

# BMJ Open Live birth after additional tubal flushing with oil-based contrast versus no additional flushing: a randomised, multicentre, parallel-group pragmatic trial in infertile women with at least one patent tube at hysterosalpingo-foam sonography (HYFOIL study)

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## ABSTRACT

**Introduction** Tubal patency testing is an essential part in the fertility workup of many subfertile women. Hysterosalpingography (HSG) has long been the test of choice in many clinics. There is evidence from a large randomised multicentre trial and from a recent meta-analysis that women who had HSG using oil soluble contrast medium (OSCM) had higher rates of ongoing pregnancy compared with women who underwent this procedure using water contrast. However, the field is moving away from HSG and nowadays hysterosalpingo-foam sonography (Hyfosalpingo) using ultrasound guidance is considered as the first line office tubal patency test. Therefore, a large multicentre randomised clinical trial (RCT) will be initiated to evaluate if flushing the fallopian tubes with OSCM after a normal Hyfosalpingo showing at least one patent fallopian tube will increase the live birth rate as compared with no flushing.

**Methods and analysis** We plan a multicentre two arm, 1:1 randomised, open-label pragmatic comparative trial in 12 Belgian centres. After informed consent, we will randomise infertile women between 18 and 40 years of age, undergoing Hyfosalpingo as part of the fertility workup to Hyfosalpingo with additional tubal flushing with OSCM versus Hyfosalpingo without additional flushing. Infertility is defined as lack of conception despite 12 months of unprotected intercourse, or three cycles of donor insemination without pregnancy or three ovulatory ovulation induction cycles without pregnancy, all three in combination with at least one patent tube on Hyfosalpingo. Primary endpoint will be live birth with conception within 6 months after randomisation.

**Ethics and dissemination** Approval on 11 May 2021 by the Ethics Committee from ZNA Hospital Antwerp who was the central Ethics Committee for the Clinical Trial Regulation Pilot (Pilot 412) in the 12 centres. The findings from this RCT will be disseminated in peer-reviewed publications and presentations at scientific international meetings.

## Strengths and limitations of this study

- This is the first adequately powered randomised controlled trial.
- Patients from 12 Belgian university hospital and private fertility centres reflect the whole fertility population.
- The pragmatic nature of the trial mirrors the current fertility treatment in the centres.
- Potential maternal and neonatal hypothyroidism will be assessed.
- Both hysterosalpingo-foam sonography and subsequent Lipiodol Ultra Fluide flushing need to be performed.

**Trial registration numbers** EudraCT number: 2020-002135-30 and NCT04379973.

## INTRODUCTION

A total of 10%–15% of couples in their reproductive lifetime face fertility problems which is defined by WHO as the absence of pregnancy after 12 months of unprotected sexual intercourse. The three most frequent causes of infertility are: sperm defects, ovulation disorders and tubal pathology.<sup>1</sup> To diagnose tubal pathology, various diagnostic tests are available. In case of suspected tubal pathology a diagnostic laparoscopy (DLS) will be performed in most cases as this technique allows direct visualisation of the pelvis including fallopian tubes, ovaries and uterus and offers the possibility to restore abnormalities of tubal function to increase chances of spontaneous conception.



Hysterosalpingography (HSG) has long been the test of choice in many clinics. Compared with DLS, there is no need for general anaesthesia and there is no risk for visceral damage and intra-abdominal bleeding. HSG is performed at the radiology department where a water-soluble contrast medium (WSCM) or oil-soluble contrast medium (OSCM) is injected via the cervical canal under radiographic visualisation. Since more than 50 years, the possible beneficial therapeutic effects of OSCM have been debated.<sup>2,3</sup> Many trials have compared the effect of OSCM and WSCM. A Cochrane review published in 2015 concluded that there is evidence suggesting that tubal flushing with OSCM may increase the pregnancy rate and live birth compared with no intervention, but that further robust randomised controlled trials are needed.<sup>4</sup> This was confirmed in the systematic review and network meta-analysis by Wang *et al.*<sup>5</sup> A large randomised multicentre trial was initiated in The Netherlands that compared ongoing pregnancy rates after OSCM and WSCM in infertile women undergoing HSG. This trial showed that OSCM increased the ongoing pregnancy rate from 29.1% to 39.7% (Relative risk (RR) 1.37, 95% CI 1.16 to 1.61).<sup>6</sup> This also translated in higher live birth rates (28.1% vs 38.8%). The results were confirmed in a recent meta-analysis, that showed that women who had HSG using oil contrast had higher rates of ongoing pregnancy compared with women who underwent this procedure using water contrast (OR 1.47, 95% CI 1.12 to 1.93).<sup>7</sup>

