Oil- based versus water-based contrast media for hysterosalpingography (HSG) in infertile women with unevaluated indications: a randomized controlled trial

H2Oil2 study

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PROTOCOL TITLE Oil- based versus water-based contrast media for hysterosalpingography (HSG) in infertile women with unevaluated indications: a randomized controlled trial

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)**
AE	**Adverse Event**
AR	**Adverse Reaction**
ART	**Assisted Reproductive Technology**
CA	**Competent Authority**
CAT	**Chlamydia Antibody Titre**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
HSG	**Hysterosalpingography/hysterosalpingogram**
IB	**Investigator's Brochure**
IC	**Informed Consent**
ICSI	**Intra Cytoplasmatic Sperm Injection**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
IUI	**Intra Uterine Insemination**
IVF	**In Vitro Fertilization**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
NVOG	**Dutch society of Obstetrics and Gynaecology (in Dutch: Nederlandse Vereeniging Obstetrie en Gynaecologie)**
PCOS	**Polycystic Ovary Syndrome**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical**

	company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
TMSC	**Total Motile Sperm Count**
Wbp	**Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: We hypothesize that tubal flushing at hysterosalpingography (HSG) with oil-based contrast will result in higher pregnancy and live birth rates as compared to tubal flushing at HSG with water-based contrast in women: with an ovulation disorder, at high risk for tubal pathology and/or ≥38 years of age, which will lead to a reduction in the need for expensive fertility treatments like IVF and/or ICSI, and will therefore be a cost effective strategy.

Objective: The objective of the proposed study is to assess the effectiveness and cost-effectiveness of the use of oil versus water-based contrast medium in terms of live birth in women undergoing HSG, who:

1: have ovulation disorders or;

2: are at high risk for tubal pathology or;

3: are 39 years of age or over

Study design: Multicenter, randomized controlled trial with a cost-effectiveness analysis alongside it.

Study population: We will study women:

1: with ovulation disorders or;

2: at high risk for tubal pathology or;

3: above 38 years of age.

Intervention (if applicable): We will compare tubal flushing at HSG with oil-based contrast (intervention) versus tubal flushing with water-based contrast (control).

Main study parameters/endpoints: The primary outcome is conception leading to live birth, with a positive pregnancy test preceding the pregnancy within 6 months after randomization. We will also study time-to-pregnancy. Our hypothesis is that HSG with oil-based contrast will increase pregnancy rates (and time to event (pregnancy)) and thus reducing the need for ART and reducing costs.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: As we compare strategies (HSG with oil-based contrast versus HSG with water-based contrast) that are already applied in current practice, no additional risks or burdens are expected from the study.

1. INTRODUCTION AND RATIONALE

Staying childless, due to the inability to conceive, is one of life's great misfortunes. Infertility, defined as the inability to conceive within 1 year of unprotected intercourse, affects 1 out of 6 couples[1].The causes of infertility can be roughly classified as anovulation, poor sperm quality and tubal pathology, with unexplained infertility as a large fourth group.

Hysterosalpingography (HSG), a test to assess tubal patency, is commonly part of the fertility work-up offered to patients presenting with infertility. HSG was initially introduced as a diagnostic test to evaluate the patency of the Fallopian tubes. Debates of therapeutic effects of tubal flushing during HSG started over six decades ago[2,3]. The first meta-analysis on this topic included both observational and randomized controlled trials (RCTs) and the conclusion favoured the use of oil-based contrast media to improve fertility outcomes[4]. The latest Cochrane systematic reviews showed a non-significant difference in ongoing pregnancies in favour of tubal flushing with oil-based contrast in infertile women [5].

To illuminate the uncertainty on the use of oil- or water-based contrast for HSG, our group conducted a large robust multicentre randomized controlled trial, the H2Oil study, in which 1.119 infertile women participated. This landmark study, published last year in the NEJM, showed significantly more ongoing pregnancies in the first 6 months following HSG with oil-based contrast as compared to HSG with water-based contrast (RR 1.38; 95% CI, 1.17 to 1.64; P<0.001)[6]. Publication of the study generated a world-wide renewed interest in tubal flushing and the use of oil-based contrast for fertility enhancement.
However, the H2Oil study was limited by inclusion of women between 18 and 38 years of age, with a spontaneous regular menstrual cycle and at low risk for tubal pathology. As indicated above however, anovulation and tubal pathology are important causes of infertility. Furthermore, increasing female age is one of the main causes of infertility in the 21st century, with > 500 of the women undergoing IVF being over 35 years of age. As a consequence, the results of our H2Oil study are not applicable to more than 50% of the population of infertile women seen in fertility clinics in The Netherlands.

A cost-effectiveness analysis based on Euro 2017 prices for oil- and water-based contrast showed that the mean costs per couple within 6 months after randomization were €448 for oil-based contrast and €377 for water-based contrast, resulting in an incremental cost-effectiveness ratio (ICER) for oil-based contrast compared to water-based contrast of €670 for an additional ongoing pregnancy[7].
This is very cost-effective as compared to for example In Vitro Fertilization (IVF). IVF generates a 25-30% live birth rate for €3000 euro per patient, resulting in a cost-effectiveness ratio of

approximately €10.000 for an additional ongoing pregnancy. Thus, there is a strong argument to incorporate tubal flushing with oil-based contrast in clinical practice.

In fact, the literature that reports on the effectiveness of tubal flushing in women with anovulation or tubal pathology is very limited. Only one study reported on women with oligo-ovulation undergoing HSG with oil- and water-based contrast medium, but as the study had extremely small samples (17 versus 18 women) the results lacked statistical power (pregnancy rates 53% versus 28%; RR, 0.53; 95% CI, 0.22 to 1.26)[8]. The same study reported on 12 versus 19 women with tubal pathology (pregnancy rates 17% versus 16%; RR, 0.95; 95% CI, 0.18 to 4.87) with a sample size again far too small to allow any firm conclusions. In later studies, such as Dreyer et al. (2017) or the one from Johnson et al. (2004), oligo-/anovulation and tubal pathology were exclusion criterion[6,9]. In all those studies women over 39 years of age were excluded.

