How long does the fertility-enhancing effect of hysterosalpingography with oil-based contrast last?

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ARTICLE

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BIOGRAPHY

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KEY MESSAGE

The therapeutic effect of oil-based versus water-based contrast on ongoing pregnancy was high immediately after hysterosalpingography and decreased in the first year to no effect after approximately 2 years. In women who experienced pain during hysterosalpingography, and perhaps had debris or mucus flushed away, the effect might last longer.

ABSTRACT

Research question: Does the fertility-enhancing effect of tubal flushing during hysterosalpingography (HSG) with oil-based contrast change over time?

Design: This was a secondary analysis of the H2Oil (long-term follow-up) study, a multicentre randomized controlled trial evaluating the effectiveness of oil-based and water-based contrast during HSG. The main outcome was ongoing pregnancy. Cox proportional hazards models for time to ongoing pregnancy were fitted over 3 years of follow-up.

Results: Data on 1107 couples were available; 550 couples had oil-based contrast and 557 water-based contrast at HSG. Ongoing pregnancy rates after 3 years were 77% and 71%, respectively. Median follow-up was 9–10 months (5th—95th percentile: <1 to 36). The hazard ratio for ongoing pregnancy for oil versus water over 3 years of follow-up was 1.26 (95% confidence interval [CI] 1.10–1.45). The scaled Schoenfeld residual plots showed a decrease in hazard ratio that was linear with log-transformed time. After including an interaction with log-transformed time, the hazard ratio immediately after HSG was 1.71 (95% CI 1.27–2.31) and reduced to no effect (hazard ratio of 1) at approximately 2 years. There was no evidence for a change in hazard ratio over time in a subgroup of women who experienced pain during HSG.

Conclusions: The hazard ratio for ongoing pregnancy of oil-based versus water-based contrast was 1.71 immediately after HSG, gradually decreasing and plateauing towards a hazard ratio of 1 (indicating no effect) after approximately 2 years. This supports the hypothesis that oil-based contrast might dislodge debris or mucus plugs from the Fallopian tubes, but this has yet to be definitively proved.

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KEY WORDS

Female infertility
Hysterosalpingography
Oil-based contrast medium
Ongoing pregnancy
Water-based contrast medium

INTRODUCTION

Hysterosalpingography (HSG) is a commonly applied tubal patency test during fertility workup (*ACOG, 2019*; *NICE, 2013*). Although it was first introduced as a diagnostic test, therapeutic effects have been debated in studies for many years, especially regarding HSG with use of oil-based contrast (*Mohiyiddeen et al., 2015*).

In 2017, a multicentre randomized controlled trial (RCT) (under the name of the H2Oil study) showed that HSG using oil-based contrast resulted in a 10% higher absolute ongoing pregnancy rate within 6 months compared with the use of water-based contrast (relative risk 1.37, 95% CI 1.16–1.61) (*Dreyer et al., 2017*). Two subsequent meta-analyses confirmed these findings (*Fang et al., 2018*; *Wang et al., 2019*). The most recent meta-analysis aimed to evaluate the long-term effects of tubal flushing; however, only three studies reported a follow-up of more than 12 months, so no definitive conclusions could be drawn (*Wang et al., 2019*). This emphasized the need for long-term follow-up studies.

Recently, the long-term reproductive outcomes of HSG with oil-based versus water-based contrast have been published (under the name of the H2Oil follow-up study). Over a 5-year follow-up period, HSG with oil-based contrast during fertility workup resulted in more ongoing pregnancies, more live births and a shorter time to pregnancy compared with HSG with water-based contrast (*van Rijswijk et al., 2020*). However, it remained uncertain whether the 5-year effect was explained by the initial effect of oil-based contrast immediately after HSG or whether the effect was long(er) lasting. Exploring the duration of this fertility-enhancing effect might provide more information on the mechanism of effect of oil-based contrast. To date, this has remained unclear.

