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ARTICLE

The therapeutic effect of hysterosalpingography in couples with unexplained subfertility: a post-hoc analysis of a prospective multi-centre cohort study

BIOGRAPHY

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KEY MESSAGE

In this secondary analysis of a large Dutch prospective cohort, an association was found between hysterosalpingography and increased ongoing pregnancy rates compared with no hysterosalpingography, regardless of the contrast medium used. These findings support the hypothesis that hysterosalpingography is not only a diagnostic but also a therapeutic intervention.

ABSTRACT

Research question: Hysterosalpingography (HSG) with an oil-based contrast has been shown to increase ongoing pregnancy rates compared with HSG with water-based contrast, but it remains unclear if an effect of HSG occurs compared with no HSG.

Design: A secondary data-analysis of a prospective cohort study among 4556 couples that presented with unexplained subfertility in 38 clinics in the Netherlands between January 2002 and December 2004. A time-varying Cox regression with inverse probability of treatment weighing was used to analyse ongoing pregnancy rates in women after undergoing the HSG procedure (with the use of either water- or oil-based contrast media) compared with women who did not undergo HSG.

Results: The probability of natural conception within 24 months after first presentation at the fertility clinic was increased after HSG, regardless of the type of contrast medium used, compared with no HSG (adjusted hazard ratio 1.48, 95% CI 1.26 to 1.73, corresponding to an absolute increase in 6-month pregnancy rate of +6%). When this analysis was limited to HSGs that were made with water-contrast, the treatment effect remained (adjusted hazard ratio 1.40, 95% CI 1.16 to 1.70).

Conclusions: HSG increases the ongoing pregnancy rate of couples with unexplained subfertility compared with no HSG, regardless of the contrast medium used. Results need to be validated in future, preferably randomized, studies.

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KEYWORDS

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INTRODUCTION

About 10% of couples who wish to have a child, fail to conceive within 1 year of regular unprotected intercourse (*Gnoth et al., 2005*). The assessment of the tubal patency is traditionally an important part in the fertility work-up for subfertile couples. Hysterosalpingography (HSG) is one of the most widely used outpatient methods for tubal patency testing in the Netherlands (*Cary Hollenback, 1973; NVOG, 2010*). An HSG examination involves the infusion of contrast medium into the uterine cavity and fallopian tubes, with subsequent radiography to evaluate patency of the tubes (*Schoemaker, 1973*).

Although initially developed as a diagnostic test, a Cochrane review found an increase in ongoing pregnancy rates after HSG with oil-based contrast medium compared with no HSG during the first months after the HSG (OR 3.59, 95% CI 2.06 to 6.26) (*Mohiyiddeen et al., 2015*). The trials included in the review, however, were of low methodological quality, had relatively small sample sizes leading to imprecise estimates and followed couples for a relatively short amount of time after randomization (Ogata *et al.*, 1993; Nugent *et al.*, 2002; *Johnson et al., 2004*). Overall, the quality of the evidence is low. Therefore, it is, at present, unclear whether a therapeutic effect of HSG occurs, i.e. if it increases ongoing pregnancy rates compared with no HSG. It is also unclear whether this would be solely the case when using an oil-based contrast medium, as a recent study showed increased ongoing pregnancy rates after HSG with oil-based contrast compared with after HSG with water-based contrast (*Dreyer et al., 2017*), or that the HSG procedure itself regardless of contrast medium used contributes to the effect. We, therefore, conducted a secondary analysis on a large nationwide prospective cohort to evaluate if HSG has a therapeutic effect and whether this is mediated by the medium used.

MATERIALS AND METHODS

This prospective cohort study was carried out between January 2002 and February 2004 in 38 clinics in the Netherlands. The study was approved by the Institutional Review Board of the Academic Medical Centre, Amsterdam, the Netherlands (reference number MEC01/204) on 24 January 2002 and approved by the Board of Directors of each of the participating clinics. All couples gave informed consent. The study has been described earlier in more detail (*van der Steeg et al., 2007*).

