Safety and efficacy of tubal flushing with ethiodized oil

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INEZ ROEST

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SAFETY AND EFFICACY OF **TUBAL FLUSHING** WITH **ETHIODIZED OIL**

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Prof. dr. V. Mijatovic (Amsterdam UMC)

Copromotoren
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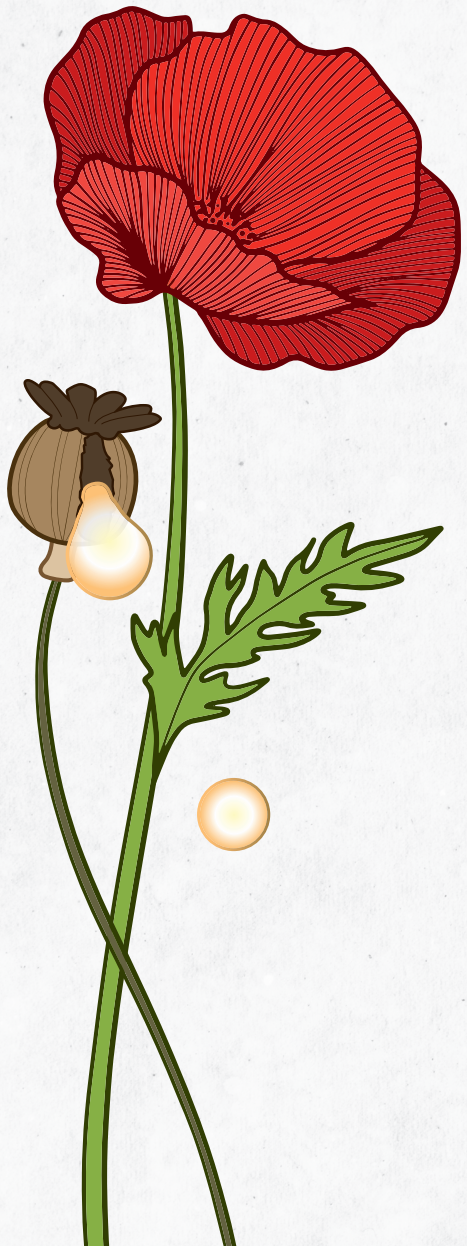

CHAPTER 1

GENERAL INTRODUCTION

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Epidemiology

In the process of evolution, the human species developed a relatively low fecundability, which is the probability of achieving a pregnancy within one menstrual cycle (Zegers-Hochschild *et al.*, 2017). The average human fecundability ranges from 13-20% in women between 30 and 39 years old and decreases with advancing age (Steiner and Jukic, 2016). The cumulative pregnancy rate after 12 menstrual cycles is around 80%, most of these couples conceive within the first six cycles (Steiner and Jukic, 2016). A multiple pregnancy only occurs in around 1.5% of all pregnancies (CBS, 2019). These numbers are low compared to other species, as a striking example, the pregnancy rate in dogs after one fertile cycle is around 90% and the average number of offspring during birth is 5.4 puppies (Chastant-Maillard *et al.*, 2017).

Unwilling childlessness is a global health issue in humans. Worldwide prevalence calculations estimate that 48.5 million couples suffer from unwilling childlessness, defined as the lack of live birth after 5 years of trying to achieve a pregnancy (Mascarenhas *et al.*, 2012). In clinical practice, subfertility is defined as the lack of conception after 12 months of timed unprotected intercourse (Zegers-Hochschild *et al.*, 2017). The prevalence of subfertility varies across regions in the world and is estimated to be around 15.5% (Thoma *et al.*, 2013). The different categories of subfertility are tubal factor, uterine or peritoneal disorders, ovulatory disorders, male factor and unexplained subfertility (NICE, 2017).

The impact of subfertility on women and men is far-reaching. Studies have shown that 57% of women and 32% of men undergoing fertility investigations and/or treatment have significant depressive symptoms, and respectively 76% and 61% experience anxiety symptoms. About a quarter of these women and men consult mental health services (Pasch *et al.,* 2016). Understandably, couples who do not choose and/or receive fertility treatment also suffer from fertility-specific distress. However, the level of fertility-specific distress has been shown to be the highest in women undergoing fertility treatment (Greil *et al.*, 2011).

Fallopian tubes

Historical background

The structure which is now called Fallopian tubes was already briefly mentioned in Hindu texts around 1000-800 B.C., though its function was still unclear. Around the 3rd century B.C. Herophilus, an anatomist, assumed that the ducts transferred “female

semen" from the ovaries to the urinary bladder. Not until around 150 A.D., a Greek physician described that the ducts ended into the uterus. Fallopius started naming the tubes "tuba uteri" around 1560, and lent his name to them. However, their currently known function was still not determined. In 1668, van Horne was the first to write down the thought that Fallopian tubes have a transmitting function of vesicles (i.e. follicles) from the ovary to the uterus, which was later proven by other scientists to be the transport of the content of the follicles, the egg. In 1880, Pinner reported that a suspension, India ink, introduced to the tubes at the ovarian side could reach the vagina (Hunter, 1988). After the discovery of the fundamentals of the tubal transporting function for sperm and (fertilized) ovum, the scientific interest did thankfully not fade. The following paragraphs provide a summary of the current knowledge on the anatomy, histology and physiology of the Fallopian tubes, however there is still a lot more to be discovered and clarified.

Anatomy of the Fallopian tubes

The Fallopian tubes (also called oviducts) are both about 12 cm in length. They can be divided into four parts, from medial to lateral; the intramural, isthmic, ampullary and infundibulum part (See Figure 1). The intramural section is narrow as it passes through the uterine wall, moving lateral, the Fallopian tube becomes slightly wider, forming the isthmic section. The ampullary section widens and terminates as the fimbriated infundibulum, which is visually similar to a sea anemone and may also be seen as a fifth, separate section of the Fallopian tube (Lyons *et al.*, 2006; Roger P. Smith and Turek, 2011; White *et al.*, 2019).

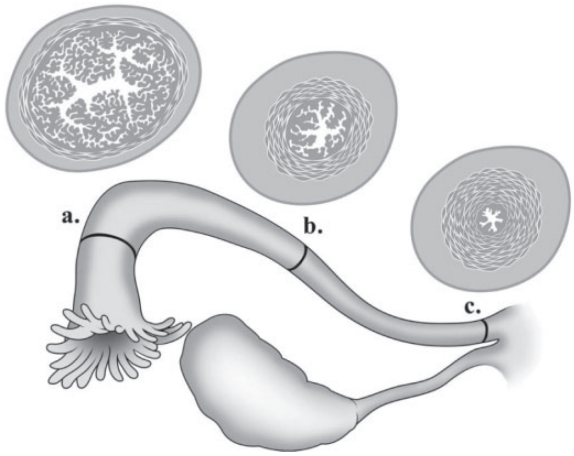

Figure 1. *Illustration of the human Fallopian tube, showing the longitudinal folds in cross-section at the (A) infundibulum, (B) ampulla and (C) isthmus (Lyons et al., 2006). Approval to use this image has been obtained from Oxford University Press.*

Histology and physiology of the Fallopian tubes

From the outside to the inside, the Fallopian tube consists of the perisalpinx, the myosalpinx and the endosalpinx.

The outer layer, perisalpinx (serosal coat, mesosalpinx), consists of connective tissue and is part of the lining of the peritoneal cavity. This layer has an abundance of blood vessels, especially in the fimbriated infundibulum. When those blood vessels are engorged, together with the muscular contractions, the fimbriae may sweep over the ovarian surface to aid the pickup of the cumulus-oocyte complex (Roger P. Smith and Turek, 2011; White *et al.*, 2019). The vascular system is also important for the generation of the tubal fluid (Winuthayanon and Li, 2018).

The myosalpinx, (tunica muscularis, the middle layer), consists of circular and longitudinal smooth muscle fibres. The muscular layer is thicker in the medial part compared to the ampullary section (Roger P. Smith and Turek, 2011; White *et al.*, 2019). Prostaglandins, progesterone, oxytocin and hCG (human Chorionic Gonadotrophin) regulate the contractility of these smooth muscle fibres, which aid the sperm and (fertilized) ovum transport (Wånggren *et al.*, 2008). Muscular contractions at the ampullary-isthmic junction create a sperm reservoir in the isthmic section and ensure that sperm enter the ampulla gradually to prevent polyspermy (Lyons *et al.*, 2006). On the other side, the fertilized ovum is retained in the ampullary part of the tube, where it is exposed to the tubal fluid with its developmental factors and nutrients, until the conditions in the uterus (i.e. endometrial receptivity) are optimal for implantation (Lyons *et al.*, 2006; Ezzati *et al.*, 2014).

The inner layer (mucosa, endosalpinx) consists of a single layer of columnar cells, partly ciliated and partly non-ciliated secretory cells (see Figure 2). This mucosal layer forms dense internal folds which increase the surface area. The folds are more complex, labyrinth-like, in the ampulla compared to the medial part of the tubes, to increase the number of cells on the surface and to achieve a high tubal fluid secretion rate (see Figure 1) (Lyons *et al.*, 2006; Roger P. Smith and Turek, 2011; Winuthayanon and Li, 2018; White *et al.*, 2019). The complex physiology of both cell types are separately discussed in the section below.

Physiology of the ciliated cells

A cilium consists of a central strand with two single microtubules, the axoneme, surrounded by nine pair of microtubules (See Figure 3) (Winuthayanon and Li, 2018). The sliding of the doublet microtubules, which requires hydrolysis of ATP (Adenosine Triphosphate), drives the rhythmical beating movement of the cilia. The beating of the

cilia generates intratubal flow in the direction of the uterus and contributes to the transportation of the ovum in addition to the ciliary beating itself and the muscular contractions of the tunica muscularis (Gaddum-Rosse *et al.*, 1973; Jansen, 1984; Satir and Matsuoka, 1989; Shi *et al.*, 2011; Ezzati *et al.*, 2014).

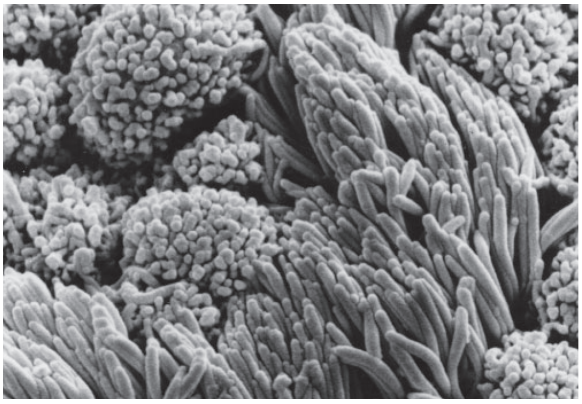

Figure 2. *Scanning electron microscope image of ciliated and secretory cells of the Fallopian tube epithelium (Lyons et al., 2006). Approval to use this image has been obtained from Oxford University Press.*

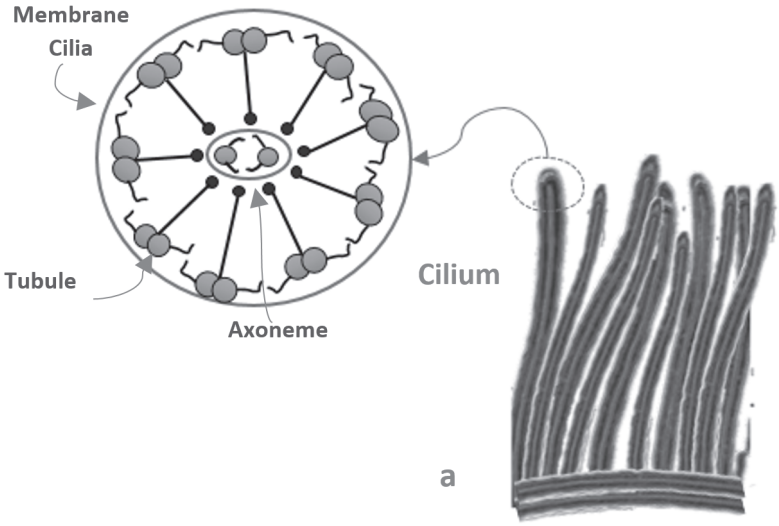

Figure 3. *The cilia and a schematic image of the axoneme structure and its microtubules (Roest et al., 2022).*

The ciliary beating frequency is presumably regulated by different factors; ovarian steroids, tubal fluid viscosity, angiotensin system, autonomic nervous system, prostaglandins, endometriosis, and smoking (Lyons *et al.*, 2002; Ezzati *et al.*, 2014; Jackson-Bey *et al.*, 2020). However, for most of these influencing factors the evidence is still limited. But, the negative influence of smoking on the ciliary beating frequency has been clearly shown (Shao *et al.*, 2012). The ciliary dysfunction caused by smoking results in a higher rate of ectopic pregnancies, clinical studies show a fourfold increase in women who smoke over 20 cigarettes a day (Bouyer *et al.*, 2003).

There is a rare, highly heterogeneous, inherited syndrome, called primary ciliary dyskinesia (PCD), which causes abnormalities to the cilia in the whole body, ranging from immotility to abnormal beating (dyskinesia) or loss of cilia. Patients mainly suffer from diseases of the upper and lower airways, due to defective mucociliary clearance. In almost 50% of the patients the embryonal organ distribution is changed due to dysfunction of the nodal monocilia leading to sinus inversus totalis. As a result of dyskinesia of the sperm flagella, asthenozoospermia and thereby malefactor infertility often occurs. Data on female fertility vary, probably due to the highly heterogeneous properties of PCD, as case reports of subfertility and ectopic pregnancies as well as spontaneous pregnancies have been described (Halbert *et al.*, 1997; Raidt *et al.*, 2015; Paff *et al.*, 2021). To diagnose PCD, respiratory ciliated cells are sampled from the nose or bronchus (Bricmont *et al.*, 2021). Fallopian cilia can only be tested after removing the Fallopian tube during e.g. a sterilization (Raidt *et al.*, 2015).

