BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-039166
Article Type:	Protocol
Date Submitted by the Author:	07-Apr-2020
Complete List of Authors:	Liang, Guiling; International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Department of Obstetrics and Gynecology Zhu, Qian; International Peace Maternity and Child Health Hospital/School of Medicine, Shanghai Jiaotong University, Department of Obstetrics and Gynecology He, Xiaoqing; International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Department of Obstetrics and Gynecology Wang, Xiaofeng; International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Department of Obstetrics and Gynecology Jiang, Ling; International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Department of Radiology Zhu, Chenfeng; International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Department of Obstetrics and Gynecology Zhang, Jian; International Peace Maternity and Child Health Hospital/School of Medicine, Shanghai Jiaotong University, Department of Obstetrics and Gynecology
Keywords:	Subfertility < GYNAECOLOGY, Diagnostic radiology < RADIOLOGY & IMAGING, GYNAECOLOGY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

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

Guiling Liang[1,2,3], Qian Zhu[1,2,3,] Xiaoqing He[1,2,3,] Xiaofeng Wang[1,2,3,] Ling Jiang[2,3,4,] Chenfeng Zhu[1,2,3,] Jian Zhang[1,2,3]*

[1] Department of Obstetrics and Gynecology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

[2] Shanghai Key Laboratory Embryo Original Diseases, Shanghai, China

[3] Shanghai Municipal Key Clinical Specialty, Shanghai, China

[4] Department of Radiology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

* Corresponding author:

Jian Zhang, Department of Obstetrics and Gynecology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China;

Email: zhangjian_sjtu@126.com

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

pathology. HSG is a commonly practiced diagnostic procedure in the fertility work-up. However, there is no uniform conclusion on the choice of contrast agents and their effects. This study 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.

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

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

Trial registration number ChiCTR2000031612.

Strengths and limitations of this study

- 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.
- This is the first time to assess the thyroid function of newborns and patients at different time points who underwent HSG.
- The trial is based in a single centre, which might limit the generalisability of the findings.
- Owing to the difference in imaging between the use of oil-based contrast and water-

based contrast, and, since our outcome of ongoing pregnancy was objective, the trial was not blinded with respect to participants and caregivers.

Introduction

Infertility is defined as the failure to establish a pregnancy after 12 months of regular, unprotected sexual intercourse or an impairment of a person's capacity to reproduce either as an individual or with his/her partner [1]. 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. In China, the majority of infertility is caused by tubal pathology [2].

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

Ultra-fluid lipiodol, one kind of oil-soluble contrast media, uses poppy seed oil as raw materials. Due to less absorption of contrast, the occurrence of adverse events reduced notably [9,10]. Water-soluble contrast media is represented by meglumine diatrizoate and ioversol. The advantages of them are as follows: low viscosity, good fluidity and rapid diffusion into the fallopian tubes, which can clearly show the anatomy of the fallopian tubes. But rapid flow may result in insufficient observation of fallopian tube lesions and poor fallopian tube dredging [9].

Some studies showed significantly higher pregnancy rates after tubal flushing with oil contrast [11,12]. This might be caused by flushing debris and dislodge mucus plugs

from otherwise undamaged tubes. Also, the oil contrast might have an effect on peritoneal macrophage activity and on endometrial receptivity, thereby enhancing fertility [13,14]. To date, in the largest multi-centre (only conducted in the Netherlands) randomised clinical trial (RCT) [7], a total of 1119 women were randomly assigned to hysterosalpingography with oil contrast (557 women) or water contrast (562 women). This study showed higher ongoing pregnancy rates among women who underwent hysterosalpingography with oil-based contrast as compared to water-based contrast.

However, there are numerous studies did not show a significant difference in rates of ongoing pregnancy between OSCM (oil-soluble contrast media) and WSCM (water-soluble contrast media) groups. The results of the study carried out by Lindquist showed that compared with WSCM, the rate of ongoing pregnancy in OSCM group was slightly increased within 20 months after randomization, but there was no statistical difference between the two groups [15]. In a multicentre RCT carried out by Spring [16], a total of 666 women were randomly assigned to WSCM group (260 women), OSCM group water contrast (273 women) or both WSCM and OSCM group (133 women). Differences in reproductive outcome among contrast material groups were not statistically significant.

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

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

use of water-based contrast media.

Objective and hypothesis

The objective of the trial is to determine whether at HSG the use of oil-based contrast media results in higher ongoing pregnancy rates compared to the use of water-based contrast media during 6 months after randomisation. Our hypothesis is that in women undergoing HSG the use of oil-based contrast media results in higher ongoing pregnancy compared to the use of water-based contrast media.

Methods and analysis

Study design and setting

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

Participants

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

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

insemination treatment.

7. Signed informed consent.

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

1. Irregular menstrual cycle, less than eight menstrual cycles per year.
2. Endocrino-pathological disease as: Polycystic ovary syndrome, Cushing syndrome, adrenal hyperplasia, hyperprolactinemia, acromegaly, hypothalamic amenorrhea, hypothyroidy, diabetes mellitus, thyroid dysfunction.
3. Known or high risk for tubal pathology, Chlamydia trachomatis positive of vaginal secretion culture, Chlamydia infection in the history.
4. Known contrast (iodine) allergy
5. Male subfertility defined as a post-wash total motile sperm count < 3 x10^6 spermatozoa/ml
6. A contra-indication for HSG.
7. Not willing or able to sign the consent form.

Randomization

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

Intervention

All patients will undergo a HSG. Patients allocated to WSCM undergo the HSG with Ioversol Injection, a solution of low osmolar contrast medium which contains iodine

(320mg I/ml) (Heng Rui Pharmaceuticals, Jiangsu, China). Patients allocated to OSCM will undergo HSG with Iodinated Oil Injection, a solution of Ethiodol and contains poppy seed oil and iodine (480 I mg/ml) (Heng Rui Pharmaceuticals, Jiangsu, China). The HSG will be performed by a radiologist. HSG will be performed without premedication, in 3-7th day after complete cessation of menstrual bleeding, and before the 14th day in the ovulatory cycle. After cleansing the vagina and cervix, a vacuum cervix adapter will be applied to the cervix or a balloon catheter will be inserted according to local protocol. Up to 10 ml of contrast medium will be slowly injected into the uterus and directly monitored by fluoroscopy. Four to six X-ray photos will be taken and assessed by a fertility specialist and radiologist. Visual analogue and qualitative scales will be used after the procedure to assess study participants pain perception.

Withdrawal of participants

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

1. Participant withdraw informed consent;
2. Participant requests to withdraw from the study;
3. If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation;
4. Violation of research protocol that may interfere with the health of the patient if the study is continued; however data collection will continue if this can safely be acquired and the data will be used for the ITT analyses
5. Subject intervention termination criteria: those who have safety problems in treatment and need to terminate treatment. Investigator can decide to withdraw a participant from the study for urgent medical reasons, however data collection will continue if this can safely be acquired and the data will be used for the ITT analyses.

