Human Reproduction, Vol.34, No.12, pp. 2391–2398, 2019
Advance Access Publication on December 30, 2019 doi:10.1093/humrep/dez206

human reproduction

ORIGINAL ARTICLE *Infertility*

Treatment effect of oil-based contrast is related to experienced pain at HSG: a post-hoc analysis of the randomised H2Oil study

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Submitted on April 11, 2019; resubmitted on August 30, 2019; editorial decision on September 3, 2019

STUDY QUESTION: Does pain or volume of used contrast medium impact the effectiveness of oil-based contrast during hysterosalpingography (HSG)?

SUMMARY ANSWER: In women who report moderate to severe pain during HSG, the use of oil-based contrast resulted in more ongoing pregnancies compared to the use of water-based contrast, whereas in women who reported mild or no pain, no difference in ongoing pregnancies was found.

WHAT IS KNOWN ALREADY: We recently showed that in infertile women undergoing HSG, the use of oil-based contrast results in more ongoing pregnancies within 6 months as compared to the use of water-based contrast. However, the underlying mechanism of this fertility-enhancing effect remains unclear.

STUDY DESIGN, SIZE, DURATION: We performed a post-hoc analysis of the H2Oil study, a multicentre randomised controlled trial (RCT) evaluating the therapeutic effect of oil- and water-based contrast at HSG. Here, we evaluated the impact of pain experienced at HSG and volume of used contrast media during HSG on ongoing pregnancy.

PARTICIPANTS/MATERIALS, SETTING, METHODS: In a subset of 400 participating women, pain during HSG by means of the Visual Analogue Scale (VAS) (range: 0.0–10.0 cm) was reported, while in 512 women, we registered the volume of used contrast (in millilitres). We used logistic regression analyses to assess whether pain and volume of used contrast media modified the effect of oil-based contrast on ongoing pregnancy rates. Data were analysed according to intention-to-treat principle.

MAIN RESULTS AND THE ROLE OF CHANCE: In 400 women in whom pain scores were reported, the overall median pain score was 5.0 (Interquartile range (IQR) 3.0–6.8) (oil group ($n = 199$) 4.8 (IQR 3.0–6.4); water group ($n = 201$) 5.0 (IQR 3.0–6.7); *P*-value 0.28). There was a significant

interaction between pain (VAS ≤5 versus VAS ≥6) and the primary outcome ongoing pregnancy (*P*-value 0.047). In women experiencing pain (VAS ≥6), HSG with oil-based contrast resulted in better 6-month ongoing pregnancy rates compared to HSG with water-based contrast (49.4% versus 29.6%; RR 1.7; 95% CI, 1.1–2.5), while in women with a pain score ≤5, 6-month ongoing pregnancy rates were not significantly different between the use of oil- (28.8%) versus water-based contrast (29.2%) (RR 0.99; 95% CI, 0.66–1.5). In the 512 women in whom we recorded contrast, median volume was 9.0 ml (IQR 5.7–15.0) in the oil group versus 8.0 ml (IQR 5.9–13.0) in the water group, respectively (*P*-value 0.72). Volume of used contrast was not found to modify the effect of oil-based contrast on ongoing pregnancy (*P*-value for interaction 0.23).

LIMITATIONS, REASONS FOR CAUTION: This was a post-hoc analysis that should be considered as hypothesis generating. The RCT was restricted to infertile ovulatory women, younger than 39 years of age and with a low risk for tubal pathology. Therefore, our results should not be generalised to infertile women who do not share these features.

WIDER IMPLICATIONS OF THE FINDINGS: The underlying mechanism of the fertility-enhancing effect induced by HSG with the use of oil-based contrast remains unclear. However, these findings suggest a possible mechanistic pathway, that is increasing intrauterine pressure occurring prior to dislodging pregnancy hindering debris or mucus plugs from the proximal part of otherwise normal fallopian tubes. This information might help in the search of the underlying fertility-enhancing mechanism found by using oil-based contrast during HSG.