Physiology of the non-ciliated secretory cells

The oviductal fluid (tubal fluid), produced by secretion of the secretory cells and transudate from circulating plasma is a protein-rich mucus. Its composition is complex and includes growth factors, enzymes and glycoproteins. The production is altered during the menstrual cycle and its changing hormone levels (see Figure 4). Elevated oestrogen levels during the follicular phase lead to an increase in size and height of epithelial cells and cause an increase in tubal fluid secretion. Around the ovulation the viscosity of the tubal fluid secretion is the highest, as a result of the oestrogen stimulus. The successive rise in progesterone and fall in oestrogen leads to cellular atrophy and therefore a decrease in tubal mucus secretion and its viscosity (Winuthayanon and Li, 2018; White et al., 2019). Additionally, oviductal fluid secretion is drastically risen after sexual stimulation due to prolactin secretion (Miki and Clapham, 2013).

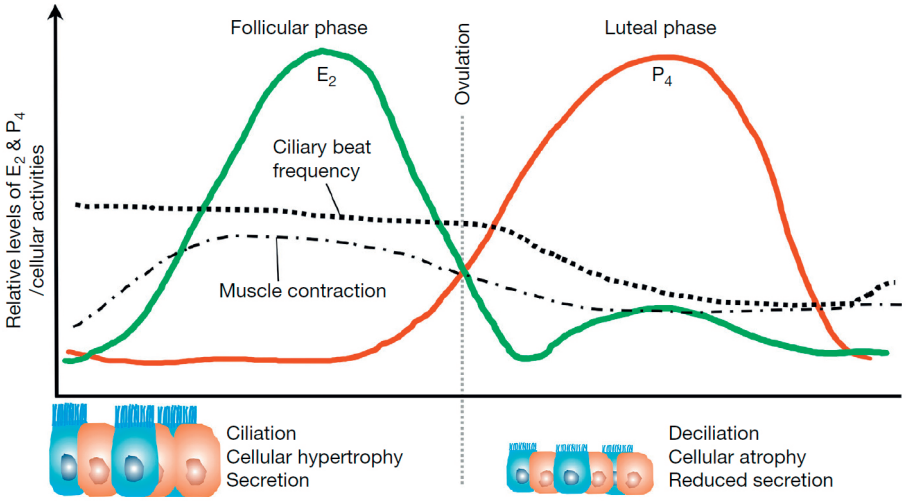

Figure 4. *The cyclic variations on epithelial cell morphology, ciliary beat frequency and muscle contraction in the Fallopian tubes (Winuthayanon and Li, 2018). Approval to use this image has been obtained from Elsevier.*

The oviductal fluid has various physiologic functions:

- The oviductal secretions coat and infuse the cumulus-oocyte complex, which may be necessary for viability and/or fertilizability (Coy *et al.*, 2008; White *et al.*, 2019).
- The spermatozoa are kept viable by adhering to the epithelial lining of the isthmus and can therefore be stored for days. The oviductal secretions induce capacitation and hyperactivity of the spermatozoa (Suarez and Pacey, 2006; White *et al.*, 2019).
- Additionally, the oviductal secretions during the periovulatory period, with its increased viscosity, form a buffer for the embryo to be protected against osmotic changes (Leese *et al.*, 2001). Furthermore, it provides nutritional support to the preimplantation-embryo during the development until the blastocyst stage, after which it may enter the uterine cavity and implant (Li and Winuthayanon, 2017; White *et al.*, 2019).
- Finally, the oviductal fluid clears cell debris, such as dead epithelial cells, bacteria, macrophages and sperm from the oviduct, especially during increased secretion in the follicular phase (Miki and Clapham, 2013).

Physiology of the spermatozoa transport

Almost directly after intravaginal ejaculation spermatozoa start to “swim” into the cervical canal. Only a fraction of the spermatozoa crosses the cervical mucus and continues its way through the female reproductive tract (Suarez and Pacey, 2006). The cervical mucus undergoes cyclic changes and is especially a barrier to less motile spermatozoa (Katz *et al.*, 1990). It is assumed that spermatozoa reach the Fallopian

tubes within minutes after entering the cervix (Settlage *et al.*, 1973). This transport is assisted by peristaltic waves of uterine smooth muscle contractions in the upward direction of the uterus (Kunz *et al.*, 1996). The flow of oviductal fluid, which secretion is increased after coitus due to an increase in prolactin, is of guidance to the spermatozoa. The spermatozoa have positive rheotaxis, which means that they move in the opposite direction of surrounding fluid flow (Miki and Clapham, 2013; Zhang *et al.*, 2016). Additionally, chemotaxis by progesterone, secreted from the cumulus cells around the oocyte, guide the spermatozoa (Teves *et al.*, 2006; Oren-Benaroya *et al.*, 2008). It has been shown that spermatozoa can bind intermittently to the endosalpingeal epithelium to be kept viable (Pacey *et al.*, 1995). Secretions from the epithelium, oviductal fluid, may trigger the spermatozoa capacitation process, which reduces its binding affinity to the epithelium. However, the detachment pull is not generated until the hyperactivation of the spermatozoa, which is triggered by a rise in cytoplasmic Ca^{2+}. Hyperactivation leads to a change in the beating pattern of the spermatozoa flagellum, and is also essential to penetrate the zona pellucida of the ovum (Chang and Suarez, 2010; Suarez and Pacey, 2006) .

Tubal patency testing

The Fallopian tubes can be permanently damaged by an ascending urogenital sexually transmitted infection (STI), nowadays most frequently Chlamydia Trachomatis. The presence of Chlamydia Trachomatis antibodies is standardly evaluated as part of the fertility workup, because subfertile women with positive Chlamydia Trachomatis serology have a higher risk of tubal pathology compared to subfertile women with negative serology (86% vs. 49% laparoscopically confirmed tubal damage) (Keltz *et al.*, 2013).

As previously mentioned, already in 1880 India ink was used to show the patency of the Fallopian tubes (Hunter, 1988). Soon afterwards, in 1895, X-rays were invented by Wilhelm Conrad Röntgen. The first hysterosalpingography (HSG), the use of roentgenology for tubal imaging, was performed in 1910 by Rindfleisch. Rindfleisch used a bismuth solution for the diagnosis of a tubal pregnancy (Rindfleisch, 1910). In the years following, various other water-based contrast media including Umbrenal, Collargol and Argyrol were developed and implemented. Later these water-based media were found to be irritative to the peritoneum and from 1914 ethiodized oils were used as an alternative, non-irritative contrast media for HSGs (Cary, 1914; Nielsen, 1946). Currently, the ethiodized oils used are Lipiodol Ultra Fluid® (Guerbet) and its biosimilar ethiodized Poppyseed Oil (Jiangsu Hengrui Medicine Co., Ltd).

Lipiodol® was developed in 1901 by Guerbet and Lafay for its ability to contain more iodine than other oils. Iodine was used at that time for a wide range of indications, including; antistrumous, antiseptic and antitoxic. It was administered through different routes; oral, rectal, parenteral and cutaneous. Later, after the discovery of its radiological property, it became used for among others myelography, visualisation of bronchopulmonary cavities, the exploration of the genital tract of woman and man and later lymphography. From 1981 Lipiodol® combined with cytostatics has also been used for the treatment of primary tumors of the liver (Bonnemain and Guerbet, 1995). Nowadays, Lipiodol® is still used for HSG, treatment of liver tumors and treatment of endemic goitre, the last being one of its original purposes (Bonnemain and Guerbet, 1995; Simescu *et al.*, 2002).

Also, over the years, various non-irritative water-based contrast media with diverse osmolality and viscosity have been developed. First, water-based contrasts of ionic monomers, with a very high osmolality compared to blood plasma, were used. Later, non-ionic water-based contrasts with a low osmolality became widely available. See Table 1 for a summary of the chemical and physical characteristics of different contrast media that have been used in recent RCT's.

Table 1. *Chemical and physical characteristics of contrast media used in recent RCTs*

	Ethiodized Poppyseed Oil[a]	**Telebrix Hystero**	**Visipaque 270**	**Optiray 320**	**Omnipaque 300**	**Ultravist 300**
Iodine amount (mg I/mL)	480	250	270	320	300	300
Viscosity (mPa.s)						
- At 15C - 25C	70	220	11.3	10	11.6	8.9
- At 37C	25	100	5.8	6.0	6.1	4.7
Osmolality (mOsm/kg)	Unknown	2260	290	700	640	590
Density (g/cm3)						
- At 15C or 20C	1.28	1.33				1.33
- At 37C	Unknown	1.32	Unknown	Unknown	Unknown	1.32
Ingredients	Ethylesters of fatty-acids of poppy seed oil	Ioxitalamate acid meglumine	Iodixanol	Joversol	Iohexol	Jopromide
Used in clinical trial	(Dreyer *et al.*, 2017; Zhang *et al.*, 2022)	(Dreyer *et al.*, 2017)	(Rosielle *et al.*, 2022)	(Zhang *et al.*, 2022)	(Zhang *et al.*, 2022)	(Zhang *et al.*, 2022)

Source: corresponding SPC of the different contrast media. [a] Generic contrast and Lipiodol Ultra Fluid. NA: not applicable. RCT: randomized controlled trial.

A few years after the introduction of HSGs, around 1920, an alternative, safer, method for tubal patency testing was developed, the tubal insufflation (Rubin) test. During this procedure, CO2 (Carbon dioxide) was insufflated via the cervix into the uterine cavity and through the Fallopian tubes in the abdominal cavity in case of tubal patency. Tubal patency was diagnosed if the pressure dropped after the initial build-up. Additional diagnostic criteria were; auscultation of airflow in the abdomen (Jet sound), shoulder/referred pain and visualization of abdominal gas under the diaphragm on X-ray (Ansari, 1979; Rubin, 1983). Due to i.e. further development of safe contrast media and the use of fluoroscopy as a safety precaution during HSGs, the Rubin test lost its importance in the 1960's and was replaced by HSG. Furthermore, between 1910 and 1930 diagnostic laparoscopy (DLS) was developed. From 1946 onwards, DLS was being performed in subfertile women to have direct visualization of the pelvis, including the Fallopian tubes, ovaries and uterus and its possible abnormalities such as adhesions. Tubal patency was evaluated by chromopertubation, in which a water-soluble blue dye is injected into the uterine cavity, in a similar manner as during an HSG (Palmer, 1947).

Hysterosalpingo-contrast-sonography (HyCoSy) was introduced by Deichter in 1986 as an alternative to the HSG. During HyCoSy an echogenic contrast medium (Echovist®) is injected into the uterine cavity by using the same transcervical catheters as during HSG, simultaneous transvaginal ultrasound is performed to evaluate the tubal patency. One of the benefits of HyCoSy is that it does not require any radiation exposure (Schlief and Deichert, 1991; Reis *et al.*, 1998). Unfortunately, the Echovist® contrast could lead to allergic reactions and its use was halted (Heikkinen *et al.*, 1995). As an alternative to Echovist®, different contrast media have been developed consisting of air/saline mixtures, however, all those turned out to be instable and therefore provided an insufficient time period for conducting the ultrasonic investigation. At the moment, ExEm-foam® or a lidocaine/air/saline-mixture is being used as a sonographic tubal patency test (HyFoSy, hysterosalpingo-foam-sonography) (Emanuel and Exalto, 2011; Ludwin *et al.*, 2017).

In 1998, the transvaginal hydrolaparoscopy (THL) was developed as an alternative to the DLS. The THL is a minimally invasive diagnostic procedure, which can be performed under local anaesthesia. Access to the pelvic cavity is obtained by a single needle puncture technique through the pouch of Douglas, after which the pelvic cavity is filled with warm saline. Tubal patency is evaluated through chromopertubation, similar as during DLS. Additionally, the tuba-ovarian structures are also directly visualized and evaluated (Gordts et al., 1998).

Evaluation of the Fallopian tubes in-vivo is mostly limited to testing tubal patency and in case of DLS or THL diagnosing peri-tubal and peri-ovarian adhesions and/or endometriosis. However, there is also the possibility of salpingoscopy to evaluate the inside of the Fallopian tube, its ampullary part, via a thin fibreoptic scope, to obtain additional information on the Fallopian tubes besides its patency. Abnormalities that can be seen are: adhesions, loss of mucosal folds, rounded edges of mucosal folds, debris, foreign bodies and abnormal vessels. It has been shown that multiple abnormalities present in the inner part of the ampulla, correlate with a decreased fecundability (Nakagawa *et al.*, 2010).

Fertility-enhancing effect of tubal testing procedures

The hypothesis that tubal flushing with ethiodized oils may also have a therapeutic value was postulated as early as 1928 (Jarcho, 1928). Already from 1948 onwards, the therapeutic value of HSG, and especially with ethiodized oil, became a research topic for different clinicians (Rutherford, 1948; William *et al.*, 1951). After that, different RCTs studied the possible fertility-enhancing effect of oil-based contrast (ethiodized oil) during HSG compared to water-based contrast, but the discussion on this topic persisted. On the other hand, throughout the years, the use of oil-based contrast for HSGs had been gradually replaced by water-based contrast for multiple reasons: lower costs, enhanced visibility of the ampullary rugae, faster excretion from the body and therefore reducing the risk of side effects, and the fact that a delayed radiograph was no longer necessary as the viscosity of water-based contrast was lower (Soules and Spadoni, 1982).

Decennia after the start of the discussion on fertility enhancement through tubal flushing, two well-powered RCT's have been published, which show a positive effect of oil-based contrast compared to water-based contrast in women with unexplained subfertility. The first RCT compared ethiodized oil (Lipiodol Ultra Fluid®) to a high-osmolality water-based contrast (Telebrix Hystero) (Dreyer *et al.*, 2017). In the second RCT three different types of low-osmolality water-based contrasts (Optiray 320, Omnipaque 300 and Ultravist 300) were compared to ethiodized poppyseed oil (Jiangsu Hengrui Medicine Co., Ltd., Lianyungang, Jiangsu Province, China) (Zhang *et al.*, 2022). See Table 1 for all the chemical and physical characteristics of these contrast media. These two studies showed, respectively, a 10% and 9% higher ongoing pregnancy rate after tubal flushing during HSG with oil-based contrast compared to water-based contrast (Dreyer *et al.*, 2017; Zhang *et al.*, 2022). Additionally, the long term follow-up of the RCT by Dreyer et al., showed significantly more naturally conceived pregnancies in women who underwent an HSG with oil-based contrast compared to water-based contrast (van Rijswijk *et al.*, 2020). Even though the fertility-enhancing effect of oil-

based contrast during HSG in women with unexplained subfertility has been shown, the mechanism behind this remains unclear after a century of speculation.

