, Ji Xi<sup>5</sup>, Yun Sun<sup>4</sup>, Li Jin<sup>5</sup>, Xiaoxi  
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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



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4 Gynecology Hospital, Fudan University and the participating hospitals. Written  
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6 informed consent will be obtained from each couple before any study procedure is  
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8 performed. Data from this study will be stored in the Research Electronic Data Capture  
9  
10 (REDCap). The results of this trial will be presented and published via peer-reviewed  
11  
12 publications and presentations at international conferences.  
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17 **Trial registration number**

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19 NCT03214185; Pre-results.  
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### 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,<sup>1 2</sup> 24 weeks of gestation in European countries,<sup>3</sup> or 22 weeks gestation according to the international glossary on infertility and fertility care.<sup>4</sup> It also includes non-visualised PLs (biochemical PLs or resolved and treated pregnancies of unknown location), and excludes ectopic and molar pregnancies.<sup>3</sup> Recurrent pregnancy loss (RPL) is defined as two or more PLs.<sup>3 5</sup> Approximately 1–5% of couples trying to conceive experience RPL.<sup>6</sup> 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,<sup>7</sup> which can either be of parental origin or arise *de novo* in the embryo from parents with normal karyotypes,<sup>8</sup> 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).<sup>9</sup> 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.<sup>10</sup> The association between aneuploidy and increasing maternal age has been recognised for a long time,<sup>11</sup> 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.<sup>12</sup> However, when the female patient in couples with a history of RPL is of relatively

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4 young age, the reasons for frequent aneuploidy cannot be attributed to advanced age  
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6 alone, and the mechanisms remain unclear.  
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9 Owing to the high frequency of aneuploidy in RPL patients, pre-implantation  
10 genetic screening (PGS)—now called preimplantation genetic testing-aneuploidy  
11 (PGT-A)—which aims to detect aneuploidy before transfer, is applied to these patients.  
12  
13 In the past two decades, fluorescence in-situ hybridisation (FISH) technology using  
14 limited probes has been applied to detect the five to ten most common aneuploidies in  
15 one or two blastomeres biopsied at day 3 in cleaving embryos. Although this has been  
16 applied to reduce the miscarriage rate and increase the live-birth rate (LBR) in IVF  
17 (PGS1.0), a few randomised clinical trials have shown a significant decrease in  
18 pregnancy outcomes after PGS1.0.<sup>13 14</sup> This disappointing result might be due to three  
19 reasons: first, the cleavage stage biopsy harms the embryo development potential;<sup>15</sup>  
20 second, FISH can detect only a limited number of aneuploidies; third, mosaicism of the  
21 cleaving embryo leads to incorrect assessment of the embryo. Therefore, a new  
22 generation of preimplantation genetic screening (PGS2.0) has been introduced to IVF  
23 centres; this favours trophectoderm biopsy and comprehensive chromosome  
24 aneuploidy screening,<sup>16 17</sup> Hence, many reports of PGS2.0 have shown increased  
25 ongoing pregnancy rates (OPRs) and LBRs.<sup>18-20</sup> However, the beneficial effect of  
26 PGS2.0 has not been proven yet in randomised controlled trials (RCTs).<sup>21</sup>  
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53 Conventional morphological blastocyst grading systems recommended by Gardner  
54 and Schoolcraft, which include the degree of blastocoel expansion, inner cell mass  
55 (ICM), and trophectoderm cells (TE), are used to predict the ploidy status of blastocysts,  
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4 <sup>9</sup> More importantly, this grading is completely non-invasive and has no adverse effects  
5  
6 on implantation. Observational studies report a correlation between good morphology  
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8 and euploidy embryos,<sup>22 23</sup> and many researchers propose embryo morphology as an  
9  
10 alternative marker of chromosomal status,<sup>24</sup> given the positive correlation between  
11  
12 morphologic grading and the euploid state of the embryo. However, it has been reported  
13  
14 that morphology analysis cannot accurately predict the genetic status of embryos,  
15  
16 because about 50–60% of excellent and good quality embryos are aneuploid.<sup>25</sup>  
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22 In Europe in 2012, the reported mean delivery rates per aspiration for IVF,  
23  
24 intracytoplasmic sperm injection (ICSI), and frozen-thawed transfer (FET) were 21.9%,  
25  
26 20.1%, and 16.0%, respectively.<sup>26</sup> In 2013, the rates were 22.2%, 20.1%, and 18.0%,  
27  
28 respectively.<sup>27</sup> In Europe in 2017, delivery rates after PGS per oocyte retrieval and per  
29  
30 embryo transfer were 13% and 22%, respectively.<sup>28</sup> These data might be analysed by  
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32 FISH (PGS1.0). Simon et al. reported LBR per transfer of 64.5% and per retrieval of  
33  
34 45.1% in 1,621 nondonor frozen cycles with PGS in 2018.<sup>29</sup> Lee et al. also reported  
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36 LBR per initiated cycle of 46.3% in 82 cycles of RPL couples with PGS in 2019.<sup>30</sup>  
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38 These data might be analysed by comprehensive chromosome testing (PGS2.0). We  
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40 have conducted a retrospective analysis and found LBR per initiated cycle of 26.6% in  
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42 RPL couples with PGS, and 15.4% in RPL couples without PGS.<sup>31</sup>  
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51 For RPL couples who require IVF to help them conceive, we know that PGS might  
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53 increase the LBR per transfer, but whether PGS2.0 could increase the LBR per start  
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55 cycle or the cumulative LBR remains unknown. PGS2.0 is thought to be a good  
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57 treatment for RPL patients, but whether it should be routinely applied for all couples  
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4 with RPL remains controversial. The present protocol describes a multicentre,  
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6 randomised trial assessing PGS2.0 in the treatment of RPL patients. The results are very  
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8 important for clinicians involved in RPL treatment, and for patients who experience  
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10 RPL.  
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## 13 14 **METHODS AND ANALYSIS**