STUDY FUNDING/COMPETING INTEREST(S): The original H2Oil RCT was an investigator-initiated study that was funded by the two academic institutions (AMC and VUmc) of the Amsterdam UMC. The funders had no role in study design, collection, analysis and interpretation of the data. K.D. reports consultancy for Guerbet. H.V. reports consultancy fees from Ferring. C.B.L. reports speakers' fees from Ferring and research grants from Ferring, Merck and Guerbet. V.M. reports receiving travel and speakers fees as well as research grants from Guerbet. B.W.M. is supported by an NHMRC Practitioner Fellowship (GNT1082548). B.W.M. reports consultancy for ObsEva, Merck KGaA and Guerbet and travel and research grants from Merck KGaA and Guerbet. The other authors do not report conflict of interests.

TRIAL REGISTRATION NUMBER: The H2Oil study was registered at the Netherlands Trial Registry (NTR 3270).

TRIAL REGISTRATION DATE: 1 February 2012.

DATE OF FIRST PATIENT'S ENROLMENT: 3 February 2012.

Key words: hysterosalpingography / tubal flushing / oil-based contrast / pain score / ongoing pregnancy rate / female infertility

Introduction

Assessment of the fallopian tubes is an important part of the fertility work-up. Hysterosalpingography (HSG) is the oldest tubal patency test and is still commonly applied in many countries. While HSG was introduced as a diagnostic test, it was, more than 50 years ago, also suggested to directly lead to an increased pregnancy rate (King and Herring, 1949; Weir and Weir, 1951). However, high-quality evidence supporting this fertility-enhancing effect was lacking. Also, it was unclear whether the type of contrast medium used affects this potential therapeutic effect (Watson *et al.*, 1994; Mohiyiddeen *et al.*, 2015).

In 2017, we compared oil- and water-based contrast in a large multicentre randomised controlled trial (RCT) performed in the Netherlands under the acronym the H2Oil study (Dreyer *et al.*, 2017). A total of 1119 women were randomised and allocated to tubal flushing at HSG with the use of oil-based contrast medium ($n = 557$) or water-based contrast medium ($n = 562$). The 6-month ongoing pregnancy rates were higher after tubal flushing at HSG with oil-based contrast (39.7%) compared to the use of water-based contrast (29.1%) (relative risk (RR), 1.37; 95% confidence interval (CI), 1.16–1.61; $P < 0.001$). Recent updated systematic reviews and meta-analyses have confirmed these findings (Fang *et al.*, 2018; Wang *et al.*, 2019).

Several potential theories have been proposed to elucidate the fertility-enhancing mechanism of tubal flushing, especially with oil-based contrast, including a mechanistic pathway dislodging non-occlusive but pregnancy-hindering debris or mucus plugs from otherwise undamaged fallopian tubes (Gillespie, 1965; Kerin *et al.*, 1992), enhancement of ciliary activity (Soules and Spadoni, 1982) and immunobiological effects on the endometrium (Yun and Lee, 2004; Johnson, 2014) and/or in the peritoneal cavity (Sawatari *et al.*, 1993; Mikulska *et al.*, 1994; Izumi *et al.*, 2017).

In the H2Oil study, we measured in a subset of women for pain experienced during HSG and registered the used volume of contrast. While both experienced pain and used contrast volume were comparable between the two groups, we did not study whether the treatment effect of oil-based contrast versus water-based contrast on ongoing pregnancy was associated with the pain that women experienced and/or the volume of contrast that was used. Moreover, previous oil-water studies have not addressed this (de Boer *et al.*, 1988; Rasmussen *et al.*, 1991). Two studies reported on volume of used contrast medium and pain scores, but did not relate them to the treatment effect (Alper *et al.*, 1986; Lindequist *et al.*, 1994).

Here, we investigate whether pain and used volume of contrast medium during HSG were associated with the treatment effect of oil-based contrast on ongoing pregnancy in the H2Oil study.

Materials and Methods

Study design and patients

This is a post-hoc data analysis of our multicentre RCT, the H2Oil study (NTR 3270) comparing ongoing pregnancy rates after the use of oil-based contrast versus water-based contrast in infertile women undergoing HSG. The study was approved by the Institutional Review Board of the Amsterdam UMC, University of Amsterdam, the Netherlands. The original study has been described in detail previously (Dreyer *et al.*, 2017). In summary, women were eligible for the study if they

had been trying to conceive for at least 1 year, were between 18 and 39 years of age, had spontaneous ovulatory cycles, and were at low risk for tubal pathology and if the male partner did not have severe male infertility, defined as a total motile sperm count (TMSC) after sperm wash of <3 million sperm per millilitre (ml).