The reasons for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF).

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/Baseline 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

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

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

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

Primary outcome measure

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

Secondary outcome measure

1. The rate of clinical pregnancy/ miscarriage/ ectopic/ live birth/ pregnancy leading to live birth between two groups. 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;
2. The thyroid function of patients and neonatal thyroid function between two groups;
3. The pain scores after the procedure between two groups, measured by means of the Visual-Analogue Scale for Pain (scores range from 0.0 to 10.0 cm, with higher scores indicating more severe pain);
4. The costs and cost-effectiveness of OSCM / WSCM and assisted reproductive

technology (ART) treatments in Chinese context

5. The side effects or complications after therapy in both arms.

Statistical considerations

sample size calculation

The primary objective of this study is to determine 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 randomisation. In Dreyer's research, the rate of ongoing pregnancy in 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 at a significance level of two-sided 5%, we calculated that 467 women for each group, a total of 934 would need to be enrolled. Anticipating lost-to-follow up or protocol violation, an additional 10% is needed. Thus, 1040 women (520 in each group) would need to be randomised. Sample size estimation curve under difference circumstance is showed in Figure 2.

Statistical analysis

The primary analysis will be done according to the intention to treat principle. We will compare the primary outcome of rate of ongoing pregnancy between WSCM group and OSCM group by using the chi-square test to assess statistical significance.

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

Univariate rate ratios or relative risks and 95% confidence intervals were calculated for the primary and other binary outcome measures, and the chi-square test was used to assess statistical significance. Continuous outcomes were analysed with the use of an independent t-test or the Mann–Whitney U-test as appropriate. We used Kaplan–Meier curves with a log-rank test to compare the groups with respect to the time to pregnancy

resulting in an ongoing pregnancy. Two-sided P values of less than 0.05 were considered to indicate statistical significance. No adjustment was made for multiple comparisons. SPSS software, version 22.0 (IBM), and R software, version 3.3.1 (R Project for Statistical Computing), were used for statistical analyse.

Interim monitoring and analysis

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

Patient and public involvement

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

Discussion

With the increasement of infertile population, HSG is widely used in the fertility assessment. Most clinics in China use water-based contrast media but some use oil-based contrast media. Up to now, no final conclusion has yet been reached on this matter that which reagent is better in some cases. Dreyer's research [7] was the largest RCT about the effects between the two kinds of contrast at HSG, however it was not sure that the results applied to Chinese population. Therefore, this study is to 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 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.

REFERENCES

1. Hochschild FZ, Adamson D, Dyer S, et al. The International Glossary on Infertility and Fertility Care. Fertil Steril 2017;108:393-406.

2. Farquhar CM, Bhattacharya S, Repping S, et al. Female subfertility. Nat Rev Dis Primers 2019;5(1):7.

3. William L. Simpson, Jr, Laura G, et al. Hysterosalpingography: A Reemerging Study. Radiographics 2006;26(2):419-31.

4. Martí-Bonmatí L. Hysterosalpingography and fertility: a technical relationship. Fertil Steril 2018;110(4):642.

5. Quaas A, Dokras A. Diagnosis and Treatment of Unexplained Infertility. Reviews in Obstetrics & Gynecology 2008;1:69-76.

6. National Collaborating Centre for Women's and Children's Health (UK. Fertility: Assessment and treatment for people with fertility problems | Information for the public | NICE. Rcog Press 2013;1(4): 21-8.

7. Dreyer K, Van Rijswijk J, Mijatovic V, et al. Oil-Based or Water-Based Contrast for Hysterosalpingography in Infertile Women. New England Journal of Medicine 2017;NEJMoa1612337.

8. Yun AJ, Lee PY. Enhanced fertility after diagnostic hysterosalpingography using oil -based contrast agents may be attributable to immunomodulation. AJR Am J Roentgenol 2004;183(6):1725-27.

9. Sueoka K, Asada H, Tsuchiya S, et al. Falloposcopic tuboplasty for bilateral tubal occlusion. A novel infertility treatment as an alternative for in-vitro fertilization?. Human Reproduction 1998;1(1):71-4.

10. Lindequist S, Justesen P, Larsen C, et al. Diagnostic quality and complications of hysterosalpingography: oil- versus water-soluble contrast media--a randomized prospective study. Radiology 1991;179(1):69-74.

11. Johnson NP, Farquhar CM, Hadden WE, et al. The FLUSH trial — flushing with lipiodol for unexplained (and endometriosis-related) subfertility by hysterosalpingography: a randomized trial. Hum Reprod 2004;19:2043-51.

12. Ogata R, Nakamura G, Uchiumi Y, et al. Therapeutic efficacy of hysterosalpingography (HSG) in infertility: a prospective, randomized, clinical study. Jap J Fertil Steril 1993;38:91-4.

13. Johnson JV, Montoya IA, Olive DL. Ethiodol oil contrast medium inhibits macrophage phagocytosis and adherence by altering membrane electronegativity and microviscosity. Fertil Steril 1992;58:511-17.

14. Johnson NP. Review of lipiodol treatment for infertility — an innovative treatment for endometriosis-related infertility? Aust N Z J Obstet Gynaecol 2014;54:9-12.

15. Lindequist S, Rasmussen F, Larsen C. Use of iotrolan versus ethiodized poppy-seed oil in hysterosalpingography. Radiology 1994;191:513–17.

16. Spring DB, Barkan HE, Pruyn SC. Potential therapeutic effects of contrast materials in hysterosalpingography: a prospective randomized clinical trial.Kaiser Permanente

Infertility Work Group. Radiology 2000;214:53–57.

17. Shuhei S, Wakasa Y, Hiroko T, et al. The effect of oil and water-soluble contrast medium in hysterosalpingography on thyroid function. Gynecological Endocrinology 2017;33(9):682-85.

18. Miyamoto Y, Tsujimoto T, Iwai K, et al. Safety and pharmacokinetics of iotrolan in hysterosalpingography. Retention and irritability compared with lipiodol. Invest Radiol 1995;30:538–43.

19. Satoh M, Aso K, Katagiri Y. Thyroid Dysfunction in Neonates Born to Mothers Who Have Undergone Hysterosalpingography Involving an Oil-Soluble Iodinated Contrast Medium. Hormone Research in Paediatrics 2015;84(6):370-75.

20. Kaneshige T, Arata N, Harada S, et al. Changes in Serum Iodine Concentration, Urinary Iodine Excretion and Thyroid Function After Hysterosalpingography Using an Oil-Soluble Iodinated Contrast Medium (Lipiodol). J Clin Endocrinol Metab 2015;100(3):E469–72.

Author affiliations

[1] Department of Obstetrics and Gynecology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

[2] Shanghai Key Laboratory Embryo Original Diseases, Shanghai, China

[3] Shanghai Municipal Key Clinical Specialty, Shanghai, China

[4] Department of Radiology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

Authors' contributions

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

Funding

This work was supported by Shanghai Municipal Key Clinical Specialty (GFY1808001), International peace maternal and child health hospital clinical applied research program (YN201915).

