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## Oil-based contrast versus water-based contrast media in the diagnosis of tubal patency at hysterosalpingography: study protocol for a randomized controlled trial

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# Oil-based contrast versus water-based contrast media in the diagnosis of tubal patency at hysterosalpingography: study protocol for a randomized controlled trial

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## Abstract

**Introduction** In recent years, due to the liberalization of the second child policy in China, the increasing age of reproductive women, environmental pollution, and working pressure, the rate of infertility in China has increased and now accounts for over 10% in reproductive women and the majority of infertility is caused by tubal

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4 pathology. HSG is a commonly practiced diagnostic procedure in the fertility work-up.  
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6 However, there is no uniform conclusion on the choice of contrast agents and their  
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8 effects. This study will answer the question whether at HSG the use of oil-based  
9  
10 contrast media results in higher ongoing pregnancy compared to the use of water-based  
11  
12 contrast media during 6 months after randomization.

13 **Methods and analysis** This study is a single-centre, randomized, controlled, parallel  
14  
15 group, superiority trial that will be carried out at the International Peace Maternity and  
16  
17 Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai,  
18  
19 China. A total of 1040 eligible patients will be randomized (1:1) to undergo HSG using  
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21 Ioversol Injection or Iodinated Oil Injection. The primary outcome measure is the rate  
22  
23 of ongoing pregnancy. The secondary outcomes will consist of live birth rate, clinical  
24  
25 pregnancy rate, miscarriages, ectopic pregnancy, pain scores, pregnancy leading to live  
26  
27 birth, thyroid function of patients, neonatal thyroid function, cost calculations of OSCM  
28  
29 / WSCM and assisted reproductive technology (ART) treatments between two groups.

30  
31 **Ethics and dissemination** This protocol received authorisation from the Medical  
32  
33 Research Ethics Committee International Peace Maternity and Child Health Hospital  
34  
35 on 18th January 2020 (approval no. GKLW2020-02). The findings will be reported in  
36  
37 peer-reviewed publications and presentations at international scientific meetings.

38  
39 **Trial registration number** ChiCTR2000031612.

## 40 41 42 43 **Strengths and limitations of this study**

- 44  
45 ● The trial will answer the question whether at HSG the use of oil-based contrast  
46  
47 media results in higher ongoing pregnancy compared to the use of water-based  
48  
49 contrast media during 6 months after randomization in Chinese women.
- 50  
51 ● This is the first time to assess the thyroid function of newborns and patients at  
52  
53 different time points who underwent HSG.
- 54  
55 ● The trial is based in a single centre, which might limit the generalisability of the  
56  
57 findings.
- 58  
59 ● Owing to the difference in imaging between the use of oil-based contrast and water-  
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3 based contrast, and, since our outcome of ongoing pregnancy was objective, the  
4 trial was not blinded with respect to participants and caregivers.  
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## 10 **Introduction**

11  
12 Infertility is defined as the failure to establish a pregnancy after 12 months of regular,  
13 unprotected sexual intercourse or an impairment of a person's capacity to reproduce  
14 either as an individual or with his/her partner [1]. Due to the liberalization of the second  
15 child policy in China, the increasing age of reproductive women, environmental  
16 pollution, and working pressure, the rate of infertility in China has increased and now  
17 accounts for over 10% in reproductive women. In China, the majority of infertility is  
18 caused by tubal pathology [2].  
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25  
26 Hysterosalpingography (HSG) refers to the radiographic evaluation of the uterine  
27 cavity and fallopian tubes after the injection of an oil-/water-soluble contrast medium  
28 through the cervical canal [3]. HSG plays an important role in the evaluation of female  
29 fallopian tubal reproductive function. HSG is a commonly practiced diagnostic  
30 procedure in the fertility work-up [4,5]. In China, it is the preferred method to check  
31 tubal patency. Compared with laparoscopy, it is less expensive and has fewer  
32 complications [6]. In addition to diagnostic information, HSG has therapeutic effects,  
33 which are associated with increased fecundability in the months after the procedure  
34 [7,8].  
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44 Ultra-fluid lipiodol, one kind of oil-soluble contrast media, uses poppy seed oil as raw  
45 materials. Due to less absorption of contrast, the occurrence of adverse events reduced  
46 notably [9,10]. Water-soluble contrast media is represented by meglumine diatrizoate  
47 and ioversol. The advantages of them are as follows: low viscosity, good fluidity and  
48 rapid diffusion into the fallopian tubes, which can clearly show the anatomy of the  
49 fallopian tubes. But rapid flow may result in insufficient observation of fallopian tube  
50 lesions and poor fallopian tube dredging [9].  
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56 Some studies showed significantly higher pregnancy rates after tubal flushing with oil  
57 contrast [11,12]. This might be caused by flushing debris and dislodge mucus plugs  
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4 from otherwise undamaged tubes. Also, the oil contrast might have an effect on  
5 peritoneal macrophage activity and on endometrial receptivity, thereby enhancing  
6 fertility [13,14]. To date, in the largest multi-centre (only conducted in the Netherlands)  
7 randomised clinical trial (RCT) [7], a total of 1119 women were randomly assigned to  
8 hysterosalpingography with oil contrast (557 women) or water contrast (562 women).  
9  
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13 This study showed higher ongoing pregnancy rates among women who underwent  
14 hysterosalpingography with oil-based contrast as compared to water-based contrast.  
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17 However, there are numerous studies did not show a significant difference in rates of  
18 ongoing pregnancy between OSCM (oil-soluble contrast media) and WSCM (water-  
19 soluble contrast media) groups. The results of the study carried out by Lindquist showed  
20 that compared with WSCM, the rate of ongoing pregnancy in OSCM group was slightly  
21 increased within 20 months after randomization, but there was no statistical difference  
22 between the two groups [15]. In a multicentre RCT carried out by Spring [16], a total  
23 of 666 women were randomly assigned to WSCM group (260 women), OSCM group  
24 water contrast (273 women) or both WSCM and OSCM group (133 women).  
25 Differences in reproductive outcome among contrast material groups were not  
26 statistically significant.  
27  
28

29 Some previous studies showed that women with subclinical hypothyroidism were  
30 thought to be more prone to lipiodol induced overt hypothyroidism [17], which may be  
31 related to the long half-life of lipiodol excretion [18]. It is known that excess iodine  
32 intake during pregnancy can adversely affect thyroid function in both the mother and  
33 fetus [19]. Nevertheless, there are no large-scale, prospective studies exploring the  
34 relationship between HSG contrast agents and the occurrence of thyroid dysfunction in  
35 patients and neonates so far.  
36  
37

38 In summary, there is no uniform conclusion on the choice of contrast agents and their  
39 effects. In view of this uncertainty, we plan a single-centre, randomized, controlled,  
40 parallel group, superiority trial that will compare ongoing pregnancy rates among  
41 women who undergo hysterosalpingography with oil contrast or water contrast during  
42 6 months after randomization. Our hypothesis is that in women undergoing HSG the  
43 use of oil-based contrast media results in higher ongoing pregnancy compared to the  
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4 use of water-based contrast media.  
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## 8 **Objective and hypothesis**

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10 The objective of the trial is to determine whether at HSG the use of oil-based contrast  
11 media results in higher ongoing pregnancy rates compared to the use of water-based  
12 contrast media during 6 months after randomisation. Our hypothesis is that in women  
13 undergoing HSG the use of oil-based contrast media results in higher ongoing  
14 pregnancy compared to the use of water-based contrast media.  
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## 23 **Methods and analysis**

### 24 **Study design and setting**

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26 The study is a single-centre randomized controlled superiority trial and will be  
27 performed in the International Peace Maternity and Child Health Hospital, School of  
28 Medicine, Shanghai Jiao Tong University, Shanghai, China. The hospital has one  
29 experienced radiologist in HSG. Consecutive patients will be asked to participate in this  
30 study after receiving oral and written information when tubal testing is indicated and  
31 will be planned. A written informed consent will be requested. Randomization will take  
32 place on the day of HSG. The details of the study design are shown in Figure 1.  
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### 44 **Participants**

45 Patients will be included in this study if they meet all the following criteria:

- 46 1. Age 20 to 39 years old
- 47 2. Spontaneous menstrual cycles (cycle length between 25 and 35 days);
- 48 3. Subfertility of at least one year;
- 49 4. Chlamydia trachomatis negative of vaginal secretion culture or no Chlamydia  
50 infection in the history
- 51 5. Low risk for tubal pathology according to the medical history.
- 52 6. Valid indication for HSG in the fertility work-up or before intra uterine  
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4 insemination treatment.

5  
6 7. Signed informed consent.

7  
8 Patients will be excluded from participating in the study if one of the following criteria  
9 is met:

- 10  
11 1. Irregular menstrual cycle, less than eight menstrual cycles per year.  
12  
13 2. Endocrino-pathological disease as: Polycystic ovary syndrome, Cushing syndrome,  
14 adrenal hyperplasia, hyperprolactinemia, acromegaly, hypothalamic amenorrhea,  
15 hypothyroidy, diabetes mellitus, thyroid dysfunction.  
16  
17 3. Known or high risk for tubal pathology, Chlamydia trachomatis positive of vaginal  
18 secretion culture, Chlamydia infection in the history.  
19  
20 4. Known contrast (iodine) allergy  
21  
22 5. Male subfertility defined as a post-wash total motile sperm count  $< 3 \times 10^6$   
23 spermatozoa/ml  
24  
25 6. A contra-indication for HSG.  
26  
27 7. Not willing or able to sign the consent form.  
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### 35 **Randomization**

36  
37 Demographic, medical, gynecological and infertile information will be collected at  
38 baseline. The patient will then be randomised between two groups (OSCM vs WSCM).  
39 Randomisation will be performed using a web-based Research Electronic Data Capture  
40 (REDCap) system. Allocation ratio will be 1:1 and permuted blocks of 4-6 will be used.  
41  
42 Prior to randomization, clinical data will be entered in the digital platform. Owing to  
43 the difference in imaging between the use of oil-based contrast and water-based  
44 contrast, and, since our outcome of ongoing pregnancy was objective, the trial was not  
45 blinded with respect to participants and caregivers.  
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### 55 **Intervention**

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57 All patients will undergo a HSG. Patients allocated to WSCM undergo the HSG with  
58 Ioversol Injection, a solution of low osmolar contrast medium which contains iodine  
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4 (320mg I/ml) (Heng Rui Pharmaceuticals, Jiangsu, China). Patients allocated to OSCM  
5 will undergo HSG with Iodinated Oil Injection, a solution of Ethiodol and contains  
6 poppy seed oil and iodine (480 I mg/ml) (Heng Rui Pharmaceuticals, Jiangsu, China).  
7  
8 The HSG will be performed by a radiologist. HSG will be performed without  
9  
10 premedication, in 3-7th day after complete cessation of menstrual bleeding, and before  
11  
12 the 14th day in the ovulatory cycle. After cleansing the vagina and cervix, a vacuum  
13  
14 cervix adapter will be applied to the cervix or a balloon catheter will be inserted  
15  
16 according to local protocol. Up to 10 ml of contrast medium will be slowly injected into  
17  
18 the uterus and directly monitored by fluoroscopy. Four to six X-ray photos will be taken  
19  
20 and assessed by a fertility specialist and radiologist. Visual analogue and qualitative  
21  
22 scales will be used after the procedure to assess study participants pain perception.  
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### 28 **Withdrawal of participants**

29  
30 Participants are free to withdraw from participation in the study at any time upon  
31  
32 request. An investigator may discontinue or withdraw a participant from the study for  
33  
34 the following reasons:

- 35 1. Participant withdraw informed consent;
- 36 2. Participant requests to withdraw from the study;
- 37 3. If the participant meets an exclusion criterion (either newly developed or not  
38  
39 previously recognized) that precludes further study participation;
- 40 4. Violation of research protocol that may interfere with the health of the patient if the  
41  
42 study is continued; however data collection will continue if this can safely be  
43  
44 acquired and the data will be used for the ITT analyses
- 45 5. Subject intervention termination criteria: those who have safety problems in  
46  
47 treatment and need to terminate treatment. Investigator can decide to withdraw a  
48  
49 participant from the study for urgent medical reasons, however data collection will  
50  
51 continue if this can safely be acquired and the data will be used for the ITT analyses.  
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57 The reasons for participant discontinuation or withdrawal from the study will be  
58  
59 recorded on the Case Report Form (CRF).  
60

## Participant timeline

The schedules for enrolment, interventions and assessments are summarised in Table 1.

**Table 1 Schedule of Activities**

Procedures	Screening Day -7 to -1	Enrollment/Ba seline Visit 1 Day 1	Study Visit 2 Months 6	Study Visit 3 Months 12	Study Visit 3 Months 24	Study Visit 3 Months 36
Informed consent	X					
Demographics	X					
Medical history	X					
Randomization		X				
Study intervention		X				
VAS for pain scores during the procedure		X				
Clinical pregnancy			X			X
Miscarriage			X			X
Ectopic pregnancy			X			X
Ongoing pregnancy			X			X
Live birth				X		X
Safety of intervention		X				X
Thyroid function of patients		X	X	X		
Neonatal thyroid function				X	X	
Side effects/complication		X				X
Additional therapies (assisted reproductive technology)			X			
Cost calculation		X			X	
Adverse event review and evaluation		X				X
Complete Case Report Files (CRFs)	X					X

The randomization and HSG will be performed on Day 1. A review of patient information should be done prior to enrolment to determine preliminary eligibility according to patient inclusion and exclusion criteria. When a patient signs an informed consent, she is considered to be enrolled in the study. Detailed clinical information

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2  
3 including age, body mass, duration of infertility, previous surgery, information of male  
4 partner will be collected. Follow-up and measurements will be same in both groups  
5  
6 with a total follow-up of 3 years and we will register data at baseline, 6, 12, 24 and 36  
7  
8 months. Procedure steps, pain scores and complications will be recorded. All additional  
9  
10 therapies or transfer to other treatments after first intervention will be registered in both  
11  
12 the two groups, such as Intrauterine insemination, IVF or ICSI and operation.