After written informed consent, women were randomly assigned in a 1:1 ratio to the use of oil-based contrast (Lipiodol Ultra-Fluid®, Guerbet) (the oil group) or water-based contrast (Telebrix Hystero®, Guerbet) (the water group) during HSG. Telebrix Hystero® was the most commonly used and registered water-based contrast for HSG in the Netherlands. Lipiodol Ultra-Fluid® was the only available oil-based contrast in the Netherlands. Table I shows the chemical and physical characteristics of the two contrast media. HSG was performed according to local protocols of the participating hospitals by a gynaecologist. The use of pain medication depended on local protocols (i.e. paracetamol, ibuprofen, naproxen or diclofenac). Contrast medium could be infused into the uterus with the use of a cervical vacuum cup, a metal cannula (hysterophore) or a balloon catheter, depending on preference and/or experience of the performing gynaecologist. Usually, 5–10 ml of contrast medium was infused and four to six radiographs, obtained to evaluate patency of both fallopian tubes, were examined by a gynaecologist and/or radiologist.

Couples were managed based on their prognosis for natural conception. In case of a prognosis for natural conception resulting in a life birth ≥30% in 12 months, couples were counselled for expectant management. When the prognosis for natural conception was <30%, treatment with intrauterine insemination was performed (Hunault *et al.*, 2005). In case of bilateral tubal occlusion on HSG, confirmed at laparoscopy, or when the male partner had very poor semen quality at repeated semen analysis, couples were advised to start *in vitro* fertilisation or intracytoplasmic sperm injection treatment. The primary outcome was ongoing pregnancy, defined as a viable pregnancy at 12-week ultrasound, with the first day of the last menstrual cycle before pregnancy within 6 months after randomisation.

In seven clinics (two academic and five teaching hospitals), pain scores were recorded immediately after completion of the HSG procedure by means of the Visual-Analogue Scale (VAS) for pain (scores range from 0.0 to 10.0 cm, with higher scores indicating more severe pain), which is a valid and reliable pain scale (Karcioglu *et al.*, 2018). As VAS scores were rounded to integers before data entry in some, we decided to round all VAS scores to integers for statistical analyses. In 16 clinics (three academic, ten teaching and three general hospitals), the volume of used contrast was recorded. Volume was measured by subtracting the remaining volume of contrast in ml after HSG was completed from the volume of contrast in ml at the beginning of the procedure, based on 10 ml per ampule. The subsets of women in which pain scores and volume of used contrast were measured and reported were randomly selected.

Statistical analysis

In this post-hoc analysis, we evaluated whether pain and volume of used contrast medium during HSG could modify the treatment effect of type of contrast (oil versus water) on 6-month ongoing pregnancy rates. Effect-modification was tested separately for pain and volume of contrast using logistic regression models with ongoing pregnancy as the dependent variable and type of contrast (oil versus water), the candidate effect modifier (pain or contrast volume) and their two-way interaction as independent variables. In case of a statistically significant two-way interaction, the treatment effect of oil-based contrast was subsequently quantified in the two separate strata defined by the effect modifier by calculating strata-specific Relative Risks (RR) for ongoing pregnancy. Pain score and volume were dichotomised using a median split due to lack of a clinical cut-off point. All analyses of this study were exploratory post-hoc analyses and performed based on the intention-to-treat analysis. We used SPSS version 22.0 (IBM SPSS Statistics for Windows, 2016) for analysis.

Table I Chemical and physical characteristics of contrast media.

	Lipiodol Ultra Fluid®	Telebrix Hystero®
Iodine amount (mg I/ml)	480	250
Viscosity		
- At 15°C or 20°C (mPa·s)	70	220
- At 37°C (mPa·s)	25	100
Osmolality (mOsm/kg)	NA[a]	2260
Density		
- At 15°C or 20°C (g/cm^3)	1.28[b]	1.33[c]
- At 37°C (g/cm^3)	Unknown[d]	1.32
Ingredients	Ethyl esters of fatty acids of poppy seeds oil	Ioxitalamate acid meglumine

Source: SPC Lipiodol Ultra Fluid® and Telebrix Hystero® Guerbet France.
[a]No osmolality for Lipiodol since it is an oil.
[b]Density at 15°C.
[c]Density at 20°C.
[d]Unknown whether density of Lipiodol changes with the temperature.
NA, not applicable.