Thus, while we have reported a very strong treatment effect in women with an ovulatory cycle, with unexplained infertility and younger than 38 years, it is unknown whether this treatment effect is applicable to more than 50% of the women presenting with infertility, i.e. women with an ovulation disorders, women at high risk of tubal pathology and women ≥38 years of age, in whom infertility is driven by decreased ovarian reserve.

In this perspective, it is important to consider that the potential mechanism underlying the treatment effect of flushing with oil-based contrast medium has not yet been elucidated. One theory is that tubal flushing with oil-based contrast flushes accumulated debris and mucous plugs from undamaged tubes, which will enhance patency[10]. Another theory is that the oil-based contrast affects the receptivity of the endometrium, which enhances embryo implantation[11].
A third theory is modulation of peritoneal macrophage activity by oil contrast which affects the implantation mediated mechanism positively[11]. In vitro studies have demonstrated that oil contrast inhibits phagocytosis of macrophages in humans and rats, perchance phagocytosis of sperm[12,13]. A recent study by Izumi et al. supposes a fourth theory, that oil contrast is incorporated by dendritic cells in the peritoneal cavity. This modulates the immunological environment in the peritoneal cavity by promoting more mature dendritic cells, altering cytokine and chemokines profiles in dendritic cells and increasing the number of T-cells. These results may contribute to the fertility enhancing effect of HSG with oil contrast[14]. While further research regarding the mechanism of oil-based contrast is necessary, it is unclear whether the fertility enhancing effect of HSG with oil- based contrast, as proven in the H2Oil study, is also present in women with other causes of infertility. Also, the mechanisms that make oil-flushing effective

in unexplained and mild male infertility, might not help in women with other types of infertility. Thus, the empirical research that we propose in this project is also very urgent.

It is important to know that before publication of the H2Oil study in 2017, clinical practice moved away from tubal patency testing. While unaware of the potential treatment effect of tubal flushing, HSG became less relevant as diagnostic test. First, the incidence of tubal pathology in women presenting with infertility has reduced over the decades *(Landelijke IVF-rapportage 2017)*. Second, IVF has become more important, and since patients will get IVF one way or the other, less emphasis has been put on the diagnosis tubal pathology.
As a consequence, tubal testing in patients at low risk for tubal pathology, based on risk prediction[15], a negative chlamydia antibody titre[16] or the presence of another infertility diagnosis (anovulation) has limited the use of tubal testing to women at high risk for tubal pathology. The 2015 NVOG guideline therefore advises to limit tubal testing to women at high risk for tubal pathology (which is defined as a positive Chlamydia Antibody Test, PID, abdominal surgery and/or peritonitis in the medical history).
The guideline does not recommend the type of contrast medium for tubal flushing during HSG in women with a high risk for tubal pathology and takes no account for the fertility enhancing effect of tubal flushing with oil contrast in unexplained infertility, and the possible fertility enhancing effects in women with other causes of infertility. Therefore it is important to evaluate the effect of oil- and water-based contrast for tubal flushing in couples with other fertility problems such as ovulation disorders and tubal pathology. Since oil-based contrast is more expensive than water-based contrast, oil-based contrast should not be used if it is not more effective than water-based contrast.

In summary, while tubal flushing has been long neglected, the beneficial results of our recently published H2Oil study, has re-introduced tubal flushing for infertile women with an ovulatory cycle at low risk for tubal pathology and younger than 38 years. However, there is a clear knowledge gap with respect to women not included in our study, i.e. women who have ovulation disorders, or women who are at high risk for tubal pathology, or women who are above 38 years of age, in whom infertility is driven by decreased ovarian reserve. Since the mechanism of infertility in these women is completely different, it is unknown if tubal flushing with oil-based contrast increases fertility chances in these women. We therefore propose a multicenter randomized controlled trial to test the hypothesis that HSG with oil-based contrast will increase the pregnancy- and life birth rate as compared to HSG with water-based contrast in above mentioned groups of infertile women.

2. OBJECTIVES

Primary Objective: conception leading to live birth, with a positive pregnancy test preceding the pregnancy within 6 months after randomization.