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

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

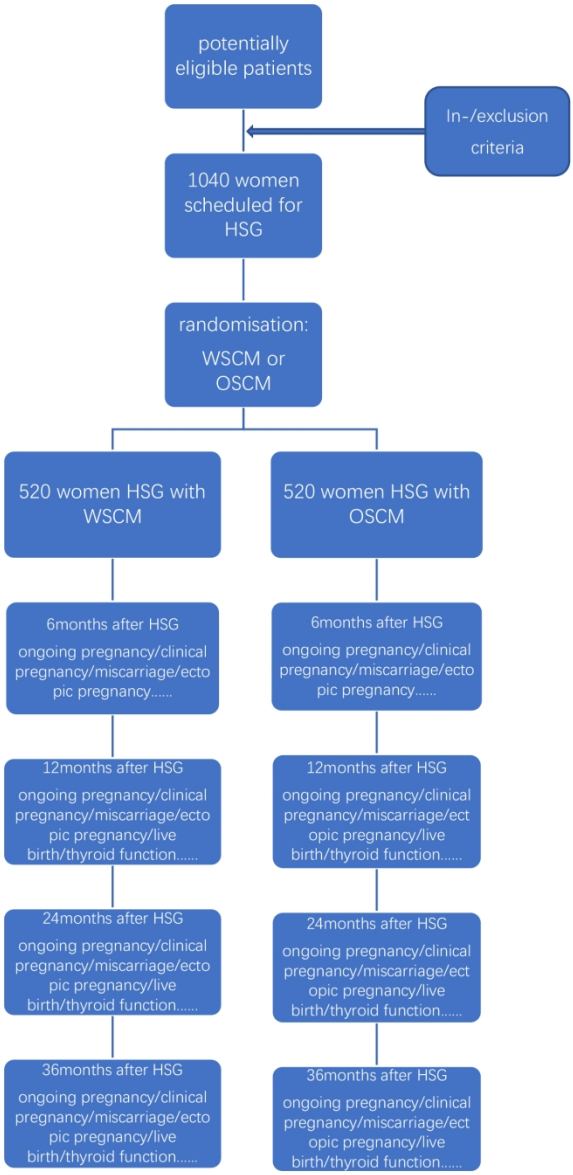

Figure 1 Flowchart

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.

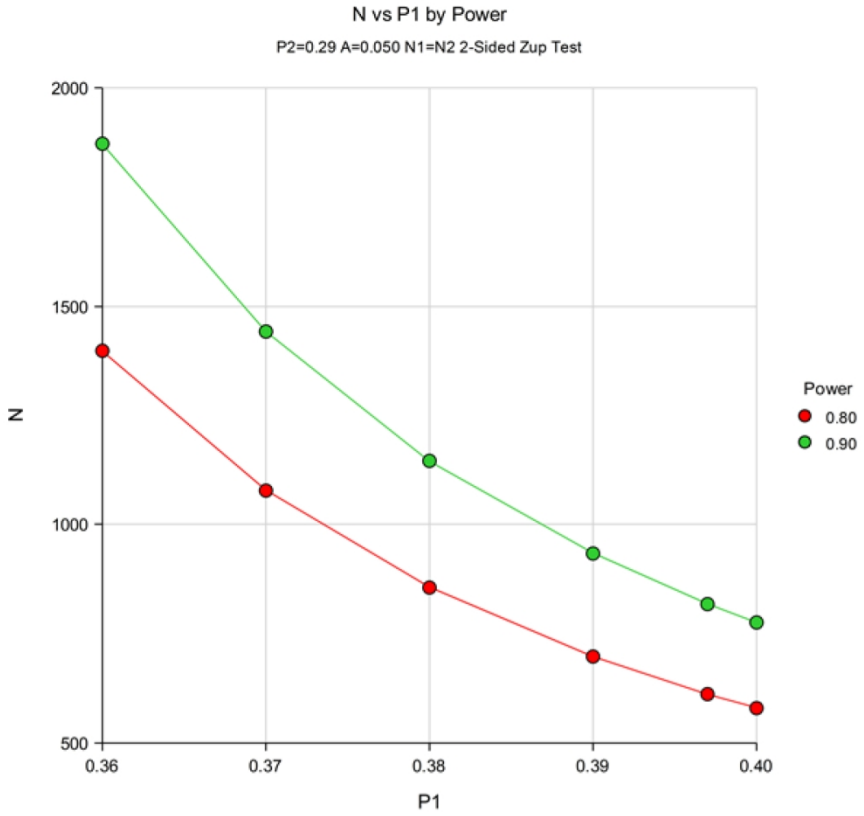

Figure 2 Sample size estimation curve under difference circumstance
N is the total sample size, N(Group OSCM) + N(Group WSCM). P1 is the proportion for Group OSCM at which power and sample size calculations are made. P2 is the proportion for Group WSCM. This is referred to Dreyer's research [7]. Target Power is the desired power value (or values) entered in the procedure. Power is the probability of rejecting a false null hypothesis. The red line means power 0.80 and the green line 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 SPIRITreporting 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

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	-
Protocol version	#3	Date and version identifier	-
Funding	#4	Sources and types of financial, material, and other support	14
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	14
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	-
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	-
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	-

Introduction			3-4
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			5-9
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	5-6

		individuals who will perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6-7
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	-
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	-
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	-
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended	7-9

		(see Figure)	
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	-
Methods: Assignment of interventions (for controlled trials)			-
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	-
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	-
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	-

Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	-
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	-
Methods: Data collection, management, and analysis			8-10
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	8-9
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	-
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (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	
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	-
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	-
Methods: Monitoring			11
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	-
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	11
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial	-

		conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	-
Ethics and dissemination			15
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	15
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	-
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	15
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	-
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	-
Declaration of	#28	Financial and other competing interests for principal	15

interests		investigators for the overall trial and each study site	
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	-
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	-
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	-
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	-
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	-
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	-

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:	*BMJ Open*
Manuscript ID	bmjopen-2020-039166.R1
Article Type:	Protocol
Date Submitted by the Author:	20-Jun-2020
Complete List of Authors:	Liang, Guiling; International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Department of Obstetrics and Gynecology Zhu, Qian; International Peace Maternity and Child Health Hospital/School of Medicine, Shanghai Jiaotong University, Department of Obstetrics and Gynecology He, Xiaoqing; International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Department of Obstetrics and Gynecology Wang, Xiaofeng; International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Department of Obstetrics and Gynecology Jiang, Ling; International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Department of Radiology Zhu, Chenfeng; International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Department of Obstetrics and Gynecology XIE, LI; Shanghai Jiao Tong University School of Medicine, Clinical Research Institute Qian, Zhaoxia; International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Department of Radiology Zhang, Jian; International Peace Maternity and Child Health Hospital/School of Medicine, Shanghai Jiaotong University, Department of Obstetrics and Gynecology
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Obstetrics and gynaecology
Keywords:	Subfertility < GYNAECOLOGY, Diagnostic radiology < RADIOLOGY & IMAGING, GYNAECOLOGY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