### 15 16 17 **Study design**

18  
19 This is a multicentre, randomised controlled clinical trial which is designed to  
20  
21 compare LBR per initiated oocyte retrieval cycle, per patient (cumulative LBR), and  
22  
23 per embryo transfer in 268 RPL couples undergoing ICSI. Participants will be enrolled  
24  
25 at three hospitals in Shanghai, China. This study has been approved by the ethics  
26  
27 committees at the three hospitals. Informed consent will be obtained from the enrolled  
28  
29 couples before any study procedures are performed. Reporting of the study results will  
30  
31 follow the 2010 revised CONSORT statement<sup>32</sup> and updated guidelines, 2012.<sup>33</sup>  
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### 37 38 **Study population/participants and recruitment**

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40 The following inclusion criteria will be applied:

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

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52 The exclusion criteria will include:

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

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6 2. Females with medical conditions that contraindicate ART or pregnancy such as  
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8 deep vein thrombosis, pulmonary embolism, cardiac disease, carcinoma, and severe  
9  
10 anaemia.  
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13  
14 In order to achieve adequate participant enrolment to reach the target sample size, we  
15  
16 will use the following strategies:  
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- 18  
19 1. at the waiting rooms of the three IVF centers, posters will be put to let more people  
20  
21 know this study.  
22  
23 2. the doctors at the three IVF centers will be encouraged to introduce the study to  
24  
25 their patients to let more people know this study.  
26  
27 3. a study contact will be designated for any person who want to know details of this  
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29 study.  
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### 34 35 *Interventions*

36  
37 Randomisation will take place during the couple's first visit to the clinic or on the  
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39 first day of stimulation. All included couples will be informed of the study procedures  
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41 and written informed consent will be signed before controlled ovarian stimulation  
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43 (COH) is implemented and any procedures are performed. The included couples will  
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45 be randomised 1:1 into either of two groups: group A (PGS2.0 group) and group B  
46  
47 (non-PGS group, conventional embryo morphology evaluation group). Group A will  
48  
49 undergo conventional embryo morphology evaluation and trophectoderm biopsy before  
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51 blastocyst cryopreservation, and group B will undergo conventional embryo  
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53 morphology evaluation before blastocyst cryopreservation. All patients will undergo a  
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4 frozen-thawed embryo transfer once a good quality embryo or an euploid embryo after  
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6 PGS2.0 is chosen. Evaluation of blastocyst stage embryos are based on three aspects:  
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8 the expansion of the blastocoele cavity (EH stage), the number and cohesiveness of the  
9  
10 inner cell mass (ICM grade) and trophoctodermal cells (TE grade) according to the  
11  
12 Gardner and Schoolcraft grading system<sup>34-36</sup>. The EH stage is assessed as one of the  
13  
14 following: (1) an early blastocyst with the volume of the blastocoele is less than half of  
15  
16 that of an embryo; (2) a blastocyst with the volume of the blastocoele is at least half  
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18 that of the embryo; (3) a full blastocyst with a completely filling blastocoele of the  
19  
20 embryo; (4) an expanded, thinning zona blastocyst with the volume of the blastocoele  
21  
22 larger than that of the full blastocyst; (5) a hatching blastocyst with the TE starting to  
23  
24 herniate through the zona; and (6) a hatched blastocyst completely escaped from the  
25  
26 zona. ICM and TE grade are evaluated after EH stage is assessed. The ICM is assessed  
27  
28 as one of the following: (A) tightly packed, many cells; (B) loosely grouped, several  
29  
30 cells; and (C) very few cells. The TE is assessed as one of the following: (A) many cells  
31  
32 forming a cohesive epithelium; (B) few cells forming a loose epithelium; and (C) very  
33  
34 few, large cells.  
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### 45 **Randomisation**