In short, 7860 couples underwent the basic fertility work-up according to the guidelines of the Dutch Society of Obstetrics and Gynaecology (*NVOG, 2004*). This assessment included a medical history, cycle monitoring, semen analysis and investigation of tubal status. In this analysis, couples were excluded if women had an ovulation disorder, a history of tubal surgery, underwent an HSG before first consultation at the fertility clinic, if timing of HSG was unknown, if no follow-up data were available, if they tried to conceive for less than 10 months or if the partner had a total motile sperm count (TMSC) of less than 1×10^6. Ovulation was confirmed by a basal body temperature chart, an elevation of serum progesterone in the luteal phase or by sonographic monitoring of the menstrual cycle. Ovulation disorder was defined as a cycle length of less than 21 days or more than 37 days. In this way we selected couples with unexplained subfertility who were not receiving any treatment, be it assisted reproductive techniques or other medical treatments.

Evaluation of the fallopian tubes during the basic fertility work-up was by measurement of chlamydia antibody titres or HSG, depending on the local protocols of the participating clinics. Serum chlamydia antibody titres (CAT) were measured by immune fluorescence technique or with enzyme immune assays. For immune fluorescence, the CAT was considered to be positive if the titre was greater than 1:16 and for enzyme-linked immunosorbent assay if the immunoglobulin G chlamydia antibody titre was greater than 1.1. A positive CAT is an indication that the woman had a previous infection with chlamydia. Some of the participating clinics planned an HSG in all subfertile women during the routine fertility work-up, regardless of the CAT outcome, whereas other clinics only planned HSG when the CAT was positive. All HSGs were performed according to the local protocols of the participating clinics. During HSG, a radiopaque contrast medium was infused through the cervix into the uterine cavity and Fallopian tubes. At the same time, radiographs were made to observe whether the infused contrast medium flowed through the fallopian tubes and subsequently into the abdominal cavity in case of patent tubes. In most of the participating clinics, water-based contrast medium was used for HSGs, whereas some clinics used oil-based contrast as the standard medium for HSG.

Female age was calculated at the first visit at the fertility clinic. The duration of subfertility was defined as the period between the time the couple had an active child wish and the first visit at the fertility clinic. If the couple had a previous pregnancy that did not result in a live birth, the duration of subfertility was defined as the period between the end of this pregnancy and the first visit at the fertility clinic. Subfertility was considered to be primary if a woman had never conceived in the current or previous relationship and secondary if a woman had ever conceived, regardless of pregnancy outcome. The semen quality was expressed in TMSC (volume of the ejaculate in millilitres times the concentration of spermatozoa times the percentage of progressive motile spermatozoa). A semen analysis was carried out at least once. In the case of two semen analyses, the mean TMSC of both samples was calculated.

The model developed by *Hunault et al. (2004)* was used to calculate a prognosis of natural conception over the year after the fertility work-up. This model comprises female age, duration of subfertility, primary or secondary subfertility, percentage of motile sperm and referral by either a general practitioner or an obstetrician or gynaecologist.

Outcome measurements

The main outcome measure was time to ongoing pregnancy, defined as a positive heartbeat on ultrasound beyond 12 weeks gestation. Time to pregnancy was censored at the time intrauterine insemination or IVF was started, when women underwent laparoscopy, at the last date of contact during follow-up when the couple did not conceive or at a maximum of 24 months after the first visit at the fertility clinic. Time to pregnancy was not censored in case of a miscarriage or ectopic pregnancy, as in those situations a woman was followed thereafter until an ongoing pregnancy or the last date of contact.