Results

Between February 2012 and October 2014, a total of 1119 women were randomised for the use of oil-based contrast ($n = 557$) or water-based contrast ($n = 562$) during HSG in the H2Oil study (Supplementary Figure S1). In a randomly selected subset of 400 women, pain during HSG was recorded (199 women in the oil group versus 201 women in the water group). Baseline characteristics of this subset are shown in Table II.

Table III shows the number of women with an ongoing pregnancy in the oil versus water group categorised per pain score.

The overall median pain score was 5.0 (IQR 3.0–6.8), 4.8 (IQR 3.0–6.4) in the oil group versus 5.0 (IQR 3.0–6.7) in the water group (*P*-value 0.28). The overall median value that was used to split was 5. Logistic regression analysis indicated a significant interaction between HSG with the use of oil-based contrast, ongoing pregnancy and pain during HSG (*P*-value 0.047). In women who scored pain during HSG as a VAS of 6 or more, ongoing pregnancy rates during HSG with oil contrast were found to be significantly higher compared to HSG with water contrast (oil group versus water group RR, 1.7; 95% CI, 1.1–2.5), while in women with a pain score of 5 or less, there was no difference in ongoing pregnancy rates between the use of oil- or water-based contrast (RR, 0.99; 95% CI, 0.66–1.5).

In both groups, 91% of the women had a normal HSG with bilateral patent tubes. In 169 women with a VAS of 6 or higher, 13 had an abnormal or inconclusive HSG and underwent a diagnostic laparoscopy in the first 6 months following HSG (six in the oil group versus seven in the water group); of these, two women had endometriosis ASRM

Table II Baseline characteristics of women within the pain score subset.

	Oil-based contrast ($n = 199$)	Water-based contrast ($n = 201$)
Age (years)	33.9 (31.3–36.6)	33.7 (31.6–36.4)
BMI[a] (kg/m^2)	22.2 (20.6–24.6)	22.4 (20.9–24.9)
Duration of infertility (months)	19.2 (16.0–25.7)	19.8 (15.7–26.9)
Cycle duration (days)	28.0 (27.0–30.0)	28.0 (27.0–30.0)
Ethnicity[b]		
- Caucasian	168 (84.4)	157 (78.1)
- Non-Caucasian	26 (13.1)	39 (19.4)
- Unknown	5 (2.5)	5 (2.5)
Smoking[c]	19 (9.5)	30 (14.9)
Previous large loop excision of the transformation zone or conisation of the cervix	9 (4.5)	6 (3.0)
Previous myoma or polyp resection or cystectomy	0 (0.0)	1 (0.5)
Previous tubal surgery	0 (0.0)	0 (0.0)
Previous intestinal surgery	10 (5.0)	14 (7.0)
Primary infertility	135 (67.8)	139 (69.2)
Total motile sperm count (million/ml)	75.4 (28.2–174.7)	71.8 (28.5–126.8)

Data presented as median (IQR) or number of women (%).
[a]The body-mass index is the weight in kilograms divided by the square of the height in meters.
[b]Ethnicity was reported by the clinicians.
[c]Data on maternal smoking were missing for 10 women in the oil group and 8 women in the water group.

Table III Ongoing pregnancies (%) after the use of oil- and water-based contrast during HSG stratified for pain score (VAS).

VAS (in cm)	Oil-based contrast ($n = 199$)	Water-based contrast ($n = 201$)	RR (95% CI)
0–2	7/19 (36.8)	3/17 (17.6)	1.8 (0.5–6.1)
2–4	12/55 (21.8)	12/47 (25.5)	0.9 (0.4–1.8)
4–6	19/52 (36.5)	22/59 (37.3)	1.0 (0.6–1.7)
6–8	27/58 (46.6)	17/58 (29.3)	1.4 (0.8–2.4)
8–10	9/15 (60.0)	5/20 (25.0)	1.9 (0.7–4.8)
≤5.0	34/118 (28.8)	33/113 (29.2)	0.99 (0.66–1.5)
≥6.0	40/81 (49.4)	26/88 (29.6)	1.7 (1.1–2.5)

Data presented as number of women (%).
VAS, visual analogue scale.

Table IV Ongoing pregnancies (%) after the use of oil- and water-based contrast during HSG stratified for volume of contrast.