However, the field is moving away from HSG to hysterosalpingo-foam sonography (Hyfosalpingography) as an alternative for HSG as a first line office tubal patency test under ultrasound guidance. The accuracy of Hyfosalpingography is comparable to that of HSG.<sup>8,9</sup> The advantages of Hyfosalpingography over HSG are manifold. With Hyfosalpingography there is no need for radiation exposure which makes it a less expensive and more patient-friendly examination compared with HSG. Moreover, the risks for an undetected early pregnancy will be much less in the absence of the use of radiation. In addition, a Hyfosalpingography can be performed by the gynaecologist during regular office hours, establishing the fertility workup in a one-stop clinic. Since the introduction of Hyfosalpingography, it became clear that the procedure is generally well tolerated. Dreyer *et al.*<sup>10</sup> concluded that the Hyfosalpingography procedure is a less painful and less time-consuming tubal patency test compared with HSG. Similar results were generated by Van Schoubroeck *et al.*<sup>11</sup> Thus far, a potential therapeutic benefit of the tubal flushing in itself—apart from the type of contrast medium used—has not been proven. In the case of Hyfosalpingography, only some observational studies have described subsequent pregnancies after Hyfosalpingography. A retrospective study on 359 women with variable follow-up period from 3 to 42 months reported a 55% pregnancy rate.<sup>12</sup> A retrospective cohort study of 111 women reported that 48 women (43.2%) conceived, of whom 24 naturally within 6 months after Hyfosalpingography.<sup>13</sup> Exacoustos *et al.*<sup>14</sup> received information by phone of 157 of 294 subfertile women who underwent Hyfosalpingography. The cumulative spontaneous pregnancy rate in this retrospective

observational study was 29.9% within 6 months and 34.4% within 12 months.

A disadvantage of OSCM is that it could lead to more maternal subclinical hypothyroidism than WSCM<sup>15,16</sup> and high dosages of oil-soluble iodinated contrast medium could lead to foetal or neonatal thyroid dysfunction.<sup>17</sup> However, in the meantime, reassuring data emerge from a retrospective study on the follow-up of 140 neonates from the H2oil study by Dreyer *et al.*<sup>6</sup> where none of the neonates tested positive for the screening of congenital hypothyroidism.<sup>18</sup> In the H2oil trial<sup>6</sup> there was no difference in miscarriage and stillbirth between the two groups. Thyroid function in all neonates conceived after HSG with iodinated contrast media was normal.<sup>18</sup>

In view of the data above, there is a large group of clinicians that is convinced that tubal flushing with OSCM is an effective treatment. The problem is, however, that OSCM is administered through HSG, while the field has completely moved away from HSG and is massively using Hyfosalpingography as a first line test to assess tubal pathology and that the use of OSCM is off-label in this indication.

Therefore, it is proposed to set up a large multicentre randomised clinical trial on the subject. This trial will evaluate if flushing the fallopian tubes with OSCM after a normal Hyfosalpingography showing at least one patent fallopian tube will increase the live birth rate as compared with no flushing. If a positive effect of tubal flushing with OSCM can be found, then this treatment will be implemented prior to in vitro fertilisation (IVF).

## METHODS AND ANALYSIS

### Trial design

A multicentre two arm, 1:1 randomised, open-label pragmatic comparative trial evaluating Hyfosalpingography with additional tubal flushing with OSCM versus Hyfosalpingography without additional flushing in infertile women between 18 and 40 years of age, undergoing Hyfosalpingography as part of the fertility workup who have infertility defined as lack of conception despite 12 months of unprotected intercourse, or three cycles of donor insemination without pregnancy or three ovulatory ovulation induction cycles without pregnancy, all three in combination with at least one patent tube on Hyfosalpingography. To show the superiority of the additional tubal flushing with OSCM compared with no additional flushing, a parallel group design will be used.