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

Guiling Liang[1,2,3], Qian Zhu[1,2,3], Xiaoqing He[1,2,3], Xiaofeng Wang[1,2,3], Ling Jiang[2,3,4], Chenfeng Zhu[1,2,3], Li Xie[5], Zhaoxia Qian[2,3,4]* and Jian Zhang[1,2,3]*

[1] Department of Obstetrics and Gynecology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

[2] Shanghai Key Laboratory Embryo Original Diseases, Shanghai, China

[3] Shanghai Municipal Key Clinical Specialty, Shanghai, China

[4] Department of Radiology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

[5] Clinical Research Institute, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

* Corresponding author:

Jian Zhang, Department of Obstetrics and Gynecology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China;

Email: zhangjian_sjtu@126.com

Zhaoxia Qian, Department of Radiology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China;

Email: zhaoxiaqian@163.com

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

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

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

Trial registration number ChiCTR2000031612.

STRENGTHS AND LIMITATIONS OF THIS STUDY

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

INTRODUCTION

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

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

Oil-soluble contrast media (OSCM) is represented by iodinated oil injection, which uses poppy seed oil as the raw material. It provides clear images and may have some anti-inflammatory effects that perhaps enhance fertility [10]. Due to less absorption of the contrast media, the occurrence of adverse events is notably reduced [11,12]. Water-soluble contrast media (WSCM) is represented by meglumine diatrizoate and ioversol. The advantages of WSCM are as follows: low viscosity, good fluidity, and

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

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

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

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

Previous studies showed that women with subclinical hypothyroidism were more prone to lipiodol induced overt hypothyroidism [22], which may be due to the long half-life of lipiodol excretion [23]. It is known that excess iodine intake during pregnancy can adversely affect thyroid function in both the mother and the foetus [24]. Nevertheless, there have been no large-scale, prospective studies exploring the relationship between HSG contrast agents and the occurrence of thyroid dysfunction in patients and neonates. In summary, there is no consensus on the choice of contrast agents and their effects. In view of this uncertainty, we plan a single-centre, randomized, controlled, parallel group, superiority trial in infertile Chinese women with a low a priori chance of tubal pathology.

OBJECTIVE AND HYPOTHESIS

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

METHODS AND ANALYSIS

Study design and setting

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

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

Recruitment

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

Participants

Inclusion criteria are as follows:

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

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

6. Valid indication for HSG in the fertility examination or before intra uterine insemination treatment.
7. Signed informed consent.

Exclusion criteria are as follows:

1. Irregular menstrual cycle, less than eight menstrual cycles per year.
2. Endocrino-pathological diseases such as: Polycystic ovary syndrome, Cushing syndrome, adrenal hyperplasia, hyperprolactinemia, acromegaly, hypothalamic amenorrhea, hypothyroidy, diabetes mellitus, and thyroid dysfunction.
3. Known or at high risk for tubal pathology, Chlamydia trachomatis positive of vaginal secretion culture, history of Chlamydia infection.
4. Known contrast (iodine) allergy
5. Male subfertility defined as a post-wash total motile sperm count $< 3 \times 10^6$ spermatozoa/mL
6. A contra-indication for HSG.
7. Not willing or able to sign the consent form.

Randomization

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

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

Blinding

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

Intervention

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

days after complete cessation of menstrual bleeding, and before the 14th day in the ovulatory cycle.

HSG will be performed as follows: 1) the patient will be placed in the supine position; 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

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

Participant timeline

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

Table 1 Schedule of Activities

Procedures	Screening Day -7 to -1	Enrollment/Baseline 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				
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					

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

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

OUTCOME MEASURES

Primary outcome measures

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

Secondary outcome measures

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

different time-points [26,27].

2. The thyroid function of the neonates will be tested within 3 to 7 days after birth. TT4 (total thyroxine), FT4, and TSH will be detected [27].

3. Pain scores during hysterosalpingography will be measured by means of the Visual-Analogue Scale for Pain (scores range from 0.0 to 10.0 cm, with higher scores indicating more severe pain). The pain scores will be recorded by a trained nurse. Meanwhile, the time from the beginning of the contrast injection to the occurrence of pain will be recorded.

4. The rates of live birth rate/ clinical pregnancy / miscarriages/ ectopic pregnancy/ pregnancy leading to live birth will be assessed. Live birth is defined as the birth of at least one living child; 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 implanted outside the uterine cavity. Pregnancy leading to live birth is defined as the ratio of live births to clinical pregnancies. Each occurrence of one of these events will be recorded during the three-year follow-up.

5. The time to pregnancy resulting in an ongoing pregnancy is defined as the time from randomization to the first day of the last menstrual period plus 4 weeks. It will be considered when ongoing pregnancy occurs.

6. The costs and cost-effectiveness of OSCM / WSCM and assisted reproductive technology (ART) treatments in Chinese context.

7. The side effects or complications after therapy in both groups.

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.

Statistical analysis

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

All data will be analysed according to the intention-to-treat principle. Categorical data will be reported as absolute numbers and percentages. Normally distributed continuous variables will be summarized as means with standard deviations, and nonnormally distributed continuous variables will be reported as medians with interquartile ranges. Univariate rate ratios or relative risks and 95% confidence intervals will be calculated for the primary and other binary outcome measures, and the chi-square test will be used to assess statistical significance. Continuous outcomes will be analysed with the use of an independent t-test or the Mann–Whitney U-test, as appropriate. We will use Kaplan–Meier curves with a log-rank test to compare the groups with respect to the time to pregnancy resulting in an ongoing pregnancy. Two-sided P values of less than 0.05 will indicate statistical significance. No adjustment will be made for multiple comparisons. SPSS software, version 22.0 or higher (IBM; Armonk, NR), and R version 3.3.1 (R Project for Statistical Computing, Vienna, Austria), will be used for statistical analyses.

Data monitoring and auditing

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

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

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

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

Ethics and dissemination

This protocol received authorisation from the Medical Research Ethics Committee

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

DISCUSSION

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

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

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

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

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

Trial status

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

Patient and public involvement

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

Acknowledgments

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

REFERENCES

1. Hochschild FZ, Adamson D, Dyer S, et al. The International Glossary on Infertility and Fertility Care. Fertil Steril 2017;108:393-406.

2. Zhenwu Z, Wenli L. Estimating the Prevalence of Infertility in China Using Census

Data. Population Research 2020;44(02):3-17.

3. William L. Simpson, Jr, Laura G, et al. Hysterosalpingography: A Reemerging Study. Radiographics 2006;26(2):419-31.

4. Martí-Bonmatí L. Hysterosalpingography and fertility: a technical relationship. Fertil Steril 2018;110(4):642.

5. Quaas A, Dokras A. Diagnosis and Treatment of Unexplained Infertility. Reviews in Obstetrics & Gynecology 2008;1:69-76.