Volume of used contrast	Oil-based contrast (*n* = 255)	Water-based contrast (*n* = 257)	RR (95% CI)
<5 mL	12/36 (33.3)	9/38 (23.7)	1.3 (0.6–2.8)
5–10 ml	40/107 (37.4)	28/106 (26.4)	1.3 (0.9–2.0)
10–15 mL	21/49 (42.9)	13/51 (25.5)	1.5 (0.8–2.7)
15–20 mL	5/17 (29.4)	4/9 (44.4)	0.7 (0.2–2.3)
20–25 mL	19/42 (45.2)	8/45 (17.8)	2.1 (0.99–4.3)
25–30 mL	1/2 (50.0)	0/2 (0.0)	2.3 (0.1–38.1)
30–35 mL	0/0 (0.0)	1/4 (25.0)	2.0 (0.2–22.1)
>35 ml	1/1 (100.0)	0/2 (0.0)	3.0 (0.2–48.0)
≤8.3 ml	45/123 (36.6)	36/133 (27.1)	1.4 (0.9–1.9)
>8.3 ml	54/132 (40.9)	27/124 (21.8)	1.9 (1.3–2.8)

Data presented as number of women (%).

grade 2–3 diagnosed (one in the oil group versus one in the water group). Also, in 231 women with a VAS score of 5 or lower, 13 underwent a diagnostic laparoscopy in the first 6 months following HSG, which resulted for five women in the diagnosis endometriosis ASRM grade 1–4 (three in the oil group versus two in the water group).

Volume of used contrast medium during HSG was recorded in a randomly selected subset of 512 women, 255 women in the oil group versus 257 women in the water group, respectively. Baseline characteristics of this subset of women are shown in Supplementary Table I.

Table IV shows the number of women with an ongoing pregnancy in the oil versus water group categorised per volume of contrast. The overall median of used contrast volume was 8.3 ml (IQR 5.8–14.0), 9.0 ml (IQR 5.7–15.0) in the oil group versus 8.0 ml (IQR 5.9–13.0) in the water group (*P*-value 0.72), with a median split for the whole population of 8.3 ml. A logistic regression analysis showed no interaction between types of contrast used during HSG, ongoing pregnancy and used volume of contrast ($P = 0.23$).

Discussion

Main findings

In this post-hoc analysis of the multicentre randomised controlled H2Oil study, we found that the treatment effect of oil-based contrast during HSG only occurred in those women who experienced pain during HSG. In women with pain scores of 6 and higher, HSG with oil contrast significantly increased the 6-month ongoing pregnancy rate from 30% to almost 50% as compared to the use of water contrast (RR 1.7; 95% CI, 1.1–2.5), while in women with a pain score of 5 or lower, there was no effect of oil contrast compared to water contrast (6-month ongoing pregnancy rate 30% versus 30%, RR, 1.0; 95% CI, 0.7–1.5). The volume of used contrast was not found to modify the treatment effect.

Strengths and limitations

This study is based on data of a large robust RCT. For this post-hoc analysis, we used a subset of randomly selected women in which pain scores and volume of used contrast prospectively were reported and measured. Randomisation was also successful within the subsets of women. Pain scores and volume of used contrast were registered prospectively at the day of HSG independent of any knowledge of the outcomes.

Our study also has limitations. We studied a subset of women from the original H2Oil study, as not every woman had a pain score and/or used volume of contrast measured. No formal power analyses were performed for these post-hoc analyses. This trial was limited to infertile women younger than 39 years of age, with a spontaneous ovulatory cycle and with a low risk for tubal pathology. Consequently, our findings cannot be generalised to infertile women who do not share these features. While additional treatments were allowed, the number of women who underwent artificial reproductive technologies were similar in the both groups. As this analysis was post-hoc and not pre-specified, our findings should be regarded exploratory and validated in future studies.

In this study, only one type of oil-based contrast and one type of water-based contrast were used to create two homogenous groups, which mirrored daily Dutch practice. However, it is questionable whether these findings are generalisable for other types of contrasts.