13  
14 We examined thyroid function of patients before HSG, and at 4, 8, 12, and 24 weeks,  
15  
16 and 9–12 months after HSG. We examined FT3, FT4, TSH, antithyroglobulin  
17  
18 antibodies (TgAb), and antithyroid peroxidase antibodies (TPOAb) levels [20].  
19

20  
21 We examined thyroid function of children when they are born 3 days, according to the  
22  
23 Newborn Screening in our country.  
24

### 25 26 27 **Primary outcome measure**

28  
29 The primary outcome is the rate of ongoing pregnancy in each treatment group.  
30  
31 Ongoing pregnancy will be defined as a registered heartbeat on ultrasound beyond 12  
32  
33 weeks of gestation with the first day of the last menstrual cycle for the pregnancy within  
34  
35 6 months after randomization.  
36

### 37 38 39 **Secondary outcome measure**

- 40  
41  
42 1. The rate of clinical pregnancy/ miscarriage/ ectopic/ live birth/ pregnancy leading  
43  
44 to live birth between two groups. Clinical pregnancy is defined as an ultrasound  
45  
46 visible gestational sac. Miscarriage is defined as a spontaneous loss off pregnancy.  
47  
48 Ectopic pregnancy is defined as an embryo implants outside the uterine cavity. Live  
49  
50 birth is defined as the birth of at least one living child;
- 51  
52 2. The thyroid function of patients and neonatal thyroid function between two groups;
- 53  
54 3. The pain scores after the procedure between two groups, measured by means of the  
55  
56 Visual-Analogue Scale for Pain (scores range from 0.0 to 10.0 cm, with higher  
57  
58 scores indicating more severe pain);
- 59  
60 4. The costs and cost-effectiveness of OSCM / WSCM and assisted reproductive

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4 technology (ART) treatments in Chinese context

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6 5. The side effects or complications after therapy in both arms.  
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## 10 **Statistical considerations**

### 11 **sample size calculation**

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14 The primary objective of this study is to determine whether at HSG the use of oil-based  
15 contrast media results in higher ongoing pregnancy compared to the use of water-based  
16 contrast media during 6 months after randomisation. In Dreyer's research, the rate of  
17 ongoing pregnancy in WSCM group was 29 %. To detect a difference of 10% between  
18 the OSCM group in rates of ongoing pregnancies as compared with WSCM group, with  
19 1:1 allocation ratio, 90% statistical power and at a significance level of two-sided 5%,  
20 we calculated that 467 women for each group, a total of 934 would need to be enrolled.  
21 Anticipating lost-to-follow up or protocol violation, an additional 10% is needed. Thus,  
22 1040 women (520 in each group) would need to be randomised. Sample size estimation  
23 curve under difference circumstance is showed in Figure 2.  
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### 36 **Statistical analysis**

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38 The primary analysis will be done according to the intention to treat principle. We will  
39 compare the primary outcome of rate of ongoing pregnancy between WSCM group and  
40 OSCM group by using the chi-square test to assess statistical significance.  
41  
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43 All data were analysed according to the intention-to-treat principle. Categorical data  
44 were reported as absolute numbers and percentages. Normally distributed continuous  
45 variables were summarized as means with standard deviations, and nonnormally  
46 distributed continuous variables were reported as medians with interquartile ranges.  
47  
48

49 Univariate rate ratios or relative risks and 95% confidence intervals were calculated for  
50 the primary and other binary outcome measures, and the chi-square test was used to  
51 assess statistical significance. Continuous outcomes were analysed with the use of an  
52 independent t-test or the Mann–Whitney U-test as appropriate. We used Kaplan–Meier  
53 curves with a log-rank test to compare the groups with respect to the time to pregnancy  
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4 resulting in an ongoing pregnancy. Two-sided P values of less than 0.05 were  
5 considered to indicate statistical significance. No adjustment was made for multiple  
6 comparisons. SPSS software, version 22.0 (IBM), and R software, version 3.3.1 (R  
7 Project for Statistical Computing), were used for statistical analyse.  
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### 13 **Interim monitoring and analysis**

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16 An Independent Data Monitoring Committee (IDMC) will review data annually during  
17 the accrual period and around the time that the planned for interim analyses. At each  
18 meeting, the IDMC will be asked to give advice on whether the accumulated data from  
19 the trial, together with results from other relevant trials, justifies continuing recruitment  
20 of further patients or further follow-up. An interim analyse on the ongoing pregnancy  
21 rate was planned at the time of approximately 50% participants were recruited. To  
22 control the overall type I error, the stopping boundaries for interim and final analyses  
23 are to be computed with use of the Lan-DeMets approximation to the O'Brien-Fleming  
24 boundary.  
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### 36 **Patient and public involvement**

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38 Neither the patients nor the public will be involved in the study design. They will also  
39 not be involved in the recruitment process or conduct of the study. The results will be  
40 disseminated to patients via an open access publication and our local trials teams.  
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### 46 **Discussion**

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48 With the increasement of infertile population, HSG is widely used in the fertility  
49 assessment. Most clinics in China use water-based contrast media but some use oil-  
50 based contrast media. Up to now, no final conclusion has yet been reached on this  
51 matter that which reagent is better in some cases. Dreyer's research [7] was the largest  
52 RCT about the effects between the two kinds of contrast at HSG, however it was not  
53 sure that the results applied to Chinese population. Therefore, this study is to answer  
54 the question whether at HSG the use of oil-based contrast media results in higher  
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ongoing pregnancy compared to the use of water-based contrast media during 6 months after randomization and explore the difference of neonatal thyroid function between the two groups.

The strengths of this study are as follows: First, the trial will answer the question whether at HSG the use of oil-based contrast media results in higher ongoing pregnancy compared to the use of water-based contrast media during 6 months after randomization in Chinese women. Second, this is the first time to assess the thyroid function of newborns and patients at different time points who underwent HSG. Final, compared to other studies, we are going to extend the follow-up periods to evaluate the long-term impacts of HSG with different agents.

The main shortcomings of this study are that the trial was not blinded with respect to participants and caregivers because of the difference in imaging between the use of oil-based contrast and water-based contrast and the objectivity of our outcomes. Moreover, another limitation is that the trial is based in a single center, which might limit the generalisability of the findings.

Therefore, we design this study to provide high-level evidence-based medical evidence for the future clinical application of hysterosalpingography.

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25 Oil-Soluble Iodinated Contrast Medium (Lipiodol). *J Clin Endocrinol Metab*  
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### 44 45 46 47 48 **Authors' contributions**

49  
50 Jian Zhang was conceived of the study and participated in its design, as well as  
51 supervised the study and critically revised the manuscript. Guiling Liang participated  
52 in writing the manuscript; Ling Jiang contributed to HSG examination; Qian zhu and  
53 Xiaoping He contribute to follow up the patients; Chenfeng Zhu and Xiaofeng Wang  
54 contributed to data analysis. All authors read and approved the final version of the  
55 manuscript.

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## **Competing interests**

The authors declare that they have no competing interests.

## **Patient consent for publication**

Patients' consent obtained.

## **Ethics approval**

The study was approved by the Medical Research Ethics Committee of International Peace Maternity and Child Health Hospital (approval no.GKLW2020-02).

## **Dissemination**

The findings will be reported in peer-reviewed publications and presentations at international scientific meetings.

## **Figure Legends**

### **Figure 1 Flowchart**

HSG: hysterosalpingography; Ongoing pregnancy will be defined as a registered heartbeat on ultrasound beyond 12 weeks of gestation; Clinical pregnancy is defined as an ultrasound visible gestational sac. Miscarriage is defined as a spontaneous loss off pregnancy. Ectopic pregnancy is defined as an embryo implants outside the uterine cavity. Live birth is defined as the birth of at least one living child.

### **Figure 2 Sample size estimation curve under difference circumstance**

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4 N is the total sample size,  $N(\text{Group OSCM}) + N(\text{Group WSCM})$ . P1 is the proportion  
5 for Group OSCM at which power and sample size calculations are made. P2 is the  
6 proportion for Group WSCM. This is referred to Dreyer's research [7]. Target Power  
7 is the desired power value (or values) entered in the procedure. Power is the probability  
8 of rejecting a false null hypothesis. The red line means power 0.80 and the green line  
9 means power 0.90.  
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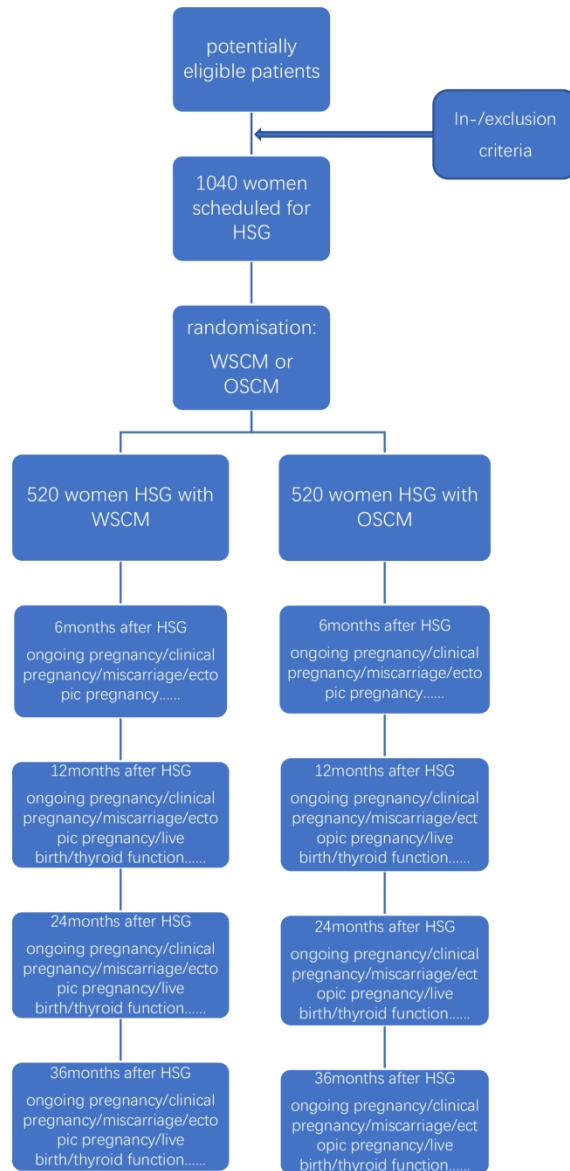
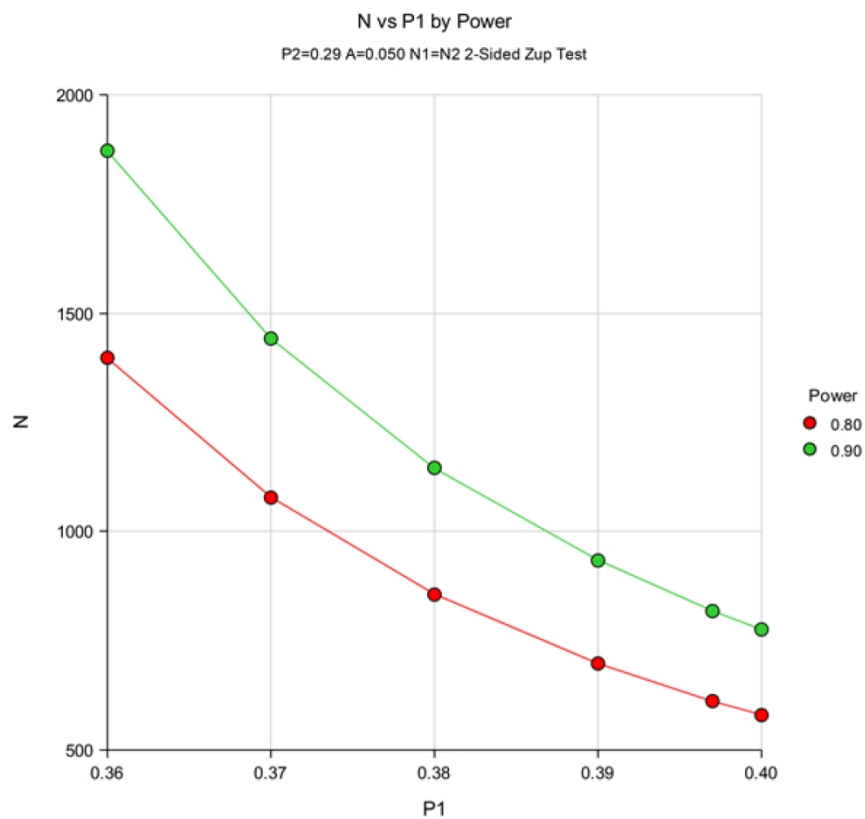


Figure 1 Flowchart

HSG: hysterosalpingography; Ongoing pregnancy will be defined as a registered heartbeat on ultrasound beyond 12 weeks of gestation; Clinical pregnancy is defined as an ultrasound visible gestational sac. Miscarriage is defined as a spontaneous loss off pregnancy. Ectopic pregnancy is defined as an embryo implants outside the uterine cavity. Live birth is defined as the birth of at least one living child.



35 Figure 2 Sample size estimation curve under difference circumstance  
36 N is the total sample size,  $N(\text{Group OSCM}) + N(\text{Group WSCM})$ . P1 is the proportion for Group OSCM at which  
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38 Dreyer's research [7]. Target Power is the desired power value (or values) entered in the procedure. Power  
39 is the probability of rejecting a false null hypothesis. The red line means power 0.80 and the green line  
40 means power 0.90.

# Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

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		Page
	Reporting Item	Number
<b>Administrative information</b>		
Title	<a href="#">#1</a> Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

1	Trial registration	<a href="#">#2a</a>	Trial identifier and registry name. If not yet registered,	2
2				
3			name of intended registry	
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6	Trial registration: data	<a href="#">#2b</a>	All items from the World Health Organization Trial	-
7				
8	set		Registration Data Set	
9				
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12	Protocol version	<a href="#">#3</a>	Date and version identifier	-
13				
14				
15	Funding	<a href="#">#4</a>	Sources and types of financial, material, and other support	14
16				
17				
18	Roles and	<a href="#">#5a</a>	Names, affiliations, and roles of protocol contributors	14
19				
20	responsibilities:			
21				
22	contributorship			
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25				
26	Roles and	<a href="#">#5b</a>	Name and contact information for the trial sponsor	-
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28	responsibilities:			
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30	sponsor contact			
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32	information			
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36	Roles and	<a href="#">#5c</a>	Role of study sponsor and funders, if any, in study design;	-
37				
38	responsibilities:		collection, management, analysis, and interpretation of	
39				
40	sponsor and funder		data; writing of the report; and the decision to submit the	
41				
42			report for publication, including whether they will have	
43				
44			ultimate authority over any of these activities	
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48	Roles and	<a href="#">#5d</a>	Composition, roles, and responsibilities of the coordinating	-
49				
50	responsibilities:		centre, steering committee, endpoint adjudication	
51				
52	committees		committee, data management team, and other individuals	
53				
54			or groups overseeing the trial, if applicable (see Item 21a	
55				
56			for data monitoring committee)	
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1	<b>Introduction</b>			3-4
2				
3				
4	Background and	<a href="#">#6a</a>	Description of research question and justification for	3
5	rationale		undertaking the trial, including summary of relevant studies	
6			(published and unpublished) examining benefits and harms	
7			for each intervention	
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11	Background and	<a href="#">#6b</a>	Explanation for choice of comparators	4
12	rationale: choice of			
13	comparators			
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17	Objectives	<a href="#">#7</a>	Specific objectives or hypotheses	5
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22	Trial design	<a href="#">#8</a>	Description of trial design including type of trial (eg, parallel	5
23			group, crossover, factorial, single group), allocation ratio,	
24			and framework (eg, superiority, equivalence, non-inferiority,	
25			exploratory)	
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34	<b>Methods:</b>			5-9
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36	<b>Participants,</b>			
37	<b>interventions, and</b>			
38	<b>outcomes</b>			
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44	Study setting	<a href="#">#9</a>	Description of study settings (eg, community clinic,	5
45			academic hospital) and list of countries where data will be	
46			collected. Reference to where list of study sites can be	
47			obtained	
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54	Eligibility criteria	<a href="#">#10</a>	Inclusion and exclusion criteria for participants. If	5-6
55			applicable, eligibility criteria for study centres and	
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1		individuals who will perform the interventions (eg,	
2			
3		surgeons, psychotherapists)	
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5			
6	Interventions:	<a href="#">#11a</a> Interventions for each group with sufficient detail to allow	6-7
7			
8	description	replication, including how and when they will be	
9			
10		administered	
11			
12			
13	Interventions:	<a href="#">#11b</a> Criteria for discontinuing or modifying allocated	-
14			
15	modifications	interventions for a given trial participant (eg, drug dose	
16			
17		change in response to harms, participant request, or	
18			
19		improving / worsening disease)	
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22			
23	Interventions:	<a href="#">#11c</a> Strategies to improve adherence to intervention protocols,	-
24			
25	adherence	and any procedures for monitoring adherence (eg, drug	
26			
27		tablet return; laboratory tests)	
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31	Interventions:	<a href="#">#11d</a> Relevant concomitant care and interventions that are	-
32			
33	concomitant care	permitted or prohibited during the trial	
34			
35			
36	Outcomes	<a href="#">#12</a> Primary, secondary, and other outcomes, including the	9
37			
38		specific measurement variable (eg, systolic blood	
39			
40		pressure), analysis metric (eg, change from baseline, final	
41			
42		value, time to event), method of aggregation (eg, median,	
43			
44		proportion), and time point for each outcome. Explanation	
45			
46		of the clinical relevance of chosen efficacy and harm	
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48		outcomes is strongly recommended	
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53	Participant timeline	<a href="#">#13</a> Time schedule of enrolment, interventions (including any	7-9
54			
55		run-ins and washouts), assessments, and visits for	
56			
57		participants. A schematic diagram is highly recommended	
58			
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(see Figure)