6. Jinxia B, Jing H, Shengli W. Clinical Progress of Tubal Patency Tests. J Int Obstet Gynecol 2020;47:111-4.

7. National Collaborating Centre for Women's and Children's Health (UK. Fertility: Assessment and treatment for people with fertility problems | Information for the public | NICE. Rcog Press 2013;1(4): 21-8.

8. Dreyer K, Van Rijswijk J, Mijatovic V, et al. Oil-Based or Water-Based Contrast for Hysterosalpingography in Infertile Women. New England Journal of Medicine 2017;NEJMoa1612337.

9. Yun AJ, Lee PY. Enhanced fertility after diagnostic hysterosalpingography using oil -based contrast agents may be attributable to immunomodulation. AJR Am J Roentgenol 2004;183(6):1725-27.

10. Zijun D. Study on Clinical Effect of Ioversol and Iodized Oil in the Hysterosalpingography. China &Foreign Medical Treatment 2018;12:175-7.

11. Sueoka K, Asada H, Tsuchiya S, et al. Falloposcopic tuboplasty for bilateral tubal occlusion. A novel infertility treatment as an alternative for in-vitro fertilization?.

Human Reproduction 1998;1(1):71-4.

12. Lindequist S, Justesen P, Larsen C, et al. Diagnostic quality and complications of hysterosalpingography: oil- versus water-soluble contrast media--a randomized prospective study. Radiology 1991;179(1):69-74.

13. Johnson NP, Farquhar CM, Hadden WE, et al. The FLUSH trial — flushing with lipiodol for unexplained (and endometriosis-related) subfertility by hysterosalpingography: a randomized trial. Hum Reprod 2004;19:2043-51.

14. Ogata R, Nakamura G, Uchiumi Y, et al. Therapeutic efficacy of hysterosalpingography (HSG) in infertility: a prospective, randomized, clinical study. Jap J Fertil Steril 1993;38:91-4.

15. Johnson JV, Montoya IA, Olive DL. Ethiodol oil contrast medium inhibits macrophage phagocytosis and adherence by altering membrane electronegativity and microviscosity. Fertil Steril 1992;58:511-17.

16. Johnson NP. Review of lipiodol treatment for infertility — an innovative treatment for endometriosis-related infertility? Aust N Z J Obstet Gynaecol 2014;54:9-12.

17. Fang F, Yu B, Yu Z, et al. Oil-based versus water-based contrast for hysterosalpingography in infertile women: a systematic review and meta-analysis of randomized controlled trials. Fertility & Sterility 2018:S0015028218302760-.

18. Alper MM, Garner PR, Spence JE, et al. Pregnancy rates after hysterosalpingography with oil- and water-soluble contrast media. Obstet Gynecol 1986;68:6–9.

19. de Boer AD, Vemer HM, Willemsen WN, et al. Oil or aqueous contrast media for

hysterosalpingography: a prospective, randomized, clinical study. Eur J Obstet Gynecol Reprod Biol 1988;28:65–8.

20. Lindequist S, Rasmussen F, Larsen C. Use of iotrolan versus ethiodized poppy-seed oil in hysterosalpingography. Radiology 1994;191:513–17.

21. Spring DB, Barkan HE, Pruyn SC. Potential therapeutic effects of contrast materials in hysterosalpingography: a prospective randomized clinical trial.Kaiser Permanente Infertility Work Group. Radiology 2000;214:53–57.

22. Shuhei S, Wakasa Y, Hiroko T, et al. The effect of oil and water-soluble contrast medium in hysterosalpingography on thyroid function. Gynecological Endocrinology 2017;33(9):682-85.

23. Miyamoto Y, Tsujimoto T, Iwai K, et al. Safety and pharmacokinetics of iotrolan in hysterosalpingography. Retention and irritability compared with lipiodol. Invest Radiol 1995;30:538–43.

24. Satoh M, Aso K, Katagiri Y. Thyroid Dysfunction in Neonates Born to Mothers Who Have Undergone Hysterosalpingography Involving an Oil-Soluble Iodinated Contrast Medium. Hormone Research in Paediatrics 2015;84(6):370-75.

25. Coppus SF, Verhoeve HR, Opmeer BC, et al. Identifying subfertile ovulatory women for timely tubal patency testing: a clinical decision rule based on medical history. Hum Reprod 2007;22(10):2685-92.

26. Kaneshige T, Arata N, Harada S, et al. Changes in Serum Iodine Concentration, Urinary Iodine Excretion and Thyroid Function After Hysterosalpingography Using an Oil-Soluble Iodinated Contrast Medium (Lipiodol). J Clin Endocrinol Metab

2015;100(3):E469–72.

27. Ad Hoc Writing Committee for Guideline on Diagnosis and Management of Thyroid Diseases during Pregnancy and Postpartum (2nd edition); Chinese Society of Endocrinology, Chinese Medical Association; Chinese Society of Perinatology, Chinese Medical Association. Guideline on Diagnosis and Management of Thyroid Diseases during Pregnancy and Postpartum (2nd edition). 2019;22(8):505-39.

Date and version

06-05-2020; Version 1.1.

Author affiliations

[1] Department of Obstetrics and Gynecology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

[2] Shanghai Key Laboratory Embryo Original Diseases, Shanghai, China

[3] Shanghai Municipal Key Clinical Specialty, Shanghai, China

[4] Department of Radiology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

[5] Clinical Research Institute, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Authors' contributions

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

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

Funding

This work was supported by Shanghai Municipal Key Clinical Specialty (GFY1808001), International peace maternal and child health hospital clinical applied research program (YN201915).

Competing interests

The authors declare that they have no competing interests.

Patient consent for publication

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

Ethics approval

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

FIGURE LEGENDS

Figure 1 Flowchart

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.