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48 At the start of the study, the grouping results will be generated by random digital  
49  
50 software corresponding to a unique grouping number. The couples will be given a  
51  
52 unique grouping number when they have signed the informed consent form;  
53  
54 subsequently, they will be randomly divided into group A or group B. Both the  
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56 investigators and patients will be aware of the grouping information and interventions.  
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4 There will be no blinding of the treatment allocation to the doctors and participants in  
5  
6 the study. The embryologist performing the embryo quality evaluation will be blinded  
7  
8 to the allocated treatment.  
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## 10 11 12 **Questionnaire**

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14 A questionnaire will be developed for collating the basic characteristics of the  
15  
16 couple; this will include the date of birth of the couple, ethnicity, education, annual  
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18 income level, occupation, and lifestyle. The participants will address these questions on  
19  
20 the Research Electronic Data Capture (REDCap) platform. REDCap is a widely used  
21  
22 secure web interface for ensuring data quality; it checks data accuracy during data entry.  
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## 26 27 **Patient and Public Involvement**

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29  
30 Patients or the public were not involved in the design, or conduct, or reporting, or  
31  
32 dissemination plans of our trial.  
33

## 34 35 **COH protocol**

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38 1. All patients will undergo up to three COH cycles unless they indicate that they wish  
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40 to stop treatment. If the patient is not pregnant after three COH cycles and has no  
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42 surplus embryos for transfer, she will be automatically withdrawn from the study.  
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45 2. A 2D ultrasound pelvic ultrasound will be performed before the start of COH, and  
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47 basal hormone levels, including serum follicle stimulating hormone (FSH), luteinising  
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49 hormone (LH), prolactin (PRL), oestradiol (E2), progesterone (P4), testosterone (T),  
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51 and anti-Mullerian hormone (AMH), will be examined.  
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55 3. Conventional GnRH antagonist COH protocols will be used in all patients either by  
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57 using daily recombinant follicle-stimulating hormone (rFSH) or human menopausal  
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gonadotropin (hMG).<sup>37</sup> 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.<sup>38</sup> 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 E<sub>2</sub>, 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 E<sub>2</sub> 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  $\geq 18$  mm or two follicles are  $\geq 16$  mm, an intramuscular injection of hCG (hCG, HCG, Zhuhai Livzon Pharmaceutical Group, Zhuhai, China) 5000–10000 IU or Triptorelin (Triptorelin

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4 Pamoate, Ferring, Switzerland) 0.1 mg will be administered to the patient.  
5  
6 Subsequently, 36 hours after hCG or Triptorelin injection, the oocytes will be retrieved  
7  
8 under transvaginal ultrasound guidance. On the trigger day, the endometrial thickness  
9  
10 and morphology, as well as the number and size of follicles ( $\geq 15$  mm, 10–15 mm and  
11  
12 <10 mm) will be documented.  
13  
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15

### 16 **ICSI and embryo culture**

17  
18  
19 A single sperm will be injected within 4 h after the follicular aspiration. Embryos  
20  
21 will be cultured in sequential medium with 5% CO<sub>2</sub> in the atmosphere. The fertilisation  
22  
23 state of the embryo will be observed 16–18 hours after ICSI. The observation of  
24  
25 blastomere formation (cleavage rate) and scoring of the effective cleavage stage  
26  
27 embryos will be performed 72 hours after ICSI; however, the day 3 cleaving embryos  
28  
29 will continue to be cultured to blastocysts.  
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### 35 **Good quality embryo evaluation**