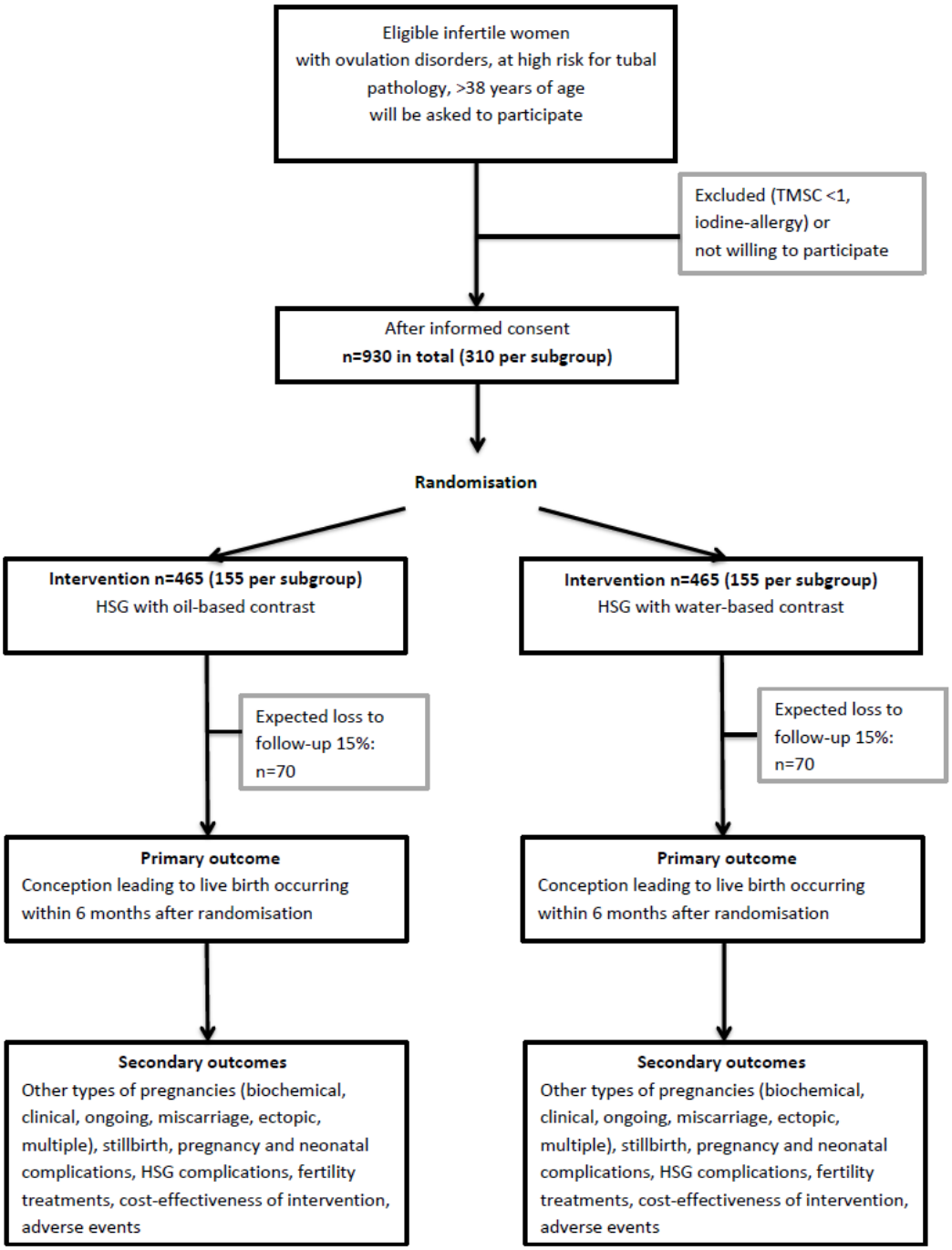
Secondary Objective(s):

- Biochemical pregnancy
- Clinical pregnancy
- Ongoing pregnancy
- Miscarriage
- Ectopic pregnancy
- Multiple pregnancy
- Time to Event (pregnancy)
- Complications following HSG (infection, intravastion)
- Pregnancy outcomes (f.e. birth weight)
- Pregnancy complications
- Stillbirth
- Thyroid function of the woman (before and 1 month after HSG)
- Neonatal outcomes
- Additional fertility treatments (Intra-uterine insemination, IVF, IVF/ICSI)
- Costs within 6 months after randomization
- Thyroid function of neonate (determined by heelprick)

3. STUDY DESIGN

We plan an investigator initiated multicenter randomized controlled trial with a cost-effectiveness analysis alongside it. Infertile women with ovulation disorders, at high risk for tubal pathology and/or above 38 years of age will be randomized to HSG with oil-based contrast or HSG with water-based contrast. 930 women (310 per subgroup) will be included in the study.

Figure 1: Flowchart



4. STUDY POPULATION

4.1 Population (base)

Couples should have tried to conceive for at least 12 months, or have oligo- or anovulation. Our H2Oil2 study will focus on the following categories of women:

1: with ovulation disorders or;

2: at high risk for tubal pathology or;

3: 39 years of age or over.

Women can participate if they undergo a HSG as part of the fertility work-up, or when a HSG is performed at a later stage, for example after multiple cycles of ovulation induction not resulting in a pregnancy.

4.2 Inclusion criteria

In order to be eligible to participate in this study, women must meet one of the following criteria:

1: with ovulation disorders (ovulation disorders will be defined as less than 8 menstrual cycles per year) or;

2: at high risk for tubal pathology (high risk for tubal pathology will be defined as a positive chlamydia infection, a pelvic inflammatory disease, known endometriosis, abdominal surgery (including tubectomy for ectopic pregnancy and appendectomy) and/or peritonitis in the medical history) or;

3: 39 years of age or over

4.3 Exclusion criteria

- Iodinated contrast agent allergy
- Male subfertility defined as a post-wash total motile sperm count < 1 x10^6 spermatozoa/ml (or pre-wash <3x10^6 spermatozoa/ml)
- Not willing or able to sign the consent form
- Women with known endocrine disorders (e.g. diabetes, hyperthyroidism and hyperprolactinemia) except for well managed hypothyroidism prior to the fertility work, as defined by TSH level between 0.3 and 2.5 mIU/l
- Insufficient ability to talk and/or read Dutch or English language

4.4 Sample size calculation

Sample size calculation was done in PASS 15.0.6 (NCSS Statistical Software LCC, Utah, USA). Group sample sizes of 395 in group 1 and 395 in group 2 achieve 80% power to detect an absolute difference between the group proportions of 10%. The proportion in group 1 (the treatment group) is assumed to be 17% under the null hypothesis and 27% under the alternative hypothesis. The proportion in group 2 (the control group) is 17%. The test statistic used is the two-sided Z-Test with unpooled variance. The significance level of the test is 0,01.