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4	Sample size	<a href="#">#14</a>	Estimated number of participants needed to achieve study 10
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6			objectives and how it was determined, including clinical and
7			
8			statistical assumptions supporting any sample size
9			
10			calculations
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13	Recruitment	<a href="#">#15</a>	Strategies for achieving adequate participant enrolment to -
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16			reach target sample size
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19	<b>Methods: Assignment</b>		-
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21	<b>of interventions (for</b>		
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23	<b>controlled trials)</b>		
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26	Allocation: sequence	<a href="#">#16a</a>	Method of generating the allocation sequence (eg, -
27			
28	generation		computer-generated random numbers), and list of any
29			
30			factors for stratification. To reduce predictability of a
31			
32			random sequence, details of any planned restriction (eg,
33			
34			blocking) should be provided in a separate document that is
35			
36			unavailable to those who enrol participants or assign
37			
38			interventions
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42			
43	Allocation	<a href="#">#16b</a>	Mechanism of implementing the allocation sequence (eg, -
44			
45	concealment		central telephone; sequentially numbered, opaque, sealed
46			
47	mechanism		envelopes), describing any steps to conceal the sequence
48			
49			until interventions are assigned
50			
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53	Allocation:	<a href="#">#16c</a>	Who will generate the allocation sequence, who will enrol -
54			
55	implementation		participants, and who will assign participants to
56			
57			interventions
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1	Blinding (masking)	<a href="#">#17a</a>	Who will be blinded after assignment to interventions (eg,	-
2			trial participants, care providers, outcome assessors, data	
3			analysts), and how	
4				
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8	Blinding (masking):	<a href="#">#17b</a>	If blinded, circumstances under which unblinding is	-
9	emergency		permissible, and procedure for revealing a participant's	
10			allocated intervention during the trial	
11	unblinding			
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16	<b>Methods: Data</b>			8-10
17	<b>collection,</b>			
18	<b>management, and</b>			
19	<b>analysis</b>			
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26	Data collection plan	<a href="#">#18a</a>	Plans for assessment and collection of outcome, baseline,	8-9
27			and other trial data, including any related processes to	
28			promote data quality (eg, duplicate measurements, training	
29			of assessors) and a description of study instruments (eg,	
30			questionnaires, laboratory tests) along with their reliability	
31			and validity, if known. Reference to where data collection	
32			forms can be found, if not in the protocol	
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43	Data collection plan:	<a href="#">#18b</a>	Plans to promote participant retention and complete follow-	-
44	retention		up, including list of any outcome data to be collected for	
45			participants who discontinue or deviate from intervention	
46			protocols	
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53	Data management	<a href="#">#19</a>	Plans for data entry, coding, security, and storage,	-
54			including any related processes to promote data quality	
55			(eg, double data entry; range checks for data values).	
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1		Reference to where details of data management	
2			
3		procedures can be found, if not in the protocol	
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6	Statistics: outcomes	<a href="#">#20a</a> Statistical methods for analysing primary and secondary	10
7			
8		outcomes. Reference to where other details of the	
9			
10		statistical analysis plan can be found, if not in the protocol	
11			
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13	Statistics: additional	<a href="#">#20b</a> Methods for any additional analyses (eg, subgroup and	-
14			
15	analyses	adjusted analyses)	
16			
17			
18	Statistics: analysis	<a href="#">#20c</a> Definition of analysis population relating to protocol non-	-
19			
20	population and	adherence (eg, as randomised analysis), and any statistical	
21			
22	missing data	methods to handle missing data (eg, multiple imputation)	
23			
24			
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26	<b>Methods: Monitoring</b>		11
27			
28			
29	Data monitoring:	<a href="#">#21a</a> Composition of data monitoring committee (DMC);	-
30			
31	formal committee	summary of its role and reporting structure; statement of	
32			
33		whether it is independent from the sponsor and competing	
34			
35		interests; and reference to where further details about its	
36			
37		charter can be found, if not in the protocol. Alternatively, an	
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39		explanation of why a DMC is not needed	
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44	Data monitoring:	<a href="#">#21b</a> Description of any interim analyses and stopping	11
45			
46	interim analysis	guidelines, including who will have access to these interim	
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48		results and make the final decision to terminate the trial	
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51	Harms	<a href="#">#22</a> Plans for collecting, assessing, reporting, and managing	-
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53		solicited and spontaneously reported adverse events and	
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55		other unintended effects of trial interventions or trial	
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1		conduct	
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4	Auditing	<a href="#">#23</a> Frequency and procedures for auditing trial conduct, if any,	-
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6		and whether the process will be independent from	
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8		investigators and the sponsor	
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11	<b>Ethics and</b>		15
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13	<b>dissemination</b>		
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16	Research ethics	<a href="#">#24</a> Plans for seeking research ethics committee / institutional	15
17			
18	approval	review board (REC / IRB) approval	
19			
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22	Protocol	<a href="#">#25</a> Plans for communicating important protocol modifications	-
23			
24	amendments	(eg, changes to eligibility criteria, outcomes, analyses) to	
25			
26		relevant parties (eg, investigators, REC / IRBs, trial	
27			
28		participants, trial registries, journals, regulators)	
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32	Consent or assent	<a href="#">#26a</a> Who will obtain informed consent or assent from potential	15
33			
34		trial participants or authorised surrogates, and how (see	
35			
36		Item 32)	
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38			
39	Consent or assent:	<a href="#">#26b</a> Additional consent provisions for collection and use of	-
40			
41	ancillary studies	participant data and biological specimens in ancillary	
42			
43		studies, if applicable	
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46			
47	Confidentiality	<a href="#">#27</a> How personal information about potential and enrolled	-
48			
49		participants will be collected, shared, and maintained in	
50			
51		order to protect confidentiality before, during, and after the	
52			
53		trial	
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57	Declaration of	<a href="#">#28</a> Financial and other competing interests for principal	15
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1	interests		investigators for the overall trial and each study site	
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4	Data access	<a href="#">#29</a>	Statement of who will have access to the final trial dataset,	-
5			and disclosure of contractual agreements that limit such	
6			access for investigators	
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11	Ancillary and post	<a href="#">#30</a>	Provisions, if any, for ancillary and post-trial care, and for	-
12			compensation to those who suffer harm from trial	
13	trial care		participation	
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19	Dissemination policy:	<a href="#">#31a</a>	Plans for investigators and sponsor to communicate trial	15
20			results to participants, healthcare professionals, the public,	
21	trial results		and other relevant groups (eg, via publication, reporting in	
22			results databases, or other data sharing arrangements),	
23			including any publication restrictions	
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31	Dissemination policy:	<a href="#">#31b</a>	Authorship eligibility guidelines and any intended use of	-
32			professional writers	
33	authorship			
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36	Dissemination policy:	<a href="#">#31c</a>	Plans, if any, for granting public access to the full protocol,	-
37			participant-level dataset, and statistical code	
38	reproducible research			
39				
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42	<b>Appendices</b>			
43				
44				
45	Informed consent	<a href="#">#32</a>	Model consent form and other related documentation given	-
46			to participants and authorised surrogates	
47	materials			
48				
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50	Biological specimens	<a href="#">#33</a>	Plans for collection, laboratory evaluation, and storage of	-
51			biological specimens for genetic or molecular analysis in	
52			the current trial and for future use in ancillary studies, if	
53			applicable	
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4  
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# BMJ Open

## Effects of oil-soluble contrast versus water-soluble contrast media at hysterosalpingography on pregnancy outcomes in Chinese women: study protocol for a randomized controlled trial

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2020-039166.R1
Article Type:	Protocol
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1 **Effects of oil-soluble contrast versus water-soluble**  
2 **contrast media at hysterosalpingography on**  
3 **pregnancy outcomes in Chinese women: study**  
4 **protocol for a randomized controlled trial**

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## ABSTRACT

**Introduction:** In recent years, due to various factors, the infertility rate in China has increased and now affects more than 10% of women of reproductive age.

Hysterosalpingography (HSG) is a common diagnostic procedure during fertility examinations. However, there is no consensus on the choice of contrast agents and their effects. As the largest multi-centre randomised controlled trial (H2Oil trial from the Netherlands) has shown the oil contrast at HSG can enhance the fertility, we propose this study to answer the question of whether the use of oil-soluble contrast media results in increased pregnancy rates in Chinese women undergo HSG.

**Methods and analysis** This study is a single-centre, randomized, controlled, parallel group, superiority trial. The patients will be randomized to undergo HSG using iodinated oil injection (OSCM group, oil-soluble contrast media) or ioversol injection (WSCM group, water-soluble contrast media). To evaluate the potential superiority of OSCM group, with 1:1 allocation ratio, 90% statistical power and a two-sided significance level of 5%, and considering 10% lost-to-follow up or protocol violation, we have calculated a sample of 520 women per group for a total of 1040 would need to be enrolled. The primary outcome is the rate of ongoing pregnancy during 6 months after randomisation. The secondary outcomes will consist of thyroid function of patients, neonatal thyroid function, pain scores during HSG, live birth rate, clinical pregnancy rate, miscarriages, ectopic pregnancies, pregnancy leading to live birth, the

1 time to pregnancy resulting in an ongoing pregnancy, cost calculations of the OSCM  
2 group/WSCM group, and assisted reproductive technology (ART) treatments between  
3 two groups.

4 **Ethics and dissemination** This protocol received authorisation from the Medical  
5 Research Ethics Committee International Peace Maternity and Child Health Hospital  
6 on 18th January 2020 (approval no. GKLW2020-02). The findings will be reported in  
7 peer-reviewed publications and presentations at international scientific meetings.

8 **Trial registration number** ChiCTR2000031612.

## 10 **STRENGTHS AND LIMITATIONS OF THIS STUDY**

- 11 ● This is the first study to assess the thyroid function of patients at different time  
12 points who undergo HSG and their newborns' thyroid function in an RCT  
13 (randomized controlled trial).
- 14 ● This is the first study with long-term follow-up of patients who undergo HSG in an  
15 RCT.
- 16 ● This is the first study to record the pain intensity and when the pain occurs during  
17 HSG.
- 18 ● The trial is based in a single centre, which might limit the generalisability of the  
19 findings.
- 20 ● The trial will not be blinded with respect to participants and caregivers.

## 1 INTRODUCTION

2 Infertility is defined as the failure to establish a pregnancy after 12 months of regular,  
3 unprotected sexual intercourse or an impairment of a person's capacity to reproduce  
4 either as an individual or with his/her partner [1]. Due to the enactment of the second  
5 child policy in China, the increasing age of mothers, environmental pollution, and  
6 workplace pressures, the infertility rate in China has increased and now affects more  
7 than 10% of reproductive women [2].

8 Hysterosalpingography (HSG) refers to the radiographic evaluation of the uterine  
9 cavity and fallopian tubes after the injection of an oil-/water-soluble contrast medium  
10 through the cervical canal [3]. HSG plays an important role in the evaluation of female  
11 fallopian tube reproductive function and is a common diagnostic procedure in the  
12 fertility examinations [4,5]. In China, it is the preferred method to investigate tubal  
13 patency [6]. Compared with laparoscopy, it is less expensive and has fewer  
14 complications [7]. In addition to diagnostic information, HSG has therapeutic effects,  
15 which are associated with increased fecundability in the months after the procedure  
16 [8,9].

17 Oil-soluble contrast media (OSCM) is represented by iodinated oil injection, which  
18 uses poppy seed oil as the raw material. It provides clear images and may have some  
19 anti-inflammatory effects that perhaps enhance fertility [10]. Due to less absorption of  
20 the contrast media, the occurrence of adverse events is notably reduced [11,12].

21 Water-soluble contrast media (WSCM) is represented by meglumine diatrizoate and  
22 ioversol. The advantages of WSCM are as follows: low viscosity, good fluidity, and

1 rapid diffusion into the fallopian tubes, which can clearly show the anatomy of the  
2 fallopian tubes. However, the rapid flow may result in insufficient observation of  
3 fallopian tube lesions and may result in poor fallopian tube dredging [11].

4 Some studies showed significantly higher pregnancy rates after tubal flushing with  
5 OSCM [13,14]. This might be caused by flushing debris and dislodging mucus plugs  
6 from otherwise undamaged tubes. Additionally, OSCM might have an effect on  
7 peritoneal macrophage activity and on endometrial receptivity, thereby enhancing  
8 fertility [15,16]. Fang's systematic review showed that HSG using OSCM may promote  
9 the ongoing pregnancy rate through a comprehensive analysis of six studies. However,  
10 the review did not include Chinese population and did not pay close attention to the  
11 thyroid function or long-term effects [17]. To date, in the largest multi-centre  
12 randomised controlled trial (RCT) [8], a total of 1119 women were randomly assigned  
13 to hysterosalpingography with OSCM (557 women) or WSCM (562 women). The  
14 study, which was conducted in the Netherlands, showed higher ongoing pregnancy  
15 rates among women who underwent hysterosalpingography with OSCM as compared  
16 to WSCM.