Possible adverse events

Due to the worldwide renewed interest in the use of ethiodized oils for tubal flushing during HSGs a discussion concerning safety has started as well. Most importantly, the focus was directed towards the risk of inflow of the ethiodized oil into the venous or lymphatic system, called intravasation. Because ethiodized oil is highly hydrophobic, it forms droplets and intravasation may lead to the formation of an oil emboli to i.a. the lungs, eyes and/or brain. Furthermore, the Lipiodol Ultra Fluid® contains a higher amount of iodine than water-based contrast (480 mg Iodine/mL in Lipiodol Ultra Fluid® versus 240 to 300 mg Iodine/mL in water-based contrast). When there is an excess of iodine in the body, the transport of iodine into the thyroid gland is increased. A negative feedback system causes a decreased production of thyroid hormone, which could lead to the development of (subclinical) hypothyroidism. Most individuals are able to escape from this so-called acute Wolff-Chaikoff effect and restore normal thyroid hormone production within 24 to 48 hours. However, individuals with pre-existing thyroid dysfunction may not be able to do so (Wolff and Chaikoff, 1948). A persistent (subclinical) hypothyroidism is associated with pregnancy complications (van den Boogaard *et al.*, 2011).

OUTLINE OF THE THESIS AND RESEARCH QUESTIONS

The research questions of this thesis can be divided into two different themes. Firstly, we aimed to provide clarity on the topic of the safety of ethiodized oil during tubal patency testing for the women and their offspring. Secondly, we studied the underlying fertility-enhancing effect of ethiodized oil and the feasibility of the implementation of ethiodized oil during different methods of tubal flushing. The objective of this thesis is to answer the following research questions :

1. What are the possible adverse events, and their clinical consequences, during or after an HSG with the use of ethiodized oil in subfertile women and their offspring?
2. What is currently the risk of adverse events in subfertile women and their offspring after an HSG using ethiodized oil compared to water-based contrast?
3. Which mechanisms form the potential root cause of the fertility-enhancing effect of tubal flushing, and how can we further investigate these mechanisms?
4. Can ethiodized oil also be used during other methods of tubal flushing than HSG?

Part I – What is the safety of ethiodized oil during tubal patency testing?

Chapter 2 discusses the safety of ethiodized oil for HSG, based on a systematic review of the literature published from 1928 onwards.

Chapter 3 discusses the current adverse events after HSG with the use of ethiodized oil, compared to water-based contrast, based on a national survey performed in the Netherlands.

Chapter 4 discusses the thyroid function of neonates conceived after a preconceptional HSG with ethiodized oil, based on a retrospective data analysis of a RCT comparing HSG with the use of oil- versus water-based contrast during fertility workup.

Part II – What is the fertility-enhancing effect and feasibility of ethiodized oil during different methods of tubal flushing?

Chapter 5 discusses five separate hypotheses, divided into the biochemical and interfacial effects derived from the contrast properties, on the root cause of the fertility-enhancing effect of tubal flushing.

Chapter 6 discusses the feasibility of the analysis of the in-vivo pressure build-up within the reproductive tract during HSG, which is essential to further investigate the interfacial effects of tubal flushing and its fertility-enhancing effect.

Chapter 7 discusses a video case report of tubal flushing with ethiodized oil at THL, in which there is direct observation of the interaction between ethiodized oil and human tissue. This interaction may be important for the fertility-enhancing interfacial effects of ethiodized oil.

Chapter 8 discusses the feasibility of additional tubal flushing with ethiodized oil after establishing at least unilateral tubal patency at THL in 50 subfertile women.

Chapter 9 provides a general discussion on the clinical implications of this thesis and suggestions for further research.

Chapter 10 and 11 summarize the results of the thesis in English and Dutch.

Chapter 12 contains the impact paragraph.

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CHAPTER 2

SAFETY OF OIL-BASED CONTRAST MEDIUM FOR HYSTEROSALPINGOGRAPHY: A SYSTEMATIC REVIEW

I. Roest, K. Rosielle, N. van Welie,
K. Dreyer, M. Bongers, V. Mijatovic,
B.W. Mol, C. Koks.

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ABSTRACT

Recent meta-analyses have shown that a hysterosalpingography (HSG) with oil-based contrast increases pregnancy rates in subfertile women. However, the frequency of complications during or after an HSG with oil-based contrast in subfertile women and/or their offspring is still unclear. We performed a systematic review and meta-analysis, without restrictions in language, publication date or study design to fill this knowledge gap. The results show that the most frequently reported complication was intravasation of contrast, which occurred in 2.7% with the use of oil-based contrast (31 cohort studies and RCTs, 95% CI, 1.7–3.8, absolute event rate 664/19,339), compared to 2.0% with the use of water-based contrast (8 cohort studies and RCTs, 95% CI, 1.2–3.0, absolute event rate 18/1,006). In the cohort studies and RCTs there were 18 women with an oil-embolism (18/19,339 HSGs), all without serious lasting consequences. Four cases with serious consequences of an oil-embolism were described (retinal oil-embolism [n=1] and cerebral complaints [n=3]), these reports did not describe the use of adequate fluoroscopy guidance during HSG. In conclusion, the most frequently reported complication after an HSG with oil-based contrast is intravasation occurring in 2.7%. In total four cases with serious consequences of oil-embolisms in subfertile women were published.

KEY MESSAGE

The most frequently reported complication after an HSG with oil-based contrast is intravasation, occurring in 2.7% of HSG procedures. In total only four cases with serious consequences of oil embolisms in subfertile women were published. Therefore, safety concerns should not be the reason to deny the use of oil-based contrast for tubal testing in women with unexplained subfertility.

INTRODUCTION

Hysterosalpingography (HSG) to assess tubal patency is an essential part of the workup for subfertile couples (NICE, 2017). The first HSG was performed in 1910 by Rindfleisch (Rindfleisch, 1910). From 1914 iodized oils were used as alternative to the water-based contrasts which were irritative to the peritoneum (Cary, 1914; Nielsen, 1946; Soules and Spadoni, 1982). Different iodized oils have been introduced: Lipiodol®, Iodochlorol, Ethiodol, Jodipin, Jodumbrin, Lipiodol® Ultra Fluid. The oil-based contrasts available nowadays are Lipiodol® Ultra Fluid (Guerbet, France) and ethiodized Poppyseed Oil (Heng Rui Pharmaceuticals, Jiangsu, China), the latter being currently only available in Asia.

Lipiodol® was developed in 1901 as a solution containing Iodine, and was used for a wide range of indications, including the reduction of struma and infection prevention. After the discovery of its radiological qualities, it was used for the visualization of the uterine cavity and Fallopian tubes, but also in myelography, bronchography and later in lymphography. In 1960 a transesterified version of Lipiodol® was developed, Lipiodol® Ultra Fluid, which had a lower viscosity (Bonnemain and Guerbet, 1995; Simescu et al., 2002).

For nearly seven decades, the therapeutic effect of oil-based contrast during HSG in the fertility workup has been debated. Recently two meta-analyses have shown a favourable effect of oil-based contrast on fertility outcomes, with an odds ratio of 1.47 (95% CI 1.12–1.93) for ongoing pregnancy and 2.18 (95% CI 1.30–3.65) for live birth when comparing HSG with oil-based contrast to water-based contrast (Fang et al., 2018; Wang et al., 2019). This generated a world-wide renewed interest in the use of oil-based contrast for fertility enhancement. However, some clinicians are still hesitant towards its use because of complications that have been reported in the past.

In 1929 the first report of intravasation of oil-based contrast during HSG was published (Pujol y Brull et al., 1929). Intravasation is the inflow of contrast in the venous or lymphatic system, and is visualized by radiography, ideally with the use of fluoroscopy screening. Even though water-based contrast can intravasate as well, only oil-based contrast is known to enter the circulation as droplets because of its hydrophobic qualities. These oil-droplets can reach organs such as the lungs or brain as oil-emboli and cause inflammation and/or occlusion of the vasculature (Uzun et al., 2004). After this first case, more reports of intravasation followed, most patients had only minor symptoms and recovered after observation. Intravasation was therefore regarded as innocuous (Soules and Spadoni, 1982). Currently, intravasation with the use of oil-based contrast is estimated to occur in around 5% of the HSGs in the Netherlands (Roest et al., 2020).

In spite of this, a recent case report describes a patient falling into a comatose state as a result of an oil embolus after HSG (Uzun et al., 2004). Although this might be a rare complication, it does emphasize the importance of safety and knowledge on the complication rates after HSGs with the use of oil-based contrast.

As previously mentioned, Lipiodol® contains iodine, the iodine concentration in Lipiodol® is higher than in water-based contrast (480 mg Iodine/mL in Lipiodol® versus 240 to 300 mg Iodine/mL in water-based contrast). Iodine exposure can cause a transient decrease in the synthesis of thyroid hormone (Wolff and Chaikoff, 1948). Subclinical hypothyroidism is associated with pregnancy complications (van den Boogaard et al., 2011). Furthermore, the HSG procedure has a risk of infection.

The systematic reviews and meta-analyses to date have primarily focused on fertility outcomes and have excluded case reports. This systematic review and meta-analysis included all study types, to provide an overview of the frequency and clinical consequences of all possible complications during or after HSG with the use of oil-based contrast in subfertile women.

MATERIALS AND METHODS

The protocol of this review was prospectively registered on PROSPERO (https://www.crd.york.ac.uk/prospero/, registration ID: CRD42018102382, registration date: 24th of July 2018). The methodology used was as described in the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (Moher et al., 2009).

Information sources and search strategies

Electronic databases including MEDLINE, EMBASE and the Cochrane Central Register of Controlled Trials (CENTRAL) were searched up to June 2020. Textbooks as well as reference lists of identified publications were also manually screened. The key search items included “hysterosalpingography”, “oil contrast”, “ethiodized oil”, “ethiodol”, “lipiodol”, “adverse effect”, “side effect”, “complication”, “thyroid”, “intravasation”, “embolization”, “granuloma”, “anaphylaxis”, “pelvic inflammatory disease”, “adnexitis” (Supplementary Table 1, 2 and 3).

Eligibility criteria

All types of studies were included, randomized controlled trials (RCT), prospective- and retrospective cohort studies, case series and case reports that report complications occurring during or after HSG with the use of oil-based contrast, with or without comparison to water-based contrast, in women trying to conceive or their offspring.

No limitation on language or publication period were applied. Colleagues who were fluent in the foreign languages assisted in translating.

Outcomes

The outcomes included adverse events of HSG with the use of oil-based contrast (versus water-based contrast) in subfertile women and their offspring, such as; intravasation of the contrast medium, embolization of the contrast medium, pelvic-inflammatory disease, lipogranuloma formation, retention of contrast, maternal or fetal thyroid dysfunction, and anaphylactic reactions. The clinical consequences included additional treatments, hospital stay, morbidity and mortality.

Study selection, data collection and quality assessment

Study eligibility was evaluated by two reviewers (IR and KR) independently; disagreements between the two reviewers were solved by consensus or by consultation with another reviewer (CK) when necessary. A predesigned form was used to extract the data and assess the quality of the included studies. The following information was collected: name of the first author, publication year, study design, study population, participants' characteristics, types of contrast, details of interventions and co-interventions, sample sizes and outcomes. Full text articles of English cohort- and randomized studies were screened by a second reviewer (KR).

Risk of bias was assessed for all studies, excluding the case reports/series, in accordance with the quality assessment checklist for prevalence studies (Hoy et al., 2012) (Supplementary Table 4). This checklist contains nine questions, each scored with 0 or 1 points. A total of 0 – 3 points is classified as an overall low risk of study bias, 4 – 6 points as moderate risk and 7 – 9 points as high risk. The risk of bias was assessed by two reviewers independently for the English studies.

Statistical analysis

The prevalence of complications occurring with the use of oil-based contrast was calculated, and where possible comparisons were made to the use of water-based contrast. Meta-analyses were performed using Review Manager Version 5.3. Statistical heterogeneity was estimated by performing a chi squared test and calculating the I-square. Pooled weighted prevalences and the 95% CI were calculated using the MetaXL tool (Version 5.3, 2016 EpiGear International Pty Ltd, Queensland, Australia). A non-pre-specified sensitivity analysis was performed selecting the cohorts and RCT to calculate the prevalence of complications. Case reports and case series were included to report all (and rare) complications.