### Population and setting

Infertile women undergoing Hyfosalpingography as part of the fertility workup will be recruited in 12 fertility clinics throughout the country both in a university and private setting. The centres should use Hyfosalpingography as a routine procedure for tubal patency testing. In practice, usually a dedicated gynaecologist is appointed but the procedure can be executed by every trained gynaecologist.

Women will be eligible if they

- ▶ Are ≥18 years and <40 years.
- ▶ Have infertility defined as

- Lack of conception despite 12 months of unprotected intercourse.
- If they are taking part in a donor sperm insemination programme: three cycles of donor insemination without pregnancy.
- In case of treated ovulation disorder: three ovulatory ovulation induction cycles without pregnancy.
- ▶ Have tubal patency of at least one fallopian tube on Hyfosal. Women will be excluded if they have.
- ▶ Known iodine allergy.
- ▶ Allergy to poppy seed oil.
- ▶ Ovulation disorders defined as less than eight menstrual cycles per year who did not have three ovulatory cycles after ovulation induction.
- ▶ Contraindication to have tubal patency testing according to the site's indication.
- ▶ Active thyroid disorders.
- ▶ Untreated subclinical hypothyroidism (Thyroid Stimulating Hormone (TSH) >2.5mIU/L) in case of autoimmune thyroid disease.
- ▶ Uterine surgery in the past 2 months before Hyfosal.
- ▶ A male partner (if applicable) with a post-wash Total Motile Sperm Count (TMC) <math>1 \times 10^6</math> and/or TMC <math>3 \times 10^6</math> in the native sperm analysis (TMC is defined as volume X concentration X (A (progressive) +B (non -progressive) motility in %) divided by 100).
- ▶ An indication for IVF.
- ▶ No Belgian national number.

### Intervention

All women will undergo a Hyfosal. Women allocated to the intervention group will undergo a tubal flush with 5–10 mL OSCM (Lipiodol Ultra Fluide, Guerbet, France). Lipiodol is available in Belgium, but is not registered for this indication, hence the use will be off-label. The intervention will be performed in an outpatient setting, immediately after the Hyfosal has been performed. Women allocated to the control group will undergo no additional treatment.

A patient who has no patent tubes at the time of Hyfosal will appear ineligible at that time, but will still be followed up following the trial procedures. In case the patient belongs to the intervention group, she will not be treated with the investigational medicinal product (IMP) for safety reasons.

After the Hyfosal patients will receive follow-up and/or fertility treatment according to the standard of care in the respective fertility centre.

### Outcomes and timeline

#### Primary outcome

To determine if the additional tubal flushing with oil-based contrast after undergoing Hyfosal significantly improves the occurrence rate of live birth, counting from the first day of the last menstrual cycle in which the patient conceives within 6 months after Hyfosal compared

with no additional flushing after undergoing Hyfosal (see table 1).

#### Secondary outcomes

- ▶ Reproductive outcomes: clinical pregnancy, ongoing pregnancy, ectopic pregnancy, multiple pregnancy and miscarriage.
- ▶ For women achieving a pregnancy >22 weeks:
  - Gestational age at delivery.
  - Birth weight.
  - Neonatal mortality.
  - Major congenital anomaly.
  - Neonatal outcomes and thyroid function of the child(ren).
  - Pregnancy complications.
- ▶ Maternal thyroid function: TSH, Free Thyroxine 4 (FT4) at 4 weeks, 26 weeks for all patients and at delivery or end of pregnancy in case of pregnancy.
- ▶ Number of complications during or immediately after the intervention (embolism, anaphylactic shock, pelvic inflammatory disease).
- ▶ Pain score of the Hyfosal (as measured by Visual Analogue Scale (VAS)).
- ▶ Pain score of the additional flush (as measured by VAS) in the intervention group.
- ▶ General (Euro Quality - 5 dimensions -5 levels (EQ-5D-5L) questionnaire) and disease-specific quality of life (FertiQoL questionnaire).<sup>19 20</sup>

#### Exploratory outcomes

- ▶ Long-term follow-up using administrative data from the Belrap registry ([www.belrap.be](http://www.belrap.be)) and the national institute for health and disability insurance: reproductive outcomes and fertility treatments at 2 and 3 years after Hyfosal. If trials results are positive, a health economy analysis will be performed.