17 However, numerous studies have not shown a significant difference in rates of ongoing  
18 pregnancy between OSCM and WSCM groups [18-21]. Lindquist found that the rate  
19 of ongoing pregnancy in OSCM group was slightly higher than the WSCM group,  
20 within 20 months after randomization, but the difference was not statistically significant  
21 [20]. In a multicentre RCT carried out by Spring [21], a total of 666 women were  
22 randomly assigned to WSCM group (260 women), OSCM group (273 women) or both

1 WSCM and OSCM group (133 women). Differences in reproductive outcomes among  
2 the groups were not statistically significant.

3 Previous studies showed that women with subclinical hypothyroidism were more prone  
4 to lipiodol induced overt hypothyroidism [22], which may be due to the long half-life  
5 of lipiodol excretion [23]. It is known that excess iodine intake during pregnancy can  
6 adversely affect thyroid function in both the mother and the foetus [24]. Nevertheless,  
7 there have been no large-scale, prospective studies exploring the relationship between  
8 HSG contrast agents and the occurrence of thyroid dysfunction in patients and neonates.

9 In summary, there is no consensus on the choice of contrast agents and their effects. In  
10 view of this uncertainty, we plan a single-centre, randomized, controlled, parallel  
11 group, superiority trial in infertile Chinese women with a low a priori chance of tubal  
12 pathology.

## 13 **OBJECTIVE AND HYPOTHESIS**

14 The objective of the trial is to determine whether the use of OSCM during HSG results  
15 in a higher ongoing pregnancy rate compared to the use of WSCM for 6 months after  
16 randomisation. Our hypothesis is that in women undergoing HSG, the use of OSCM  
17 will result in a higher ongoing pregnancy rate compared to the use of WSCM.

## 19 **METHODS AND ANALYSIS**

### 20 **Study design and setting**

21 The study is a single-centre randomized controlled superiority trial and will be  
22 performed in the International Peace Maternity and Child Health Hospital, School of



1 Medicine, Shanghai Jiao Tong University, Shanghai, China. The hospital has two  
2 experienced radiologist in HSG. The details of the study design are shown in Figure 1.

## 4 **Recruitment**

5 Before participation, patients need to complete a screening questionnaire. Consecutive  
6 patients will be asked to participate in this study after receiving oral and written  
7 information from a gynaecologist in outpatient services when tubal testing has been  
8 indicated and will be scheduled. A research nurse will administer oral and written  
9 informed consent. To reach the target sample size, we will advertise our clinical trial  
10 using the hospitals' official accounts on WeChat and distribute brochures in the  
11 outpatient department.

## 13 **Participants**

14 Inclusion criteria are as follows:

- 15 1. Age 20 to 39 years old
- 16 2. Spontaneous menstrual cycles (cycle length between 25 and 35 days);
- 17 3. Subfertility of at least one year and a fertile partner (defined as a post-wash total  
18 motile sperm count  $> 3 \times 10^6$  spermatozoa/mL)
- 19 4. Chlamydia trachomatis negative via vaginal secretion culture (through Chlamydia  
20 antigen detection) and no history of Chlamydia infection.
- 21 5. Low risk for tubal pathology according to the medical history. (The patient has not  
22 been exposed to high risk factors of tubal pathology, such as history of chlamydia

1 infection, pelvic inflammatory disease, known endometriosis or adenomyosis,  
2 pelvic abdominal surgery [including salpingostomy or salpingectomy for ectopic  
3 pregnancy and complicated appendectomy] and/or peritonitis) [25].

4 6. Valid indication for HSG in the fertility examination or before intra uterine  
5 insemination treatment.

6 7. Signed informed consent.

7 Exclusion criteria are as follows:

8 1. Irregular menstrual cycle, less than eight menstrual cycles per year.

9 2. Endocrino-pathological diseases such as: Polycystic ovary syndrome, Cushing  
10 syndrome, adrenal hyperplasia, hyperprolactinemia, acromegaly, hypothalamic  
11 amenorrhea, hypothyroidy, diabetes mellitus, and thyroid dysfunction.

12 3. Known or at high risk for tubal pathology, Chlamydia trachomatis positive of  
13 vaginal secretion culture, history of Chlamydia infection.

14 4. Known contrast (iodine) allergy

15 5. Male subfertility defined as a post-wash total motile sperm count  $< 3 \times 10^6$   
16 spermatozoa/mL

17 6. A contra-indication for HSG.

18 7. Not willing or able to sign the consent form.

19

## 20 **Randomization**

21 Randomization will take place on the day of HSG. Demographic, medical,  
22 gynaecological and infertility information will be collected at baseline. The patient will

1 then be randomised between two groups (OSCM or WSCM). Randomisation will be  
2 performed by an independent statistician using a web-based Research Electronic Data  
3 Capture (REDCap) system. The allocation ratio will be 1:1 and permuted blocks of 4-  
4 6 will be used. Prior to randomisation, screening data will be entered in the digital  
5 platform. The randomisation list will be sealed in sequentially numbered opaque  
6 envelopes. The envelopes will be stored in a double-locked cabinet and will only be  
7 opened by the practitioner to assign participants to each group after obtaining informed  
8 consent and having been screened for eligibility. The opened envelopes will again be  
9 separately stored in a double-locked cabinet.

## 11 **Blinding**

12 Owing to the difference in imaging between the use of OSCM and WSCM, and since  
13 the outcome measure of ongoing pregnancy will be an objective measure, the trial will  
14 not be blinded with respect to participants and caregivers.

## 16 **Intervention**

17 All patients will undergo HSG. Patients allocated to OSCM will undergo HSG with  
18 iodinated oil injection, a solution of Ethiodol that contains poppy seed oil and iodine  
19 (480 I mg/ml) (Heng Rui Pharmaceuticals, Jiangsu, China). Patients allocated to  
20 WSCM will undergo HSG with ioversol injection, a solution of low osmolar contrast  
21 medium that contains iodine (320 mg I/mL) (Heng Rui Pharmaceuticals, Jiangsu,  
22 China). HSG will be performed by a radiologist, without premedication, on the 3rd-7th

1 days after complete cessation of menstrual bleeding, and before the 14th day in the  
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1 HSG will be performed as follows: 1) the patient will be placed in the supine position;  
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2) Routine disinfection will be performed in the bladder lithotomy area of the patient;  
3) Vaginal speculum will be used to expose the vagina and cervix uteri, then  
disinfection will be implemented; 4) A rubber double-lumen tube or a special catheter  
will be inserted into the cervix uteri and will then be fixated; 5) Up to 10 ml of contrast  
medium will be slowly injected into the uterus under appropriate pressure until  
adequate uterine filling has occurred or contrast medium has flowed into the pelvic  
cavity. This will be directly monitored by fluoroscopy; 6) During the infusion, the  
dynamic flow of the course of the contrast medium into the uterine cavity and fallopian  
tube will be observed, and images will be captured before the contrast agent is injected  
and after the uterine cavity is filled, while the Fallopian tube appears; 7) When the  
images overlap, the tube or bed position will be changed as needed. After the procedure,  
images will be reviewed by a radiologist, and a diagnosis will be established.

### **Withdrawal of participants**

Participants will be free to withdraw from the study at any time upon request. An  
investigator may discontinue or withdraw a participant from the study for the following  
reasons: the participant meets an exclusion criterion; violation of research protocol; the  
patient is experiencing an urgent medical situation.

The reasons for participant discontinuation or withdrawal from the study will be

1 recorded on the Case Report Form (CRF). Data collection will continue if data can be  
2 safely acquired, and the data will be used for the ITT (intention-to-treat) analyses.

### 4 **Participant timeline**

5 The schedules for enrolment, interventions and assessments are summarised in Table  
6 1. The investigators will record the information in the CRFs and source documents.

7 **Table 1 Schedule of Activities**

Procedures	Screening Day -7 to -1	Enrollment/Ba seline Visit 1 Month 1	Study Visit 2 Months 6	Study Visit 3 Months 12	Study Visit 3 Months 24	Study Visit 3 Months 36
Informed consent	X					
Demographics	X					
Medical history	X					
Randomization		X				
Study intervention		X				
Ongoing pregnancy			X			
Thyroid function of patients	X		X	X		
Neonatal thyroid function				X	X	X
VAS for pain scores during the procedure		X				
Live birth				X		X
Clinical pregnancy			X			X
Miscarriage			X			X
Ectopic pregnancy			X			X
Safety of intervention		X				X
Additional therapies (assisted reproductive technology/ operation)			X			
Cost calculation		X			X	
Complete Case Report Files (CRFs)	X					X

8  
9 The randomisation and HSG will be performed on Day 1. A review of patient  
10 information will be done prior to enrolment to determine preliminary eligibility  
11 according to the inclusion and exclusion criteria. When a patient provides informed

1 consent, she will be considered to be enrolled in the study. Detailed clinical information  
2 including age, body mass, duration of infertility, previous surgery, and information on  
3 her partner will be collected. Follow-up and measurements will be the same for both  
4 groups with a total follow-up of 3 years. Data will be collected at baseline, 6, 12, 24,  
5 and 36 months. Procedure steps, pain scores, and complications will be recorded. All  
6 additional therapies or transfer to other treatments after the first intervention will be  
7 recorded in both groups, such as Intrauterine insemination, in vitro IVF (in vitro  
8 fertilization), ICSI (Intracytoplasmic sperm injection), and operation (laparoscopic or  
9 hysteroscopic surgery).

## 11 **OUTCOME MEASURES**

### 12 **Primary outcome measures**

13 The primary outcome is the rate of ongoing pregnancy in each treatment group.  
14 Ongoing pregnancy will be defined as a registered heartbeat on ultrasound beyond 12  
15 weeks of gestation with the first day of the last menstrual cycle as the beginning of the  
16 pregnancy within 6 months after randomisation.

### 18 **Secondary outcome measures**

- 19 1. The thyroid function of patients will be examined before HSG, and at 4, 8, 12, and  
20 24 weeks, and 9–12 months after HSG. We will determine free triiodothyronine  
21 (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH), antithyroglobulin  
22 antibodies (TgAb), and antithyroid peroxidase antibodies (TPOAb) levels at

- 1 different time-points [26,27].
- 2 2. The thyroid function of the neonates will be tested within 3 to 7 days after birth.
- 3 TT4 (total thyroxine), FT4, and TSH will be detected [27].
- 4 3. Pain scores during hysterosalpingography will be measured by means of the Visual-
- 5 Analogue Scale for Pain (scores range from 0.0 to 10.0 cm, with higher scores
- 6 indicating more severe pain). The pain scores will be recorded by a trained nurse.
- 7 Meanwhile, the time from the beginning of the contrast injection to the occurrence
- 8 of pain will be recorded.
- 9 4. The rates of live birth rate/ clinical pregnancy / miscarriages/ ectopic pregnancy/
- 10 pregnancy leading to live birth will be assessed. Live birth is defined as the birth of
- 11 at least one living child; clinical pregnancy is defined as an ultrasound visible
- 12 gestational sac; miscarriage is defined as a spontaneous loss of pregnancy; ectopic
- 13 pregnancy is defined as an embryo implanted outside the uterine cavity. Pregnancy
- 14 leading to live birth is defined as the ratio of live births to clinical pregnancies. Each
- 15 occurrence of one of these events will be recorded during the three-year follow-up.
- 16 5. The time to pregnancy resulting in an ongoing pregnancy is defined as the time
- 17 from randomization to the first day of the last menstrual period plus 4 weeks. It will
- 18 be considered when ongoing pregnancy occurs.
- 19 6. The costs and cost-effectiveness of OSCM / WSCM and assisted reproductive
- 20 technology (ART) treatments in Chinese context.
- 21 7. The side effects or complications after therapy in both groups.

22

## **Safety assessments**

All adverse events (AEs) will be recorded during the entire study period. An adverse event is defined as an event during or following hysterosalpingography or follow-up which was not intended to happen and is suspected to be a complication of the intervention performed. Common adverse events of HSG include allergic reactions, artificial abortion syndrome, abdominal pain, and intravasation. The severity of the AEs will be primarily ranked as 'mild', 'moderate', or 'severe'. The causes of the AEs will be rated as 'definitely related', 'probably related', 'possibly related', 'probably not related', 'definitely not related', or 'unknown'.

A severe adverse event (SAE) is defined as death, illness necessitating hospitalization, disability, or congenital malformation. All SAEs will be reported to the ethics committee that approved the protocol within 24 hours.

## **STATISTICAL CONSIDERATIONS**

### **Sample size calculation**

In Dreyer's research, the rate of ongoing pregnancy in the WSCM group was 29 %. To detect a difference of 10% between the OSCM group in rates of ongoing pregnancies as compared with WSCM group, with 1:1 allocation ratio, 90% statistical power, and a two-sided significance level of 5%, we calculated that 467 women per group for a total of 934 will need to be enrolled. Anticipating lost-to-follow-up or protocol violation, an additional 10% is needed. Thus, 1040 women (520 in each group) will need to be randomised.



1

## 2 **Statistical analysis**

3 We will compare the primary outcome of rate of ongoing pregnancy between the  
4 OSCM group and the WSCM group using the chi-square test to assess statistical  
5 significance.

6 All data will be analysed according to the intention-to-treat principle. Categorical data  
7 will be reported as absolute numbers and percentages. Normally distributed continuous  
8 variables will be summarized as means with standard deviations, and nonnormally  
9 distributed continuous variables will be reported as medians with interquartile ranges.

10 Univariate rate ratios or relative risks and 95% confidence intervals will be calculated  
11 for the primary and other binary outcome measures, and the chi-square test will be used  
12 to assess statistical significance. Continuous outcomes will be analysed with the use of  
13 an independent t-test or the Mann–Whitney U-test, as appropriate. We will use Kaplan–  
14 Meier curves with a log-rank test to compare the groups with respect to the time to  
15 pregnancy resulting in an ongoing pregnancy. Two-sided P values of less than 0.05 will  
16 indicate statistical significance. No adjustment will be made for multiple comparisons.  
17 SPSS software, version 22.0 or higher (IBM; Armonk, NR), and R version 3.3.1 (R  
18 Project for Statistical Computing, Vienna, Austria), will be used for statistical analyses.

19

## 20 **Data monitoring and auditing**

21 Data monitoring and auditing will be conducted for quality assurance. Monitoring staff  
22 will consist of an Independent Data Monitoring Committee (IDMC) and an ethics

1 committee. They will visit the institutions at important time points throughout the trial,  
2 for example, at participant enrolment, at the study interim point, and at study  
3 completion. Monitoring staff will ensure consistency concerning data documented in  
4 both the CRF and the source document and will ensure that the entire study process is  
5 in accordance with the approved protocol.

6 An Independent Data Monitoring Committee (IDMC) and ethics committee will review  
7 data annually during the accrual period and near the time that is planned for interim  
8 analyses. At each meeting, the IDMC will be asked to give advice on whether the  
9 accumulated data from the trial, together with results from other relevant trials, justifies  
10 continuing recruitment of further patients or further follow-up. An interim analysis on  
11 the ongoing pregnancy rate is planned for the time when approximately 50%  
12 participants will be recruited. To control for overall type I error, the stopping boundaries  
13 for interim and final analyses will be computed using the Lan-DeMets approximation  
14 to the O'Brien-Fleming boundary.