Statistical analysis

The ongoing pregnancy rates were compared between two periods of follow-up time: 'no HSG period' and 'HSG period' formed by women who had an HSG in the preceding 6 months. Women who did not undergo HSG within 24 months after the first visit at the fertility clinic were analysed in the 'no HSG period'. Women who underwent HSG were analysed in the 'no HSG period' during the period between first visit at the fertility clinic and the moment they had the HSG examination, in the 'HSG period' during a period of maximally 6 months after their HSG procedure, and in the 'no HSG period' from 6 months after HSG up to a maximum of 24 months after first visit at the fertility clinic. This was because the therapeutic effect of HSG was assumed to last for about 6 months after HSG.

An iterative inverse probability of treatment weighing (IIPTW) was applied to correct for possible unbalance in prognostic factors between women who had an HSG and those who did not (*van der Wal, 2011*). A Cox proportional hazards model was used to calculate propensity scores that predict the probability of receiving HSG for each woman over time and updated them every 2 weeks. The following prognostic factors were included in the propensity model: female age at registration, duration of subfertility at registration, total motile sperm count, referral by a specialist or general practitioner, female subfertility being primary or secondary and the result from the CAT being categorized as positive, negative or not conducted. In a sensitivity analysis using only couples from the 15 largest fertility clinics, clinic was also included as a factor to the propensity model. The IIPTW method weighs the couples by dividing outcomes of couples who received HSG or not by an individual's propensity score for their treatment status. After weighing, the possible imbalance between couples with and without HSG in terms of these prognostic factors is reduced. An iterative estimation procedure for the weights was used: the weights were modified iteration after iteration within the IIPTW routine until a maximum of 2000 iterations or when the weights did not change anymore, meaning balance was achieved, which was defined as a variance of the log of newly derived weights less than 1×10^{-7} (*van der Wal, 2011*). After IIPTW, the balance in the patient characteristics that were included in the propensity model was checked between women who did and those who did not undergo HSG using the standardized mean difference. A standardized mean difference below 0.10 is considered no relevant difference (*Austin, 2011; Austin and Stuart, 2015*).

The data were analyed using a Cox proportional hazards model with a time-varying covariate for HSG i.e. 'HSG period' versus 'no HSG period' and we calculated a hazard ratio with and without applying the weights representing the crude and adjusted therapeutic effect of HSG. Using the weighted model, the absolute probabilities were calculated of natural conception leading to ongoing pregnancy over the following 6 months if a woman would receive HSG immediately at registration compared with if she would not receive HSG.

Secondary analysis

To compare ongoing pregnancy rates between women who had an HSG with water-based contrast medium only and women who had not undergone an HSG, couples that received an HSG with oil-based contrast, iso viscose contrast or when the contrast medium was unknown were excluded. The IIPTW procedure was repeated for this selection of couples.

Missing data

Missing data in the dataset were accounted for in a previous study using multiple imputation, creating 10 imputation sets (*van Eekelen et al., 2017a*). Only 3.8% of patient characteristics were missing in the dataset; therefore, one randomly selected imputation set was selected for our analyses. All reported *P*-values are two-sided and $P < 0.05$ was considered to indicate statistical significance.

The following programme was used for statistical analysis: R version 3.3.2 (*R Core Team, 2013*). Source code of our analyses are available upon request.

RESULTS

A total of 4556 couples were included in this study, of whom 2196 underwent HSG during the follow-up period of maximally 24 months after the first visit at the fertility clinic and 2360 couples who did not receive HSG (FIGURE 1).

Baseline characteristics of the included subfertile couples are presented in TABLE 1 and stratified for those who did and those who did not undergo HSG within 24 months after the first visit at the fertility clinic. Couples who did not undergo HSG during the fertility work-up were more often referred by another specialist and more often received a positive CAT result compared with women who underwent HSG (11% versus 3% for specialist referral, 31% versus 13% for positive CAT).