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38 Group A: Blastocysts in group A will first be evaluated according to a widely used  
39  
40 grading system (Gardner and Schoolcraft) as previously described.<sup>35 39</sup> Subsequently,  
41  
42 three to ten trophectoderm cells will be biopsied and immediately transported to the  
43  
44 PGD lab for chromosome screening analysis. The day of trophectoderm biopsy will be  
45  
46 dependent upon blastocyst development and recorded as day 5 or day 6. The amplified  
47  
48 products will be preserved according to the requirements of the genetic laboratory.  
49  
50  
51 Blastocysts will be cryopreserved immediately after the biopsy procedure is finished.  
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53  
54 Embryos will be classified as euploid, aneuploid, mosaic, or not classifiable.  
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57 Consequently, only one euploid and good morphology embryo will be transferred. If  
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4 no euploid embryo is detected, the transfer cycle will be cancelled.  
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6       Group B: Blastocysts in group B will be evaluated according to the Gardner  
7 grading system as described above and then cryopreserved. One good quality embryo  
8 will be transferred in the next frozen-thawed cycle.  
9  
10

11       The freeze-all strategy used here is to reduce the potential risk of ovarian hyper-  
12 stimulation syndrome which could happen on some of these patients. If that was  
13 happened, we will record these adverse events and give appropriate and timely  
14 treatment.  
15  
16

#### 17 **Embryo transfer and luteal phase support**

18       Endometrial preparation will be hormonally induced. Oral E2 valerate (E2V,  
19 Progynova, Bayer Schering Pharma, Shanghai, China) will be given to patients at a  
20 dose of 4 mg daily from menstrual day 3. The E2V dose will remain unchanged for 10  
21 days and will then be increased to approximately 6–8 mg/day if the endometrial  
22 thickness is still less than 8 mm. When the endometrial thickness is  $\geq 8$  mm, 60 mg of  
23 progesterone (progesterone injection, Xianju pharma, Zhejiang, China) will be injected  
24 intramuscularly per day. Six days after the progesterone injections, the blastocyst will  
25 be frozen-thawed and transferred. One good quality embryo will be transferred through  
26 a catheter guided by transabdominal ultrasound. The patients will lie in bed for half an  
27 hour or be free to walk around after transfer. The dose of E2V and progesterone will be  
28 unchanged until the day on which serum  $\beta$ -hCG levels are measured. If the patient is  
29 pregnant, luteal phase support will continue until 11 weeks of gestation and 8%  
30 progesterone sustained-release vaginal gel (Crinone, Merck Serono, Shanghai, China;  
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4 90 mg per day) will be added.  
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### 6 **Pregnancy evaluation** 7

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9 Serum  $\beta$ -hCG will be measured to determine pregnancy 14 days after embryo  
10 transfer. If a biochemical pregnancy has been detected, a transvaginal ultrasound scan  
11 will be performed 28 days after embryo transfer. If a gestational sac is detected and a  
12 heartbeat is seen, a clinical pregnancy is confirmed. The ultrasound scan will be  
13 repeated every 2 weeks until 11 weeks. Ongoing pregnancy will be confirmed if the  
14 foetal heartbeat is confirmed at 12 weeks of gestation.  
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### 24 **Follow-up evaluation** 25

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27 At 12 weeks of gestation, first-trimester pregnancy complications (miscarriage,  
28 ectopic pregnancy and gestational trophoblastic neoplasia) will be documented in the  
29 case report form (CRF) for the first pregnancy follow-up time point. Antenatal care will  
30 be referred for these women when the ongoing pregnancy is beyond 12 weeks.  
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38 At 28 weeks of gestation, the situation of mothers and foetuses will be documented  
39 in the CRF at the second pregnancy follow-up time point. If the patient fails to have a  
40 live birth, another frozen-thawed transfer will be arranged and followed up. Perinatal  
41 care will be introduced to these mothers when the pregnancy is beyond 28 weeks.  
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49 At 42 weeks of gestation, delivery information (gestational age, delivery mode,  
50 placenta abnormality, and delivery complications), and the newborn information (baby  
51 sex, birth weight, Apgar score, and birth defects) will be documented in the CRF for  
52 the third pregnancy follow-up time point. Postpartum care will be introduced to these  
53 mothers to help with postpartum recovery.  
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4 Six weeks after delivery, the postpartum information and neonatal disease  
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6 information will be documented in the CRF for the fourth and final pregnancy follow-  
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8 up time points.  
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### 10 11 **Primary objective**