15 Maintenance of participant confidentiality will involve: (1) asking subjects to only  
16 share personal and study-related information during our study; (2) storing data in  
17 password-protected files on a designated specific computer with restricted access; (3)  
18 only the research-related person will have access to personal identifiable information,  
19 which will be destroyed once the study is completed.

## 20 21 **Ethics and dissemination**

22 This protocol received authorisation from the Medical Research Ethics Committee

1 International Peace Maternity and Child Health Hospital on 18th January 2020  
2 (approval no. GKLW2020-02). The findings will be reported in peer-reviewed  
3 publications and presentations at international scientific meetings. The papers will be  
4 written by the major researchers. The public can obtain access to the full protocol,  
5 participant-level dataset, and statistical code via emailing the major researchers.

6

## 7 **DISCUSSION**

8 HSG is widely used during the fertility assessment. Most clinics in China use WSCM  
9 but some use OSCM. Until now, no consensus has been reached on which reagent is  
10 better and for which patients. Dreyer's research [8] was the largest RCT about the  
11 effects between the two kinds of contrast at HSG; however, it remains unclear if the  
12 results apply to the Chinese population. Therefore, the aim of this study is to determine  
13 whether at HSG using OSCM results in higher ongoing pregnancy rates compared to  
14 the use of WSCM during 6 months after randomisation and to explore the difference of  
15 maternal and neonatal thyroid function between the two groups.

16 The strengths of this study are as follows: First, this will be the first study to assess  
17 thyroid function of patients at different time points who undergo HSG and assess their  
18 newborns' thyroid function in a RCT. Second, compared to other studies, we will  
19 extend the follow-up period to evaluate the long-term impacts of HSG using different  
20 reagents. Finally, this will be the first record of pain intensity during HSG and the time  
21 interval of when pain occurs.

22 The main shortcomings of this study are that the trial will not be blinded with respect

1 to participants and caregivers because of the difference in imaging between OSCM and  
2 WSCM and the objectivity of our outcomes. Another limitation is that the trial is based  
3 in a single centre, which might limit the generalisability of the findings.

4 Therefore, we have designed this study to provide rigorous medical evidence for the  
5 future clinical application of hysterosalpingography.

6

### 7 **Trial status**

8 The recruitment has not started yet. We are going to begin the recruitment in June 2020  
9 and expect to complete the recruitment in June 2021.

10

### 11 **Patient and public involvement**

12 Neither the patients nor the public will be involved in the study design. They will also  
13 not be involved in the recruitment process or conduct of the study. The results will be  
14 disseminated to patients via an open access publication and our local trials teams.

15

### 16 **Acknowledgments**

17 The authors thank Elsevier Language Editing Services for polishing the manuscript.

18

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### 8 **Date and version**

9 06-05-2020; Version 1.1.

### 10 **Author affiliations**

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18 China.

### 19 **Authors' contributions**

20 Jian Zhang was conceived of the study and participated in its design, as well as  
21 supervised the study and critically revised the manuscript. Zhaoxia Qian critically  
22 revised the manuscript and contributed to the HSG examination. Guiling Liang



1 participated in writing the manuscript; Ling Jiang contributed to HSG examination;  
2 Qian Zhu and Xiaoqing He contribute to follow up the patients; Chenfeng Zhu and  
3 Xiaofeng Wang contributed to data collection; Li Xie contributed to revised the  
4 statistical methods. All authors read and approved the final version of the manuscript.

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7 (GFY1808001), International peace maternal and child health hospital clinical applied  
8 research program (YN201915).

### 10 **Competing interests**

11 The authors declare that they have no competing interests.

### 13 **Patient consent for publication**

14 Patients' consent will be obtained for this trial. We may get an extra informed consent  
15 for other studies. We have uploaded model consent form in the register website (Trial  
16 registration number ChiCTR2000031612).

### 18 **Ethics approval**

19 The study was approved by the Medical Research Ethics Committee of International  
20 Peace Maternity and Child Health Hospital (approval no.GKLW2020-02).

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#### 4 **FIGURE LEGENDS**

##### 5 **Figure 1 Flowchart**

6 HSG: hysterosalpingography. Ongoing pregnancy is defined as a registered heartbeat  
7 on ultrasound beyond 12 weeks of gestation. Clinical pregnancy is defined as an  
8 ultrasound visible gestational sac. Miscarriage is defined as a spontaneous loss of  
9 pregnancy. Ectopic pregnancy is defined as an embryo implants outside the uterine  
10 cavity. Live birth is defined as the birth of at least one living child.

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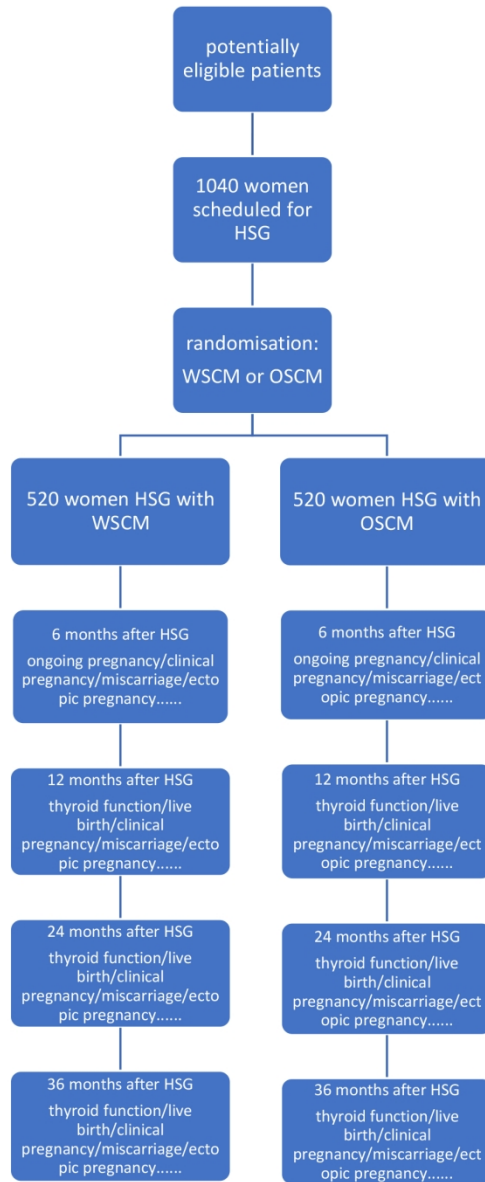


Figure 1 Flowchart

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HSG: hysterosalpingography. Ongoing pregnancy is defined as a registered heartbeat on ultrasound beyond 12 weeks of gestation. Clinical pregnancy is defined as an ultrasound visible gestational sac. Miscarriage is defined as a spontaneous loss of pregnancy. Ectopic pregnancy is defined as an embryo implants outside the uterine cavity. Live birth is defined as the birth of at least one living child.

# Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

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	Reporting Item	Page Number
<b>Administrative information</b>		
Title	<a href="#">#1</a> Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

1	Trial registration	<a href="#">#2a</a>	Trial identifier and registry name. If not yet registered,	3
2			name of intended registry	
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6	Trial registration: data	<a href="#">#2b</a>	All items from the World Health Organization Trial	-
7	set		Registration Data Set	
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12	Protocol version	<a href="#">#3</a>	Date and version identifier	22
13				
14				
15	Funding	<a href="#">#4</a>	Sources and types of financial, material, and other support	23
16				
17				
18	Roles and	<a href="#">#5a</a>	Names, affiliations, and roles of protocol contributors	22
19	responsibilities:			
20	contributorship			
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26	Roles and	<a href="#">#5b</a>	Name and contact information for the trial sponsor	1
27	responsibilities:			
28	sponsor contact			
29	information			
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36	Roles and	<a href="#">#5c</a>	Role of study sponsor and funders, if any, in study design;	22
37	responsibilities:		collection, management, analysis, and interpretation of	
38	sponsor and funder		data; writing of the report; and the decision to submit the	
39			report for publication, including whether they will have	
40			ultimate authority over any of these activities	
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48	Roles and	<a href="#">#5d</a>	Composition, roles, and responsibilities of the coordinating	15-16
49	responsibilities:		centre, steering committee, endpoint adjudication	
50	committees		committee, data management team, and other individuals	
51			or groups overseeing the trial, if applicable (see Item 21a	
52			for data monitoring committee)	
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1	<b>Introduction</b>		3-6
2			
3			
4	<b>Background and</b>	<a href="#">#6a</a>	3-6
5	<b>rationale</b>	Description of research question and justification for	
6		undertaking the trial, including summary of relevant	
7		studies (published and unpublished) examining benefits	
8		and harms for each intervention	
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14	<b>Background and</b>	<a href="#">#6b</a>	3-6
15	<b>rationale: choice of</b>	Explanation for choice of comparators	
16	<b>comparators</b>		
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21	<b>Objectives</b>	<a href="#">#7</a>	6
22		Specific objectives or hypotheses	
23			
24			
25	<b>Trial design</b>	<a href="#">#8</a>	6
26		Description of trial design including type of trial (eg,	
27		parallel group, crossover, factorial, single group),	
28		allocation ratio, and framework (eg, superiority,	
29		equivalence, non-inferiority, exploratory)	
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35	<b>Methods: Participants,</b>		6-14
36	<b>interventions, and</b>		
37	<b>outcomes</b>		
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42	<b>Study setting</b>	<a href="#">#9</a>	6
43		Description of study settings (eg, community clinic,	
44		academic hospital) and list of countries where data will be	
45		collected. Reference to where list of study sites can be	
46		obtained	
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52	<b>Eligibility criteria</b>	<a href="#">#10</a>	7-8
53		Inclusion and exclusion criteria for participants. If	
54		applicable, eligibility criteria for study centres and	
55		individuals who will perform the interventions (eg,	
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surgeons, psychotherapists)

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4	Interventions:	<a href="#">#11a</a>	Interventions for each group with sufficient detail to allow 9-10
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6	description		replication, including how and when they will be
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8			administered
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11	Interventions:	<a href="#">#11b</a>	Criteria for discontinuing or modifying allocated 10
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13	modifications		interventions for a given trial participant (eg, drug dose
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15			change in response to harms, participant request, or
16			improving / worsening disease)
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21	Interventions:	<a href="#">#11c</a>	Strategies to improve adherence to intervention protocols, 15-16
22			
23	adherence		and any procedures for monitoring adherence (eg, drug
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25			tablet return; laboratory tests)
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29	Interventions:	<a href="#">#11d</a>	Relevant concomitant care and interventions that are 11-12
30			
31	concomitant care		permitted or prohibited during the trial
32			
33			
34	Outcomes	<a href="#">#12</a>	Primary, secondary, and other outcomes, including the 12-13
35			
36			specific measurement variable (eg, systolic blood
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38			pressure), analysis metric (eg, change from baseline, final
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40			value, time to event), method of aggregation (eg, median,
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42			proportion), and time point for each outcome. Explanation
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44			of the clinical relevance of chosen efficacy and harm
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46			outcomes is strongly recommended
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51	Participant timeline	<a href="#">#13</a>	Time schedule of enrolment, interventions (including any 11-12
52			
53			run-ins and washouts), assessments, and visits for
54			
55			participants. A schematic diagram is highly recommended
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57			(see Figure)
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1	Sample size	<a href="#">#14</a>	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	14
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11	Recruitment	<a href="#">#15</a>	Strategies for achieving adequate participant enrolment to reach target sample size	7
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16	<b>Methods: Assignment</b>			8-9
17	<b>of interventions (for</b>			
18	<b>controlled trials)</b>			
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24	Allocation: sequence	<a href="#">#16a</a>	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	8-9
25	generation			
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41	Allocation	<a href="#">#16b</a>	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8-9
42	concealment			
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45	mechanism			
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51	Allocation:	<a href="#">#16c</a>	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8-9
52	implementation			
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1	Blinding (masking)	<a href="#">#17a</a>	Who will be blinded after assignment to interventions (eg,	9
2			trial participants, care providers, outcome assessors, data	
3				
4			analysts), and how	
5				
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7				
8	Blinding (masking):	<a href="#">#17b</a>	If blinded, circumstances under which unblinding is	-
9	emergency unblinding		permissible, and procedure for revealing a participant's	
10			allocated intervention during the trial	
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16	<b>Methods: Data</b>			10,15-
17	<b>collection,</b>			16
18	<b>management, and</b>			
19	<b>analysis</b>			
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26	Data collection plan	<a href="#">#18a</a>	Plans for assessment and collection of outcome, baseline,	10, 15
27			and other trial data, including any related processes to	
28			promote data quality (eg, duplicate measurements,	
29			training of assessors) and a description of study	
30			instruments (eg, questionnaires, laboratory tests) along	
31			with their reliability and validity, if known. Reference to	
32			where data collection forms can be found, if not in the	
33			protocol	
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45	Data collection plan:	<a href="#">#18b</a>	Plans to promote participant retention and complete	15
46	retention		follow-up, including list of any outcome data to be	
47			collected for participants who discontinue or deviate from	
48			intervention protocols	
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55	Data management	<a href="#">#19</a>	Plans for data entry, coding, security, and storage,	15-16
56			including any related processes to promote data quality	
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(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