Several potential mechanisms have been suggested. These can be categorized according to their location of action: the Fallopian tube, the endometrium and the peritoneum. First, tubal flushing, i.e. mechanical flushing of debris or mucus plugs or unblocking of peritubal adhesions, can clear the passageway of otherwise normal Fallopian tubes (*Kerin et al., 1992*). Second, uterine bathing with oil-based contrast can enhance endometrial receptivity. Oil-based contrast is produced from poppy seeds and contains opium alkaloids, which potentially interact with opioid receptors in the endometrium (*Totorikaguena et al., 2017*) or through alterations of the uterine immune response (*Johnson et al., 2005*). A third potential mechanism is that oil-based contrast reduces peritoneal macrophage phagocytosis and macrophage adherence by forming an oily layer over the macrophages, changing their shape and surface configuration (*Johnson et al., 1992*). Previous studies have shown that sperm phagocytosis is inhibited *in vitro* by oil-based contrast (*Boyer et al., 1986*; *Mikulska et al., 1994*).

More knowledge on the duration of the fertility-enhancing effect of oil-based contrast might contribute to the understanding of the underlying mechanism. It was postulated that an effect on the endometrium or on the immune response in the peritoneum would be short lasting, and that dislodging of mucus or debris from the proximal parts of the Fallopian tubes might be painful but longer lasting (i.e. over multiple cycles). This information can contribute to the search for the mechanism underlying the fertility-enhancing effect of oil-based contrast. The present study investigated whether or not the fertility-enhancing effect of HSG using oil- versus water-based contrast would changed over time.

MATERIALS AND METHODS

The H2Oil study was a multicentre RCT comparing oil-based and water-based contrast in women scheduled for HSG during their fertility workup (Netherlands Trial Register [NTR] 3270) and was approved by the Institutional Review Board of the Amsterdam University Medical Centre – Academic Medical Centre (reference 2008.362, dated 12 February 2009). The H2Oil follow-up study assessed the long-term outcomes of the H2Oil trial (NTR 6577) and was approved by the Institutional Review Board of the Amsterdam University Medical Centre – VU University Medical Centre (reference 2017.221, dated 14 June 2017).

Study details and results have previously been published (*Dreyer et al., 2017*; *van Rijswijk et al., 2020*). In short, the H2Oil trial recruited a total of 1119 participants in a network of 27 hospitals in the Netherlands between 3 February 2012 and 29 October 2014 (*Dreyer et al., 2017*). Participating infertile women were aged between 18 and 39 years, had an ovulatory cycle, had a low risk of tubal pathology according to their medical history, were without known endocrine disorders and had partners had a total motile sperm count after sperm wash of more than 3 million/ml. They had been trying to conceive for at least 1 year and were scheduled for tubal patency testing with HSG at the end of the fertility workup. After informed consent, couples were randomized for HSG with oil-based contrast or water-based contrast. In the H2Oil follow-up study, data regarding fertility treatments and pregnancies were collected until 3–5 years after randomization (*van Rijswijk et al., 2020*).

Study outcomes

The main outcome was ongoing pregnancy, defined as an ultrasound-confirmed positive heartbeat beyond 12 weeks of gestation. Additional to various other pregnancy outcomes, data on fertility treatments were collected. The start of follow-up was defined as 2 weeks before HSG (reflecting the first day of the menstruation before HSG). Time to pregnancy was defined as 2 weeks before HSG to the first day of menstruation before conception leading to an ongoing pregnancy, loss to follow-up or end of study follow-up, whichever occurred first. Median follow-up was calculated as the 50th percentile in all numerical follow-up values. Pain experienced during HSG was reported using a visual analogue scale (VAS) score (range 0.0 to 10.0 in centimetres).

Primary analysis

For long-term follow-up up to 3 years, first a Cox proportional hazards model was fitted for time to pregnancy data with the randomization allocation, i.e. oil versus water, and the overall hazard. Scaled Schoenfeld residuals were then derived and plotted to visualize the change in log hazard ratio over time; a chi-squared test was applied to the residuals to test the plausibility of the proportional hazards assumption that 'the relative effect is stable over time' (*Grambsch and Therneau, 1994*). For these tests and plots, both regular time and log-transformed time were used. The non-linear and linear interactions

between allocation and regular or log-transformed time were added to the Cox model, and the best fitting model was determined by looking at the *P*-value for the interaction and/or whether the model had lowest Akaike information criterion (AIC) (*Akaike, 1974*; *Harrell et al., 1996*). This best-fitting model was used to quantify the change of effect of oil- versus water-based contrast over time by estimating hazard ratios at different time points during follow-up: at 2 weeks of follow-up (which is directly after HSG), and after 1 month (which is the start of the next menstrual cycle after HSG), 3 months, 6 months, 9 months, 1 year, 2 years and 3 years.