This study was not a blinded trial, as pain scores were recorded immediately after completion of the HSG procedure and volume of contrast was documented by the doctor who administered the contrast. However, we render it unlikely that any of the participating women or the doctors were aware of a possible effect of pain or volume, and our primary endpoint, ongoing pregnancy, was objective, which makes it unlikely that a lack of blinding influenced our findings. The use of pain medication depended on the local protocol of the participating hospitals and was not registered. Furthermore, it has been suggested that the pre-procedural anxiety level affects pain scores in women undergoing HSG (Tokmak *et al.*, 2015). As we did not report pain medication and/or anxiety, this may influence our outcomes.

Furthermore, the type of HSG technique used (Semm Cup, metal cannula (hysterophore) or balloon catheter) depended on preference and/or experience of the performing gynaecologist. A few small studies have reported less pain during HSG with the use of a balloon catheter, as compared to a metal cannula or cervical cup; however, the differences have been minimal (Tur-Kaspa *et al.*, 1998; Cohen *et al.*, 2001; Ricci *et al.*, 2007). In our study, the numbers of used techniques were comparable between the two groups and within the pain subset; however, the technique could possible influence pain scores. Finally, it has been suggested that pre-warmed contrast medium (to 37°C) compared to contrast medium at room temperature alleviates the pain associated with HSG (Zhu *et al.*, 2012). In this study, the use of pre-warmed contrast depended on the local protocol; this might have impacted the pain scores in this study.

Implications

As mentioned earlier, several potential theories have been suggested to clarify the fertility-enhancing mechanism of tubal flushing, especially with the use of oil-based contrast. Independent of the type of contrast, tubal flushing itself has a treatment effect in women undergoing tubal assessment (Dreyer *et al.*, 2019; Wang *et al.*, 2019). An immunobiological effect of oil-based contrast on endometrium receptivity with enhanced implantation has been suggested (Yun and Lee, 2004; Johnson, 2005; Johnson, 2014). Another possible explanation is modulation of peritoneal macrophage activity by oil-based contrast, resulting in alteration of cytokine production, inhibition of sperm phagocytosis and maturation of dendritic cells and regulatory T cells in the peritoneal cavity (Sawatari *et al.*, 1993; Mikulska *et al.*, 1994; Izumi *et al.*, 2017). Other proposed explanations are stimulation of ciliary activity in the tubes, improvement of cervical mucus and iodine-induced bacteriostatic action on mucus membranes (Soules and Spadoni, 1982; Mohiyiddeen *et al.*, 2015).

Also, oil-based contrast is derived from poppy seed oil, which contains opium alkaloids. Recent research demonstrated that receptors for these opioids are present and expressed in human endometrial cells during the menstrual cycle showing that the opioid receptor expression changes during the menstrual cycle: its mRNA expression increased during the proliferative phase and decreased during the secretory phase of the menstrual cycle with maximum values around the time of ovulation (Totorikaguena *et al.*, 2017). The cyclic upregulation of the opioid receptor, especially during the period from the mid-proliferative to the mid-secretory phase of the menstrual, suggests a role in implantation and could explain, at least in part, for the aforementioned increased pregnancy rates after use of oil-based contrast.

The association found between pain and treatment effect of oil-based contrast during HSG on ongoing pregnancy might be explained by a higher intrauterine pressure induced by mucus plugs or debris in the proximal part of the tubes. By flushing the fallopian tubes, pregnancy-hindering mucus plugs or debris might be dislodged and flushed away from otherwise undamaged fallopian tubes, therefore, enhancing fertility. The higher intrauterine pressure associated with the dislodgement of mucus plugs and debris might cause more pain. The existence of obstructive debris in the proximal parts of the tube was confirmed in 1992 by using falloposcopy and found to histologically consist of casts of debris containing aggregates of histiocyte-like cells probably of endometrial stromal or mesothelial origin (Kerin *et al.*, 1992). They found in 6% of the tubes obstructive debris, which could be washed away by hydro-dissection. However, falloposcopy may under-diagnose the occurrence of obstructive debris because of easily mobilised debris, which is washed away before the falloposcope is in place (Kerin *et al.*, 1992). As shown in Table I, there are multiple differences between the chemical and physical characteristics of oil-based and water-based contrast. Oil-based contrast has a lower viscosity and higher iodine concentration as compared to water-based contrast. There is currently very limited evidence regarding the impact of these characteristics, although these differences in chemical and physical characteristics of both contrast media will most probably contribute to the observed treatment effect. Future studies are needed.