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8	Statistics: outcomes	<a href="#">#20a</a>	15
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16	Statistics: additional	<a href="#">#20b</a>	15
17	analyses		
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21	Statistics: analysis	<a href="#">#20c</a>	10
22			
23	population and		
24	missing data		
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31	<b>Methods: Monitoring</b>		13-16
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34	Data monitoring:	<a href="#">#21a</a>	-15
35	formal committee		
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48	Data monitoring:	<a href="#">#21b</a>	16
49	interim analysis		
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56	Harms	<a href="#">#22</a>	13-14
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1		solicited and spontaneously reported adverse events and	
2		other unintended effects of trial interventions or trial	
3		conduct	
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8	<b>Auditing</b>	<a href="#">#23</a> Frequency and procedures for auditing trial conduct, if	15
9		any, and whether the process will be independent from	
10		investigators and the sponsor	
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15	<b>Ethics and</b>		16
16	<b>dissemination</b>		
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21	Research ethics	<a href="#">#24</a> Plans for seeking research ethics committee / institutional	16
22	approval	review board (REC / IRB) approval	
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26	Protocol amendments	<a href="#">#25</a> Plans for communicating important protocol modifications	15-16
27		(eg, changes to eligibility criteria, outcomes, analyses) to	
28		relevant parties (eg, investigators, REC / IRBs, trial	
29		participants, trial registries, journals, regulators)	
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36	Consent or assent	<a href="#">#26a</a> Who will obtain informed consent or assent from potential	7,23
37		trial participants or authorised surrogates, and how (see	
38		Item 32)	
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44	Consent or assent:	<a href="#">#26b</a> Additional consent provisions for collection and use of	23
45	ancillary studies	participant data and biological specimens in ancillary	
46		studies, if applicable	
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51	Confidentiality	<a href="#">#27</a> How personal information about potential and enrolled	16
52		participants will be collected, shared, and maintained in	
53		order to protect confidentiality before, during, and after the	
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4	Declaration of	<a href="#">#28</a>	Financial and other competing interests for principal
5	interests		investigators for the overall trial and each study site
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9	Data access	<a href="#">#29</a>	Statement of who will have access to the final trial dataset,
10			and disclosure of contractual agreements that limit such
11			access for investigators
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16	Ancillary and post trial	<a href="#">#30</a>	Provisions, if any, for ancillary and post-trial care, and for
17	care		compensation to those who suffer harm from trial
18			participation
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24	Dissemination policy:	<a href="#">#31a</a>	Plans for investigators and sponsor to communicate trial
25	trial results		results to participants, healthcare professionals, the public,
26			and other relevant groups (eg, via publication, reporting in
27			results databases, or other data sharing arrangements),
28			including any publication restrictions
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36	Dissemination policy:	<a href="#">#31b</a>	Authorship eligibility guidelines and any intended use of
37	authorship		professional writers
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42	Dissemination policy:	<a href="#">#31c</a>	Plans, if any, for granting public access to the full protocol,
43	reproducible research		participant-level dataset, and statistical code
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47	<b>Appendices</b>		
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50	Informed consent	<a href="#">#32</a>	Model consent form and other related documentation
51	materials		given to participants and authorised surrogates
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56	Biological specimens	<a href="#">#33</a>	Plans for collection, laboratory evaluation, and storage of
57			biological specimens for genetic or molecular analysis in
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1 the current trial and for future use in ancillary studies, if  
2  
3 applicable  
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6 None The SPIRIT checklist is distributed under the terms of the Creative Commons Attribution

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# BMJ Open

## Effects of oil-soluble versus water-soluble contrast media at hysterosalpingography on pregnancy outcomes in women with a low risk of tubal disease : study protocol for a randomized controlled trial

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1 **Effects of oil-soluble versus water-soluble contrast**  
2 **media at hysterosalpingography on pregnancy**  
3 **outcomes in women with a low risk of tubal disease:**  
4 **study protocol for a randomized controlled trial**

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### 3 **ABSTRACT**

4 **Introduction:** In recent years, due to various factors, the infertility rate in China has  
5 increased and now affects over 10% women of reproductive age.  
6 Hysterosalpingography (HSG) is a common diagnostic procedure during fertility  
7 examinations. However, there is no consensus on the choice of contrast agents and their  
8 effects. As the largest multi-centre randomized controlled trial (H2Oil trial from the  
9 Netherlands) has shown the oil-soluble contrast at HSG can enhance the fertility  
10 comparing to the water-soluble contrast, we propose this study to answer the question  
11 whether the use of oil-soluble contrast media results in increased pregnancy rates in  
12 Chinese women undergo HSG.

13 **Methods and analysis** This study is a single-centre, randomized, controlled, parallel  
14 group, superiority trial. The patients with a low risk of tubal disease will be randomized  
15 to undergo HSG using iodinated oil injection (OSCM group, oil-soluble contrast media)  
16 or ioversol injection (WSCM group, water-soluble contrast media). To evaluate the  
17 potential superiority of OSCM group, with 1:1 allocation ratio, 90% statistical power  
18 and a two-sided significance level of 5%, we have calculated a sample of 520 women  
19 per group for a total of 1040 including 10% loss to follow-up or protocol variation to  
20 be enrolled. The primary outcome is the rate of ongoing pregnancy during 6 months  
21 after randomization. The secondary outcomes will consist of thyroid function of  
22 patients and newborns, pain scores during HSG, live birth rate, clinical pregnancies,

1 miscarriages, ectopic pregnancies, the time to ongoing pregnancy, the time to live birth,  
2 cost calculations of the OSCM group/WSCM group, and assisted reproductive  
3 technology treatments between two groups.

4 **Ethics and dissemination** This protocol received authorisation from the Medical  
5 Research Ethics Committee International Peace Maternity and Child Health Hospital  
6 on 18th January 2020 (approval no. GKLW2020-02). The findings will be reported in  
7 peer-reviewed publications and presentations at international scientific meetings.

8 **Trial registration number** ChiCTR2000031612.

## 10 **STRENGTHS AND LIMITATIONS OF THIS STUDY**

- 11 ● This is a study to assess the thyroid function of patients at different time points who  
12 undergo HSG and their newborns' thyroid function in an RCT (randomized  
13 controlled trial).
- 14 ● This is a study with long-term follow-up of patients who undergo HSG in an RCT  
15 which may explore the cumulative pregnancy rate and the cumulative live birth.
- 16 ● This study will record the pain intensity and when the pain occurs during HSG.
- 17 ● The trial is based in a single centre, which might limit the generalisability of the  
18 findings.
- 19 ● The trial will not be blinded with respect to participants and caregivers.

## 21 **INTRODUCTION**

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4 1 Infertility is defined as the failure to establish a pregnancy after 12 months of regular,  
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6 2 unprotected sexual intercourse or an impairment of a person's capacity to reproduce  
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9 3 either as an individual or with his/her partner [1]. Due to the enactment of the second  
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11 4 child policy in China, the increasing age of mothers, environmental pollution, and  
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14 5 workplace pressures, the infertility rate in China has increased and now affects more  
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17 6 than 10% of reproductive women [2].

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19 7 Hysterosalpingography (HSG) refers to the radiographic evaluation of the uterine  
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22 8 cavity and fallopian tubes after the injection of an oil-/water-soluble contrast medium  
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25 9 through the cervical canal [3]. HSG plays an important role in the evaluation of female  
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28 10 fallopian tube reproductive function and is a common diagnostic procedure in the  
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31 11 fertility examinations [4,5]. In China, it is the preferred method to investigate tubal  
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34 12 patency [6]. Compared with laparoscopy, it is less expensive and has fewer  
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37 13 complications [7]. In addition to diagnostic information, HSG has therapeutic effects,  
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40 14 which are associated with increased fecundability in the months after the procedure  
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43 15 [8,9].

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46 16 Oil-soluble contrast media (OSCM) is represented by iodinated oil injection, which  
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49 17 uses poppy seed oil as the raw material. It provides clear images and may have some  
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52 18 anti-inflammatory effects that perhaps enhance fertility [10]. Due to less absorption of  
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55 19 the contrast media, the occurrence of adverse events is notably reduced [11,12].

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58 20 Water-soluble contrast media (WSCM) is represented by meglumine diatrizoate and  
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61 21 ioversol. The advantages of WSCM are as follows: low viscosity, good fluidity, and  
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64 22 rapid diffusion into the fallopian tubes, which can clearly show the anatomy of the

1 fallopian tubes. However, the rapid flow may result in insufficient observation of  
2 fallopian tube lesions and may result in poor fallopian tube dredging [11].

3 Some studies showed significantly higher pregnancy rates after tubal flushing with  
4 OSCM [13,14]. This might be caused by flushing debris and dislodging mucus plugs  
5 from otherwise undamaged tubes. Additionally, OSCM might have an effect on  
6 peritoneal macrophage activity and on endometrial receptivity, thereby enhancing  
7 fertility [15,16]. Fang's systematic review showed that HSG using OSCM may promote  
8 the ongoing pregnancy rate through a comprehensive analysis of six studies. However,  
9 the review did not include Chinese population and did not report relevant contents about  
10 the thyroid function or long-term effects [17]. To date, in the largest multi-centre  
11 randomized controlled trial (RCT) [8], a total of 1119 women were randomly assigned  
12 to hysterosalpingography with OSCM (557 women) or WSCM (562 women). The  
13 study, which was conducted in the Netherlands, showed higher ongoing pregnancy  
14 rates among women who underwent hysterosalpingography with OSCM as compared  
15 to WSCM.

16 However, numerous studies have not shown a significant difference in rates of ongoing  
17 pregnancy between OSCM and WSCM groups [18-21]. Lindquist found that the rate  
18 of ongoing pregnancy in OSCM group was slightly higher than the WSCM group,  
19 within 20 months after randomization, but the difference was not statistically significant  
20 [20]. In a multicentre RCT carried out by Spring [21], a total of 666 women were  
21 randomly assigned to WSCM group (260 women), OSCM group (273 women) or both  
22 WSCM and OSCM group (133 women). Differences in reproductive outcomes among

1 the groups were not statistically significant.

2 Previous studies showed that women with subclinical hypothyroidism were more prone

3 to OSCM induced overt hypothyroidism [22], which may be due to the long half-life of

4 OSCM excretion [23]. It is known that excess iodine intake during pregnancy can

5 adversely affect thyroid function in both the mother and the foetus [24]. Nevertheless,

6 there have been no large-scale, prospective studies exploring the relationship between

7 HSG contrast agents and the occurrence of thyroid dysfunction in patients and neonates.

8 In summary, there is no consensus on the choice of contrast agents and their effects. In

9 view of this uncertainty, we plan a single-centre, randomized, controlled, parallel

10 group, superiority trial in infertile Chinese women with a low a priori chance of tubal

11 pathology.

## 12 **OBJECTIVE AND HYPOTHESIS**

13 The objective of the trial is to determine whether the use of OSCM during HSG results

14 in a higher ongoing pregnancy rate compared to the use of WSCM for 6 months after

15 randomization. Our hypothesis is that in women undergoing HSG, the use of OSCM

16 will result in a higher ongoing pregnancy rate compared to the use of WSCM.

17

## 18 **METHODS AND ANALYSIS**

### 19 **Study design and setting**

20 The study is a single-centre randomized controlled superiority trial and will be

21 performed in the International Peace Maternity and Child Health Hospital, School of

22 Medicine, Shanghai Jiao Tong University, Shanghai, China. The hospital has two

1 experienced radiologist in HSG. The details of the study design are shown in Figure 1.

2

### 3 **Recruitment**

4 Before participation, patients need to complete a screening questionnaire. Eligible  
5 patients will be asked to participate in this study after receiving oral and written  
6 information from a gynaecologist in outpatient services when tubal testing has been  
7 indicated and will be scheduled. A research nurse will administer oral and written  
8 informed consent. To reach the target sample size, we will advertise our clinical trial  
9 using the hospitals' official accounts on WeChat and distribute brochures in the  
10 outpatient department.

11

### 12 **Participants**

13 Inclusion criteria are as follows:

- 14 1. Age 20 to 39 years old
- 15 2. Spontaneous menstrual cycles (cycle length between 25 and 35 days);
- 16 3. Subfertility of at least one year and a fertile partner (defined as sperm count  $> 15 \times 10^6$   
17 spermatozoa/mL or a post-wash total motile sperm count  $> 3 \times 10^6$   
18 spermatozoa/mL before Intrauterine insemination (IUI)) [25].
- 19 4. Chlamydia trachomatis negative via vaginal secretion culture (through Chlamydia  
20 antigen detection) and no history of Chlamydia infection.
- 21 5. Low risk for tubal pathology according to the medical history. (The patient has not  
22 been exposed to high risk factors of tubal pathology, such as history of chlamydia

1 infection, pelvic inflammatory disease, known endometriosis or adenomyosis,  
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4 1 pelvic abdominal surgery [including salpingostomy or salpingectomy for ectopic  
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6 2 pregnancy and complicated appendectomy] and/or peritonitis) [26].  
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12 4 6. Valid indication for HSG in the fertility examination or before intra uterine  
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14 5 insemination treatment.  
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17 6 7. Signed informed consent.  
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20 7 Exclusion criteria are as follows:  
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23 8 1. Irregular menstrual cycle, less than eight menstrual cycles per year.  
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26 9 2. Endocrino-pathological diseases such as: Polycystic ovary syndrome, Cushing  
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28 10 syndrome, adrenal hyperplasia, hyperprolactinemia, acromegaly, hypothalamic  
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30 11 amenorrhea, hypothyroidy, diabetes mellitus, and thyroid dysfunction.  
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33 12 3. Known or at high risk for tubal pathology, Chlamydia trachomatis positive of  
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35 13 vaginal secretion culture, history of Chlamydia infection.  
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38 14 4. Known contrast (iodine) allergy  
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41 15 5. Male subfertility defined as sperm count  $< 15 \times 10^6$  spermatozoa/mL or a post-wash  
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43 16 total motile sperm count  $< 3 \times 10^6$  spermatozoa/mL before IUI.  
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46 17 6. A contra-indication for HSG.  
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49 18 7. Not willing or able to sign the consent form.  
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## 53 20 **Randomization**

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56 21 Randomization will take place on the day of HSG. Demographic, medical,  
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58 22 gynaecological and infertility information will be collected at baseline. The patient will  
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1 then be randomized between two groups (OSCM or WSCM). Randomization will be  
2 performed by an independent statistician using a web-based Research Electronic Data  
3 Capture (REDCap) system. The allocation ratio will be 1:1 and permuted blocks of 4-  
4 6 will be used. Prior to randomization, screening data will be entered in the digital  
5 platform. The randomization list will be sealed in sequentially numbered opaque  
6 envelopes. The envelopes will be stored in a double-locked cabinet and will only be  
7 opened by the practitioner to assign participants to each group after obtaining informed  
8 consent and having been screened for eligibility. The opened envelopes will again be  
9 separately stored in a double-locked cabinet.

### 11 **Blinding**

12 Owing to the difference in imaging between the use of OSCM and WSCM, and since  
13 the outcome measure of ongoing pregnancy will be an objective measure, the trial will  
14 not be blinded with respect to participants and caregivers.

### 16 **Intervention**

17 All patients will undergo HSG. Patients allocated to OSCM will undergo HSG with  
18 iodinated oil injection, a solution of Ethiodol that contains poppy seed oil and iodine  
19 (480 I mg/ml) (Heng Rui Pharmaceuticals, Jiangsu, China). Patients allocated to  
20 WSCM will undergo HSG with ioversol injection, a solution of low osmolar contrast  
21 medium that contains iodine (320 mg I/mL) (Heng Rui Pharmaceuticals, Jiangsu,  
22 China). HSG will be performed by a radiologist, without premedication, on the 3rd-7th



1 days after complete cessation of menstrual bleeding, and before the 14th day in the  
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1 days after complete cessation of menstrual bleeding, and before the 14th day in the  
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1 HSG will be performed as follows: 1) the patient will be placed in the supine position;  
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2) Routine disinfection will be performed in the bladder lithotomy area of the patient;  
3) Vaginal speculum will be used to expose the vagina and cervix uteri, then  
disinfection will be implemented; 4) A rubber double-lumen tube or a special catheter  
will be inserted into the cervix uteri and will then be fixated; 5) Up to 10 ml of contrast  
medium will be slowly injected into the uterus under appropriate pressure until  
adequate uterine filling has occurred or contrast medium has flowed into the pelvic  
cavity. This will be directly monitored by fluoroscopy; 6) During the infusion, the  
dynamic flow of the course of the contrast medium into the uterine cavity and fallopian  
tube will be observed, and images will be captured before the contrast agent is injected  
and after the uterine cavity is filled, while the Fallopian tube appears; 7) When the  
images overlap, the tube or bed position will be changed as needed. After the procedure,  
images will be reviewed by a radiologist, and a diagnosis will be established.