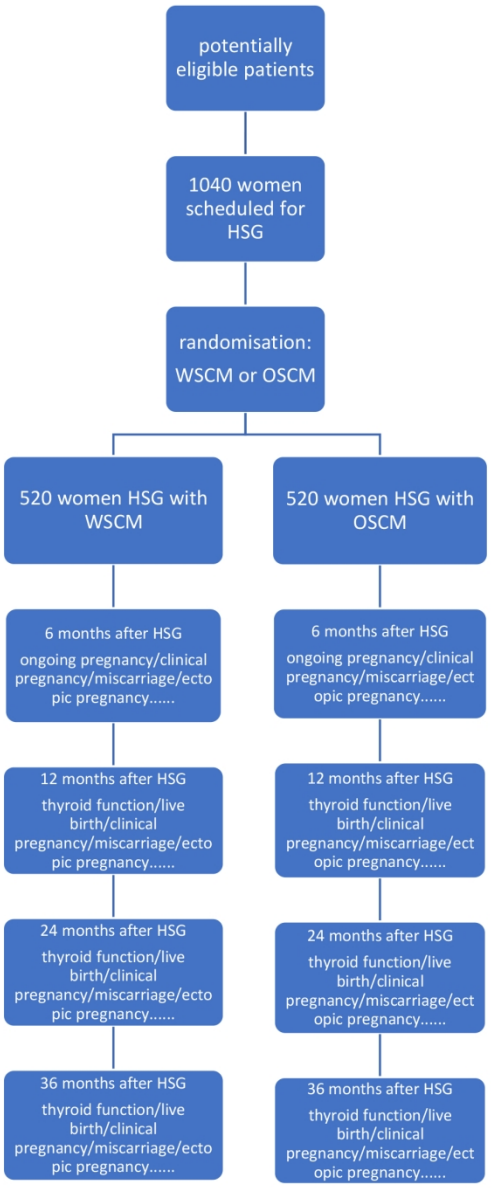

Figure 1 Flowchart

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 SPIRITreporting 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

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	-
Protocol version	#3	Date and version identifier	22
Funding	#4	Sources and types of financial, material, and other support	23
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	22
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	22
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	15-16

Introduction			3-6
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-6
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3-6
Objectives	#7	Specific objectives or hypotheses	6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			6-14
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg,	7-8

		surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-10
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	10
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	15-16
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	11-12
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	12-13
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	11-12

Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	14
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	7
Methods: Assignment of interventions (for controlled trials)			8-9
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8-9
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8-9
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8-9

Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	-
Methods: Data collection, management, and analysis			10,15-16
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10, 15
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	15
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality	15-16

		(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	
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	15
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	15
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	10
Methods: Monitoring			13-16
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	-15
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	16
Harms	#22	Plans for collecting, assessing, reporting, and managing	13-14

		solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	15
Ethics and dissemination			16
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	16
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	15-16
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7,23
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	23
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the	16

		trial	
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	-
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	16,18
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	16-17
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	17
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	23
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in	-

the current trial and for future use in ancillary studies, if applicable

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-039166.R2
Article Type:	Protocol
Date Submitted by the Author:	30-Aug-2020
Complete List of Authors:	Liang, Guiling; International Peace Maternity and Child Health Hospital, Department of Obstetrics and Gynecology Zhu, Qian; International Peace Maternity and Child Health Hospital, Department of Obstetrics and Gynecology He, Xiaoqing; International Peace Maternity and Child Health Hospital, Department of Obstetrics and Gynecology Wang, Xiaofeng; International Peace Maternity and Child Health Hospital, Department of Obstetrics and Gynecology Jiang, Ling; International Peace Maternity and Child Health Hospital, Department of Radiology Zhu, Chenfeng; International Peace Maternity and Child Health Hospital, Department of Obstetrics and Gynecology XIE, LI; Shanghai Jiao Tong University School of Medicine, Clinical Research Institute Qian, Zhaoxia; International Peace Maternity and Child Health Hospital, Department of Radiology Zhang, Jian; International Peace Maternity and Child Health Hospital, Department of Obstetrics and Gynecology
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Obstetrics and gynaecology
Keywords:	Subfertility < GYNAECOLOGY, Diagnostic radiology < RADIOLOGY & IMAGING, GYNAECOLOGY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

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

Guiling Liang[1,2,3], Qian Zhu[1,2,3], Xiaoqing He[1,2,3], Xiaofeng Wang[1,2,3], Ling Jiang[2,3,4], Chenfeng Zhu[1,2,3], Li Xie[5], Zhaoxia Qian[2,3,4]* and Jian Zhang[1,2,3]*

[1] Department of Obstetrics and Gynecology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

[2] Shanghai Key Laboratory Embryo Original Diseases, Shanghai, China

[3] Shanghai Municipal Key Clinical Specialty, Shanghai, China

[4] Department of Radiology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

[5] Clinical Research Institute, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

* Corresponding author:

Jian Zhang, Department of Obstetrics and Gynecology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China;

Email: zhangjian_sjtu@126.com

Zhaoxia Qian, Department of Radiology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China;

Email: zhaoxiaqian@163.com

ABSTRACT

Introduction: In recent years, due to various factors, the infertility rate in China has increased and now affects over 10% 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 randomized controlled trial (H2Oil trial from the Netherlands) has shown the oil-soluble contrast at HSG can enhance the fertility comparing to the water-soluble contrast, we propose this study to answer the question 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 with a low risk of tubal disease 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%, we have calculated a sample of 520 women per group for a total of 1040 including 10% loss to follow-up or protocol variation to be enrolled. The primary outcome is the rate of ongoing pregnancy during 6 months after randomization. The secondary outcomes will consist of thyroid function of patients and newborns, pain scores during HSG, live birth rate, clinical pregnancies,

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

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

Trial registration number ChiCTR2000031612.

STRENGTHS AND LIMITATIONS OF THIS STUDY

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

INTRODUCTION

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

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

Oil-soluble contrast media (OSCM) is represented by iodinated oil injection, which uses poppy seed oil as the raw material. It provides clear images and may have some anti-inflammatory effects that perhaps enhance fertility [10]. Due to less absorption of the contrast media, the occurrence of adverse events is notably reduced [11,12]. Water-soluble contrast media (WSCM) is represented by meglumine diatrizoate and ioversol. The advantages of WSCM are as follows: low viscosity, good fluidity, and rapid diffusion into the fallopian tubes, which can clearly show the anatomy of the

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

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

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

the groups were not statistically significant.

Previous studies showed that women with subclinical hypothyroidism were more prone to OSCM induced overt hypothyroidism [22], which may be due to the long half-life of OSCM excretion [23]. It is known that excess iodine intake during pregnancy can adversely affect thyroid function in both the mother and the foetus [24]. Nevertheless, there have been no large-scale, prospective studies exploring the relationship between HSG contrast agents and the occurrence of thyroid dysfunction in patients and neonates. In summary, there is no consensus on the choice of contrast agents and their effects. In view of this uncertainty, we plan a single-centre, randomized, controlled, parallel group, superiority trial in infertile Chinese women with a low a priori chance of tubal pathology.

OBJECTIVE AND HYPOTHESIS

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

METHODS AND ANALYSIS

Study design and setting

The study is a single-centre randomized controlled superiority trial and will be performed in the International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China. The hospital has two

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

Recruitment

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

Participants

Inclusion criteria are as follows:

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

infection, pelvic inflammatory disease, known endometriosis or adenomyosis, pelvic abdominal surgery [including salpingostomy or salpingectomy for ectopic pregnancy and complicated appendectomy] and/or peritonitis) [26].

6. Valid indication for HSG in the fertility examination or before intra uterine insemination treatment.
7. Signed informed consent.