With a drop-out rate of 15% we need to included 930 couples. For the whole population the difference between the two treatment arms will be expressed as relative risk with both a corresponding 99% and a 95% confidence boundary. We will stratify by indication of treatment into three groups: 1: women with ovulation disorders, 2: women at high risk for tubal pathology, 3: women above 38 years of age. The sample size of 930 allows to do a stratified analysis of the data. As a secondary analysis, we will do a cox proportional hazard analysis to evaluate the difference in primary outcomes over time while accounting for the subgroups. We will also evaluate presence of interactions between subgroups and treatment. The differences in the subgroups will be expressed as Hazard Ratios with corresponding 95% confidence boundaries.

5. TREATMENT OF SUBJECTS

Women will be randomized for tubal flushing at HSG with oil-based contrast medium or tubal flushing at HSG with water-based contrast medium.

5.1 Investigational product/treatment

Experimental intervention:
Tubal flushing at HSG with oil-based contrast medium (Lipiodol®, by Guerbet) (max. 15mL).

Control:
Tubal flushing at HSG with water-based contrast medium (Visipaque®, by GE Healthcare) (max. 15mL).

HSG:
HSG will be done in the follicular phase of the cycle. During HSG a maximum amount of 15cc of contrast medium will be infused into the uterine cavity through a cervical vacuum cup, a metal cannula (hysterophore) or a special HSG-balloon catheter. The contrast medium contains Iodine and will be visible on radiography. During instillation of the contrast medium (oil- or water-based contrast), 4-6 radiographs will be taken to see if the fallopian tubes are patent. Test results of the HSG will be classified as normal, one-sided tubal pathology or double-sided tubal pathology.

The planned fertility treatment will be based on the results of the fertility work-up. Women with patent tubes at HSG will be treated according to their prognosis for natural conception based on the model of Hunault[17]. In case the prognosis for natural conception within 12 months ≥30%, women will be counselled for expectant management. In case the prognosis is <30%, women will be treated with Intra-Uterine Insemination (IUI) eventually followed by IVF. In case of suspected uni- or bilateral tubal occlusion or pathology, women will be scheduled for Diagnostic Laparoscopy (DLS) according to the local protocol, followed by IVF in case bilateral tubal occlusion is conformed. In case of uni- or bilateral tubal patency during DLS the subsequent fertility treatment will also be based on the Hunault prognosis for natural conception.[17] Calculation of Hunault score is not validated for women over 38 years of age. In women over 38 years of age management will be based on local protocols. Women with ovulation disorders will start or continue with ovulation induction treatment.

5.2 Use of co-intervention (if applicable)

Antibiotic will be prescribed (for instance Doxycyclin 200mg BDS for 7 days) to women with suspicion of intra-abdominal adhesions or hydrosalpinx following HSG, according to the local protocols of the participating centres. Women who have a high perceived risk for tubal pathology prior to the HSG may receive prophylactic antibiotics according to local protocols.

5.3 Escape medication (if applicable)

Not applicable.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product

The investigational product is an oil-based contrast medium, Lipiodol® (Guerbet). Lipiodol® is a solution of ethyl esters of iodized fatty acids of poppy seed oil equivalent to 480mg I /ml and is a licensed contrast agent in the Netherlands (RVG 02806, see SmPC D2). The maximum amount of Lipiodol® per HSG procedure is 15 ml. Based on the H2Oil study the medium volume of contrast medium used in HSG procedures was 8-9 ml per participant[6].

The water-based contrast medium, that serves as a control is Visipaque 270® (GE Healthcare), contains 550 mg/ml Iodixanol equivalent to 270mg I/ml and is a licensed contrast agent in the Netherlands (RVG 17664, see SmPC D2).

6.2 Summary of findings from non-clinical studies

This section is not applicable. Both contrast media are routinely used in daily practice and there is more than 50 years of experience with both contrast media in humans.

6.3 Summary of findings from clinical studies

Recently, a meta-analysis of six RCT's (N=2,562) was published. This meta-analysis showed that the use of oil-based contrast medium at HSG was associated with significantly higher rates of ongoing pregnancy compared with the use of water based contrast medium at HSG (OR 1.47, 95% CI 1.12–1.93), based on moderate-quality evidence. Three trials reported live birth, but they were not pooled owing to extreme statistical heterogeneity There was no difference in incidence of miscarriage (OR 0.83, 95% CI 0.56–1.24) or ectopic pregnancy (OR 0.65, 95% CI 0.18–2.36) between oil-based contrast medium and water-based contrast medium groups. However, most of the studies included women at low risk for tubal pathology and with idiopathic subfertility[18].
Our study group recently submitted a network meta-analysis regarding the effectiveness of tubal flushing with different contrast media on fertility outcomes, we concluded that tubal flushing with oil-based contrast increases short-term (6 months) clinical pregnancy rates and may increase live birth rates compared to tubal flushing with water-based contrast and no tubal flushing, but it is not certain whether such potential superiority persists after 6 months. The effect of oil-based contrast has not been evaluated outside HSG. A limitation of this study was that the majority of the participants of the included studies were couples with unexplained infertility. The is lack of evidence to determine whether the observed treatment effect holds for other populations such as women with advanced age, anovulation or tubal factor infertility. Results of the combination group should be interpreted with caution due to the high risk of selection, attrition or other bias in the including studies[19].

6.4 Summary of known and potential risks and benefits

The possible fertility enhancing mechanism of HSG with oil-based contrast, as showed in the above described Systematic Reviews, is the most important possible benefit in our study.