The number of pregnancies per group per cycle during the first 6 months after HSG and their relative risks were tabulated to look for a trend over time shortly after HSG.

Sensitivity analyses

Three sensitivity analyses were conducted. For the first, it was postulated that women might experience pain at HSG when the contrast fluid removed debris or mucus plugs from their Fallopian tubes. If this were the mechanism of action, there might not be any change over time in the effect of oil-based contrast versus water-based contrast in this group. To test this, the steps from the primary statistical analysis were repeated in the subgroup of women who experienced pain during the HSG procedure, defined as a pain score of 6 points or more on the VAS.

Second, it was postulated that starting IVF at some point during follow-up might distort the effect of oil versus water given the hypothesis that oil-based contrast would flush debris or mucus plugs, as IVF bypasses the Fallopian tubes. For this reason, follow-up was censored, i.e. stopped, when couples started IVF, and the steps in the primary statistical analysis were repeated.

Third, as patient characteristics were similar between allocation groups at randomization but might differ later in follow-up, the following characteristics were adjusted for, and the following steps from the primary statistical analysis repeated: female age, duration of infertility, primary or secondary infertility, percentage of progressive motile spermatozoa, volume of semen sample, referral status by general practitioner or specialist, abnormal HSG result in terms of blockages, female smoking status and female body mass index.

Supplementary analysis

As a supplementary analysis, the study continued with the question of whether the effect of oil contrast versus water contrast would be different for couples undergoing expectant management or receiving medically assisted reproduction (MAR), i.e. intrauterine insemination (IUI)/intrauterine insemination with ovarian stimulation (IUI-OS) or IVF/intracytoplasmic sperm injection (ICSI) as the Fallopian tubes were bypassed in IVF/ICSI. First, the follow-up data were reformatted by dividing the follow-up time into periods when couples where either pursuing expectant management, receiving IUI/IUI-OS or receiving IVF/ICSI. The start of follow-up for MAR was defined as 14 days before the first day of last menstruation previous to commencing treatment. The end of MAR follow-up was defined as the first day of the last menstruation before either ongoing pregnancy or the final insemination or embryo transfer. This aligned with the definition of time to natural conception. A treatment indicator was created that denoted which treatment (expectant, IUI/IUI-OS or IVF/ICSI) was received in which time period. Next, a Cox model was fitted with the oil versus water allocation, the MAR indicator and the interaction between these two. This model estimates the effect of oil versus water separately for expectant management, IUI/IUI-OS and IVF/ICSI.

RESULTS

The H2Oil study randomized 1119 couples. After excluding couples who conceived before receiving HSG and couples with missing follow-up or pregnancy data, data on 1107 couples were available, of which 550 couples received oil-based contrast and 557 couples received HSG with water-based contrast. Ongoing pregnancy rates at