RESULTS

Characteristics of included studies

The search identified 492 records. A total of 8 RCT, 41 cohort studies (4 prospective cohorts, 24 retrospective cohorts, 13 cohort-studies which were not further specified) and 59 case reports/case series were included within the review. In these studies, a total of 23,536 HSG procedures were performed with the use of oil-based contrast (23,298 HSGs in cohort studies / RCTs). Sixteen of the included studies reported on HSGs with water-based contrast as well, with a total of 1,975 HSGs with water-based contrast (1,973 HSGs in cohort studies / RCTs) (For flowchart see Supplementary Figure 1). The included studies were published between 1928 and 2020 (See Supplementary Table 5) for the characteristics of the included studies) (Alper et al., 1986; Aznar et al., 1969; Bang, 1950; Barqawi et al., 2007; Bateman et al., 1980; Bergin, 1951; Bersi, 1977; Binder et al., 1976; Bohm and Seewald, 1972; Böttger and Fleck, 1955; Brent et al., 2006; Brown et al., 1949; Buytaert and Meulyzer, 1977; Charawanamuttu et al., 1973; Claus and Dochez, 1966; Coventry, 1934; Dan et al., 1990; Dreyer et al., 2017; Drukman and Rozin, 1951; Effkemann, 1935; Eisen and Goldstein, 1945; Elliott et al., 1965; Faris and McMurrey, 1947; Feiner, 1942; Flew, 1944; Fochem and Ulm, 1954; Frischkorn, 1958; Geary et al., 1969; Gotoh et al., 2010; Grant et al., 1957; Grosskinsky et al., 1994; Grossmann, 1946; Gunsberger, 1958; Heinen and Schussler, 1966; Hemmeler, 1938; Hirst, 1928; Hohlbein, 1965; Ishizuki et al., 1992; Johnson et al., 2004; Kaneshige et al., 2015; Karshmer and Stein, 1951; Kika, 1954; Kilroe and Hellman, 1933; Kuzavova, 1964; La Sala et al., 1982; Lau, 1969; Levinson, 1963; Li et al., 2018; Lin and Tsou, 1935; Lindequist et al., 1994, 1991; Liu et al., 2010; Ma et al., 2016; Mackey et al., 1971; Madsen, 1942; Malter and Fox, 1972; Meaker, 1934; Mekaru et al., 2008; Miyazaki et al., 2020; Morii et al., 2013; Netter and Weill-Fage, 1950; Nordio, 1938; Norris, 1956; Novak, 1930; Nugent et al., 2002; Nunley et al., 1987; Omoto et al., 2013; Palmer, 1960; Pear and Boyden, 1967; Piatt, 1947; Porcher, 1935; Pujol y Brull et al., 1929; Rasmussen et al., 1987; Riche and Fayot, 1931; Ries, 1929; Robins and Shapira, 1951; Rubin, 1928; Rutherford, 1948; Sappey et al., 1952; Sasaki et al., 2017; Satoh et al., 2015; Schaffer, 1954; Schultze, 1932; Schutte et al., 2006; Schwabe et al., 1983; Shapiro et al., 1957; Slater et al., 1959; So et al., 2017; Solal, 1932; Steiner et al., 2003; Stoll and Zeitz, 1956; Takeyama et al., 2014; Tan et al., 2019; Ueda et al., 2016; Uzun et al., 2004; van Welie et al., 2020; Vara, 1950; Volk, 1936; Weise et al., 1973; Weitzner, 1935; Werner, 1952; Williams, 1944; Witwer et al., 1930; Woltz et al., 1958; Wong et al., 1932; Yamazaki et al., 2019; Zachariae, 1955; Zacharin, 1933).

Quality of evidence of the studies

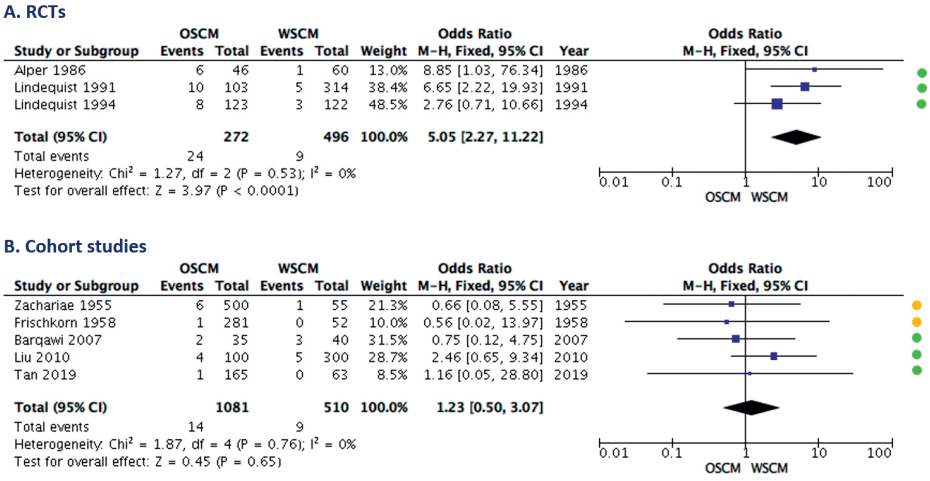
Of the 49 cohort studies and RCTs, 16 studies were classified as low risk, 31 studies as moderate risk and 2 studies as high risk of study bias. In 18 studies, there was no clear

definition of the reported complications. Mainly, there was no predefined definition of intravasation or oil-embolism. There is no reliable or valid classification method for intravasation, therefore 44 of the 48 studies were classified as high risk of bias for the reliability and validity of the study instrument that measured the parameter of interest (see Supplementary Table 6 for the classification of all studies).

Intravasation and oil-embolisms

Eight studies (three RCTs and five cohort studies), compared the frequency of intravasation between HSGs with the use of oil-based and water-based contrast (Figure 1) (Alper et al., 1986; Barqawi et al., 2007; Frischkorn, 1958; Lindequist et al., 1994, 1991; Liu et al., 2010; Tan et al., 2019; Zachariae, 1955). Rates of intravasation were 2.8% (38/1,353) after HSGs with oil-based contrast and 1.8% (18/1,006) after HSGs with water-based contrast (odds ratio 5.05 (95% CI 2.27–11.22, P < 0.0001) based on the RCTs and 1.23 (95% CI 0.50–3.07, P-value 0.65) based on the cohort studies), showing that intravasation occurs more frequently with the use of oil-based contrast.

Figure 1. *Prevalence of intravasation of oil-based contrast versus water-based contrast in HSGs for subfertility*

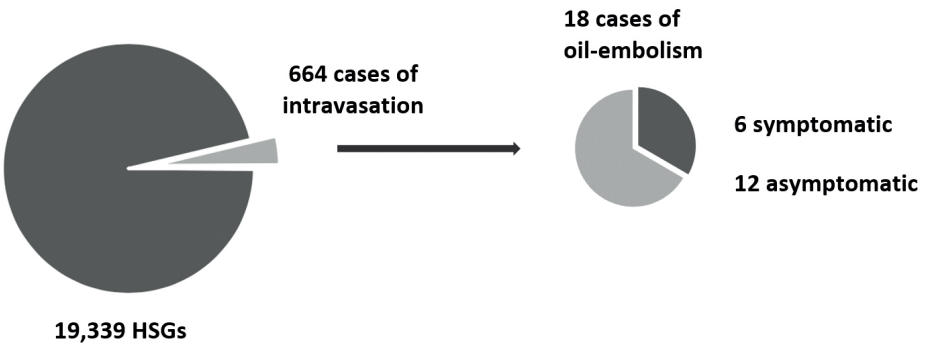


Forest plot of meta-analysis reporting on intravasation with the use of oil-based contrast compared to water-based contrast. A. RCTs. B. cohort studies. Odds ratio and 95% confidence-interval (CI). Odds ratios less than 1 favour oil-based contrast (fewer adverse events); Odds ratios greater than 1 favour water-based contrast (fewer adverse events).The risk of bias of de individual studies is represented by coloured dots, green (low risk of bias), and yellow (moderate risk of bias). HSG = hysterosalpingography; OSCM = oil-based contrast media; RCT = randomized controlled trial; WSCM = water-based contrast media.

Twenty-three additional cohort studies reported on the prevalence of intravasation with the use of oil-based contrast alone. The overall pooled weighted frequency of intravasation in the 31 RCTs and cohort studies with the use of oil-based contrast was 2.7% (95% CI 1.7–3.8, absolute event rate 664/19,339), compared with 2.0% (95% CI 1.2–3.0, absolute event rate 18/1,006) in the eight studies with the use of water-based contrast. When including only studies published from 2000 onwards, the pooled frequency of intravasation with the use of oil-based contrast was 2.8% (95% CI 1.2–5.1, absolute event rate 12/471), compared with 1.8% (95% CI 0.0–5.9, absolute event rate 8/403) with the use of water-based contrast.

In the whole group of HSGs with the use of oil-based contrast performed in RCTs and cohort studies, there were 18 women with oil-embolisms (18/19,339, 0.1% of HSGs; 18/664, 2.7% of cases with intravasation). In six of these cases pulmonary embolisms were described, while the other 12 cases only described the contrast moving rapidly out of the pelvis. The latter were all asymptomatic and, serious lasting consequences were not reported (See Figure 2).

Figure 2. *Intravasation and oil-embolisms in HSGs with oil-based contrast for subfertility in cohort studies and RCTs.*



HSG = hysterosalpingography; RCT = randomized controlled trial.

Additionally, there were 197 cases of intravasation after an HSG with the use of oil-based contrast in the case reports/series. In 22 of these women this led to the formation of an oil-embolism (22/197, 11.2%). Four of these women were asymptomatic, 18 were symptomatic. Symptoms included a transient cough and/or dyspnea and neurological symptoms. Four cases were described of women with serious consequences of an oil-embolism (Table 1) (Charawanamuttu et al., 1973; Dan et al., 1990; Flew, 1944; Uzun et al., 2004).

Table 1. *Characteristics of serious consequences of oil-embolism after HSGs with the use of oil-based contrast for subfertility*

Study	Contrast	Risk factors	Organ system involved	Consequences
Flew, 1944	Lipiodol (not specified)	HSG on day 24 of menstrual cycle. Use of fluoroscopy not reported.	Pulmonary and cerebrum	Hemiplegia, survived
Charawanamuttu et al., 1973	Lipiodol® Ultra Fluid	>20 mL of contrast, poor definition of fluoroscopy images	Pulmonary and retina	3 months impaired vision
Dan et al., 1990	Lipiodol® Ultra Fluid	Use of fluoroscopy not reported	Pulmonary, central nervous system	Comatose for 11 days, afterwards normal mental/ motor function
Uzun et al, 2004	Lipiodol (not specified)	Use of fluoroscopy not reported	Pulmonary, central nervous system	Comatose for 10 days, afterwards mental/ motor function progressively improved

When including only the studies (including the case reports) that used fluoroscopy screening, there were 250 women with intravasation after an HSG with the use of oil-based contrast. In this group there were 16 women with oil-embolisms (16/250, 6.4%), of which two had symptoms of coughing and one temporary impaired vision as a result of a retinal oil-embolism (3/16, 18.8%). The authors reported that the fluoroscopy images were of poor quality, and over 20 mL of contrast was used during this last procedure (Charawanamuttu et al., 1973).

When excluding the studies with known fluoroscopy guidance, there were 611 women with intravasation after an HSG with the use of oil-based contrast. In this group there were 24 women with oil-embolisms (24/611, 3.9%), of which 19 (19/24, 79.2%) had, mostly transient, pulmonary symptoms. Of the 24 women with oil-embolisms there were three women with serious lasting consequences of cerebral complaints after an oil-embolism (See Table 1) (Dan et al., 1990; Flew, 1944; Uzun et al., 2004).

Infection

Two RCTs and 18 cohort studies reported on the frequency of infection after HSGs with the use of oil-based contrast. The overall pooled weighted frequency of infection was 0.90% (95% CI 0.47–1.5, 70/11,287 women). Two RCTs and two cohort studies compared HSGs with the use of oil-based contrast to HSGs using water-based contrast. The

frequency of infection with the use of water-based contrast was 1.9% (95% CI 0.27–4.6, 17/564 women). Including only the studies published in or after 1960, the overall pooled frequency of infection was 0.55% (95% CI 0.23–1.0) after HSGs with the use of oil-based contrast and 0.35% (95% CI 0.00–7.3) with the use of water-based contrast. The use of antibiotic prophylaxis was not systematically reported.

Mortality

Five cases of mortality were reported after HSGs with the use of oil-based contrast in subfertile women. Four of these cases were infection-related, and they were published in the period between 1942 and 1950 (Bang, 1950; Feiner, 1942; Rutherford, 1948). The fifth case described a woman that passed away minutes after a recurrent HSG with 9 mL of lipoiodine under light cyclopropane anaesthesia, possibly due to an allergic reaction to the oil-based contrast or the used anaesthesia (Faris and McMurrey, 1947). Additionally, two cases were reported in 1928 and 1930 where tubal blockage was found on the HSG. These women underwent subsequent surgery, one and five days later, and died shortly after, presumably from infectious complications of the surgery (Hirst, 1928; Novak, 1930).

Lipogranuloma and oil-remnants

Eleven studies reported on 41 women with lipogranuloma formation after an HSG with the use of different types of oil-based contrast. These included three cohort studies, one case series and seven case reports. The contrasts used were; Lipiodol not further specified (33 cases), oil-based/iodized contrast not further specified (five cases), Jodipin (two cases), Ethiodol (one case). In nine cases histology examination was mentioned, in 32 cases this was not mentioned.

Additionally, there were 85 reports of oil remnants after an HSG with the use of oil-based contrast. These were reported in nine studies; three cohort-studies and six case reports. Forty-four cases were discovered within two weeks after the procedure, while 41 were discovered up to 27 years after the HSG-procedure. Fifty-six cases were diagnosed after laparoscopy, 29 cases were diagnosed after radiology-imaging. Histological examination was only reported in one case.

Thyroid dysfunction

Table 2 shows four cohort studies and four case reports/series on maternal thyroid function after HSG with the use of oil-based contrast.

Table 2. *Maternal thyroid function after HSGs*

Study design	Country	Procedure	Thyroid function pre-HSG	Outcome
Case reports				
Li, 2018	China	Oil-based contrast	Unknown	Fourteen women with increased urinary iodine content: 50% (7/14) (subclinical) hypothyroidism. All neonates tested negative during congenital thyroid screening.
Sasaki, 2017	Japan	Oil-based contrast	Unknown	Case of hypothyroidism, no treatment. Fetal goitre.
Ma, 2016	China	Oil-based contrast 100 mL	Euthyroid	Hyperthyroidism, no treatment, resolved spontaneously after 1.5 months.
Ishizuki, 1992	Japan	Lipiodol	Graves' disease	Thyroiditis, goiter, treated with steroids for 2 months.
Cohorts/RCTs				
So, 2017	Japan	Lipiodol Max 5 mL	Euthyroid	Oil-based contrast: 22.6% subclinical hypothyroidism after 1-30d, 24.4% after 31-180d. Water-based contrast: 9.5% subclinical hypothyroidism after 1-30d, 3.6% after 31-180d.
Kaneshige, 2015	Japan	Lipiodol 6.1 mL (4.0 – 9.0)	Euthyroid: 27% goitre palpable	0% hypothyroidism (0/22). 13.6% (3/22) transient subclinical hypothyroidism.
Mekaru, 2008	Japan	Lipiodol 5-10 mL	76% euthyroid 12% subclinical hypothyroidism 12% subclinical hyperthyroidism	• Euthyroid: 4/180 (2.2%) hypothyroidism, 28/180 (15.6%) subclinical hypothyroidism, 2/180 (1.1%) subclinical hyperthyroidism. • Subclinical hypothyroidism: 10/28 (35.7%) hypothyroidism, 3 required thyroid hormone replacement. 1/28 (3.6%) subclinical hyperthyroidism. • Subclinical hyperthyroidism: 4/12 (33.3%) normalization, 2/12 (16.7%) unchanged.
Slater, 1959	USA	Lipiodol	Clinically euthyroid	Oil-based contrast: 80% depression of iodine uptake, increase in protein bound iodine for 4 months. Water-based contrast: no depression of iodine uptake. Increase in protein bound iodine for 24-48h.