Exclusion criteria are as follows:

1. Irregular menstrual cycle, less than eight menstrual cycles per year.
2. Endocrino-pathological diseases such as: Polycystic ovary syndrome, Cushing syndrome, adrenal hyperplasia, hyperprolactinemia, acromegaly, hypothalamic amenorrhea, hypothyroidy, diabetes mellitus, and thyroid dysfunction.
3. Known or at high risk for tubal pathology, Chlamydia trachomatis positive of vaginal secretion culture, history of Chlamydia infection.
4. Known contrast (iodine) allergy
5. Male subfertility defined as sperm count < 15×10^6 spermatozoa/mL or a post-wash total motile sperm count < 3×10^6 spermatozoa/mL before IUI.
6. A contra-indication for HSG.
7. Not willing or able to sign the consent form.

Randomization

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

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

Blinding

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

Intervention

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

days after complete cessation of menstrual bleeding, and before the 14th day in the ovulatory cycle.

HSG will be performed as follows: 1) the patient will be placed in the supine position; 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

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

Participant timeline

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

Table 1 Schedule of Activities

Procedures	Screening Day -7 to -1	Enrollment/Baseline 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				
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					

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

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

OUTCOME MEASURES

Primary outcome measures

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

Secondary outcome measures

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

antibodies (TgAb), and antithyroid peroxidase antibodies (TPOAb) levels at different time-points [27,28]. Also, we will record the thyroid function of patients as possible as we can to make the data more accurate.

2. The thyroid function of the neonates will be tested within 3 to 7 days after birth. TT4 (total thyroxine), FT4, and TSH will be detected [28].
3. Pain scores during hysterosalpingography will be measured by means of the Visual-Analogue Scale for Pain (scores range from 0.0 to 10.0 cm, with higher scores indicating more severe pain). The pain scores will be recorded by a trained nurse. Meanwhile, the time from the beginning of the contrast injection to the occurrence of pain will be recorded.
4. The rates of live birth rate/ clinical pregnancy / miscarriages/ ectopic pregnancy will be assessed. Live birth is defined as the birth of at least one living child; 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 implanted outside the uterine cavity. Each occurrence of one of these events will be recorded during the three-year follow-up.
5. The time to ongoing pregnancy is defined as the time from randomization to the first day of the last menstrual period plus 4 weeks. It will be considered when ongoing pregnancy occurs. The time to first live birth is defined as the time from randomization to the date of the firth live birth. It will be considered when live birth occurs.
6. The costs and cost-effectiveness of OSCM / WSCM and assisted reproductive

technology (ART) treatments in Chinese context.

7. The side effects or complications after therapy in both groups.

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. Thus, 1040 women (520 in each group) will need to be randomized, including 10% loss to follow-up or protocol variation.

Statistical analysis

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

Statistical analysis of the first 6 months

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

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

Statistical analysis of the 3-year follow-up

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

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

Categorical data will be described as absolute numbers and percentages. Distributed continuous variables will be reported as medians with interquartile ranges.

Subgroup analysis

To identify a subgroup effect, we plan to test for an interaction for the following subgroups: (1) age of the patients (<35years, and ≥35years); (2) primary versus secondary fertility. We may perform some other subgroups when we finally analyse data.

Missing data analysis

We will use Multiple Imputation (MI) to process the missing data.

Data monitoring and auditing

Data monitoring and auditing will be conducted for quality assurance. Monitoring staff will consist of an Independent Data Monitoring Committee (IDMC) and an ethics committee. The IDMC is composed of five members, including one statistician, three clinical experts and one ethicist. They will visit the institutions at important time points throughout the trial, for example, at participant enrolment, at the study interim point,

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

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

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

Ethics and dissemination

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

publications and presentations at international scientific meetings.

Patient and public involvement

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

DISCUSSION

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

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

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

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

REFERENCES

1. Hochschild FZ, Adamson D, Dyer S, et al. The International Glossary on Infertility and Fertility Care. Fertil Steril 2017;108:393-406.

2. Zhenwu Z, Wenli L. Estimating the Prevalence of Infertility in China Using Census Data. Population Research 2020;44(02):3-17.

3. William L. Simpson, Jr, Laura G, et al. Hysterosalpingography: A Reemerging Study. Radiographics 2006;26(2):419-31.

4. Martí-Bonmatí L. Hysterosalpingography and fertility: a technical relationship. Fertil Steril 2018;110(4):642.

5. Quaas A, Dokras A. Diagnosis and Treatment of Unexplained Infertility. Reviews in Obstetrics & Gynecology 2008;1:69-76.

6. Jinxia B, Jing H, Shengli W. Clinical Progress of Tubal Patency Tests. J Int Obstet Gynecol 2020;47:111-4.

7. National Collaborating Centre for Women's and Children's Health (UK. Fertility: Assessment and treatment for people with fertility problems | Information for the public

| NICE. Rcog Press 2013;1(4): 21-8.

8. Dreyer K, Van Rijswijk J, Mijatovic V, et al. Oil-Based or Water-Based Contrast for Hysterosalpingography in Infertile Women. New England Journal of Medicine 2017;NEJMoa1612337.

9. Yun AJ, Lee PY. Enhanced fertility after diagnostic hysterosalpingography using oil -based contrast agents may be attributable to immunomodulation. AJR Am J Roentgenol 2004;183(6):1725-27.

10. Zijun D. Study on Clinical Effect of Ioversol and Iodized Oil in the Hysterosalpingography. China &Foreign Medical Treatment 2018;12:175-7.

11. Sueoka K, Asada H, Tsuchiya S, et al. Falloposcopic tuboplasty for bilateral tubal occlusion. A novel infertility treatment as an alternative for in-vitro fertilization?. Human Reproduction 1998;1(1):71-4.

12. Lindequist S, Justesen P, Larsen C, et al. Diagnostic quality and complications of hysterosalpingography: oil- versus water-soluble contrast media--a randomized prospective study. Radiology 1991;179(1):69-74.

13. Johnson NP, Farquhar CM, Hadden WE, et al. The FLUSH trial — flushing with lipiodol for unexplained (and endometriosis-related) subfertility by hysterosalpingography: a randomized trial. Hum Reprod 2004;19:2043-51.

14. Ogata R, Nakamura G, Uchiumi Y, et al. Therapeutic efficacy of hysterosalpingography (HSG) in infertility: a prospective, randomized, clinical study. Jap J Fertil Steril 1993;38:91-4.

15. Johnson JV, Montoya IA, Olive DL. Ethiodol oil contrast medium inhibits

macrophage phagocytosis and adherence by altering membrane electronegativity and microviscosity. Fertil Steril 1992;58:511-17.

16. Johnson NP. Review of lipiodol treatment for infertility — an innovative treatment for endometriosis-related infertility? Aust N Z J Obstet Gynaecol 2014;54:9-12.

17. Fang F, Yu B, Yu Z, et al. Oil-based versus water-based contrast for hysterosalpingography in infertile women: a systematic review and meta-analysis of randomized controlled trials. Fertility & Sterility 2018:S0015028218302760-.