#### Sample size

The sample size for the primary outcome is calculated based on the result of the H2oil trial where they report a 39% live birth rate in the oil group and a 28% live birth rate in the control group. In order to demonstrate a difference of 11% in live birth rate over a control rate of 28% with a power of 80% and an alpha of 5% 288 patients are needed per group. This number was calculated using PASS V.11 software using the difference test for two proportions using Z test (Pooled) as test type.

We anticipate that 10% of the women will have no patent tubes at time of Hyfosal and will appear ineligible at that time (tubal flush with IMP not possible for safety reasons),<sup>13</sup> they will be excluded in both groups. We also expect that about 7.2% of the women will have one patent tube and one blocked tube for which possible extra procedures (HSG or laparoscopy) might cloud the effect of the IMP.<sup>6</sup> These women will be included in primary analysis but this percentage was taken into account for the secondary per-protocol analysis. Summarising we need

Table 1 Overview and timeline of the study

Phase	Fertility treatment		Multiple visits depending on treatment plan (n)				Follow-up if not pregnant at 6 months	Follow-up if not pregnant at 6 months		
	Screening visit (V1)	Start study visit (V2)	Study visit (V3)	Study visit (V4)	Study visit (V5)	Follow-up visit (V6a)			Follow-up visit (V6b)	Follow-up visit (V6c)
<b>Timing of visits</b>	-8 weeks to day 1	Day 1	+4 weeks (+2 weeks)	+26 weeks (+2 weeks)	+26 weeks (+8 weeks)	+52 weeks (+2 weeks)	15 weeks pregnancy (+2 weeks) (o)	25 weeks pregnancy (+2 weeks) (o)	35 weeks pregnancy (+2 weeks) (o)	Post partum or miscarriage (+17 weeks) (o)
Informed consent (a)	x									x
Inclusion/exclusion criteria	x									
Demographics (b)	x									
Baseline data (c)	x									
Relevant medical and surgical history (d)	x									
Current medication	x									
TSH, FT4	x		x (p)		x (p)					x
Anti-TPO, anti-TSH rec	x									
Randomisation	x (q)		x (q)							
Questionnaires: ▲ EQ-5D-5L ▲ FertQoL	x (r)		x (r)		x (t)					
Hyfosal with or without tubal flushing with Lipiodol Ultra Fluide (e)(f)	x (s)									
Questionnaire: VAS pain scores	x (u)									
Use of analgetics during Hyfosal	x									
Discussion of fertility treatment		x (g)			x					
Phone contact				x (v)		x (w)	x (x)	x (x)	x (y)	
Screening and observation presence of pregnancy (h)		x	x	x	x					

Continued

Table 1 Continued

Phase	Fertility treatment			Multiple visits depending on treatment plan (n)				Follow-up if pregnant at 6 months		Follow-up if not pregnant at 6 months	
	Screening visit (V1)	Start study visit (V2)	Study visit (V3)	Study visit (V4)	Study visit (V5)	Follow-up visit (V6a)	Follow-up visit (V6b)	Follow-up visit (V6c)	Follow-up visit (V6d)	Follow-up visit (V7)	
Pregnancy and type (i)											
Evaluation reproductive outcome (j)											x
Evaluation neonatal outcome (k)											x (z)
Adverse reactions, serious adverse events and concomitant medication (l)		x	x	x	x	x	x	x	x	x	x
Miscarriage reporting (m)		x	x	x	x	x	x	x	x	x	

Continued

Table 1 Continued

Phase	Screening visit (V1)	Fertility treatment start study visit (V2)	Multiple visits depending on treatment plan (n)	Study visit (V3)	Study visit (V4)	Study visit (V5)	Follow-up if not pregnant at 6 months		Follow-up if not pregnant at 6 months	
							Follow-up visit (V6a)	Follow-up visit (V6b)	Follow-up visit (V6c)	Follow-up visit (V6d)

(a) Patient need to sign the initial informed consent form before study-specific assessments at screening visit (V1). Additional informed consent form needs to be signed at Follow-up visit (V7).