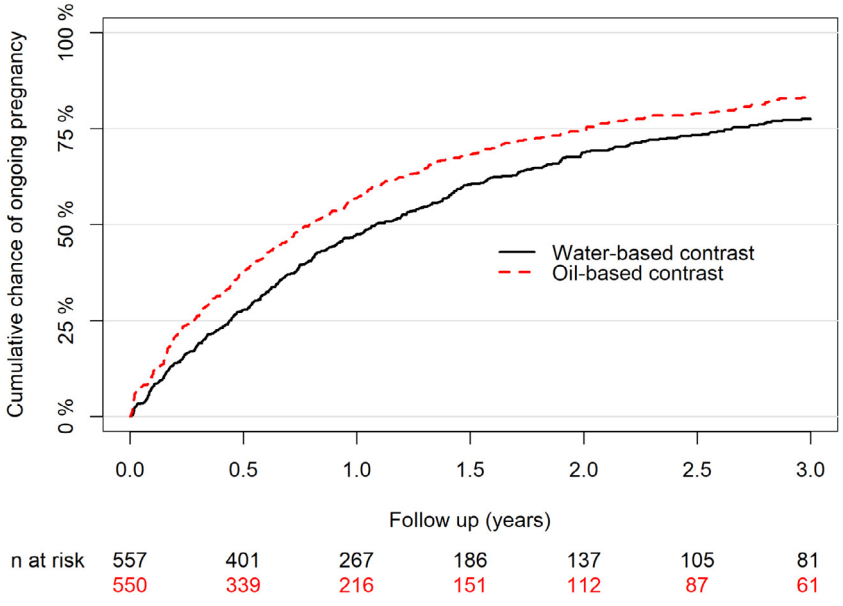

FIGURE 1 Kaplan–Meier curve for time to an ongoing pregnancy.

TABLE 1 ESTIMATED HR FOR ONGOING PREGNANCY OF OIL VERSUS WATER HSG AT DIFFERENT TIME POINTS AFTER HSG

Follow-up time	HR (95% CI) for ongoing pregnancy using log time
Immediately after HSG	1.71 (1.27–2.31)
1 month	1.57 (1.24–1.99)
3 months	1.36 (1.17–1.59)
6 months	1.25 (1.09–1.44)
9 months	1.19 (1.03–1.38)
1 year	1.15 (0.98–1.35)
2 years	1.06 (0.86–1.3)
3 years	1.00 (0.79–1.28)

CI, confidence interval; HR, hazard ratio; HSG, hysterosalpingography.

3 years were 426 (77%) and 394 (71%), respectively. FIGURE 1 displays ongoing pregnancy rates as a Kaplan–Meier curve including the sample size over time. Average female age at randomization was 32.7 years (5th–95th percentile: 26.1–38.9) and median duration of infertility was 1.61 years (5th–95th percentile: 0.91–3.89). A total of 746 (67%) couples had primary infertility. The median follow-up for all couples including up to conception was 9–10 months (5th–95th percentile: <1 to 36).

Primary analysis

The hazard ratio for ongoing pregnancy estimated over 3 years follow-up of oil-based versus water-based contrast was 1.26 (95% CI 1.10–1.45). Using regular time, the scaled Schoenfeld residuals plot showed a slight decrease in hazard ratio over time from approximately 1.6 after HSG to 1.3 after 1 year (Supplementary Figure 1, test for non-proportional hazards over time: $P = 0.10$). Using log-transformed time, the decrease in log hazard ratio over time was more pronounced shortly after HSG and was statistically significant (Supplementary Figure 2, test for non-proportional hazards over time: $P = 0.02$). There thus seems evidence that the effect of oil versus water decreases over time, and that this occurs mostly within the first year after HSG.

The best fitting model included a linear term for the interaction between log-transformed time and allocation ($P = 0.02$ for interaction, an approximately 3-point decrease in AIC compared with the model with only a main treatment effect). The estimated hazard ratios at different follow-up time points are shown in TABLE 1. The estimated hazard ratio of oil versus water started at 1.71, gradually decreased over follow-up time and eventually plateaued around 1 after approximately 2 years of follow-up.

The numbers of pregnancies for the first six cycles are shown in TABLE 2. The relative risks ranged from 1.16 to 1.83. The effect seemed to last during these six cycles but due to a small number of pregnancies per cycle, the estimates for this approach were uncertain, making it difficult to ascertain a clear trend.

Sensitivity analyses

When repeating the primary analysis only in women who were asked to judge their pain on a visual analogue scale ($n = 401$) and scored at least 6 points ($n = 152$: 73 oil and 79 water), the estimated hazard ratio over 3 years was 1.47 (95% CI 1.03–2.12). There was no evidence of a change in effect of oil versus water over time as the scaled Schoenfeld residuals plot showed a slight increase rather than a decrease, and the tests for regular and log-transformed time were not significant (Supplementary Figures 3 and 4, $P = 0.88$ and $P = 0.71$, respectively).

When censoring for IVF/ICSI, the estimated hazard ratio for oil versus water over 3 years was 1.29 (95% CI 1.11–1.50). Results from plots and tests using scaled Schoenfeld residuals were very similar to those in the primary analysis, as were the estimated hazard ratios at sequential time points (results not shown).