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14 The primary objective of the study is to investigate if the LBR per initiated cycle  
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16 after PGS is superior compared with the conventional embryo morphology evaluation  
17  
18 strategy in the treatment of RPL patients. Live birth will be defined as a live-born baby  
19  
20 with a gestational period beyond gestational week 28, and birth weight more than 1000  
21  
22 g. Investigation of the cumulative LBR, which is the LBR per patient, and LBR per  
23  
24 blastocyst transfer, is also considered a primary aim of the study.  
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### 30 31 **Secondary objectives**

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33 The secondary objectives are as follows:

- 34  
35 1. To analyse clinical pregnancy rate per transfer, per initiative and cumulative  
36  
37 pregnancy rate in the two groups. Clinical pregnancy will be defined as the presence of  
38  
39 an intrauterine gestation sac 4 weeks after embryo transfer.  
40  
41
- 42  
43 2. To measure time-to-pregnancy from the date of starting COH to the date of the first  
44  
45 ongoing pregnancy in the two groups (the longest follow-up time will be 2 years; hence,  
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47 failure will be defined as no pregnancy over the 2-year period from the start of COH).  
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- 50  
51 3. To measure the miscarriage rate in the two groups. Miscarriage will be defined as  
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53 the termination of the pregnancy at <28 weeks of gestation with a miscarried foetal  
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55 weight less than 1000 g.  
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### 58 59 **Sample size calculation**

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4 The three study centres had an average 15% LBR per initiated retrieval cycle and  
5  
6 an average 30% LBR per initiated cycle following PGS and frozen-thawed transfer  
7  
8 strategy for the last 3 years. For the sample size calculations, we aim to detect an  
9  
10 increase of 15% of LBR following PGS strategy with an alpha error level of 0.05 and  
11  
12 a beta error level of 0.2. The number will be set to 1:1 in each group, and the minimum  
13  
14 sample size will be 242 participants. Considering a dropout rate of 10%, we expect to  
15  
16 have a total of 268 participants, with 134 participants in each group.  
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### 22 **Outcome measurements (primary and secondary)**

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24 Four investigators from the three centres have composed a Data Monitoring Group  
25  
26 (DMG), that is responsible for data integrity and accuracy. All the data will be stored  
27  
28 in the REDCap, and this interface will automatically ensure accuracy during data entry.  
29  
30 We included data obtained from participants completing the self-administered basic  
31  
32 characteristics survey questionnaire. We included outcome data from the whole COH  
33  
34 cycle and follow-up evaluations. We will use the full analysis set (FAS), an intent-to-  
35  
36 treat (ITT) approach, to examine differences in the LBR per initiated cycle in the two  
37  
38 treatment arms in the primary analysis using a Pearson  $\chi^2$  test. Clinical pregnancy rate  
39  
40 and other rates will be analysed using the Pearson  $\chi^2$  test and logistic regression. Cox  
41  
42 proportional hazards models and the Kaplan-Meier method will be used to compare  
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44 differences of time to pregnancy and cumulative LBR. Multiple imputation will be  
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46 conducted for analysis of missing data. The DMG will audit the data quarterly.  
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### 56 **Ethics and dissemination**