However, several safety concerns on tubal flushing have been raised. Firstly, venous intravasation occurs in approximately 2-7% cases in HSG[20-22], and occurs more frequently when using oil-based contrast compared to water-based contrast. While intravasation can potentially result in pulmonary embolism and even death, no deaths have been reported since the 1960s[23] which is probably related to the introduction of fluoroscopy screening during HSG. Moreover, no cases of embolism were reported in the published RCTs (including the H2Oil study).
Secondly, the concern about the thyroid function of mother and child is based on the effects of iodinated contrast media[24,25] and a longer persisting time of oil-based contrast in the pelvis compared to water contrast [26]. Maternal hypothyroidism can occur after tubal flushing with oil-based contrast[25], especially in women with subclinical hypothyroidism before HSG[27]. With regards to neonatal safety, one Japanese cohort studied infants born to mothers who had become pregnant after undergoing HSG with oil-based contrast. Five out of 212 infants tested positive during congenital hypothyroidism screening (2.4%), this frequency was higher than the recall rate among first congenital hypothyroidism screening results (0.7%) in Tokyo, Japan. However, iodine-rich seaweed consumption in Japan can also be a cause of excessive iodine exposure[24]. It is also important to mention that according to the RIVM in the Netherlands, the congenital hypothyroidism (CH) background rate is much lower (0.05%) compared to the background rate in Japan (0.7%). In addition, the median dosage of oil contrast used in the thyroid dysfunction group was significantly higher than compared to the normal thyroid function group (20 ml versus 8 ml, p = 0.033)[24]. For this current study, the maximum amount of oil contrast will be 15 ml (as per SmPC).
To evaluate CH after HSG with oil or water contrast we conducted a retrospective cohort study (METc VUmc 2018.463). We are currently working on this project. However, we anticipate a very low chance of thyroid problems in the neonate.

Thirdly, pelvic infection is another potential safety concern. Data from the included trials in our network meta-analysis indicated it is a rare event and there is insufficient evidence to show a difference between oil based- and water based contrast use in HSG.
See also SmPCs under section D2 of the research file, paragraph 4.4, 4.6 and 4.8.

6.5 Description and justification of route of administration and dosage

The HSG will be performed by a gynaecologist, attending-gynaecologist or fertility doctor according to local protocols. HSG will be done in the follicular phase of the cycle. During HSG a maximum amount of 15 ml of contrast medium (in case of Lipiodol®, there is no strict in limit when using Visipaque®) will be infused into the uterine cavity through a cervical vacuum cup, a metal cannula (hysterophore) or a special HSG-balloon catheter. Both types of contrast medium for HSG contain Iodine (oil- and water-based contrast) and will be visible on radiography. All HSGs will be made with real time fluoroscopic imaging. During instillation of the contrast medium, 4-6 radiographs will be taken to see if the fallopian tubes are patent. Test results of the HSG will be classified as normal, one-sided tubal pathology or double-sided tubal pathology. The HSG procedure will be aborted if intravasation occurs, in order to minimize the risk of an embolism. The maximum amount of contrast medium is 15 ml. Based on the H2Oil

study the medium volume of water- and oil-contrast medium used in HSG procedures will be 8-9 mL per participant[6].

6.6 Dosages, dosage modifications and method of administration

The investigational product is an oil-based contrast medium, Lipiodol® (Guerbet). Lipiodol® is a solution of ethyl esters of iodized fatty acids of poppy seed oil equivalent to 480mg I /ml and is a licensed contrast agent in the Netherlands (RVG 02806, see SmPC D2). The maximum amount of Lipiodol® per HSG procedure is 15 ml. Based on the H2Oil study the medium volume of contrast medium used in HSG procedures was 8-9 ml per participant[6].

The water-based contrast medium, that serves as a control is Visipaque 270® (GE Healthcare), contains 550 mg/ml Iodixanol equivalent to 270mg I/ml and is a licensed contrast agent in the Netherlands (RVG 17664, see SmPC D2). There is no maximum limit of Visipaque® per HSG procedure. Based on the H2Oil study the medium volume of contrast medium used in HSG procedures was 8-9 mL per participant[6].

6.7 Preparation and labelling of Investigational Medicinal Product

The Investigational Medicinal Products will not be prepared and labelled according to EU GMP annex 13 regulations. Labelling as an IMP is not deemed necessary, because the IMPs will not be dispensed to the patient and administration is performed by a trained physician. We will ensure the IMP is produced and handled according to GMP and GDP regulations. Both Lipiodol Ultra Fluid® and Visipaque® are produced by a GMP approved manufacturer and are authorized in the Netherlands. Both contrast media are licensed for the indication (Hysterosalpingography) used in this study, see also SPC's under section D2 of the research file. An HSG with Lipiodol or Visipaque is normal and standard care in the Netherlands[28]. Lipiodol Ultra Fluid® is distributed by a GMP/GDP pharmaceutical company (Guerbet) stored at constant room temperature in the hospital pharmacy. Visipaque® is distributed by a GMP/GCP pharmaceutical company (GE Healthcare) stored at constant room temperature in the hospital pharmacy. Dispensing to the radiology department is documented and the contrast media are stored at constant room temperature with air conditioning, in a locked and secured room at the radiology department according to the storage conditions in the SmPC par 6.4.

6.8 Drug accountability

Both contrast media will not be prescribed for the individual patient. The (attending) gynaecologist will state in the medical file of the patient that "the patient is eligible to participate in this study" and states that "the patient will undergo an HSG with contrast medium (Lipiodol® or Visipaque®) based on randomization outcome". The (attending) gynaecologist communicates this with the researcher or research nurse of this study. The researcher or research nurse will also check whether the patient is eligible to participate in this study. Randomization will be done by either the (attending) gynaecologist, researcher or

research nurse (depending per local center and delegation log). The (attending) gynaecologist will perform the HSG with Lipiodol® or Visipaque® in concordance with the randomization. Lipiodol® or Visipaque® will only be used once during HSG and will be distributed from the pharmacy to the radiology department. The use of both contrast media are standard care, which is used in daily patient care, there the logistics will be according to standard procedures. Before opening a research site the local procedures will be checked for GDP compatibility.
To ensure traceability and accurate documentation of the intervention the (attending-) gynaecologist and investigator will note the RVG-number, batchnumber, expiration date and volume of used contrast in the register form in the CRF. Drug accountability will be monitored via the CRF based on study number. There will be a monitor plan for all participating centers to check drug traceability and accountability. Together with the CRB of the Amsterdam UMC location VUmc we will ensure the monitoring of the participating centers.