The number of women in the 'HSG period' and 'no HSG period' over time is depicted in FIGURE 2. Women who had an HSG during the fertility work-up underwent this examination after a median period of 3.0 months (quartiles: 1.9 to 4.8) after their first visit at the fertility clinic. The median follow-up in the 'no HSG period' was 4.4 months (quartiles 2.4 to 8.1) and the median follow up in the 'HSG period' was 4.0 months (quartiles: 1.5 to 6.0). A total of 662 women had a natural conception leading to ongoing pregnancy during the 'no HSG period' (rate: 0.29 per person-year) compared with 301 during the 'HSG period' (rate: 0.44 per person-year). After IIPTW, the patient characteristics were well balanced between the group who underwent HSG and the group who did not. Five months after registration, when the HSG group was the largest, none of the standardized mean differences between groups were above 0.10.

The unweighted (crude) Cox model with HSG as a time-varying covariate showed that women had a significantly higher chance of natural conception leading to ongoing pregnancy in the 6 months after HSG compared with no HSG (hazard ratio: 1.59; 95% CI 1.37 to 1.85). After applying the IIPTW weights, the adjusted hazard ratio was 1.48 (95% CI 1.26 to 1.73) (TABLE 2). A woman who would receive HSG at the time of registration had an estimated probability of 21% (95% CI 18 to 24) to conceive naturally leading to ongoing pregnancy in the following 6 months. If she would not receive HSG, the estimated probability was 15% (95% CI 14 to 16).

Of the 2196 women who underwent HSG, 1331 (61%) used a water-based contrast medium, 321 (15%) an oil-based contrast medium and 30 (1%) used an iso viscose contrast medium. For 514 (23%) women, the contrast medium used was unknown.