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58 RPL is unexplained in about 50% of young couples, and the effectiveness of  
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4 treatments, such as anticoagulation,<sup>40</sup> corticosteroids,<sup>41</sup> and other such treatments, is  
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6 controversial. In current practice, RPL is considered an issue derived mostly from  
7  
8 embryo causes. However, it is questionable whether this embryo-centred approach is  
9  
10 correct.  
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13  
14 In this trial, we hypothesise that euploid embryos will increase the LBR for young  
15  
16 RPL couples. Many observational studies have shown that PGS can increase the LBR  
17  
18 per transfer, but may decrease the LBR per initiated cycle in women of advanced age.<sup>13</sup>  
19  
20 <sup>25</sup> To the best of our knowledge, this trial is the first RCT to analyse LBR in young RPL  
21  
22 couples.  
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26  
27 The limitations of this RCT are that the sample size calculation is based on a  
28  
29 difference in the LBR per initiated cycle of 15% between the two cohorts; hence, it a  
30  
31 may not be able to detect smaller differences in LBR. Larger effect sizes may be  
32  
33 achieved in more controlled settings; however, this is a trade-off for studying the  
34  
35 complex, heterogeneous RPL population who might receive other individualised and  
36  
37 complex treatment. Additionally, the centres included in this RCT are all in Shanghai,  
38  
39 although included couples may come from all over the country. Therefore, the  
40  
41 generalisability of the results may be limited and the inclusion of sites and patient  
42  
43 populations from around the country may have provided a more diverse and larger  
44  
45 sample size. We will try to minimise this by using randomisation and by choosing  
46  
47 young couples who have travelled from other parts of China for treatment.  
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55 No blinding of the treatment allocation to the doctors in the study might cause the  
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57 doctors to choose a higher stimulation dose in the PGS2.0 group in order to get more  
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4 oocytes for selection. However, the dose of the Gonadotropins and euploidy rate is  
5  
6 controversial.<sup>38 42</sup> The initiative doses will be 150–300 IU/day according to female age,  
7  
8 BMI, number of antral follicles, and basal hormone levels. To choose PGS or not is not  
9  
10 considered when choosing the initiative stimulation dose, and the adjustment of dose  
11  
12 will be based on the women's ovarian response. We use the randomized trial to reduce  
13  
14 confounders.  
15  
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19  
20 Counselling of young couples confronted with unexplained RPL regarding its  
21  
22 aetiology and prognosis is an essential part of the treatment process, and the advice will  
23  
24 allow them to choose their treatment modalities and decide for or against future  
25  
26 attempts. This study may prove that PGS is a quick and safe future treatment option.  
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30 Amendments to the protocol will be agreed on by the ethics committee, data and  
31  
32 safety monitoring committee and will be approved by the ethics committee prior to  
33  
34 implementation.  
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36

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38 Ethical approval has been granted by the Ethics Committees of Obstetrics and  
39  
40 Gynecology Hospital, Fudan University (2017-85), the Shanghai JiAi Genetics & IVF  
41  
42 Institute (JIAI E2017-15), the coordinated centres of Renji Hospital, Shanghai Jiao  
43  
44 Tong University School of Medicine (2017072101), and The International Peace  
45  
46 Maternity & Child Health Hospital of China Welfare Institute, Shanghai Jiao Tong  
47  
48 University School of Medicine (GKLW2017-13). Written informed consent will be  
49  
50 obtained from each couple before any study procedure is performed. Data from this  
51  
52 study are/will be stored in the Research Electronic Data Capture (REDCap). To  
53  
54 improve adherence to intervention protocols, the investigators will keep the proper  
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4 scientific research attitude, and be able to answer the participants' various questions to  
5  
6 increase participants' compliance. The personal information of the enrolled participants  
7  
8 will be removed during collecting, sharing, and maintaining in order to protect  
9  
10 confidentiality of the participants, and all COH cycles assigned to the participant will  
11  
12 be identified by a consistent patient identification. There will be no interim analysis  
13  
14 during the study period. The results of this trial will be presented and published via  
15  
16 peer-reviewed publications and presentations at international conferences.  
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### 22 **Trial status**

23  
24 The study was designed in July 2017, and the first participant was randomised on  
25  
26 March 22, 2018. At the time of the manuscript preparation, we have recruited 100  
27  
28 couples and the recruitment is ongoing. Trial registration number: NCT03214185 and  
29  
30 stage: Pre-results. We aim to complete the recruitment by March 31, 2021.  
31  
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45  
46  
47

### 48 **AUTHORS' CONTRIBUTIONS**

49  
50 Contributors LCX, SYL, and SXX from the sponsor hospital have designed the whole  
51  
52 study. SXX is responsible for the whole project. LCX, SXX, SY, and JL will be  
53  
54 responsible for patient recruitment and randomisation. LCX, LY, XJ, and YJF will form  
55  
56 the data management team responsible for collecting and analysing all data. LCX, YJF,  
57  
58  
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3  
4 SXX, SY, and JL will supervise the data. The manuscript will be drafted by LCX. All  
5  
6 authors will participate in reviewing, curating, and the approval of the final manuscript.  
7  
8