### 17 **Withdrawal of participants**

18 Participants will be free to withdraw from the study at any time upon request. An  
19 investigator may discontinue or withdraw a participant from the study for the following  
20 reasons: the participant meets an exclusion criterion; violation of research protocol; the  
21 patient is experiencing an urgent medical situation.

22 The reasons for participant discontinuation or withdrawal from the study will be

1 recorded on the Case Report Form (CRF). Data collection will continue if data can be  
 2 safely acquired, and the data will be used for the ITT (intention-to-treat) analyses.

3  
 4 **Participant timeline**

5 The schedules for enrolment, interventions and assessments are summarised in Table  
 6 1. The investigators will record the information in the CRFs and source documents.

7 **Table 1 Schedule of Activities**

Procedures	Screening Day -7 to -1	Enrollment/Ba seline Visit 1 - Baseline	Study Visit 2 Months 6	Study Visit 3 Months 12	Study Visit 3 Months 24	Study Visit 3 Months 36
Informed consent	X					
Demographics	X					
Medical history	X					
Randomization		X				
Study intervention		X				
Ongoing pregnancy			X			
Thyroid function of patients	X		X	X	X	X
Neonatal thyroid function				X	X	X
VAS for pain scores during the procedure		X				
Live birth				X		X
Clinical pregnancy			X			X
Miscarriage			X			X
Ectopic pregnancy			X			X
Safety of intervention		X				X
Additional therapies (assisted reproductive technology/ operation)			X			X
Cost calculation		X				X
Complete Case Report Files (CRFs)	X					X

8  
 9 The randomization and HSG will be performed on Day 1. A review of patient  
 10 information will be done prior to enrolment to determine preliminary eligibility  
 11 according to the inclusion and exclusion criteria. When a patient provides informed

1 consent, she will be considered to be enrolled in the study. Detailed clinical information  
2 including age, body mass, duration of infertility, previous surgery, and information on  
3 her partner will be collected. Follow-up and measurements will be the same for both  
4 groups with a total follow-up of 3 years. Data will be collected at baseline, 6, 12, 24,  
5 and 36 months by telephone follow-up survey or e-questionnaire survey. Procedure  
6 steps, pain scores, and complications will be recorded. All additional therapies or  
7 transfer to other treatments after the first intervention will be recorded in both groups,  
8 such as Intrauterine insemination, in vitro IVF (in vitro fertilization), ICSI  
9 (Intracytoplasmic sperm injection), and operation (laparoscopic or hysteroscopic  
10 surgery).

## 12 **OUTCOME MEASURES**

### 13 **Primary outcome measures**

14 The primary outcome is the rate of ongoing pregnancy in each treatment group.  
15 Ongoing pregnancy will be defined as a registered heartbeat on ultrasound beyond 12  
16 weeks of gestation with the first day of the last menstrual cycle as the beginning of the  
17 pregnancy within 6 months after randomization.

### 19 **Secondary outcome measures**

20 1. The thyroid function of patients will be examined before HSG, and at 4, 8, 12, and  
21 24 weeks, and 9–12 months after HSG. We will detect free triiodothyronine (FT3),  
22 free thyroxine (FT4), thyroid stimulating hormone (TSH), antithyroglobulin

- 1 antibodies (TgAb), and antithyroid peroxidase antibodies (TPOAb) levels at  
2 different time-points [27,28]. Also, we will record the thyroid function of patients  
3 as possible as we can to make the data more accurate.
- 4 2. The thyroid function of the neonates will be tested within 3 to 7 days after birth.  
5 TT4 (total thyroxine), FT4, and TSH will be detected [28].
- 6 3. Pain scores during hysterosalpingography will be measured by means of the Visual-  
7 Analogue Scale for Pain (scores range from 0.0 to 10.0 cm, with higher scores  
8 indicating more severe pain). The pain scores will be recorded by a trained nurse.  
9 Meanwhile, the time from the beginning of the contrast injection to the occurrence  
10 of pain will be recorded.
- 11 4. The rates of live birth rate/ clinical pregnancy / miscarriages/ ectopic pregnancy  
12 will be assessed. Live birth is defined as the birth of at least one living child; clinical  
13 pregnancy is defined as an ultrasound visible gestational sac; miscarriage is defined  
14 as a spontaneous loss of pregnancy; ectopic pregnancy is defined as an embryo  
15 implanted outside the uterine cavity. Each occurrence of one of these events will be  
16 recorded during the three-year follow-up.
- 17 5. The time to ongoing pregnancy is defined as the time from randomization to the  
18 first day of the last menstrual period plus 4 weeks. It will be considered when  
19 ongoing pregnancy occurs. The time to first live birth is defined as the time from  
20 randomization to the date of the first live birth. It will be considered when live birth  
21 occurs.
- 22 6. The costs and cost-effectiveness of OSCM / WSCM and assisted reproductive

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4 1 technology (ART) treatments in Chinese context.  
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6 2 7. The side effects or complications after therapy in both groups.  
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## 11 4 **Safety assessments**

12 5 All adverse events (AEs) will be recorded during the entire study period. An adverse  
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14 6 event is defined as an event during or following hysterosalpingography or follow-up  
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16 7 which was not intended to happen and is suspected to be a complication of the  
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18 8 intervention performed. Common adverse events of HSG include allergic reactions,  
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20 9 artificial abortion syndrome, abdominal pain, and intravasation. The severity of the AEs  
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22 10 will be primarily ranked as ‘mild’, ‘moderate’, or ‘severe’. The causes of the AEs will  
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24 11 be rated as ‘definitely related’, ‘probably related’, ‘possibly related’, ‘probably not  
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26 12 related’, ‘definitely not related’, or ‘unknown’.  
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30 13 A severe adverse event (SAE) is defined as death, illness necessitating hospitalization,  
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32 14 disability, or congenital malformation. All SAEs will be reported to the ethics  
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34 15 committee that approved the protocol within 24 hours.  
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## 45 17 **STATISTICAL CONSIDERATIONS**

### 46 18 **Sample size calculation**

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48 19 In Dreyer’s research, the rate of ongoing pregnancy in the WSCM group was 29 %. To  
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50 20 detect a difference of 10% between the OSCM group in rates of ongoing pregnancies  
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52 21 as compared with WSCM group, with 1:1 allocation ratio, 90% statistical power, and a  
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54 22 two-sided significance level of 5%, we calculated that 467 women per group for a total  
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1 of 934 will need to be enrolled. Thus, 1040 women (520 in each group) will need to be  
2 randomized, including 10% loss to follow-up or protocol variation.

#### 4 **Statistical analysis**

5 All data will be analysed according to the intention-to-treat principle. SPSS software,  
6 version 22.0 or higher (IBM; Armonk, NR), and R version 3.3.1 (R Project for  
7 Statistical Computing, Vienna, Austria), will be used for statistical analyses. Two-sided  
8 P values of less than 0.05 will indicate statistical significance.

#### 9 **Statistical analysis of the first 6 months**

10 As for the primary outcome, we will use the chi-square test to compare the rate of  
11 ongoing pregnancy between the OSCM group and the WSCM. We will use Kaplan–  
12 Meier curves with a log-rank test to compare the difference of the time to ongoing  
13 pregnancy between the two groups.

14 We will use the chi-square test to compare the rate of clinical pregnancy, the rate of  
15 miscarriages and the rate of ectopic pregnancy during 6 months after randomization  
16 between the two groups. As for the pain scores during hysterosalpingography,  
17 independent t-test or the Mann–Whitney U-test will be used to compare the average  
18 pain scores and the mean interval between the beginning of the contrast injection and  
19 the occurrence of pain [29].

#### 20 **Statistical analysis of the 3-year follow-up**

21 We will calculate cumulative pregnancy rate of the two groups at one year, at two years  
22 and at three years, and use the chi-square test to assess statistical significance. The

1  
2  
3  
4 1 cumulative live birth rate will be analysed as the cumulative pregnancy rate. We will  
5  
6 2 use Kaplan–Meier curves with a log-rank test to compare the difference of the time to  
7  
8 3 first live birth between the two groups. During the 3-year follow-up, we will record  
9  
10 4 every occurrence of clinical pregnancy or miscarriage or ectopic pregnancy, which may  
11  
12 5 be considered as exploratory outcomes. We may not focus on the multiplicity of type I  
13  
14 6 error on these outcomes. As for the thyroid function of the patients, we will use  
15  
16 7 ANOVA to compare it at different time-points [27].

17  
18 8 Categorical data will be described as absolute numbers and percentages. Distributed  
19  
20 9 continuous variables will be reported as medians with interquartile ranges.

#### 21 22 23 24 25 26 27 10 **Subgroup analysis**

28  
29  
30 11 To identify a subgroup effect, we plan to test for an interaction for the following  
31  
32 12 subgroups: (1) age of the patients (<35years, and  $\geq 35$ years); (2) primary versus  
33  
34 13 secondary fertility. We may perform some other subgroups when we finally analyse  
35  
36 14 data.

#### 37 38 39 40 15 **Missing data analysis**

41  
42  
43 16 We will use Multiple Imputation (MI) to process the missing data.

#### 44 45 46 17 **Data monitoring and auditing**

47  
48 18 Data monitoring and auditing will be conducted for quality assurance. Monitoring staff  
49  
50 19 will consist of an Independent Data Monitoring Committee (IDMC) and an ethics  
51  
52 20 committee. The IDMC is composed of five members, including one statistician, three  
53  
54 21 clinical experts and one ethicist. They will visit the institutions at important time points  
55  
56 22 throughout the trial, for example, at participant enrolment, at the study interim point,  
57  
58  
59  
60

1 and at study completion. Monitoring staff will ensure consistency concerning data  
2 documented in both the CRF and the source document and will ensure that the entire  
3 study process is in accordance with the approved protocol.

4 An Independent Data Monitoring Committee (IDMC) and ethics committee will review  
5 data trimonthly during the accrual period and near the time that is planned for interim  
6 analyses. At each meeting, the IDMC will be asked to give advice on whether the  
7 accumulated data from the trial, together with results from other relevant trials, justifies  
8 continuing recruitment of further patients or further follow-up. An interim analysis on  
9 the ongoing pregnancy rate is planned for the time when approximately 50%  
10 participants will be recruited. To control for overall type I error, the stopping boundaries  
11 for interim and final analyses will be computed using the Lan-DeMets approximation  
12 to the O'Brien-Fleming boundary.

13 Maintenance of participant confidentiality will involve: (1) asking subjects to only  
14 share personal and study-related information during our study; (2) storing data in  
15 password-protected files on a designated specific computer with restricted access; (3)  
16 only the research-related person will have access to personal identifiable information,  
17 which will be destroyed once the study is completed.

## 19 **Ethics and dissemination**

20 This protocol received authorisation from the Medical Research Ethics Committee  
21 International Peace Maternity and Child Health Hospital on 18th January 2020  
22 (approval no. GKLW2020-02). The findings will be reported in peer-reviewed



1 publications and presentations at international scientific meetings.

### 3 **Patient and public involvement**

4 Neither the patients nor the public will be involved in the study design. They will also  
5 not be involved in the recruitment process or conduct of the study. The results will be  
6 disseminated to patients via an open access publication and our local trials teams.

## 8 **DISCUSSION**

9 HSG is widely used during the fertility assessment. Most clinics in China use WSCM  
10 but some use OSCM. Until now, no consensus has been reached on which reagent is  
11 better and for which patients. Dreyer's research [8] was the largest RCT about the  
12 effects between the two kinds of contrast at HSG; however, it remains unclear if the  
13 results apply to the Chinese population. Therefore, the aim of this study is to determine  
14 whether at HSG using OSCM results in higher ongoing pregnancy rates compared to  
15 the use of WSCM during 6 months after randomization and to explore the difference of  
16 maternal and neonatal thyroid function between the two groups.

17 The strengths of this study are as follows: First, this will be the first study to assess  
18 thyroid function of patients at different time points who undergo HSG and assess their  
19 newborns' thyroid function in a RCT. Second, compared to other studies, we will  
20 extend the follow-up period to evaluate the long-term impacts of HSG using different  
21 reagents. Finally, this will be the first record of pain intensity during HSG and the time  
22 interval of when pain occurs.

1 The main shortcomings of this study are that the trial will not be blinded with respect  
2 to participants and caregivers because of the difference in imaging between OSCM and  
3 WSCM and the objectivity of our outcomes. Another limitation is that the trial is based  
4 in a single centre, which might limit the generalisability of the findings.

5 Therefore, we have designed this study to provide rigorous medical evidence for the  
6 future clinical application of hysterosalpingography.

7

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2

### 3 **Date and version**

4 08-26-2020; Version 1.2.

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### 16 **Authors' contributions**

17 Jian Zhang was conceived of the study and participated in its design, as well as  
18 supervised the study and critically revised the manuscript. Zhaoxia Qian critically  
19 revised the manuscript and contributed to the HSG examination. Guiling Liang  
20 participated in writing the manuscript; Ling Jiang contributed to HSG examination;  
21 Qian Zhu and Xiaoqing He contribute to follow up the patients; Chenfeng Zhu and  
22 Xiaofeng Wang contributed to data collection; Li Xie contributed to revised the

1  
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3  
4 1 statistical methods. All authors read and approved the final version of the manuscript.

5  
6  
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8  
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10  
11 4 (shslczdzk01802), International peace maternal and child health hospital clinical  
12  
13  
14 5 applied research program (YN201915).

15  
16  
17 6 **Competing interests**

18  
19  
20 7 The authors declare that they have no competing interests.

21  
22 8 **Patient consent for publication**

23  
24  
25 9 Patients' consent will be obtained for this trial. We may get an extra informed consent  
26  
27 10 for other studies. We have uploaded model consent form in the register website (Trial  
28  
29 11 registration number ChiCTR2000031612).

30  
31  
32 12 **Ethics approval**

33  
34  
35 13 The study was approved by the Medical Research Ethics Committee of International  
36  
37 14 Peace Maternity and Child Health Hospital (approval no.GKLW2020-02).

38  
39  
40 15 **Trial status**

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42  
43 16 The recruitment starts from August 1<sup>st</sup> and we expect to complete the recruitment in  
44  
45 17 August 2021.