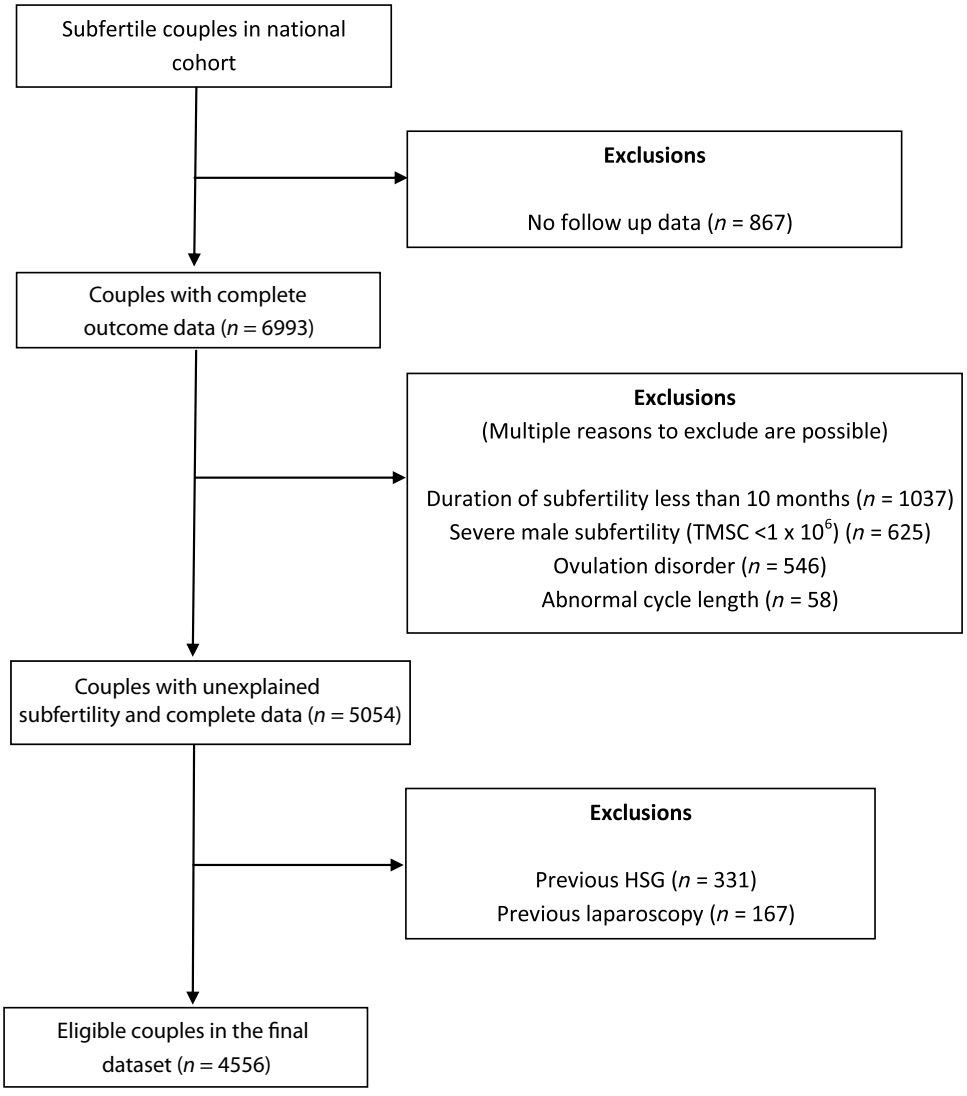

FIGURE 1 Study profile. TMSC, total motile sperm count.

TABLE 1 BASELINE CHARACTERISTICS, STRATIFIED FOR UNDERGOING HYSTEROSALPINGOGRAPHY WITHIN 24 MONTHS AFTER REGISTRATION AT THE FERTILITY CLINIC

***n* = 4556**	**No HSG (*n* = 2360)**	**HSG (*n* = 2196)**
Mean age at registration (years) (SD)	32.1 (4.4)	32.3 (4.2)
Median cycle length (days) (quartiles)	28 (26–28)	28 (26–28)
Median duration of subfertility at registration (months) (quartiles)	18.8 (14.4–27.5)	18.5 (14.4–24.8)
Primary subfertility, n (%)	1620 (69)	1473 (67)
Positive chlamydia antibody test, n (%)	730 (31)	295 (13)
Negative chlamydia antibody test, n (%)	1221 (52)	1344 (61)
No chlamydia test conducted, n (%)	409 (17)	557 (25)
Referral by specialist, n (%)	256 (11)	64 (3)
Median total motile count x10^6 (quartiles)	42 (12–107)	57 (20–126)
Mean calculated 1-year prognosis of natural conception[a] in percentage points (SD)	31.7 (11.8)	32.7 (10.9)

[a] Using the Hunault model (*Hunault et al., 2004*): chance to conceive naturally leading to live birth over the year after the work-up.
SD, standard deviation.