(b) Year of birth, age and ethnicity of the patient.

(c) BMI, smoker status, duration and type of infertility (primary–secondary) and previous results of the fertility workup of the patient. Total motile sperm count (TMC) of male partner if applicable (yes/no) and use of donor sperm (yes/no). TMC of male partner should be mentioned in patient record of female participant.

(d) Previous diagnosis of endometriosis, previous ectopic pregnancy, previous Chlamydia infection, previous pelvic inflammatory disease, previous appendicitis (ruptured/unruptured).

(e) In the intervention group, Hyfosity is followed by tubal flushing with Lipiodol Ultra Fluide if at least one patent tube is present. No tubal flushing with Lipiodol Ultra Fluide will be performed after Hyfosity in the control group. An intervention group patient with no patent tubes at the time of Hyfosity will not be treated with the IMP for safety reasons.

(f) In case of intervention group, the following Hyfosity-related aspects must be assessed: exact amount of Lipiodol Ultra Fluide injected, the presence of reflux, type of catheter used, tubal patency details, time interval between injection of ExEm Foam and Lipiodol Ultra Fluide and visualisation of Lipiodol Ultra Fluide under ultrasound guidance.

(g) Expectant management for 4–6 months and/or intrauterine insemination for 3–4 cycles, to have another three cycles in case of three cycles of donor insemination without pregnancy or three ovulatory induction cycles without pregnancy, can be proposed when at least one tube is patent. A fixed period of time to perform expectant management before starting IUI is not mandatory. If therapy does not lead to an ongoing pregnancy, IVF can be proposed. Diagnostic/therapeutic laparoscopy and/or in vitro fertilisation will be proposed in case of a bilateral tubal block.

(h) In case of pregnancy: pelvic ultrasound scan at 7–8 weeks of pregnancy at the fertility clinic or gynaecologist, and referral to the obstetric department for pregnancy follow-up (standard of care).

(i) Pregnant (yes/no), pregnancy type and date of first day of the last menstrual cycle within 6 months after Hyfosity. Pregnancy status at the end of the fertility treatment phase will define whether the patient is pregnant or not pregnant, and conclude the appropriate follow-up phase of the patient.

(j) Reproductive outcome and pregnancy complications.

(k) Delivery date, number of babies, gender and birth weight, gestational date at delivery, admission to neonatal care (if needed), full name baby(s) and birth date (to obtain thyroid function by heel prick test).

(l) Safety findings female participant and neonate (live birth till heel prick test).

(m) Contact to define date of miscarriage, performed as outpatient visit or by phone. In addition: phone contact to perform follow-up visit (V6a) at 52 weeks (+2 weeks) and if pregnant at study visit five also follow-up visit 7. Miscarriage definition: Spontaneous loss of an intrauterine pregnancy prior to 22 completed weeks of gestational age.

(n) Visits depending on treatment plan: consultation, pelvic ultrasound, serum hormonal analysis, intrauterine insemination, in vitro fertilisation (standard of care).

(o) The duration of pregnancy is calculated in weeks of amenorrhoea since first day of last menstrual period or related to expected date of delivery.

(p) If TSH and/or FT4 are abnormal, there will be a monthly ( $\pm 2$  weeks) check for TSH and FT4 until normalisation with a maximum of 6 months.

(q) Randomisation needs to be performed before Hyfosity but after successful screening by the local or central study coordinator. Patients may have no knowledge of the allocated group until all baseline assessments are performed.

(r) The questionnaires will be provided at screening electronically (or on paper as back-up), and need to be completed before Hyfosity on the start study visit (V2).

(s) A randomised patient who has no patent tube at the time of Hyfosity will appear ineligible at that time, but will still be followed up following the trial procedures.

(t) The questionnaires will be provided at study visit V5 electronically (or on paper as back-up), and need to be completed within 2 weeks after the visit.

(u) To be completed directly after Hyfosity and flush in the intervention group.