HSG = hysterosalpingography

Three cases of fetal goiter following an HSG with oil-based contrast were reported. In two of the cases the HSG had been performed in the month of conception (10 mL of Lipiodol and an unknown volume of not specified oil-based contrast was used), in the third case three HSGs had been performed in the year before conception. In one case intra-amniotic levothyroxine was administered as treatment. After birth, hypothyroidism was diagnosed in one of the newborns, which resolved by day seven. The other neonates were euthyroid. One of the mothers had hypothyroidism during pregnancy, two were euthyroid. In one of the mothers oil-remnants were present in the abdominal cavity on a postpartum X-ray (Omoto et al., 2013; Sasaki et al., 2017; Yamazaki et al., 2019).

One retrospective cohort study (Satoh et al., 2015) from Japan evaluated the neonatal thyroid function after HSGs with the use of Lipiodol. Abnormal congenital thyroid screening was seen in 2.4% (5/212); three cases of subclinical hypothyroidism and two cases of overt hypothyroidism. The median volume of contrast in the group with thyroid dysfunction was significantly higher than the group with normal thyroid function (20 mL [range 10 – 20 mL] vs 8 mL [range 3 – 25 mL], P-value 0.033). However, the volume was only reported for 3 out of 5 neonates with abnormal thyroid function test results. Another retrospective cohort study investigated the thyroid function of 140 neonates born after a preconceptional HSG with oil-based contrast, Lipiodol® Ultra Fluid (n=76) or water-based contrast, Telebrix Hystero® (n=64). None of the neonates tested positive during the congenital hypothyroidism screening. Furthermore, the volume of contrast used did not influence the thyroid function (median of 9.0 mL of oil-based contrast) (van Welie et al., 2020).

Other complications

One case of a tubal rupture, without ill effects was described. The diagnostic method was not reported (Witwer et al., 1930). Additionally, one case report described abdominal pain, Fitz-Hugh-Curtis Syndrome-like, possibly due to the chemical stimulation of the iodized oil (not further specified) used during an HSG (Morii et al., 2013).

HSGs performed for non-subfertility indications

The primary intention of this study was to take into account HSGs performed for subfertility. However, in a non-systematic way, the study also identified one case of a massive oil-embolism leading to death, published in 1931. A 60-year old received an HSG with 8 mL Jodipin for postmenopausal blood loss which was suspected for malignancy. A massive oil-embolism occurred in the cerebrum, pituitary gland, liver, spleen, kidney and heart, and the patient died within 5 hours after the procedure. The

use of fluoroscopy screening was not reported. It is likely, that no adequate fluoroscopy was performed at the time (Gajzago, 1931).

Furthermore, a case report of a woman falling into a comatose state after an HSG was reported. This woman had had two unsuccessful curettage attempts, for termination of pregnancy, after which she received an HSG with Lipiodol® Ultra Fluid. The endometrium was injured after the several curettages, and so the contrast could flow directly into the bloodstream, leading to a massive intravasation with oil-embolisms. After 81 days she was discharged with slight mental deficit (Ogihara et al., 1991).

Additionally, we identified case reports of pulmonary oil-embolisms after HSGs performed in patients with: tubal ligation (n=2) (Roblee, 1945), suspected endometrium carcinoma (n=1) (Breitländer and Hinrichs, 1941), abdominal pain (n=1) (Ingersoll and Robbins, 1947), uterus myomatosus (n=2) (Hodge and Price, 1969; Keller, 1943) and missed abortion (n=1) (Hinaut et al., 1966).

DISCUSSION

Summary of key findings

In this review of articles published from 1928 onwards, including a total of 23,536 HSGs with the use of oil-based contrast, the most frequently reported complication of HSGs performed for subfertility was intravasation of contrast. This occurred in 2.7% of the HSGs with the use of oil-based contrast (31 studies, 95% CI 1.7–3.8), compared with 2.0% with the use of water-based contrast (8 studies, 95% CI 1.2–3.0) derived from cohort studies and RCTs. Oil-embolisms occurred in 0.1% of the HSGs performed in cohort studies and RCTs. In all studies, including the case reports, the percentage of symptomatic oil-embolisms was strikingly lower in the group with fluoroscopy guidance during HSG compared with no fluoroscopy guidance (19% versus 79%). With the use of fluoroscopy guidance during HSG, no serious consequences of oil-embolisms occurred. The frequency of infection with the use of oil-based contrast was 0.90% (20 studies, 95% CI 0.47–1.50), compared with 1.9% (4 studies, 95% CI 0.27–4.60) with the use of water-based contrast. One case of non-infection related mortality after an HSG, most likely due to an anaphylactic reaction was reported. There were 85 reports of oil remnants after an HSG. Half of the cases were diagnosed within two weeks of the procedure. Furthermore, there were 41 reports of lipogranuloma formation. Women with subclinical hypothyroidism seem more likely to develop hypothyroidism after an HSG with oil-based contrast (35.7% versus 0–2.2% in euthyroid women), however this is based on only 28 and 202 women respectively (Kaneshige et al., 2015; Mekaru et al., 2008; So et al., 2017). Results on the effect on the thyroid function of the offspring

are contradicting, a Japanese study showed abnormal congenital thyroid screening in 2.4% whereas a Dutch study did not show any abnormalities (Satoh et al., 2015; van Welie et al., 2020).

Strengths and limitations

This is the first systematic review on the safety of HSGs with oil-based contrast that includes all study types. Another strength of this systematic review is that no restriction on language or publication date was applied. However, the systematic review has limitations. First, the quality of the included studies was moderate to low. This is attributable to the design and the publication year of the included studies. In most of the studies the primary outcome was pregnancy-related. Complications were often reported as secondary outcomes. Second, the development of fluoroscopy guidance during HSG has helped clinicians to diagnose intravasation and oil-embolisms leading to timely termination of the HSG procedure. This development is suggested as the reason for the increase in reported cases of intravasation and oil-embolisms, however as mentioned previously, the percentage of symptomatic oil-embolisms has therefore drastically decreased.

Clinical implications

Oil-embolisms, also known as fat-embolisms, have not only been reported in the gynecological literature. Bone marrow fat embolisms occur in 11-19% of trauma or orthopedic-surgery patients (Mellor and Soni, 2001). Fat embolisms may cause a fat embolism syndrome, with clinical symptoms varying from right heart failure and cardiovascular collapse to hypoxemia, pyrexia, petechial rash and neurological symptoms (Mellor and Soni, 2001). When reaching the lungs, the fatty substance mixes with the locally secreted lipase. Free fatty acids are released, causing inflammation to the pulmonary microvasculature and leading to a shock lung-like or acute respiratory distress syndrome-like syndrome (Duran et al., 2018). Suggested treatment is mainly supportive. Corticosteroids are proposed, for their possible beneficial effect on the pulmonary capillary membrane preventing pulmonary edema (Mellor and Soni, 2001). The pathogenesis of oil-embolisms after the use of oil-based contrast could be similar to that described after a bone marrow fat embolism, however, in the latter case it concerns autologous tissue, while in case of the use of oil-based contrast it concerns foreign material. In the four cases with severe complications of oil-embolisms that are summarized in this review, one case was treated with corticosteroids (Charawanamuttu et al., 1973), in the other cases only supportive measures were reported.

In this systematic review of HSGs with oil-based contrast for subfertility, four cases of infection-related mortality were identified. It should be noted that these cases were all

in the 1940s, when penicillin was recently introduced and the treatment for infection was completely different from the current practice (Bud, 2007). In the literature, there are also reports of infection-related mortality following HSGs with the use of water-based contrast (Lachmann, 1944). With the increased use and improvements of (prophylactic and therapeutic) antibiotics, the course of these infections has become less severe. The frequency of acute pelvic inflammatory disease after HSGs is nowadays 0.5% with antibiotic prophylaxis and 1.4% without prophylaxis (H.-M. Li et al., 2018).

There were more than twice the number of reports on oil-remnants (n=85) than lipogranuloma formation (n=41) after HSGs with the use of oil-based contrast. Lipogranuloma is a pathological diagnosis and may be missed if oil-remnants are not sent for pathological examination. Lipogranuloma may result in adhesion formation (Grosskinsky et al., 1994).

After iodine exposure, there is an excess of iodine transportation into the thyroid gland. Through negative feedback, this causes a transient decrease in the synthesis of thyroid hormone, potentially leading to the development of subclinical hypothyroidism. Within 24 to 48 hours, the level of thyroid hormone production will normally be restored. However, patients with underlying thyroid abnormalities may be unable to escape from this so-called acute Wolff-Chaikoff effect and therefore acquire an iodine-induced (transient) overt hypothyroidism (Wolff and Chaikoff, 1948). This is in line with the results of the cohort study of Mekaru et al., which showed that 35.7% of the women with a subclinical hypothyroidism develop overt hypothyroidism after an HSG with oil-based contrast, compared to 0–2.2% of euthyroid women (Kaneshige et al., 2015; Mekaru et al., 2008). Iodine-induced (transient) hyperthyroidism can also occur, in susceptible patients due to activation of quiescent nodules (Wolff and Chaikoff, 1948). This was shown in a case report of a woman with Graves' disease, who developed hyperthyroidism after an HSG (Ishizuki et al., 1992).

Five out of eight studies included in this review, on maternal thyroid dysfunction after HSGs, were performed in Japan. The effect of iodinated contrast on the thyroidal gland may vary between Japanese and Caucasian women, possibly because of a different background risk (i.e. iodine rich diet). The consumption of iodine-rich foods by mothers in Japan has shown to lead to neonatal hypothyroidism (Nishiyama et al., 2004). This may be reflected in the overall risk for congenital hypothyroidism, which is 0.7% in Japan compared to 0.04% in the Netherlands ("Activity Report Tokyo Health Service Association," 2010; Verkerk et al., 2014). Data on Asian women suggest that neonatal thyroid dysfunction after HSGs is related to the amount of oil-based contrast used

during the procedure. Although volume of contrast was not reported for all procedures (Satoh et al., 2015).

It is unclear if Caucasian women with an underlying thyroid disease are also at risk of developing hypothyroidism after an HSG with oil-based contrast. Until further studies have been performed, we suggest that women with an overt thyroid disease should not receive an HSG with oil-based contrast. In current practice, routine thyroid screening for women with subfertility varies. According to the NICE-guidelines thyroid screening is not recommended as routine measurement in asymptomatic women presenting with subfertility (National Institute Care Excellence (NICE), 2017). However, the ACOG (American College of Obstetricians and Gynecologists) committee opinion on fertility workup does recommend routine thyroid testing for all subfertile women (ACOG, 2019). Moreover, the 2017 American Thyroid Association guidelines for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum, advises to maintain serum TSH levels below 2.5 mU/L pre-conceptually in the subfertility setting (Alexander et al., 2017).

In this systematic review of complications of HSGs from 1928 onwards, the most frequently reported complication with oil-based contrast is intravasation occurring in 2.7%. Since 1928 only four cases with serious consequences of oil-embolisms in subfertile women were published. Therefore, safety concerns should not be the reason to deny the use of oil-based contrast for tubal testing in women with unexplained subfertility.

Further studies on the effect of oil-based contrast on maternal and neonatal thyroid function in Caucasian women are suggested. Furthermore, future research should investigate the mechanism of the pregnancy enhancing effect of oil-based contrast. By gaining knowledge on the mechanism of action, it would be possible to determine which women would benefit most from an HSG with the use of oil-based contrast.

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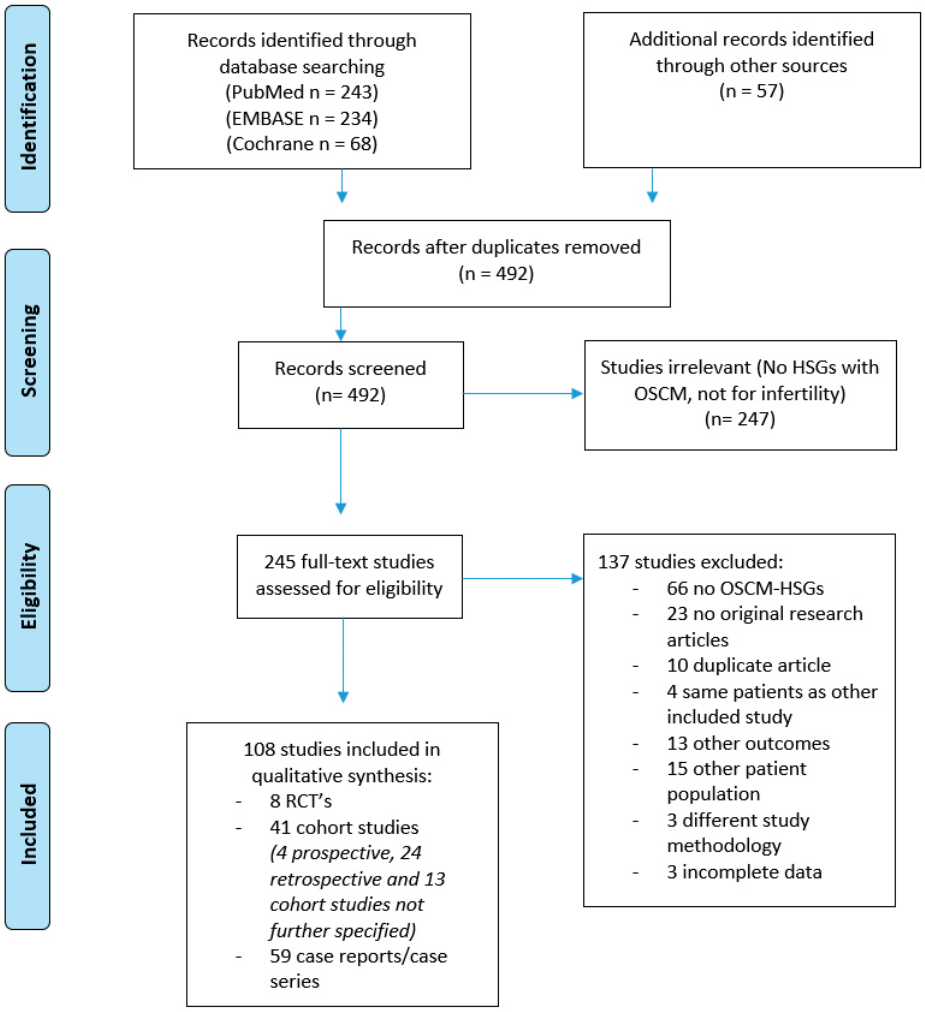
SUPPLEMENTARY DATA

Supplementary Figure 1. *PRISMA flowchart*



HSG = hysterosalpingography; OSCM = oil-soluble contrast media.