7. NON-INVESTIGATIONAL PRODUCT

7.1 Name and description of non-investigational product(s)

Not applicable.

7.2 Summary of findings from non-clinical studies

Not applicable.

7.3 Summary of findings from clinical studies

Not applicable.

7.4 Summary of known and potential risks and benefits

Not applicable.

7.5 Description and justification of route of administration and dosage

Not applicable.

7.6 Dosages, dosage modifications and method of administration

Not applicable.

7.7 Preparation and labelling of Non Investigational Medicinal Product

Not applicable.

7.8 Drug accountability

Not applicable.

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

The primary outcome is conception leading to live birth, with a positive pregnancy test preceding the pregnancy within 6 months after randomization.

8.1.2 Secondary study parameters/endpoints (if applicable)

- Biochemical pregnancy (a positive pregnancy test or an increase in HCG combined with menstrual bleeding and absence of ultrasound visible pregnancy)
- Clinical pregnancy (an ultrasound visible gestational sac with or without heartbeat)
- Ongoing pregnancy (vital intrauterine pregnancy at 12 weeks)
- Miscarriage (the presence of non-vitality on ultrasound or spontaneous loss off pregnancy)
- Ectopic pregnancy (no intrauterine gestational sac with: an ectopic gestational sac OR serum HCG >=1500 IU/L with free fluid/ectopic mass on ultrasound OR serum HCG >= 2000 IU/L without free fluid/ectopic mass on ultrasound)
- Multiple pregnancy (2 or more vital intrauterine pregnancies at 12 weeks)
- Time to Event (pregnancy) (from HSG date to positive pregnancy test of the pregnancy resulted in a live birth)
- Complications following HSG ((serious) adverse events; infection, intravasation or embolism)
- Pregnancy outcomes (f.e. birth weight, gestational age)
- Pregnancy complications (f.e. diabetes gravidarum, pregnancy induced hypertension (PIH), Pre-eclampsia, HELLP Syndrome, Placenta Praevia)
- Stillbirth
- Thyroid function of the woman (before and 1 month after HSG)
- Neonatal outcomes
- Additional fertility treatments (Intra-uterine insemination, IVF, IVF/ICSI)
- Costs within 6 months after randomization
- Thyroid function of neonate (determined by heelprick)

8.1.3 Other study parameters (if applicable)

The basic parameters which will be monitored are the following: Women's age, ethnicity, medical history, referral status, cycle duration, use of medications, intoxications, BMI.

8.1.4 Data/analysis and presentation/synthesis

Step 1: Summarizing trial data

Baseline data and outcome data will be separately summarized. For continuous variables, we will examine the distribution of the observations and if normally distributed, we will summarize them as means with standard deviations (SDs). If they are not normally distributed, then medians and inter-quartile ranges (IQRs) will be reported. For dichotomous data, we will provide proportions (with percentages). In addition, to the baseline and outcome data, we will also summarize recruitment numbers, those lost to follow-up, protocol violations and other relevant data.

Step 2: Inter-group comparisons

The analyses of all outcomes will be done on an intention-to-treat basis. The effectiveness of oil- and water-based contrast medium will be expressed as a rate for ongoing pregnancy leading to live birth with corresponding 95% confidence intervals. We will compare the ongoing pregnancy rates in both groups using Kaplan-Meier analysis. Dichotomous outcomes will be analysed using either Fisher Exact of chi-square as appropriate. Categorical data will also be analysed using chi-square. For continuous outcomes we will use t-test if the observations in each trial arm and subgroup are normally distributed, and if non-normally distributed, then Mann-Whitney-U test will be employed. Although p-values will be reported, the focus will be on providing 95% confidence intervals around point estimates as these are more useful in interpreting the findings of the trial. As analysis of all outcomes will be done on an intention-to-treat basis, all women randomized will be followed up until the occurrence of an ongoing pregnancy within 6 months after inclusion and until life birth if an ongoing pregnancy has occurred. As a secondary analysis, we will do a cox proportional hazard analysis to evaluate the difference in primary outcomes over time while accounting for the subgroups. We will also evaluate presence of interactions between subgroups and treatment. The differences in the subgroups will be expressed as relative risks with corresponding 95% confidence boundaries.

Step 3: Adjustments and sensitivity analysis

If randomization fails to achieve balanced groups, then we will perform secondary analyses in which we will adjust for unbalanced prognostic factors using procedures such as (multiple) logistic regression analyses. If the primary analysis and secondary adjusted analysis are discordant, we will give greater weighting to the primary analysis in the interpretation of trial findings. For issues such as losses to follow-up, missing data and protocol violations, we will attempt sensitivity ("worst-case scenario") analyses to explore the effect of these factors on the trial findings. As a secondary analysis, we will adjust tor missing data using imputation techniques to explore the effects of such imputations on the trial findings. The effect of baseline characteristic on the primary outcome will be explored using (multiple) logistic regression analyses.

8.1.5 Cost-effectiveness Analysis (CEA)

The average costs and effects of tubal flushing during HSG with oil-based contrast and water-based contrast during fertility work-up will be compared. Fertility treatments and pregnancy outcomes (collected using the CRF) will be evaluated after a follow-up of 6 months after randomization. These data will be used to calculate the direct medical costs. We will use the data from the iPCQ questionnaire for productivity loss (absenteeism from paid and unpaid work and presentism) for the calculation of social costs. Our study population represents 10,000 new couples each year. If we find 10% benefit from tubal flushing, this would implicate 1.000 new pregnancies each year and thus a reduction of 1,000 IVF cycles, i.e. a saving of 3 million euro annually.