(v) Contact to make appointment for study visit (V5). The patient's menstrual cycle needs to be taken into account during scheduling to be able to determine on study visit (V5) if the patient became pregnant in the fertility treatment phase (ie, the first day of the last menstrual cycle in which the patient conceives was within 6 months after Hyfosity).

(w) Provide patient with electronic treatment report (paper as back-up) to be filled out and resend within 2 weeks before follow-up visit (V6a). Thereafter, contact to discuss treatment report: determine if the participant became pregnant outside fertility treatment phase (after study visit V5), pregnancy type and treatment plan.

(x) Contact to follow-up on pregnancy and type of pregnancy. These phone calls do not need to be performed if the patient is already 15 and/or 25 weeks pregnant before study visit (V5), as the pregnancy will be followed up during the fertility treatment phase.

(y) Contact to make appointment for follow-up visit (V7).

(z) Not applicable in case of miscarriage.

Assessments and treatment IMP in bold are not standard of care and are reimbursed by the sponsor's budget.

BMI, body mass index; EQ-5D-DL, Euro Quality - 5 dimensions -5 levels; FT4, Free Thyroxine 4; IMP, Investigational Medicinal Product; IUI, Intra-uterine Insemination; TPO, Thyroid Peroxidase; TSH,

Thyroid Stimulating hormone; VAS, Visual Analogue Scale.

349 patients per group to have 288 patients that have at least one patent tube and do not have extra procedures.

Accounting for a 5% loss to follow-up rate we will need 368 patients per group or we need to randomise 736 patients in total.

### Recruitment

Patients will be recruited by the fertility centres. The recruited patient population will consist of new patients visiting the fertility centre for the first time, and patients who are already known in the centre. There are 12 recruiting centres to allow sufficient recruitment.

### Randomisation, blinding and treatment allocation

Stratified randomisation will be used according to site (12 fertility clinics) and women's age (<36 or ≥36 years). Per stratum, permuted blocked randomisation will be used with a variable block size.

As outcome is objective, the study will be open-label but the statistician will be subgroup unblinded in the analysis (knowing only A and B without knowing which is which).

The study coordinators randomise the patients in REDCap in order of creating their entry in REDCap and after eligibility has been checked. Randomisation cannot be undone. REDCap is set up to allow only the data manager to have access and oversight of the randomisation list.

### Data collection and management

An electronic case report form (eCRF) system REDCap will be used by all participating sites to collect the individual patient data required by the trial protocol. The trial participants will be pseudonymised by their unique Subject ID. The eCRF system will not be used as a primary source of data, except for trial-related questionnaires that were completed electronically per email by the patient.

Direct access will be granted on written request to authorised representatives from the Belgian Health Care Knowledge Centre (KCE), the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

### Data monitoring and auditing

Before study initiation, at a site initiation visit a representative from the sponsor will review the protocol and data capture requirements (ie, eCRFs) with the local investigators and their staff. During the study, field monitors employed by the sponsor (belonging to the clinical trial centre) will employ several methods of ensuring protocol and Good Clinical Practice compliance and the quality/integrity of the sites' data. The field monitor will visit the site to check the completeness of patient records, the accuracy of data capture and data entry, the adherence to the protocol and to Good Clinical Practice, the progress of enrolment, and to ensure that study treatment is being dispensed and accounted for according to specifications. Key study personnel must be available to assist the field monitor during these visits. Continuous remote

monitoring of each site's data may be performed by the centralised Sponsor research associate.

### Data analysis

Analysis will be according to a modified intention-to-treat principle where only the patients with at least one patent tube will be included in the primary outcome analysis. Patients with no patent tube(s) will be excluded post-randomisation in the intervention and the control group.