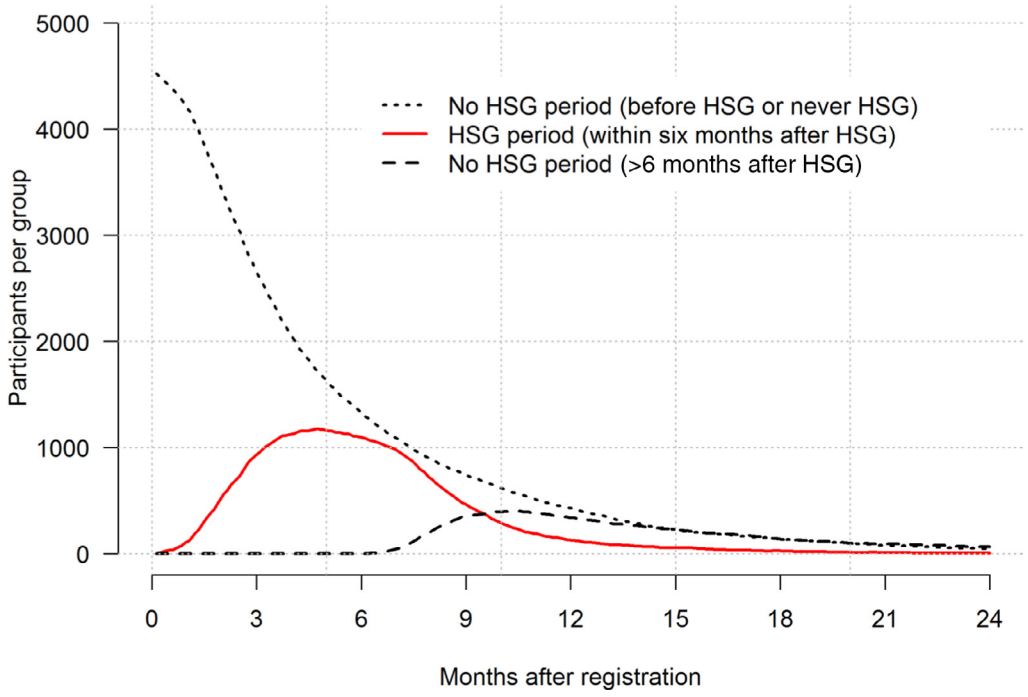

FIGURE 2 Number of participants per period over time.

The secondary analysis comparing HSG using water-based contrast media versus no HSG showed an unweighted (crude) hazard ratio of 1.49 (95% CI 1.25 to 1.79), which decreased to an adjusted hazard ratio of 1.40 (95% CI 1.16 to 1.70) after applying weights (TABLE 2).

The sensitivity analyses adding clinic to the propensity model showed similar results for both the primary and the secondary analysis.

DISCUSSION

This secondary analysis of women included in a prospective cohort study showed that HSG carried out during the basic fertility work-up was associated with a significant increase in ongoing pregnancy rates. The hazard ratio was 1.48 (95% CI 1.26 to 1.73) in favour of HSG with the use of any contrast medium. This hazard ratio corresponds to an absolute increase in 6-month pregnancy rate of +6%, an effect size that is comparable to, for instance, the prognostic effect of a woman that is 7 years older or a 2-year longer duration of subfertility (*van Eekelen et al., 2017a*). When limited to HSG conducted with water-based contrast, the treatment effect remained (hazard ratio 1.40, 95% CI 1.16 to 1.70).

Our study has some limitations. This study was a secondary analysis of a prospective cohort study. Women were not randomized for tubal patency testing by HSG versus no HSG, which might have introduced bias in terms of confounding by prognostic factors that differ between couples. The most notable difference in prognostic factors between the couples receiving HSG versus those not receiving HSG was unbalance in the proportion of couples with a positive CAT result. This indicates that the group that did not (yet) receive an HSG was more likely to have tubal disease affecting fecundity. This is in line with what could be expected from our design: during the observation period before HSG, tubal disease status was not yet verified by any visual diagnostic test. For those in the HSG group, if tubal disease was identified, couples were likely removed from follow-up and scheduled for a second diagnostic procedure (laparoscopy) or possibly for treatment. The potential effect of the observed difference in CAT infections is limited. With an anticipated prognostic hazard ratio of 0.7 for CAT positive versus CAT negative (*van Geloven et al., 2012*), the potential benefit for the HSG group with 13% CAT positive versus 31% CAT positive in the no-HSG group could roughly amount to a hazard ratio of 1.07 to the benefit of the HSG group and cannot explain the hazard ratio of 1.48 that we found.

We attempted to reduce the potential bias by conducting a time-varying inverse probability of treatment weighing (IPTW)

TABLE 2 RESULTS FROM COX MODELS: EFFECT OF HYSTEROSALPINGOGRAPHY ON ONGOING PREGNANCY

Analysis	Hazard ratio (95% CI)
Primary outcome	
HSG versus no HSG, unadjusted	1.59 (1.37 to 1.85)
HSG versus no HSG, adjusted[a]	1.48 (1.26 to 1.73)
Secondary	
Water-based contrast HSG versus no HSG, unadjusted	1.49 (1.25 to 1.79)
Water-based contrast HSG versus no HSG, adjusted[a]	1.40 (1.16 to 1.70)

[a] Adjusted by applying inverse probability of treatment weights that were estimated using female age at registration, duration of subfertility at registration, total motile count, primary or secondary subfertility, referral status and chlamydia antibody testing.
HSG, (hysterosalpingography).

analysis to balance women who did and women who did not receive HSG for these prognostic factors that might have influenced the decision to perform an HSG. Residual (unmeasured) confounding can, however, not be excluded. An example is that patients who were identified as having other pathology on HSG, e.g. from endometriosis, ruptured appendicitis, gonorrhoea, would be excluded from the HSG period, and may be more prevalent in the no-HSG period. Although we expect the effect of such rare diseases is small, we cannot rule out residual bias due to our observational design. Our findings must be validated in future studies.