8.2 Randomisation, blinding and treatment allocation

Consenting eligible women will be randomized to HSG with oil- or water based contrast. Randomization is stratified per subgroup and centre, will be performed after baseline data have been entered in a centrally web-based system (CASTOR) with the use of permuted block design. Owing to the nature of the intervention and since our primary outcome of live birth is objective, the trial is not blinded with respect to participants and caregivers. The researcher that performs the analysis will be blinded for the randomization outcome (oil- or water-based contrast).
Women who decline randomization will be offered the standard treatment in the participating clinic.
The HSG images will be judged by a gynaecologist and/or radiologist according to local protocol, and a conclusion and advised treatment will be given depending on the results and the prognosis on a natural conception based on the model of Hunault[17].

8.3 Study procedures

Eligible women for participation in the study will be counselled during their regular outpatient clinic visit by the (attending) gynaecologist or fertility doctor. To ensure that they are fully informed on the nature of the study, they will also receive written information (Participant Information Form, section E of this research file). Women who agree to participate will be asked to sign a written informed consent of which they will receive a copy. In cases where women don't have a follow-up appointment scheduled, they can be contacted by telephone by the researcher after they have given consent to be called to their physician. The researcher can answer any questions, ensure the participant is eligible and will ask them to send a (digital) copy of their signed informed consent form to the researcher after which randomization will take place. In these instances it is possible that the researcher signs the original copy of the informed consent form after the date of randomization.

Consenting eligible women will be randomized to HSG with oil- or water-based contrast. Randomization is stratified per subgroup and centre, will be performed after baseline data have been entered in a centrally web-based system. (CASTOR) with the use of permuted block

design. Owing to the nature of the intervention and since our primary outcome of life birth is objective, the trial is not blinded with respect to participants and caregivers. The researcher that performs the analysis will be blinded for the randomization outcome (oil- or water-based contrast). The following patients' characteristics of all patients will be assessed after randomisation: a complete medical history, referral status, BMI, smoking and ethnicity duration. Women who decline randomization will be offered the standard treatment in the participating clinic.

Intervention:
Women randomized to HSG with oil-based contrast (Lipiodol).

Usual care/comparison:
Women randomized to HSG with water-based contrast (Visipaque).

HSG:
Women will be asked to fill out the anxiety questionnaire prior to the HSG to assess fear or anxiety for the procedure. Directly after the procedure women will be asked to report the pain they experienced on a Visual Analogue Scale (VAS).
HSG will be done in the follicular phase of the cycle. During HSG a maximum amount of 15cc of contrast medium (oil- or water-based contrast) will be infused into the uterine cavity through a cervical vacuum cup, a metal cannula (hysterophore) or a special HSG-balloon catheter. The contrast medium contains Iodine and will be visible on radiography. During instillation of the contrast medium, 4-6 radiographs will be taken to see if the fallopian tubes are patent. Test results of the HSG will be classified as normal, one-sided tubal pathology or double-sided tubal pathology.
The planned fertility treatment will be based on the results of the fertility work-up. Women with patent tubes at HSG will be treated according to their prognosis for natural conception based on the model of Hunault.[17]In case the prognosis for natural conception within 12 months ≥30% women will be counselled for expectant management. In case the prognosis is <30%, women will be treated with Intra-Uterine Insemination (IUI) eventually followed by IVF. In case of suspected uni- or bilateral tubal occlusion or pathology, women will be scheduled for Diagnostic Laparoscopy (DLS) according to the local protocol, followed by IVF in case bilateral tubal occlusion is conformed. In case of uni- or bilateral tubal patency during DLS the subsequent fertility treatment will also be based on the Hunault prognosis for natural conception.[17] Calculation of Hunault score is not validated for women over 38 years of age. In women over 38 years of age management will be based on local protocols. Women with ovulation disorders will start or continue with ovulation induction treatment depending on local protocols.

Follow-up:
All women randomized will be followed up until the occurrence of an ongoing pregnancy within 6 months after inclusion and until life birth if an ongoing pregnancy has occurred. At 6 months,

all participating women will receive two questionnaires. One questionnaire regarding fertility treatments and pregnancy outcomes. And one questionnaire regarding production loss (iPCQ) in the past 6 months. For the CEA, the direct medical costs will be calculated from the CRF (ART treatments, pregnancy outcomes etcetera). We will use the data from the iPCQ questionnaire for productivity loss (absenteeism from paid and unpaid work, and presentism) for calculation of social costs.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.4.1 Specific criteria for withdrawal (if applicable)

Not applicable.

8.5 Replacement of individual subjects after withdrawal

According to our power calculation we need to include 930 patients, in case of withdrawal we will replace these patients with new inclusions.

8.6 Follow-up of subjects withdrawn from treatment

Patients who have been withdrawn from treatment will not be followed-up.

8.7 Premature termination of the study

Both the investigator and the Sponsor reserve the right to terminate the study for safety reasons of the subjects of if the trial becomes scientifically meaningless.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the intervention. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation; *except hospitalization for labour, diagnostic laparoscopy, vacuum curettage in case of an incomplete miscarriage or ovarian hyper stimulation syndrome (OHSS) during IVF.*
- results in persistent or significant disability or incapacity;
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

According to literature, the SAE's that are seen after an HSG are related to infection or allergic reacting, both of which are expected to occur within several days to two weeks after the procedure. In this study the abovementioned SAE's will be reported up until 1 month after the HSG. Additionally, adverse neonatal outcomes such as a congenital anomaly or birth defect will be reported as SAE.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events. SAEs that result in death or are life threatening should be reported expedited.