18. Alper MM, Garner PR, Spence JE, et al. Pregnancy rates after hysterosalpingography with oil- and water-soluble contrast media. Obstet Gynecol 1986;68:6–9.

19. de Boer AD, Vemer HM, Willemsen WN, et al. Oil or aqueous contrast media for hysterosalpingography: a prospective, randomized, clinical study. Eur J Obstet Gynecol Reprod Biol 1988;28:65–8.

20. Lindequist S, Rasmussen F, Larsen C. Use of iotrolan versus ethiodized poppy-seed oil in hysterosalpingography. Radiology 1994;191:513–17.

21. Spring DB, Barkan HE, Pruyn SC. Potential therapeutic effects of contrast materials in hysterosalpingography: a prospective randomized clinical trial.Kaiser Permanente Infertility Work Group. Radiology 2000;214:53–57.

22. Shuhei S, Wakasa Y, Hiroko T, et al. The effect of oil and water-soluble contrast medium in hysterosalpingography on thyroid function. Gynecological Endocrinology 2017;33(9):682-85.

23. Miyamoto Y, Tsujimoto T, Iwai K, et al. Safety and pharmacokinetics of iotrolan

in hysterosalpingography. Retention and irritability compared with lipiodol. Invest Radiol 1995;30:538–43.

24. Satoh M, Aso K, Katagiri Y. Thyroid Dysfunction in Neonates Born to Mothers Who Have Undergone Hysterosalpingography Involving an Oil-Soluble Iodinated Contrast Medium. Hormone Research in Paediatrics 2015;84(6):370-75.

25. Cooper TG, Noonan E, von Eckardstein S, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update. 2010;16(3):231-45.

26. Coppus SF, Verhoeve HR, Opmeer BC, et al. Identifying subfertile ovulatory women for timely tubal patency testing: a clinical decision rule based on medical history. Hum Reprod 2007;22(10):2685-92.

27. Kaneshige T, Arata N, Harada S, et al. Changes in Serum Iodine Concentration, Urinary Iodine Excretion and Thyroid Function After Hysterosalpingography Using an Oil-Soluble Iodinated Contrast Medium (Lipiodol). J Clin Endocrinol Metab 2015;100(3):E469–72.

28. Ad Hoc Writing Committee for Guideline on Diagnosis and Management of Thyroid Diseases during Pregnancy and Postpartum (2nd edition); Chinese Society of Endocrinology, Chinese Medical Association; Chinese Society of Perinatology, Chinese Medical Association. Guideline on Diagnosis and Management of Thyroid Diseases during Pregnancy and Postpartum (2nd edition). 2019;22(8):505-39.

29. van Welie N, Dreyer K, van Rijswijk J, et al. Treatment effect of oil-based contrast is related to experienced pain at HSG: a post-hoc analysis of the randomized H2Oil

study. Hum Reprod. 2019;34(12):2391-98.

Date and version

08-26-2020; Version 1.2.

Author affiliations

[1] Department of Obstetrics and Gynecology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

[2] Shanghai Key Laboratory Embryo Original Diseases, Shanghai, China

[3] Shanghai Municipal Key Clinical Specialty, Shanghai, China

[4] Department of Radiology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

[5] Clinical Research Institute, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Acknowledgments

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

Authors' contributions

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

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

Funding

This work was supported by Shanghai Municipal Key Clinical Specialty (shslczdzk01802), International peace maternal and child health hospital clinical applied research program (YN201915).

Competing interests

The authors declare that they have no competing interests.

Patient consent for publication

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

Ethics approval

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

Trial status

The recruitment starts from August 1st and we expect to complete the recruitment in August 2021.

Data sharing statement

We plan to publish the primary report when all data on pregnancy resulting from the first 6 months are available (including secondary pregnancy outcomes) and an additional report when 3-year follow-up data are available. The public can obtain access to the full protocol, participant-level dataset, and statistical code via emailing the major

researchers.

FIGURE LEGENDS

Figure 1 Flowchart

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.

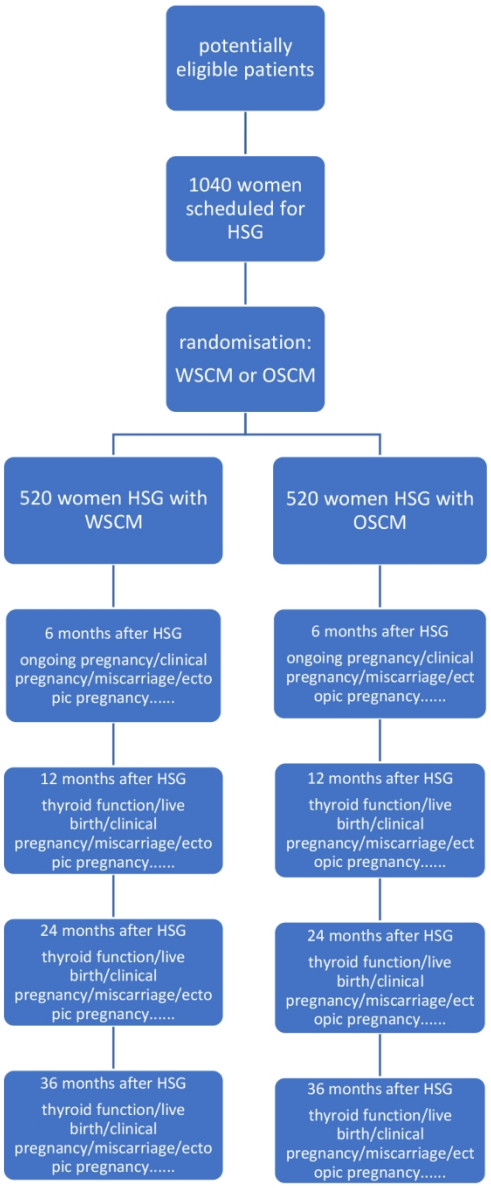

Figure 1 Flowchart

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 SPIRITreporting 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

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	-
Protocol version	#3	Date and version identifier	22
Funding	#4	Sources and types of financial, material, and other support	23
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	23
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	24
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	16-17

Introduction			3-6
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-6
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3-6
Objectives	#7	Specific objectives or hypotheses	6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			6-13
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	7-8

		individuals who will perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-10
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	10
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	16-17
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	11-12
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	12-13
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended	11-12

		(see Figure)	
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	14
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	7
Methods: Assignment of interventions (for controlled trials)			8-9
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8-9
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8-9
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8-9

Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	-
Methods: Data collection, management, and analysis			10-16
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10-12
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values).	10-11

		Reference to where details of data management procedures can be found, if not in the protocol	
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	15-16
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	16
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	10,16
Methods: Monitoring			14-17
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	16
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	17
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial	14

		conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	16
Ethics and dissemination			16
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	17
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	16-17
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	24
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	24
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	17
Declaration of	#28	Financial and other competing interests for principal	24-25

interests		investigators for the overall trial and each study site	
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	24
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	-
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	24
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	24
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	24
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	24
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	-