Supplementary Table 1. *Systematic search, PubMed*

Search	Add to builder	Query	Items found	Time
#10	Add	Search **#1 AND #4 AND #9**	243	04:02:22
#9	Add	Search **#7 OR #8**	5348646	03:50:34
#8	Add	Search **((adverse[tw] OR side[tw] OR undesirable[tw] OR injurious[tw]) AND (effect[tw] OR effects[tw])) OR risk[tw]**	4211290	03:49:14
#7	Add	Search **#5 OR #6**	1729799	03:48:50
#6	Add	Search **thyroid[tiab] OR hypothyroidism[tiab] OR intravasation[tiab] OR extravasation[tiab] OR embolization[tiab] OR embolisation[tiab] OR complication*[tiab] OR anaphylaxis[tiab] OR anaphylactic[tiab] OR pelvic inflammatory disease*[tiab] OR adnexitis[tiab] OR lipogranuloma[tiab]**	1239799	03:48:36
#5	Add	Search **"Thyroid Hormones"[Mesh] OR "Thyroid Diseases"[Mesh] OR "Thyroid Function Tests"[Mesh] OR "Hypothyroidism"[Mesh] OR "Extravasation of Diagnostic and Therapeutic Materials"[Mesh] OR "Pregnancy Complications"[Mesh] OR "Anaphylaxis"[Mesh] OR "Pelvic Inflammatory Disease"[Mesh] OR "Granuloma"[Mesh]**	691814	03:48:17
#4	Add	Search **#2 OR #3**	125755	03:47:56
#3	Add	Search **ethiodized oil*[tiab] OR lipiodol[tiab] OR ethiodol[tiab] OR iodinated contrast[tiab] OR oil contrast[tiab] OR oil soluble contrast[tiab] OR iodolipol[tiab] OR iodized oil*[tiab]**	7201	03:47:42
#2	Add	Search **"Ethiodized Oil"[Mesh] OR "Iodopyridones"[Mesh] OR "Iodized Oil"[Mesh] OR "Contrast Media" [Pharmacological Action] OR "Contrast Media"[Mesh]**	123400	03:47:25
#1	Add	Search **"Hysterosalpingography"[Mesh] OR hysterosalpingograph*[tiab] OR salpingograph*[tiab] OR HSG[tiab] OR uterosalpingograph*[tiab]**	5587	03:47:09

Search terms used for PubMed literature search performed on June 23th, 2020.

Supplementary Table 2. *Systematic search, EMBASE*

# ▲	Searches	Results
1	exp hysterosalpingography/	4219
2	(hysterosalpingograph* or salpingograph* or HSG or uterosalpingograph*).ab,ti.	3864
3	1 or 2	5869
4	exp ethiodized oil/ or exp iodopyridone/ or exp iodinated poppyseed oil/ or exp contrast medium/	163382
5	(ethiodized oil* or lipiodol or ethiodol or iodinated contrast or oil contrast or oil soluble contrast or iodolipol or iodized oil*).ab,ti.	9576
6	4 or 5	165360
7	exp thyroid hormone/ or exp thyroid disease/ or exp thyroid function test/ or exp hypothyroidism/ or exp contrast medium extravasation/ or exp pregnancy complication/ or exp anaphylaxis/ or exp pelvic inflammatory disease/ or exp lipogranuloma/ or exp adnexitis/	425013
8	(thyroid or hypothyroidism or intravasation or extravasation or embolization or embolisation or complication* or anaphylaxis or anaphylactic or pelvic inflammatory disease* or adnexitis or lipogranuloma).ab,ti.	1633099
9	7 or 8	1829337
10	adverse.mp. or exp adverse event/ or exp adverse outcome/ or exp side effect/ or exp risk/	4309515
11	(((adverse or side or undesirable or injurious) and (effect or effects)) or risk).ab,kw,ti,sh.	3761777
12	10 or 11	5634843
13	9 or 12	6786477
14	3 and 6 and 13	234

Search terms used for EMBASE (Ovid) literature search performed on June 23th, 2020.

Supplementary Table 3. *Systematic search, Cochrane*

#1	MeSH descriptor: [Hysterosalpingography] explode all trees		MeSH ▾	127
#2	(hysterosalpingograph* or salpingograph* or uterosalpingograph*):ti,ab,kw (Word variations have been searched)	S ▾	Limits	385
#3	#1 OR #2		Limits	385
#4	MeSH descriptor: [Iodized Oil] explode all trees		MeSH ▾	190
#5	MeSH descriptor: [Iodopyridones] explode all trees		MeSH ▾	0
#6	MeSH descriptor: [Contrast Media] explode all trees		MeSH ▾	2673
#7	(ethiodized oil* or lipiodol* or ethiodol or iodinated contrast or oil contrast):ti,ab,kw (Word variations have been searched)	S ▾	Limits	1636
#8	(oil soluble contrast or iodolipol or iodized oil*):ti,ab,kw (Word variations have been searched)	S ▾	Limits	216
#9	#4 or #5 or #6 or #7 or #8		Limits	3984
#10	#3 and #9		Limits	68

Search terms used for Cochrane literature search performed on June 23th, 2020.

Supplementary Table 4. *Quality assessment checklist for prevalence studies (adapted from Hoy et al.)*

Name of author(s): Year of publication: Study title:		
Risk of bias items	**Risk of bias levels**	**Points scored**
1. Was the study's target population a close representation of the national population in relation to relevant variables, e.g. age, sex, occupation?	**Yes (LOW RISK)**: The study's target population was a close representation of the national population.	0
	No (HIGH RISK): The study's target population was clearly NOT representative of the national population.	1
2. Was the sampling frame a true or close representation of the target population?	**Yes (LOW RISK)**: The sampling frame was a true or close representation of the target population.	0
	No (HIGH RISK): The sampling frame was NOT a true or close representation of the target population.	1
3. Was some form of random selection used to select the sample, OR, was a census undertaken?	**Yes (LOW RISK)**: A census was undertaken, OR, some form of random selection was used to select the sample (e.g. simple random sampling, stratified random sampling, cluster sampling, systematic sampling).	0
	No (HIGH RISK): A census was NOT undertaken, AND some form of random selection was NOT used to select the sample.	1
4. Was the likelihood of non-response bias minimal?	**Yes (LOW RISK)**: The response rate for the study was ≥75%, OR, an analysis was performed that showed no significant difference in relevant demographic characteristics between responders and non- responders	0
	No (HIGH RISK): The response rate was <75%, and if any analysis comparing responders and non-responders was done, it showed a significant difference in relevant demographic characteristics between responders and non-responders	1
5. Were data collected directly from the subjects (as opposed to a proxy)?	**Yes (LOW RISK)**: All data were collected directly from the subjects.	0
	No (HIGH RISK): In some instances, data were collected from a proxy.	1
6. Was an acceptable case definition used in the study?	**Yes (LOW RISK)**: An acceptable case definition was used. **No (HIGH RISK)**: An acceptable case definition was NOT used	0 1
7. Was the study instrument that measured the parameter of interest (e.g. prevalence of low back pain) shown to have reliability and validity (if necessary)?	**Yes (LOW RISK)**: The study instrument had been shown to have reliability and validity (if this was necessary), e.g. test-re- test, piloting, validation in a previous study, etc.	0
	No (HIGH RISK): The study instrument had NOT been shown to have reliability or validity (if this was necessary).	1
8. Was the same mode of data collection used for all subjects?	**Yes (LOW RISK)**: The same mode of data collection was used for all subjects.	0
	No (HIGH RISK): The same mode of data collection was NOT used for all subjects.	1
9. Were the numerator(s) and denominato r(s) for the parameter of interest appropriate	**Yes (LOW RISK)**: The paper presented appropriate numerator(s) AND denominator(s) for the parameter of interest (e.g. the prevalence of low back pain).	0
	No (HIGH RISK): The paper did present numerator(s) AND denominator(s) for the parameter of interest but one or more of these were inappropriate.	1
10. Summary on the overall risk of study bias	**LOW RISK**	0-3
	MODERATE RISK	4-6
	HIGH RISK	7-9

Supplementary Table 5. *Characteristics of the included studies*

							Intervention			
Reference	**Country**	**Study design**	**Purpose**	**No.**	**Population**	**Mean age at HSG (y) (SD or IQR) Median (range)**	**Contrast**	**Volume OSCM (mL) Median (range)**	**Fluoroscopy**	**HSG results**
(Hirst, 1928)	**USA**	Case report	Case of death after surgery following-HSG	OSCM = 1	-	-	Lipiodol (not spec)	6	-	-
(Ries, 1929)	**USA**	Case report	Case of lipogranuloma	OSCM = 1	Subfertility (not spec)	30	Lipiodol (not spec)	-	-	Uni/bilateral patent tubes
(Pujol y Brull et al., 1929)	**Spain**	Case series	Cases of intravasation	OSCM = 5	1 metrorrhagia, 1 possibly TBC	-	Lipiodol (not spec)	-	-	-
(Novak, 1930)	**Austria**	Case report	Case of death after surgery following-HSG	OSCM=1	Sec subfertility	36	Jodipin	-	-	Bilateral tubal occlusion
(Solal, 1932)	**France**	Case report	Case of intravasation	OSCM = 1	Subfertility (not spec)	30	Lipiodol (not spec)	8	-	Bilateral tubal occlusion Uterus infantile bicornus
(Wong et al., 1932)	**China**	Case series	Cases of intravasation	OSCM = 4	50% prim subfertility 50% sterility not spec	22, 24, 31, 35	Lipiodol (not spec)	4, 8, 9, 15	No	Bilateral tubal occlusion 75% Unilateral tubal occlusion 25%
(Kilroe and Hellman, 1933)	**USA**	Case report	Case of intravasation	OSCM = 1	Prim subfertility	30	Lipiodol (not spec)	-	-	-
(Zacharin, 1933)	**Australia**	Case report	Case of intravasation	OSCM = 1	Subfertility (not spec) HSG after Rubin's test	30	Lipiodol (not spec)	8	-	Bicornuate uterus, unilateral tubal occlusion
(Coventry, 1934)	**USA**	Case report	Case of intravasation	OSCM = 1	Prim subfertility	34	Lipiodol (not spec)	10	-	Bilateral tubal occlusion

Supplementary Table 5. *(Continued)*

							Intervention			
Reference	**Country**	**Study design**	**Purpose**	**No.**	**Population**	**Mean age at HSG (y) (SD or IQR) Median (range)**	**Contrast**	**Volume OSCM (mL) Median (range)**	**Fluoroscopy**	**HSG results**
(Meaker, 1934)	**USA**	Case report	Case of intravasation	OSCM = 1	Prim subfertility	-	Ioidized oil (not spec)	11	-	Bilateral tubal occlusion
(Effkemann, 1935)	**Germany**	Case report	Case of intravasation	OSCM = 1	Subfertility (not spec)	34	Jodipin (20%)	12	-	Unilateral tubal occlusion
(Lin and Tsou, 1935)	**China**	Case Series	Cases of intravasation	OSCM = 7	Subfertility (not spec) 1 fistula after hysterotomy	31 (27-35)	Lipiodol (not spec)	8 (8-20)	No	Bilateral tubal occlusion 14% Patent 28% Not reported 57%
(Porcher, 1935)	**France**	Case report	Case of intravasation	OSCM = 1	Subfertility (not spec)	-	Lipiodol (not spec)	7.5	No	Bilateral patency
(Weitzner, 1935)	**USA**	Case report	Case of intravasation	OSCM = 1	Prim subfertility and myoma	35	Ioidized poppy-seed oil	8	-	1 patent, 1 previously removed
(Hemmeler, 1938)	**Switzerland**	Case report	Case of pulmonary embolism	OSCM = 1	Prim subfertility	30	Lipiodol (not spec)	4	-	Tubes not visible
(Flew, 1944)	**UK**	Case report	Case of pulmonary/ cerebral embolism	OSCM = 1	Subfertility (not spec)	-	Lipiodol (not spec)	-	-	-
(Williams, 1944)	**UK**	Case series	Cases of intravasation	OSCM = 6	Subfertility (not spec)	< 36	OSCM (not spec)	-	-	Bilateral tubal occlusion 17% Not reported 83%
(Eisen and Goldstein, 1945)	**Canada**	Case report	Case of pulmonary embolism	OSCM = 1	Sec subfertility	28	Lipiodol (not spec)	15	-	1 tube patent 1 previously removed