Both for couples who did and for those who did not undergo HSG, follow-up time was censored at the time of receiving a laparoscopy. As women suspected of tubal pathology, either based on a positive CAT or a positive HSG, were more likely to undergo laparoscopy, this may have introduced informative censoring since women with tubal occlusion will have a poorer prognosis for natural conception than women with patent tubes. Informative censoring might have occurred relatively more often in the group that received an HSG, as that procedure is considered a more specific test for tubal pathology than the CAT. Our model did not capture this, which may potentially lead to an overestimation of the effect of HSG. It is rare, however, that unexplained subfertile women have tubal pathology, in particular two-sided. Therefore, the effect of informative censoring on our results is expected to be limited.

Our results are in line with the results from previous randomized trials that reported higher ongoing pregnancy rates after HSG made with oil-based contrast medium versus no intervention as pooled in a Cochrane review (*Mohiyiddeen et al., 2015*), but the estimated effect in our study was smaller. In the present study, most HSG examinations were conducted with water-based contrast medium (61%) instead of an oil-based contrast medium (15%). No randomized trials that evaluated the therapeutic effect of HSG with water-based contrast versus no HSG in women with unexplained subfertility have been published. One small trial on hysterosalpingo-contrast sonography, for which the process of tubal flushing is similar to HSG, compared water-based contrast with no flushing and did not find a significant difference in ongoing pregnancy rates (Lindborg *et al.*, 2009). Our secondary analysis showed a similar, albeit slightly lower, increase in ongoing pregnancy rates in the first 6 months after HSG with use of a water-based contrast medium (hazard ratio 1.40, 95% CI 1.16 to 1.70) compared with the analysis, including HSGs with any contrast medium (hazard ratio 1.48 95% CI 1.26 to 1.73). This suggests a therapeutic effect of the HSG procedure itself, regardless of the contrast medium. The exact underlying fertility-enhancing mechanism of HSG is unclear, but it has been suggested that tubal flushing during HSG can dislodge non-occlusive but pregnancy-hindering debris from otherwise undamaged fallopian tubes (*Kerin et al., 1991; Watson et al., 1994*). Given this hypothesis, hysterosalpingo-contrast sonography is expected to yield similar results, as the flushing procedure is the same as HSG and only the visualization of liquid flow differs between the two procedures.

The recently published H2Oil trial (*Dreyer et al., 2017*) demonstrated a substantial increase in ongoing pregnancy rates during the first 6 months after HSG with the use of oil-based contrast compared with water-based contrast (rate ratio, 1.37; 95% CI 1.16 to 1.61). A possible explanation may be a direct effect of the oil contrast on the endometrial receptivity enhancing fertility by an implantation mediated mechanism (*Johnson et al., 2004; Johnson et al., 2005; Johnson, 2014*). Another suggested explanation is an effect of oil contrast on the peritoneal macrophage activity, leading to a change in production of cytokines and an inhibition of sperm phagocytosis (*Mikulska et al., 1994*). An endometrial receptivity study is needed to gain more insight into the fertility-enhancing mechanism of oil contrast over water contrast.

In conclusion, in women with unexplained or mild male subfertility, HSG during the fertility work-up was associated with an increase of ongoing pregnancy rates after natural conception compared with no HSG. This positive effect of HSG was also present when executed with water-based contrast. These findings support the hypothesis that HSG is not only a diagnostic but also a therapeutic intervention.

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