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48 18 **Data sharing statement**

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51 19 We plan to publish the primary report when all data on pregnancy resulting from the  
52  
53 20 first 6 months are available (including secondary pregnancy outcomes) and an  
54  
55  
56 21 additional report when 3-year follow-up data are available. The public can obtain access  
57  
58 22 to the full protocol, participant-level dataset, and statistical code via emailing the major  
59  
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4 1 researchers.  
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12 4 **FIGURE LEGENDS**  
13

14 5 **Figure 1 Flowchart**  
15

16  
17 6 HSG: hysterosalpingography. Ongoing pregnancy is defined as a registered heartbeat  
18  
19 7 on ultrasound beyond 12 weeks of gestation. Clinical pregnancy is defined as an  
20  
21 8 ultrasound visible gestational sac. Miscarriage is defined as a spontaneous loss of  
22  
23 9 pregnancy. Ectopic pregnancy is defined as an embryo implants outside the uterine  
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25 10 cavity. Live birth is defined as the birth of at least one living child.  
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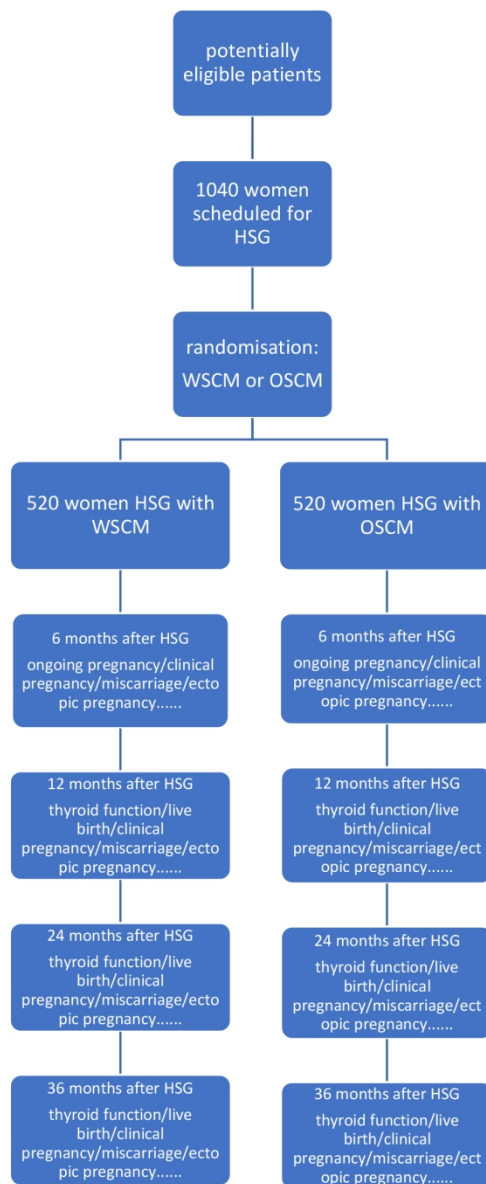


Figure 1 Flowchart

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HSG: hysterosalpingography. Ongoing pregnancy is defined as a registered heartbeat on ultrasound beyond 12 weeks of gestation. Clinical pregnancy is defined as an ultrasound visible gestational sac. Miscarriage is defined as a spontaneous loss of pregnancy. Ectopic pregnancy is defined as an embryo implants outside the uterine cavity. Live birth is defined as the birth of at least one living child.

# Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. *Ann Intern Med.* 2013;158(3):200-207

		Page
	Reporting Item	Number
<b>Administrative information</b>		
Title	<a href="#">#1</a> Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

1	Trial registration	<a href="#">#2a</a>	Trial identifier and registry name. If not yet registered,	3
2			name of intended registry	
3				
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6	Trial registration: data	<a href="#">#2b</a>	All items from the World Health Organization Trial	-
7	set		Registration Data Set	
8				
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12	Protocol version	<a href="#">#3</a>	Date and version identifier	22
13				
14				
15	Funding	<a href="#">#4</a>	Sources and types of financial, material, and other support	23
16				
17				
18	Roles and	<a href="#">#5a</a>	Names, affiliations, and roles of protocol contributors	23
19	responsibilities:			
20	contributorship			
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26	Roles and	<a href="#">#5b</a>	Name and contact information for the trial sponsor	1
27	responsibilities:			
28	sponsor contact			
29	information			
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36	Roles and	<a href="#">#5c</a>	Role of study sponsor and funders, if any, in study design;	24
37	responsibilities:		collection, management, analysis, and interpretation of	
38	sponsor and funder		data; writing of the report; and the decision to submit the	
39			report for publication, including whether they will have	
40			ultimate authority over any of these activities	
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48	Roles and	<a href="#">#5d</a>	Composition, roles, and responsibilities of the coordinating	16-17
49	responsibilities:		centre, steering committee, endpoint adjudication	
50	committees		committee, data management team, and other individuals	
51			or groups overseeing the trial, if applicable (see Item 21a	
52			for data monitoring committee)	
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1	<b>Introduction</b>			3-6
2				
3				
4	Background and	<a href="#">#6a</a>	Description of research question and justification for	3-6
5	rationale		undertaking the trial, including summary of relevant studies	
6			(published and unpublished) examining benefits and harms	
7			for each intervention	
8				
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11	Background and	<a href="#">#6b</a>	Explanation for choice of comparators	3-6
12	rationale: choice of			
13	comparators			
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16				
17	Objectives	<a href="#">#7</a>	Specific objectives or hypotheses	6
18				
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20				
21				
22	Trial design	<a href="#">#8</a>	Description of trial design including type of trial (eg, parallel	6
23			group, crossover, factorial, single group), allocation ratio,	
24			and framework (eg, superiority, equivalence, non-inferiority,	
25			exploratory)	
26				
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34	<b>Methods:</b>			6-13
35				
36	<b>Participants,</b>			
37	<b>interventions, and</b>			
38	<b>outcomes</b>			
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44	Study setting	<a href="#">#9</a>	Description of study settings (eg, community clinic,	6
45			academic hospital) and list of countries where data will be	
46			collected. Reference to where list of study sites can be	
47			obtained	
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54	Eligibility criteria	<a href="#">#10</a>	Inclusion and exclusion criteria for participants. If	7-8
55			applicable, eligibility criteria for study centres and	
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1		individuals who will perform the interventions (eg,	
2			
3		surgeons, psychotherapists)	
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6	Interventions:	<a href="#">#11a</a> Interventions for each group with sufficient detail to allow	9-10
7			
8	description	replication, including how and when they will be	
9			
10		administered	
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12			
13	Interventions:	<a href="#">#11b</a> Criteria for discontinuing or modifying allocated	10
14			
15	modifications	interventions for a given trial participant (eg, drug dose	
16			
17		change in response to harms, participant request, or	
18			
19		improving / worsening disease)	
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23	Interventions:	<a href="#">#11c</a> Strategies to improve adherence to intervention protocols,	16-17
24			
25	adherence	and any procedures for monitoring adherence (eg, drug	
26			
27		tablet return; laboratory tests)	
28			
29			
30			
31	Interventions:	<a href="#">#11d</a> Relevant concomitant care and interventions that are	11-12
32			
33	concomitant care	permitted or prohibited during the trial	
34			
35			
36	Outcomes	<a href="#">#12</a> Primary, secondary, and other outcomes, including the	12-13
37			
38		specific measurement variable (eg, systolic blood	
39			
40		pressure), analysis metric (eg, change from baseline, final	
41			
42		value, time to event), method of aggregation (eg, median,	
43			
44		proportion), and time point for each outcome. Explanation	
45			
46		of the clinical relevance of chosen efficacy and harm	
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48		outcomes is strongly recommended	
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53	Participant timeline	<a href="#">#13</a> Time schedule of enrolment, interventions (including any	11-12
54			
55		run-ins and washouts), assessments, and visits for	
56			
57		participants. A schematic diagram is highly recommended	
58			
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(see Figure)

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3			
4	Sample size	<a href="#">#14</a>	Estimated number of participants needed to achieve study 14
5			
6			objectives and how it was determined, including clinical and
7			
8			statistical assumptions supporting any sample size
9			
10			calculations
11			
12			
13	Recruitment	<a href="#">#15</a>	Strategies for achieving adequate participant enrolment to 7
14			
15			reach target sample size
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17			
18	<b>Methods: Assignment</b>		8-9
19			
20	<b>of interventions (for</b>		
21			
22	<b>controlled trials)</b>		
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26	Allocation: sequence	<a href="#">#16a</a>	Method of generating the allocation sequence (eg, 8-9
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28	generation		computer-generated random numbers), and list of any
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30			factors for stratification. To reduce predictability of a
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32			random sequence, details of any planned restriction (eg,
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34			blocking) should be provided in a separate document that is
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36			unavailable to those who enrol participants or assign
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38			interventions
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43	Allocation	<a href="#">#16b</a>	Mechanism of implementing the allocation sequence (eg, 8-9
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45	concealment		central telephone; sequentially numbered, opaque, sealed
46			
47	mechanism		envelopes), describing any steps to conceal the sequence
48			
49			until interventions are assigned
50			
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52			
53	Allocation:	<a href="#">#16c</a>	Who will generate the allocation sequence, who will enrol 8-9
54			
55	implementation		participants, and who will assign participants to
56			
57			interventions
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1	Blinding (masking)	<a href="#">#17a</a>	Who will be blinded after assignment to interventions (eg,	9
2			trial participants, care providers, outcome assessors, data	
3			analysts), and how	
4				
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7				
8	Blinding (masking):	<a href="#">#17b</a>	If blinded, circumstances under which unblinding is	-
9	emergency		permissible, and procedure for revealing a participant's	
10			allocated intervention during the trial	
11	unblinding			
12				
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15				
16	<b>Methods: Data</b>			10-16
17	<b>collection,</b>			
18	<b>management, and</b>			
19	<b>analysis</b>			
20				
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26	Data collection plan	<a href="#">#18a</a>	Plans for assessment and collection of outcome, baseline,	10-12
27			and other trial data, including any related processes to	
28			promote data quality (eg, duplicate measurements, training	
29			of assessors) and a description of study instruments (eg,	
30			questionnaires, laboratory tests) along with their reliability	
31			and validity, if known. Reference to where data collection	
32			forms can be found, if not in the protocol	
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43	Data collection plan:	<a href="#">#18b</a>	Plans to promote participant retention and complete follow-	10
44	retention		up, including list of any outcome data to be collected for	
45			participants who discontinue or deviate from intervention	
46			protocols	
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53	Data management	<a href="#">#19</a>	Plans for data entry, coding, security, and storage,	10-11
54			including any related processes to promote data quality	
55			(eg, double data entry; range checks for data values).	
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1		Reference to where details of data management	
2			
3		procedures can be found, if not in the protocol	
4			
5			
6	Statistics: outcomes	<a href="#">#20a</a> Statistical methods for analysing primary and secondary	15-16
7			
8		outcomes. Reference to where other details of the	
9			
10		statistical analysis plan can be found, if not in the protocol	
11			
12			
13	Statistics: additional	<a href="#">#20b</a> Methods for any additional analyses (eg, subgroup and	16
14			
15	analyses	adjusted analyses)	
16			
17			
18			
19	Statistics: analysis	<a href="#">#20c</a> Definition of analysis population relating to protocol non-	10,16
20			
21	population and	adherence (eg, as randomised analysis), and any statistical	
22			
23	missing data	methods to handle missing data (eg, multiple imputation)	
24			
25			
26	<b>Methods: Monitoring</b>		14-17
27			
28			
29	Data monitoring:	<a href="#">#21a</a> Composition of data monitoring committee (DMC);	16
30			
31	formal committee	summary of its role and reporting structure; statement of	
32			
33		whether it is independent from the sponsor and competing	
34			
35		interests; and reference to where further details about its	
36			
37		charter can be found, if not in the protocol. Alternatively, an	
38			
39		explanation of why a DMC is not needed	
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44	Data monitoring:	<a href="#">#21b</a> Description of any interim analyses and stopping	17
45			
46	interim analysis	guidelines, including who will have access to these interim	
47			
48		results and make the final decision to terminate the trial	
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51	Harms	<a href="#">#22</a> Plans for collecting, assessing, reporting, and managing	14
52			
53		solicited and spontaneously reported adverse events and	
54			
55		other unintended effects of trial interventions or trial	
56			
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1		conduct	
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4	<b>Auditing</b>	<a href="#">#23</a> Frequency and procedures for auditing trial conduct, if any,	16
5		and whether the process will be independent from	
6			
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8		investigators and the sponsor	
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11	<b>Ethics and</b>		16
12			
13	<b>dissemination</b>		
14			
15			
16	<b>Research ethics</b>	<a href="#">#24</a> Plans for seeking research ethics committee / institutional	17
17			
18	<b>approval</b>	review board (REC / IRB) approval	
19			
20			
21			
22	<b>Protocol</b>	<a href="#">#25</a> Plans for communicating important protocol modifications	16-17
23			
24	<b>amendments</b>	(eg, changes to eligibility criteria, outcomes, analyses) to	
25			
26		relevant parties (eg, investigators, REC / IRBs, trial	
27			
28		participants, trial registries, journals, regulators)	
29			
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31			
32	<b>Consent or assent</b>	<a href="#">#26a</a> Who will obtain informed consent or assent from potential	24
33			
34		trial participants or authorised surrogates, and how (see	
35			
36		Item 32)	
37			
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39	<b>Consent or assent:</b>	<a href="#">#26b</a> Additional consent provisions for collection and use of	24
40			
41	<b>ancillary studies</b>	participant data and biological specimens in ancillary	
42			
43		studies, if applicable	
44			
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46			
47	<b>Confidentiality</b>	<a href="#">#27</a> How personal information about potential and enrolled	17
48			
49		participants will be collected, shared, and maintained in	
50			
51		order to protect confidentiality before, during, and after the	
52			
53		trial	
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57	<b>Declaration of</b>	<a href="#">#28</a> Financial and other competing interests for principal	24-25
58			
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1	interests		investigators for the overall trial and each study site	
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4	Data access	<a href="#">#29</a>	Statement of who will have access to the final trial dataset,	24
5			and disclosure of contractual agreements that limit such	
6			access for investigators	
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11	Ancillary and post	<a href="#">#30</a>	Provisions, if any, for ancillary and post-trial care, and for	-
12			compensation to those who suffer harm from trial	
13	trial care		participation	
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19	Dissemination policy:	<a href="#">#31a</a>	Plans for investigators and sponsor to communicate trial	24
20			results to participants, healthcare professionals, the public,	
21	trial results		and other relevant groups (eg, via publication, reporting in	
22			results databases, or other data sharing arrangements),	
23			including any publication restrictions	
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31	Dissemination policy:	<a href="#">#31b</a>	Authorship eligibility guidelines and any intended use of	24
32			professional writers	
33	authorship			
34				
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36	Dissemination policy:	<a href="#">#31c</a>	Plans, if any, for granting public access to the full protocol,	24
37			participant-level dataset, and statistical code	
38	reproducible research			
39				
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42	<b>Appendices</b>			
43				
44				
45	Informed consent	<a href="#">#32</a>	Model consent form and other related documentation given	24
46			to participants and authorised surrogates	
47	materials			
48				
49				
50	Biological specimens	<a href="#">#33</a>	Plans for collection, laboratory evaluation, and storage of	-
51			biological specimens for genetic or molecular analysis in	
52			the current trial and for future use in ancillary studies, if	
53			applicable	
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