Supplementary Table 5. *(Continued)*

							Intervention			
Reference	**Country**	**Study design**	**Purpose**	**No.**	**Population**	**Mean age at HSG (y) (SD or IQR) Median (range)**	**Contrast**	**Volume OSCM (mL) Median (range)**	**Fluoroscopy**	**HSG results**
(Grossmann, 1946)	**Czech Republic**	Case series	Cases of intravasation	OSCM = 2	1 sec subfertility 1 subfertility (not spec)	34, unknown	Lipiodol (not spec) Neohydriol	-	-	1 patent tubes, 1 unknown
(Faris and McMurrey, 1947)	**USA**	Case series	Cases of intravasation / anaphylactic reaction	OSCM = 2	Prim subfertility 50% Unknown 50%	35, unknown	Lipiodine	10, 12	Yes	Bilateral tubal occlusion 50%. Not reported 50%
(Piatt, 1947)	**USA**	Case report	Case of intravasation	OSCM = 1	Sec subfertility	26	Lipiodol (not spec)	10	-	Unilateral patent tubes (1 tube previously removed)
(Netter and Weill-Fage, 1950)	**France**	Case report	Case of intravasation	OSCM = 1	Subfertility (not spec)	37	Lipiodol (not spec)	-	-	-
(Karshmer and Stein, 1951)	**UK**	Case report	Case of pulmonary emboli	OSCM = 1	Prim subfertility	29	Iodochlorol/Lipiodol	11	No	Bilateral tubal occlusion
(Sappey et al., 1952)	**France**	Case report	Case of pulmonary emboli	OSCM = 1	Subfertility (not spec)	-	Lipiodol 40%	-	-	-
(Werner, 1952)	**Germany**	Case report	Case of oil granuloma	OSCM = 1	-	24	Jodipin	-	-	-
(Fochem and Ulm, 1954)	**Germany**	Case series	Cases of intravasation	OSCM = 3	-	28, 36, 41	Jodipin	-	-	-
(Schaffer, 1954)	**Argentina**	Case report	Case of PID / foreign body granuloma	OSCM = 1	Sec subfertility	36	OSCM	-	-	Bilateral tubal occlusion + bilateral hydrosalpinx

Supplementary Table 5. *(Continued)*

							Intervention			
Reference	**Country**	**Study design**	**Purpose**	**No.**	**Population**	**Mean age at HSG (y) (SD or IQR) Median (range)**	**Contrast**	**Volume OSCM (mL) Median (range)**	**Fluoroscopy**	**HSG results**
(Böttger and Fleck, 1955)	**Germany**	Case report	Case of PID	OSCM = 1	Prim subfertility	-	OSCM	-	-	-
(Grant et al., 1957)	**Scotland**	Case report	Case of pulmonary embolism	OSCM = 1	Prim subfertility	29	Lipiodol-Lafay	7-8	-	Both tubes patent
(Shapiro et al., 1957)	**USA**	Case report	Case of intravasation	OSCM = 1	Sec subfertility and fibroids	26	Lipiodol (not spec)	-	-	-
(Gunsberger, 1958)	**Yugoslavia**	Case report	Case of pulmonary embolism	OSCM = 1	-	32	Iodized oil (not spec)	-	-	-
(Hohlbein, 1965)	**Germany**	Case report	Case of pulmonary embolism	OSCM = 1	Prim subfertility	26	40% Jodipin	20	Yes	-
(Levinson, 1963)	**USA**	Case report	Case of pulmonary embolism	OSCM = 1	Sec subfertility	49	Iodochlorol 27% iodine	-	-	-
(Elliott et al., 1965)	**USA**	Case series	Cases of oil granuloma	OSCM = 3	-	-	OSCM	-	-	-
(Claus and Dochez, 1966)	**Belgium**	Case series	Cases of pulmonary embolism	OSCM = 2	-	27, 38	Lipiodol (not spec)	-	-	-
(Pear and Boyden, 1967)	**USA**	Case report	Case of retention of OSCM	OSCM = 1	-	28	Lipiodol (not spec)	-	-	-
(Aznar et al., 1969)	**Mexico**	Case series	Cases of intravasation	OSCM = 141	Prim subfertility 21% Sec subfertility 18% Other indication 62%	26 – 30	Ioidized oil	2	Yes	Both tubes patent 40% Unilateral tubal occlusion 15% Bilateral tubal occlusion 30%

Supplementary Table 5. *(Continued)*

							Intervention			
Reference	**Country**	**Study design**	**Purpose**	**No.**	**Population**	**Mean age at HSG (y) (SD or IQR) Median (range)**	**Contrast**	**Volume OSCM (mL) Median (range)**	**Fluoroscopy**	**HSG results**
(Lau, 1969)	**Germany**	Case series	Cases of intravasation	OSCM = 1 WSCM = 2	-	24, 33, 35	Jodipin	-	-	Uncertain in all 3
(Bohm and Seewald, 1972)	**Germany**	Case series	Case series of PID/ salpingitis	OSCM = 3	-	-	Jodipin	-	-	-
(Malter and Fox, 1972)	**USA**	Case report	Case of retention of OSCM	OSCM = 1	Prim subfertility	26	OSCM	-	-	-
(Charawanamuttu et al., 1973)	**UK**	Case report	Case of retinal embolism	OSCM = 1	Sec subfertility	31	Lipiodol® Ultra Fluid 40%	>20	Yes	Both tubes patent Uterine septum
(Weise et al., 1973)	**Germany**	Case report	Case of oil granuloma	OSCM = 1	-	40	Jodipin	-	-	-
(Binder et al., 1976)	**Romania**	Case report	Case of pulmonary embolism	OSCM = 1	Prim subfertility	26	Lipiodol (not spec)	-	No	Bilateral obstruction, unilateral hydrosalpinx, uterus arcuate.
(Bersi, 1977)	**Italy**	Case report	Case of oil granuloma	OSCM = 1	Prim subfertility, myomas	35	Lipiodol (not spec)	-	-	-
(Ishizuki et al., 1992)	**Japan**	Case report	Case of thyroiditis	OSCM = 1	Subfertility (not spec). Graves' disease	28	Lipiodol (not spec)	-	-	-
(Dan* et al., *1990)	**Israel**	Case report	Case of pulmonary embolism with comatose state	OSCM = 1	Secondary subfertility	33	Lipiodol® Ultra Fluid	6	-	Both tubes patent Bicornuate uterus

Supplementary Table 5. *(Continued)*

							Intervention			
Reference	**Country**	**Study design**	**Purpose**	**No.**	**Population**	**Mean age at HSG (y) (SD or IQR) Median (range)**	**Contrast**	**Volume OSCM (mL) Median (range)**	**Fluoroscopy**	**HSG results**
(Grosskinsky et al., 1994)	**USA**	Case report	Case of oil granuloma	OSCM = 1	Secondary subfertility	29	Ethiodol	6	-	Both tubes patent
(Uzun et al., 2004)	**Turkey**	Case report	Case of pulmonary embolism with comatose state	OSCM = 1	Secondary amenorrhoea	29	Lipiodol® Ultra Fluid	-	-	Irregular endometrial cavity At least one tube patent
(Schutte et al., 2006)	**Netherlands**	Case report	Case of retention of OSCM	OSCM = 1	Subfertility (not spec)	31	-	-	-	-
(Gotoh et al., 2010)	**Japan**	Case report	Case of retention of OSCM	OSCM = 1	Subfertility (not spec)	39	OSCM	-	-	-
(Morii* et al., *2013)	**Japan**	Case report	Case of Fitz-Hugh-Curtis syndrome-like findings	OSCM = 1	Subfertility (not spec)	37	OSCM	-	-	-
(Omoto et al., 2013)	**Japan**	Case report	Case of fetal goiter	OSCM = 1	Secondary subfertility	40	Lipiodol (not spec)	10	-	Both tubes patent
(Takeyama et al., 2014)	**Japan**	Case report	Case of retention of OSCM	OSCM = 1	Subfertility (not spec)	34	OSCM	-	-	-
(Ueda et al., 2016)	**Japan**	Case report	Case of pulmonary embolism	OSCM = 1	Subfertility (not spec), previous left tubectomy	27	Lipiodol® Ultra Fluid	8	Yes	Left salpingectomy Right not described
(Ma et al., 2016)	**China**	Case report	Case of maternal thyroid dysfunction	OSCM = 1	Subfertility (not spec)	33	OSCM	100	-	Both tubes patent

Supplementary Table 5. *(Continued)*

							Intervention			
Reference	**Country**	**Study design**	**Purpose**	**No.**	**Population**	**Mean age at HSG (y) (SD or IQR) Median (range)**	**Contrast**	**Volume OSCM (mL) Median (range)**	**Fluoroscopy**	**HSG results**
(Sasaki et al., 2017)	**Japan**	Case report	Case of fetal goiter	OSCM = 1	Subfertility (not spec)	27	OSCM	-	-	-
(Li *et al.*, 2018)	**China**	Case series	Cases of (subclinical) hypothyroidism	OSCM = 14	-	-	OSCM	-	-	-
(Yamazaki *et al.*, 2019)	**Japan**	Case report	Case of fetal goiter	OSCM = 1	-	35	OSCM	3 HSGs	-	-
(Miyazaki et al., 2020)	**Japan**	Case report	Case of oil granuloma	OSCM = 1	Subfertility (not spec)	30	Lipiodol (not spec)	-	-	-
(Rubin, 1928)	**USA**	Cohort not specified	CO2-insufflation followed by HSG	OSCM = 66	-	-	Lipiodol (not spec)	-	-	Bilateral tubal occlusion 100%
(Witwer et al., 1930)	**USA**	Retrospective cohort	Evaluate complications	OSCM = 512	-	-	Lipiodol (not spec)	4 (2.5 – 5.0)	Not standard	Not described. Only 1 case with tubal rupture in which bilateral hydrosalpinx
(Riche and Fayot, 1931)	**France**	Retrospective cohort	Evaluate complications	OSCM = 120	HSGs not for infertility 21%	-	Lipiodol (not spec)	-	-	-
(Schultze, 1932)	**Germany**	Retrospective cohort	Evaluate complications	OSCM = 600	-	-	Jodipin 40%	-	-	-
(Volk, 1936)	**Germany**	Retrospective cohort	Evaluate complications	OSCM = 316	Not reported. Also for other indications	-	OSCM	-	-	-
(Nordio, 1938)	**Italy**	Retrospective cohort	Evaluate intravasation	OSCM = 106	-	-	Iodized oil	-	-	-

Supplementary Table 5. *(Continued)*

							Intervention			
Reference	**Country**	**Study design**	**Purpose**	**No.**	**Population**	**Mean age at HSG (y) (SD or IQR) Median (range)**	**Contrast**	**Volume OSCM (mL) Median (range)**	**Fluoroscopy**	**HSG results**
(Feiner, 1942)	**USA**	Cohort not specified	Cases of PID/ salpingitis/death	OSCM = 337	-	-	Lipiodol (not spec)	-	-	Bilateral tubal occlusion 44% Uni/bilateral patent tubes 56%
(Madsen, 1942)	**Denmark**	Retrospective cohort	Evaluate complications	OSCM = 490	Complicated cases, 2/6 for subfertility	Complicated cases: 45 (30-56)	Complicated cases: 5/6 Jodipin 20%, 1/6 Jodumbrin	Complicated cases: 5.3 (3.5-9)	No	Complicated cases: 4/6 bilateral tubal occlusion
(Rutherford, 1948)	**USA**	Cohort not spec	Description of technique, HSG after CO2-insufflation	OSCM = 417	Prim subfertility 75% Sec subfertility 25%	19 – 41	Lipiodal	Max 12	Yes	After failed CO2-insufflation, all bilateral tubal occlusion
(Bang, 1950)	**Denmark**	Retrospective cohort	Evaluate complications	OSCM = 900	Prim/sec subfertility 98% Referred for sterilisation 1.1%	-	Half Jodubrin, half Jodipin, 15 cases Lipiodol	-	-	-
(Vara, 1950)	**Finland**	Retrospective cohort	Evaluate intravasation	OSCM = 1119	Prim/sec subfertility	-	Jodipin, Lipiodol, Jodolja, Neo-Hydrol (20-40%)	-	No	Bilateral patent tubes 53%
(Bergin, 1951)	**UK**	Retrospective cohort	Evaluate complications	OSCM = 201 WSCM = 69	-	-	Lipiodol (not spec)	-	-	-
(Drukman and Rozin, 1951)	**Israel**	Retrospective cohort	Evaluate intravasation	OSCM = 2000	-	-	Lipiodol 40% 'Assia' and 'Laffay'	-	-	-

Supplementary Table 5. *(Continued)*

							Intervention			
Reference	**Country**	**Study design**	**Purpose**	**No.**	**Population**	**Mean age at HSG (y) (SD or IQR) Median (range)**	**Contrast**	**Volume OSCM (mL) Median (range)**	**Fluoroscopy**	**HSG results**
(Robins and Shapira, 1951)	**USA**	Retrospective cohort	Evaluate HGS and complications	OSCM = 4800	Not reported. Also for other indications	-	Lipiodol Lafay 28%	-	-	-
(Kika, 1954)	**Japan**	Retrospective cohort	Evaluate intravasation and tuberculosis	OSCM = 1200	Complicated cases: Prim subfertility 90% Sec subfertility 10%	-	20-40% Moljodol	4-8	Yes	Complicated cases: Bilateral patent tubes 11% Bilateral or unilateral tubal occlusion 89%
(Zachariae, 1955)	**Denmark**	Cohort not spec	Evaluate complications	OSCM = 505 WSCM = 55	Complicated cases: Prim subfertility 67% Non-infertility 33%	Complicated cases: 25 – 59	Various OSCM (esp iodumbrin, neohydriol)	-	Yes	-
(Norris, 1956)	**Canada**	Cohort not spec	Evaluate complications	OSCM = 961	Subfertility (not spec)	-	Lipiodol (not spec)	3.5-4 (max 10)	-	Bilateral tubal occlusion 35%
(Stoll and Zeitz, 1956)	**Germany**	Retrospective cohort	Evaluate intravasation	OSCM = 2236	-	-	Jodipin	-	-	-
(Frischkorn, 1958)	**Germany**	Retrospective cohort	Evaluate intravasation	OSCM = 281 WSCM = 52	-	-	Jodipin 40%	8	-	-
(Woltz* et al., *1958)	**USA**	Cohort not spec	Evaluate complications	OSCM = 500	-	-	Iodochlorol	Average 7-10 (3-20)	-	Bilateral tubal occlusion 12% Hydrosalpinx 4.2%
(Palmer, 1960)	**USA**	Cohort not spec	Evaluate pregnancy rate and complications	OSCM = 258	Subfertility (not spec)	-	Ethiodol	-	-	-