Two previous oil-water studies addressed interaction with pain at HSG (Alper *et al.*, 1986; Lindequist *et al.*, 1994). One study found, in 131 women, comparable pain scores in the oil group versus the water group, respectively, mean 2.9 (SD 0.9) versus 3.2 (SD 1.6), and found no clear correlation between volume of contrast administered or cycle day and pain score (Alper *et al.*, 1986). Another study reported that 98 of the 245 women (40%) experienced moderate to severe pain during HSG, independent of the contrast medium used; however, they did not link this to ongoing pregnancy (Lindequist *et al.*, 1994). None of the other oil-water RCTs explored any potential associations or interactions with the treatment effect (de Boer *et al.*, 1988; Rasmussen *et al.*, 1991; Spring *et al.*, 2000).

Finally, venous intravasation occurs in ~2–7% of the HSGs, which can be painful for women (Bateman *et al.*, 1980; Nunley *et al.*, 1987; Dusak *et al.*, 2013). No cases of venous intravasation were reported in our H2Oil study, and serious adverse events related to the HSG procedure did not occur in >1100 randomised women, indicating that none of the possible missed cases of intravasation was symptomatic or had clinical consequences (Dreyer *et al.*, 2017). Since the prevalence of more severe pain was much higher than the prevalence of intravasation, the higher pregnancy rates observed in the women with a pain score of ≥6 are not related to venous intravasation of the oil-based contrast.

Future, preferably *in vitro*, studies to confirm the association of pain during tubal flushing and treatment effect and thereby the underlying fertility-enhancing mechanism of tubal flushing with oil contrast are welcome.

Conclusion

Moderate to severe pain during HSG with the use of oil-based contrast is associated with more pregnancies compared to the use of water-based contrast (6-month ongoing pregnancy rate 49.4% versus 29.6%, RR 1.7; 95% CI, 1.1–2.5), whereas in women who reported mild or no pain (pain score of 5 or lower), no difference in pregnancies was found between the use of oil- or water-based contrast (RR 0.99; 95% CI, 0.66–1.5).

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Acknowledgements

We thank all participating women. We thank H2Oil study group collaborators: A. Hoek, M.H.A. van Hooff, A.B. Hooker, I.A.J. van Rooij,

R.J.T. van Golde, C.F. van Heteren, M.J. Pelinck, R. Tros, M. Kaplan, M.J. Lambers, G.A. van Unnik, C.H. de Koning, A. Mozes, C.C.M. Timmerman, N. van Geloven, J.W.R. Twisk, P.G.A. Hompes and their staff for their contributions to this study, especially the research nurses and other recruiting staff. We thank the staff of the nationwide consortium for women's health research (NVOG Consortium; www.zorgevaluatienederland.nl).

Authors' roles

B.W.M. designed this study and was the principle investigator of the original H2Oil study. He analysed and interpreted the data and he critically discussed and structured the manuscript. K.D. was the coordinating investigator of the original H2Oil study. N.W., K.D. and J.R. collected the data. N.W. and K.D. analysed and interpreted the data and critically discussed and structured the manuscript. N.W. is the first author of this manuscript. P.M.V. analysed and interpreted the data and critically discussed the manuscript. C.B.L. and V.M. were local investigators who coordinated recruitment of participants in the original H2Oil study. H.R.V., M.G., A.W.N., J.M.S., M.A.T., H.G.M.R.-L., A.J.C.M.D., P.B., J.P.B., A.V.S. and A.P.G. were local investigators who recruited participants in the original H2Oil study. All authors discussed and commented on the manuscript. All authors have approved the final draft of the manuscript.

Funding

The H2Oil study was an investigator-initiated study that was funded by our own academic institutions (AMC and VUmc) of the Amsterdam UMC. The funders had no role in study design, collection, analysis and interpretation of the data.

Conflict of interest

K.D. reports consultancy for Guerbet. H.V. reports consultancy fees from Ferring. C.B.L. reports speakers' fees from Ferring and research grants from Ferring, Merck and Guerbet. V.M. reports receiving travel and speakers' fees as well as research grants from Guerbet. B.W.M. is supported by a NHMRC Practitioner Fellowship (GNT1082548). B.W.M. reports consultancy for ObsEva, Merck KGaA and Guerbet and travel and research grants from Merck KGaA and Guerbet. The other authors do not report conflict of interests.

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