When adjusting for baseline characteristics, the estimated hazard ratio for oil versus water over 3 years was 1.30 (95% CI 1.13–1.50). Results from plots and tests using scaled Schoenfeld residuals were very similar to those in the primary analysis, as were the estimated hazard ratios at sequential time points (results not shown).

Supplementary analysis

There was found no evidence that the effect of oil versus water was different for expectant management, IUI/IUI-OS or IVF/ICSI: the interaction between oil versus water allocation and MAR treatment was not significant ($P = 0.39$) and did not lead to a better fit in terms of AIC.

DISCUSSION

Evidence was found that the hazard ratio for ongoing pregnancy after an HSG with oil-based contrast versus water-based contrast was highest shortly after HSG and then gradually decreased. This change was best described as linear with log-time, decreasing from a hazard ratio of 1.71 to 1, i.e. no effect, after approximately 2 years. In the subgroup

TABLE 2 NUMBER OF PREGNANCIES PER GROUP, PER CYCLE

Cycle after HSG	Ongoing pregnancies oil group	Ongoing pregnancies water group	Relative risk (95% CI)
1	47/550	31/557	1.54 (0.99–2.38)
2	39/502	30/526	1.36 (0.86–2.16)
3	41/463	24/496	1.83 (1.12–2.98)
4	24/422	23/471	1.16 (0.67–2.03)
5	22/398	19/447	1.30 (0.71–2.37)
6	23/374	19/428	1.39 (0.77–2.50)

CI, confidence interval; HSG,hysterosalpingography.

of women who experienced pain during HSG, which might be because flushing dislodged debris or mucus plugs in their Fallopian tubes, there was found no evidence for a change in hazard ratio over time.

A decreasing hazard ratio over time could be due to three potential mechanisms. The first is that, for each woman, the hazard ratio for the effect of oil-based versus water-based contrast diminishes over time. Second, a decreasing hazard ratio may also be explained by heterogeneity of treatment effect, meaning that the oil contrast may have a beneficial effect that is stable over time in only a subgroup of women. These women quickly conceived and, due to selection, at later time points the treatment effect was only evaluated in women for whom it was not beneficial, leading to a lower hazard ratio. A third possible explanation is unobserved heterogeneity, meaning that pregnancy chances varied between couples due to factors unknown to the authors. Even with a treatment effect that is constant over time and similar for all women, unobserved heterogeneity may lead to attenuation of the hazard ratio towards 1 over time (*Aalen et al., 2015*; *Hernan, 2010*). When adjusting for baseline characteristics that are known prognostic factors, the results did not differ from the primary analysis. In addition, it can be argued that, in the first year after HSG, unobserved heterogeneity might not yet play a role and that the observed decrease in hazard ratio can be explained by either one of the former mechanisms.

In terms of strengths and limitations, this secondary analysis was performed using data from a well-designed multicentre RCT with a long follow-up period of 3–5 years. Using an objective outcome measure, ongoing pregnancy, the risk of bias was minimal. Only women with unexplained or mild male infertility were included; they were below 39 years of age, did not have known endocrinological disorders and had a low risk of tubal pathology based on their medical history. Therefore, it is questionable whether the findings are generalizable to infertile women who do not share these features. Additionally, it should be noted that the main outcome in this study was ongoing pregnancy, whereas in clinical practice live birth is the desired outcome. However, there are several reasons to justify the use of ongoing pregnancy as a proxy for live birth in fertility research (*Braakhekke et al., 2014*).

The finding that the fertility-enhancing effect of oil-based contrast lasts for a substantial amount of time promotes the hypothesis that the mechanism of action lies in the Fallopian tubes, implying that tubal flushing during HSG dislodges debris, mucus plugs or small adhesions in the proximal parts of the Fallopian tubes, thereby resolving an 'unexplained' fertility factor (*Kerin et al., 1992*; *van Welie et al., 2019*). The findings are less consistent with the other suggested mechanisms, as it was postulated here that an effect in the endometrium or alteration in the immune response in the peritoneum would be a temporary effect.