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18  
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20  
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22

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

Section/item	Item No	Description	Page
<b>Administrative information</b>			
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

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	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
<b>Introduction</b>			
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**

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4	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
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9	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)
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15	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
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20		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)
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25		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
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30		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
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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
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4	Allocation	16b	Mechanism of implementing the allocation sequence	10
5	concealment		(eg, central telephone; sequentially numbered,	
6	mechanism		opaque, sealed envelopes), describing any steps to	
7			conceal the sequence until interventions are assigned	
8				
9	Implementation	16c	Who will generate the allocation sequence, who will	20
10			enrol participants, and who will assign participants to	
11			interventions	
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14	Blinding (masking)	17a	Who will be blinded after assignment to interventions	11
15			(eg, trial participants, care providers, outcome	
16			assessors, data analysts), and how	
17				
18		17b	If blinded, circumstances under which unblinding is	N/A
19			permissible, and procedure for revealing a participant's	
20			allocated intervention during the trial	
21				
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23	<b>Methods: Data collection, management, and analysis</b>			
24				
25	Data collection	18a	Plans for assessment and collection of outcome,	11
26	methods		baseline, and other trial data, including any related	
27			processes to promote data quality (eg, duplicate	
28			measurements, training of assessors) and a	
29			description of study instruments (eg, questionnaires,	
30			laboratory tests) along with their reliability and validity,	
31			if known. Reference to where data collection forms can	
32			be found, if not in the protocol	
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35		18b	Plans to promote participant retention and complete	15-16
36			follow-up, including list of any outcome data to be	
37			collected for participants who discontinue or deviate	
38			from intervention protocols	
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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
<b>Methods: Monitoring</b>			
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



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4		21b	20
5		Description of any interim analyses and stopping	
6		guidelines, including who will have access to these	
7		interim results and make the final decision to terminate	
8		the trial	
9	Harms	22	14
10		Plans for collecting, assessing, reporting, and	
11		managing solicited and spontaneously reported	
12		adverse events and other unintended effects of trial	
13		interventions or trial conduct	
14			
15	Auditing	23	18
16		Frequency and procedures for auditing trial conduct, if	
17		any, and whether the process will be independent from	
18		investigators and the sponsor	
19			
20	<b>Ethics and dissemination</b>		
21			
22	Research ethics	24	19
23	approval	Plans for seeking research ethics	
24		committee/institutional review board (REC/IRB)	
25		approval	
26	Protocol	25	19
27	amendments	Plans for communicating important protocol	
28		modifications (eg, changes to eligibility criteria,	
29		outcomes, analyses) to relevant parties (eg,	
30		investigators, REC/IRBs, trial participants, trial	
31		registries, journals, regulators)	
32			
33	Consent or assent	26a	19-20
34		Who will obtain informed consent or assent from	
35		potential trial participants or authorised surrogates, and	
36		how (see Item 32)	
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4		26b	Additional consent provisions for collection and use of	N/A
5			participant data and biological specimens in ancillary	
6			studies, if applicable	
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8	Confidentiality	27	How personal information about potential and enrolled	20
9			participants will be collected, shared, and maintained in	
10			order to protect confidentiality before, during, and after	
11			the trial	
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14	Declaration of	28	Financial and other competing interests for principal	21
15	interests		investigators for the overall trial and each study site	
16				
17	Access to data	29	Statement of who will have access to the final trial	21
18			dataset, and disclosure of contractual agreements that	
19			limit such access for investigators	
20				
21	Ancillary and post-	30	Provisions, if any, for ancillary and post-trial care, and	15-16
22	trial care		for compensation to those who suffer harm from trial	
23			participation	
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26	Dissemination	31a	Plans for investigators and sponsor to communicate	17
27	policy		trial results to participants, healthcare professionals,	
28			the public, and other relevant groups (eg, via	
29			publication, reporting in results databases, or other	
30			data sharing arrangements), including any publication	
31			restrictions	
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34		31b	Authorship eligibility guidelines and any intended use	19
35			of professional writers	
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37		31c	Plans, if any, for granting public access to the full	11
38			protocol, participant-level dataset, and statistical code	
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**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

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\*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.