All investigators of the participating hospitals will directly report the serious adverse events to the Sponsor of this study. The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
    - Summary of Product Characteristics (SPC) for an authorised medicinal product;
    - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.

The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

9.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.
This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

9.5 [Data Safety Monitoring Board (DSMB) / Safety Committee]

A DSMB will not be installed since the product and intervention used in this study are registered for the given indication and used in clinical practice for years.

10. STATISTICAL ANALYSIS

10.1 Primary study parameter(s)

The analyses of all outcomes will be done on an intention-to-treat basis. The primary outcome is conception leading to life birth, with a positive pregnancy test preceding the pregnancy within 6 months after randomization. For the whole population the difference between the two treatment arms will be expressed as relative risk with both a corresponding 99% and a 95% confidence boundary.

For the subgroups, we will do a cox proportional hazard analysis as a secondary analysis to evaluate the difference in primary outcomes over time while accounting for the subgroups. We will also evaluate presence of interactions between subgroups and treatment. The differences in the subgroups will be expressed as relative risks with corresponding 95% confidence boundaries.

10.2 Secondary study parameter(s)

All secondary outcomes (listed in section 8) will be compared in the intention-to-treat and the per protocol population. For numerical and continuous outcomes, the Student t test or Mann Whitney U test will be used. For dichotomous outcomes, the difference between two proportions will be calculated by using the Chi-square of Fishers exact test. Time-to-event (pregnancy) will be calculated. We will compare the time to ongoing pregnancy in both groups using Kaplan-Meier analysis.

For the cost effectiveness analyses a Cost Effectiveness Analysis (CEA) will be performed.

10.3 Other study parameters

In case of continuous data, group results are presented as means and standard deviations (normally distributed data) or medians and ranges (non-parametrical data). In case of categorical data number with percentages will be used. These statistics will be provided for the intention to treat (ITT) and per protocol (PP) groups.

10.4 Interim analysis (if applicable)

Not applicable.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts. The study protocol will be submitted to the ethics committee.

11.2 Recruitment and consent

Eligible patients will be informed about the study by the gynaecologist, the attending resident or the fertility doctor. The patients will also obtain written information about the study. Subsequently, the investigator, supervising gynaecologist, the attending registrar or fertility doctor must explain to each subject the nature of this study, its purpose, procedures, expected duration and the potential risks and benefits involved in study participation along with any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that withdrawal of consent will not affect her right to the most appropriate medical treatment or affect the doctor relationship. This informed consent should be given by means of a standard written statement. It should be written so as to be easily understood by the subject. The subject should be given enough time to read and understand the statement herself before signing her consent and dating the document. In case the subject wants more information, an independent doctor is available to answer her questions. The subject should receive a copy of the written statement once signed.

11.3 Objection by minors or incapacitated subjects (if applicable)

Not applicable.

11.4 Benefits and risks assessment, group relatedness

The potential benefit of direct tubal flushing with oil-based contrast during fertility work-up is a shorter time to pregnancy.

11.5 Compensation for injury

The VUmc has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

1. € 650.000,-- (i.e. six hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 5.000.000,-- (i.e. five million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;
3. € 7.500.000,-- (i.e. seven million five hundred thousand Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.6 Incentives (if applicable)

Not applicable.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Informed Consents (IC) will be stored in a locked closet on the medical researchers' room (or in the ISF on the local research nurses' room) which is locked if the medical researcher or local research nurse is not there. Randomisation will be done in a web-based registration system (Castor Electronic Data Capture, Ciwit BV, Amsterdam, The Netherlands, 2016) with the use of permuted block design and is stratified per center. The study code is a meaningless generated number and will not be based on the patient's initials or birth date. Data will be handled coded and confidentially according to the Good Clinical Practice (GCP) guideline and General Data Protection Regulation (GDPR) (in Dutch: Algemene verordening Gegevensbescherming (AVG)).

The Trial Master File (TMF) or Investigator Site File (ISF) will be stored with a password on the hard disk of the local Obs&Gyn department of the participating center. Only the medical researcher and principal investigator have access to the TMF on the hard disk at the VUmc, and are able to identify the link between study number and participant. The Investigator Site is available for the local investigator and research nurse. Data will be handled confidentially, with the link between the participant and study code only available to the local investigator and research nurse in the participating center. All data will be collected by study number in a web-based registration system (Castor Electronic Data Capture, Ciwit BV, Amsterdam, The Netherlands, 2016). Data will be stored for a maximum of 25 years after the study has been completed. Afterwards all data will be deleted. Data will only be shared with other researchers in case of valid and relevant requests, and a coded database will be shared without any linkage to individual participants.

12.2 Monitoring and Quality Assurance

An independent monitor from the Clinical Research Bureau (CRB) of the VUmc will have access to the data and source documents of the trial. Monitoring will be performed in compliance with Good Clinical Practice (GCP) and other rules and regulations in order to achieve high quality research and secure patient safety.

12.3 Amendments

All amendments will be notified to the METC and to the competent authority.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Start of study

The start of this study will be communicated to the METC.

12.6 Temporary halt and (prematurely) end of study report

The investigator will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.
The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.7 Public disclosure and publication policy

No specific arrangements will be made between any sponsors and the investigator concerning the public disclosure and publication of the research data. The principle investigator will publish the results of the study as soon as appropriate. The results of the study will be disclosed unreservedly as mentioned in the statement on publication policy of the CCMO (www.ccmo.nl).

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

Not applicable: use of registered products used within the indication and not in combination with other products.

13.2 Synthesis

Since we compare two interventions used in daily practice for years we do not expect additional risks compared to the standard practice outside the study. See also previous literature in section K of this research file.

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