Making the assumption that the HSG using oil-based contrast does dislodge debris, mucus, etc. from the Fallopian tubes, this would mean that they are essentially 'cured', which here means that their tubes are once more fully operational and they are back to their 'normal' state of fertility. However, the 'normal' fertility potential varies considerably between women (*te Velde and Pearson, 2002*; *van Eekelen et al., 2017*). This inherent difference between women in terms of their chance of conception might explain why not all women in this subgroup conceive within the first couple of cycles after being 'cured': some of them with lower potentials take much longer, for example more than 1 year, to conceive.

Additionally, it has been postulated that oil could emulsify debris in Fallopian tubes, facilitating the removal of debris more efficiently (*Watson et al., 1994*). Furthermore, the two contrast media have many differences in chemical and physical characteristics, for example oil-based contrast (Lipiodol Ultra-Fluid®, Guerbet, France) has a lower viscosity. The oil-based contrast also contains a higher iodine concentration than the water-based contrast used here (Telebrix Hystero®, Guerbet, France). There is currently very limited evidence regarding the impact of these differences, and future studies are needed.

Although a previous analysis did not identify characteristics that were associated with a greater or lesser effect of oil-based contrast compared with water-based contrast, the hypothesis regarding the Fallopian tubes is supported by a recent analysis of perceived pain during HSG (*van Rijswijk et al., 2019*; *van Welie et al., 2019*). Women who reported a VAS score of 6.0 or more were found to benefit more from oil-based contrast (49.4% versus 29.6%, Relative Risk 1.7, 95% CI 1.1–2.5) (*van Welie et al., 2019*). In the current study, there was no evidence that the effect of oil versus water contrast decreased over time for women who had a VAS score of 6 or higher. Thus, in addition to the effect of oil contrast being dependent on VAS score, this provides some evidence to support the theory of dislodging debris or mucus plugs in the Fallopian tubes, as that effect is likely to be (semi-)permanent. However, the small sample sizes for this sensitivity analysis must be acknowledged as the VAS score was not measured in all patients (n = 401) and only 152 of those scored 6 or higher. The previously mentioned second mechanism might also explain the decrease in hazard ratio that was found in the whole cohort: that there is only an effect in the group in which debris was dislodged and pain was felt, and as their chances increase, they conceive and drop out of the cohort.

This secondary analysis was performed to understand the biological mechanism underlying the fertility-enhancing effect of tubal flushing and to evaluate how long it is beneficial for infertile women. However, the study emphasized the complexity of 'unexplained infertility' with multiple unknown aspects. All studied women were below 39 years of age, had a regular ovulatory cycle and had a low perceived risk of tubal pathology, so it is unclear what causes infertility in these women. Women who did not conceive within 2 years after HSG no longer benefited from the oil-based contrast. This may support the hypothesis that tubal flushing using oil-based contrast dislodges debris or mucus plugs from the proximal parts of the tubes and that, after 2 years, most of these women conceived. This hypothesis can be further explored by studies in which the pressure build-up of oil-based and water-based contrast during HSG, resulting in dislodgment of material such as debris and mucus plugs, is investigated. Furthermore, future studies are needed to evaluate whether HSG with oil-based contrast before IVF has an fertility-enhancing effect, and to assess whether the fertility-enhancing effect of oil-based contrast is also present in women above 39 years of age with a

diminished ovarian reserve, women who have ovulation disorders or women at high risk of tubal pathology.

CONCLUSION

The fertility-enhancing effect of oil-based contrast versus water-based contrast after HSG in terms of the hazard ratio was highest shortly after HSG and then decreased, although the effect still seemed to be present for at least 1 year after tubal flushing. After approximately 2 years there was no beneficial effect. Additionally, in women who experienced pain during HSG, the effect might last longer. The current results favour the hypothesis that oil-based contrast might dislodge debris or mucus plugs from the Fallopian tubes, and contradicts other locations of action, i.e. the endometrium or the peritoneum, although this has yet to be proven definitively. The findings can be used to further investigate unexplained infertility and to counsel couples with unexplained infertility that they might still conceive naturally after HSG and that treatment could be delayed for a period of time.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2020.08.038.

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