Baseline data and patient characteristics will be reported per group. Primary outcome analysis to compare the occurrence of live birth between intervention and control group will be a  $\chi^2$  test. A multiple logistic regression model for the event of live birth will be used to assess an intervention effect with correction for covariates like women's age, subgroups of patients (12 month of infertility, use of donor sperm or treated ovulation disorder), treatment strategy (expectant management, intrauterine insemination, IVF), type of infertility (primary or secondary), duration of infertility, smoker status, body mass index as well as TMC. The same analysis will be done for clinical and ongoing pregnancy. RRs and ORs with 95% CI will be reported. Two-sided  $p < 0.05$  will be used to determine statistical significance. A multiple Cox regression model will be considered for the time to live birth and pregnancy (clinical and ongoing) with control for the same variables. Pain VAS scores of the Hyfosal with or without additional flush with oil and changes in quality of life (EQ-5D-5L and FertiQoL) from baseline to 6 months after Hyfosal will be analysed by a Student's t-test or a Mann-Whitney U test in case of a non-normal distribution of the data. A linear mixed model for the evolution of the quality of life over time using subject as a random effect will be considered. A subgroup analysis is planned in the intervention group to compare pain VAS scores between Hyfosal and additional flush and if reproductive outcomes are dependent on pain experienced during the procedure.

The main reproductive outcomes will also be considered in a per-protocol population defined as eligible women who did not undergo extra procedures like HSG or laparoscopy after Hyfosal. Besides the analysis on all eligible women, as a sensitivity analysis we will evaluate the intervention effect on the main reproductive outcomes in all randomised women (taking into account the women with no patent tube(s)).

In terms of safety of the mother, the miscarriage rate and number of complications will be compared between the two groups. In particular, the percentage of (sub) clinical hypothyroidism at 1 and 6 months after Hyfosal will be compared using a  $\chi^2$  test. Change in TSH and FT4 from baseline to 1 and 6 months will be compared as well using a Student's t-test or Mann-Whitney U test as appropriate. As we have repeated measures for TSH and FT4 we will also consider a linear mixed model for these outcomes. For the neonates, the percentage of congenital hypothyroidism as well as neonatal thyroid function will be compared between intervention and control group.

## Trial management

A trial management group (TMG) is installed for this study. The TMG meets on a weekly basis and is responsible for the daily management of the study. In addition, a trial steering committee (TSC) is installed to provide the overall supervision of the trial. The TSC will closely monitor trial progress, advise on scientific developments and discuss crucial topics in relation to the performance of the study.

## Harm

Hypothyroidism after use of Lipiodol Ultra Fluide has been described and therefore the thyroid function of mother and child are collected for safety reasons during the follow period, pregnancy and after delivery (for timing of these analyses, see [table 1](#)). Because in most studies the median dosage of ethiodised oil in the thyroid dysfunction group was significantly higher than in the normal thyroid function group (20 vs 8 mL,  $p=0.033$ ), we decided for this study that the maximum of ethiodised oil will be 10 mL. There will also be no tubal flushing with Lipiodol Ultra Fluide when there is no patent tube present.

## ETHICS AND DISSEMINATION

Informed consent will always be obtained by the gynaecologist prior to the participant undergoing procedures that are specifically for the purposes of the trial and are not standard routine care at the participating site. Potential participants will always be given ample time and possibilities to ask questions regarding participation in the trial. An additional informed consent will be obtained from the mother to retrieve heel prick data from the neonate.

Patient confidentiality must be maintained at all instances and the trial will be compliant with the requirements of the Belgian Privacy Act of 30 July 2018 on the protection of privacy in relation to the processing of personal data and the European Regulation 2016/679 of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation).

Access to the study data by KCE is fully defined and in conjunction with the research agreement between KCE and the Sponsor, which supersedes the protocol in case of contradictory statements. Prior to completion, closure and checking of the HYFOIL database, access to the HYFOIL database by third parties other than those contributing data to the HYFOIL database should only be allowed by the TSC under carefully controlled circumstances.

On study completion and finalisation of the study report, the study results will be disseminated as soon as possible by disclosing them to the public by appropriate means, including scientific publications. The final study report and any other dissemination of results of the study will be made available for review and comment by KCE before their dissemination as required by the agreement between the Antwerp University Hospital (UZA) and

KCE. UZA shall assure that any dissemination is scientifically correct, objective and unbiased.

## Public and patient involvement

The planned research and trial is warmly welcomed and supported by the patient organisation 'De Verdwaalde Ooievaar' who have agreed to be a partner in the research project. A Flemish-speaking and French-speaking patient from the fertility clinics with experience in Hyfosal are engaged in the further conduct of the trial.

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