Supplementary Table 5. *(Continued)*

							Intervention			
Reference	**Country**	**Study design**	**Purpose**	**No.**	**Population**	**Mean age at HSG (y) (SD or IQR) Median (range)**	**Contrast**	**Volume OSCM (mL) Median (range)**	**Fluoroscopy**	**HSG results**
(Kuzavova, 1964)	**Russia**	Retrospective cohort	Evaluate intravasation after HSG	OSCM = 730	Only intravasation cases: all subfertility	Intravasation cases: 20 – 37	Lipiodol (not spec)	3-5	No	Cases with intravasation: Bilateral tubal occlusion 54%
(Heinen and Schussler, 1966)	**Germany**	Retrospective cohort	Evaluate intravasation after HSG	OSCM = 122	-	-	Jodolen	-	-	-
(Geary et al., 1969)	**USA**	Retrospective cohort	Evaluate pregnancy rates and complications	OSCM = 501	Prim subfertility 55% Sec subfert 29% Other subfertility: 16%	-	Lipiodol (not spec)	10	-	Both tubes patent 79% Unilateral tubal occlusion 7.4% Bilateral tubal occlusion 8.8%
(Mackey et al., 1971)	**USA**	Retrospective cohort	Evaluate pregnancy rates	OSCM = 221 WSCM = 63	OSCM: Prim subfertility 63% Sec subfertility 37%	OSCM: 27.3 – 28.9	Ethiodol	-	Yes	-
(Buytaert and Meulyzer, 1977)	**Belgium**	Retrospective cohort	Evaluate pregnancy rates and complications	OSCM = 208	Prim subfertility 65% Sec subfertility 35%	-	Lipiodol 40%	3-6	Yes	-
(Bateman et al., 1980)	**USA**	Cohort not spec	Evaluate complications after HSG	OSCM = 533	Subfertility and preoperative HSG before tuba reconstruction	Intravasation cases: 24-33	Ethiodol (37%)	1.5 – 12	Yes	Cases with complications: Bilateral tubal occlusion 69%

Supplementary Table 5. *(Continued)*

							Intervention			
Reference	**Country**	**Study design**	**Purpose**	**No.**	**Population**	**Mean age at HSG (y) (SD or IQR) Median (range)**	**Contrast**	**Volume OSCM (mL) Median (range)**	**Fluoroscopy**	**HSG results**
(La Sala et al., 1982)	**Italy**	Retrospective cohort study	Evaluate intravasation after HSG	OSCM = 119	Sterility, suspected uterine malformations and other pathologies	-	Lipiodol® ultra fluid (40%)	5-10	Yes	-
(Rasmussen et al., 1987)	**Denmark**	Cohort not spec	Evaluate pregnancy rates after HSG	OSCM = 294	Prim subfertility 59% Sec subfertility 41%	28 (19 – 40)	Lipiodol® Ultra Fluid	5-10	Yes	Both tubes patent: 41% Unilateral tubal occlusion 14% Bilateral tubal occlusion 19%
(Nunley et al., 1987)	**USA**	Cohort not spec	Evaluate intravasation	OSCM = 593	Subfertility (not spec) Complications: 11 preoperative before tubal reconstruction	-	Ethiodol	2.0-10	Yes	Both tubes patent 41% Unilateral tubal occlusion 9.8% Bilateral tubal occlusion 44%
(Barqawi et al., 2007)	**Jordan**	Retrospective cohort study	Compare pregnancy between OSCM and WSCM	OSCM = 35 WSCM = 40	Subfertility (not spec)	OSCM 28 (3)	OSCM	10-20	Yes	None bilateral tubal occlusion
(Mekaru et al., 2008)	**Japan**	Cohort not spec	Evaluate thyroid function after HSG	OSCM = 220	Subfertility (not spec)	35 (4.6)	Lipiodol (not spec)	Average 5-10	Yes	-

Supplementary Table 5. *(Continued)*

							Intervention			
Reference	**Country**	**Study design**	**Purpose**	**No.**	**Population**	**Mean age at HSG (y) (SD or IQR) Median (range)**	**Contrast**	**Volume OSCM (mL) Median (range)**	**Fluoroscopy**	**HSG results**
(Liu* et al., *2010)	**China**	Retrospective cohort	Compare complications after HSG	OSCM = 100 WSCM = 300	-	Mean 31 (25-37)	Ioidized oil (not spec).	5-10	Yes	OSCM: Bilateral tubal occlusion 12% Unilateral tubal occlusion 15%
(Satoh et al., 2015)	**Japan**	Retrospective cohort study	Evaluate neonatal thyroid function	OSCM = 212	-	-	Lipiodol (480)	5-10	-	-
(So* et al., *2017)	**Japan**	Cohort not spec	Compare maternal thyroid function after HSG with OSCM and WSCM	OSCM = 164 WSCM = 94	OSCM: Sec subfertility 36.6% < 3 months before HSG normal thyroid function	34 (4.3)	Lipiodol (480)	5	-	OSCM: Bilateral tubal occlusion 11%
(Brown et al., 1949)	**USA**	Cohort not spec	Evaluate absorption of OSCM	OSCM = 118	-	-	Lipiodol (not spec) Iodochlorol Lipoiodine	8-12	No	-
(Slater et al., 1959)	**USA**	Prospective study	Evaluate thyroid function after HSG with OSCM and WSCM	OSCM = 10 WSCM = 18	All clinically euthyroid.	-	Ethiodol	-	-	-
(Brent et al., 2006)	**Australia**	Prospective cohort	Evaluate pregnancy rates and complications after HSG	OSCM = 100	Prim subfertility 60% Sec subfertility 40%	36.83 (4.26)	Lipiodol® Ultra Fluid	10 (sometimes >10)	Yes	Bilateral tubal patency 80% Bilateral tubal occlusion 4%

Supplementary Table 5. *(Continued)*

							Intervention			
Reference	**Country**	**Study design**	**Purpose**	**No.**	**Population**	**Mean age at HSG (y) (SD or IQR) Median (range)**	**Contrast**	**Volume OSCM (mL) Median (range)**	**Fluoroscopy**	**HSG results**
(Kaneshige et al., 2015)	**Japan**	Prospective cohort	Evaluate maternal thyroid function after HSG	OSCM = 22	Subfertility (not spec)	36+2.45	Lipiodol (not spec)	6.1 (4.0-9.0)	-	None bilateral tubal occlusion
(Tan et al., 2019)	**China**	Prospective cohort	Evaluate image quality and complications	OSCM = 165 WSCM = 63	OSCM: Prim subfertility 64%	OSCM 31.36 (4.99)	Ethiodized poppyseed oil	OSCM 6-8	Yes	-
(Welie et al., 2020)	**Netherlands**	Retrospective cohort study	Evaluate neonatal thyroid function	OSCM = 76 WSCM = 64	Subfertility (not spec)	-	Lipiodol® Ultra Fluid.	OSCM 9.0 (6.0-11.8)	Yes	-
(Schwabe et al., 1983)	**USA**	RCT	Compare pregnancy rates after HSG	OSCM = 56 WSCM = 65	Subfertility (not spec)	-	Ethiodol	-	Yes	-
(Alper et al., 1986)	**Canada**	RCT	Compare pregnancy rates after HSG	OSCM = 58 WSCM = 73	Subfertility (not spec)	OSCM 29 (2.9)	Lipiodol® Ultra Fluid	10.5 (SD 4.7)	Yes	None bilateral tubal occlusion
(Lindequist et al., 1991)	**Denmark**	RCT	Evaluate complications after HSG	OSCM = 103 WSCM = 314	Subfertility (not spec)	-	Lipiodol® Ultra Fluid	5-10	Yes	OSCM: Bilateral tubal patency 47%
(Lindequist et al., 1994)	**Denmark**	RCT	Compare pregnancy rates and complications after HSG	OSCM = 123 WSCM = 122	OSCM: Prim subfertility 60% Sec subfertility 40%	OSCM 29.9 (21-43)	Lipiodol® Ultra Fluid	5-10	Yes	OSCM: Bilateral tubal patency 54%
(Nugent et al., 2002)	**UK**	RCT	Evaluate pregnancy rates after HSG	OSCM = 17	Prim subfertility 71% Sec subfertility 29%	31 (1.1)	Lipiodol (not spec)	5.8 (0.7)	Yes	All patent

Supplementary Table 5. *(Continued)*

							Intervention			
Reference	**Country**	**Study design**	**Purpose**	**No.**	**Population**	**Mean age at HSG (y) (SD or IQR) Median (range)**	**Contrast**	**Volume OSCM (mL) Median (range)**	**Fluoroscopy**	**HSG results**
(Steiner et al., 2003)	**USA**	RCT	Evaluate pregnancy rates after HSG	OSCM = 30	All subfertility Prim subfertility 47%	33 (3.6)	Ethiodol	5-10	Yes	All patent
(Johnson et al., 2004)	**Australia**	RCT	Evaluate pregnancy rates after	OSCM = 73	All subfertility Prim subfertility 55%	33.9 (2.9)	Lipiodol® Ultra Fluid	10	Yes	Bilateral tubal occlusion 8.3%
(Dreyer et al., 2017)	**Netherlands**	RCT	Compare pregnancy rates after HSG	OSCM = 550 WSCM = 556	All subfertility Prim subfertility OSCM 67.3%	OSCM 32.8 (30.1-35.7)	Lipiodol® Ultra Fluid.	9.0 (5.7-15.0)	Yes	OSCM: Bilateral tubal patency 86% Bilateral tubal occlusion 1.6%

Not spec = not specified. OSCM = oil-soluble contrast media. WSCM = water-soluble contrast media.

Supplementary Table 6. *Classification of risk of bias*

First author, year of publication	Study type	1. Was the study's target population a close representation of the national population in relation to relevant variables	2. Was the sampling frame a true or close representation of the target population?	3. Was some form of random selection used to select the sample, OR, was a census undertaken?	4. Was the likelihood of non-response bias minimal?	5. Were data collected directly from the subjects (as opposed to a proxy)?	6. Was an acceptable case definition used in the study?	7. Was the study instrument that measured the parameter of interest shown to have reliability and validity (if necessary)?	8. Was the same mode of data collection used for all subjects?	9. Were the numerator(s) and denominator(s) for the parameter of interest appropriate?	10. Summary of the overall risk of study bias:	0-3 Low risk, 4-6 moderate risk, 7-9 High risk.
Alper, 1986	RCT	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	High risk	Low risk	Low risk	1	Low risk
Bang, 1950	Retrospective cohort	Low risk	Low risk	Unclear	Low risk	Unclear	High risk	High risk	Unclear	Low risk	5	Moderate risk
Barqawi, 2007	Retrospective cohort	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	High risk	Low risk	Low risk	1	Low risk
Bateman, 1980	Cohort not spec	High risk	Low risk	Low risk	Low risk	Low risk	Unclear	High risk	Unclear	Low risk	4	Moderate risk
Bergin, 1951	Retrospective cohort	Unclear	Unclear	Unclear	Unclear	Unclear	High risk	High risk	Unclear	Low risk	8	High risk
Brent, 2006	Prospective cohort	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	High risk	Low risk	Low risk	1	Low risk
Brown, 1949	Cohort not spec	Unclear	Unclear	Unclear	Low risk	Unclear	Low risk	High risk	Unclear	Low risk	6	Moderate risk
Buytaert, 1977	Retrospective cohort	Low risk	Unclear	Unclear	Low risk	Unclear	High risk	High risk	Unclear	Low risk	6	Moderate risk
Dreyer, 2017	RCT	Low risk	Low risk	Low risk	Low risk	Low risk	High risk	High risk	Low risk	Low risk	2	Low risk
Drukman, 1951	Retrospective cohort	High risk	Unclear	Unclear	Low risk	Unclear	Low risk	High risk	Unclear	Low risk	6	Moderate risk
Feiner, 1942	Retrospective cohort	Low risk	Unclear	Unclear	Low risk	High risk	High risk	High risk	Unclear	High risk	6	Moderate risk
Frischkorn, 1958	Retrospective cohort	Unclear	Unclear	Low risk	Low risk	Unclear	High risk	High risk	Unclear	Low risk	6	Moderate risk
Geary, 1969	Retrospective cohort	High risk	Low risk	Low risk	Low risk	Unclear	High risk	High risk	Unclear	Low risk	5	Moderate risk
Heinen, 1966	Retrospective cohort	Unclear	Unclear	Unclear	Low risk	Unclear	Low risk	High risk	Unclear	Low risk	6	Moderate risk

Supplementary Table 6. *(Continued)*

First author, year of publication	Study type	1. Was the study's target population a close representation of the national population in relation to relevant variables	2. Was the sampling frame a true or close representation of the target population?	3. Was some form of random selection used to select the sample, OR, was a census undertaken?	4. Was the likelihood of non-response bias minimal?	5. Were data collected directly from the subjects (as opposed to a proxy)?	6. Was an acceptable case definition used in the study?	7. Was the study instrument that measured the parameter of interest shown to have reliability and validity (if necessary)?	8. Was the same mode of data collection used for all subjects?	9. Were the numerator(s) and denominator(s) for the parameter of interest appropriate?	10. Summary of the overall risk of study bias:	0-3 Low risk, 4-6 moderate risk, 7-9 High risk.
Johnson, 2004	RCT	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	High risk	Low risk	Low risk	1	Low risk
Kaneshige, 2015	Prospective cohort	Low risk	Unclear	Unclear	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	2	Low risk
Kika, 1954	Retrospective cohort	Low risk	High risk	Unclear	Low risk	Unclear	Low risk	High risk	Unclear	Low risk	4	Moderate risk
Kuzavova, 1964	retrospective cohort	Low risk	Low risk	Low risk	Low risk	Unclear	Low risk	High risk	Unclear	Low risk	3	Low risk
La Sala, 1982	Retrospective cohort	High risk	Low risk	Low risk	Low risk	Unclear	High risk	High risk	Unclear	Low risk	5	Moderate risk
Lindequist, 1991	RCT	High risk	Low risk	Low risk	Unclear	Low risk	Low risk	High risk	Low risk	Low risk	3	Low risk
Lindequist, 1994	RCT	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	High risk	Low risk	Low risk	1	Low risk
Liu, 2010	Retrospective cohort	Low risk	Low risk	Low risk	Low risk	Unclear	Low risk	High risk	Unclear	Low risk	3	Low risk
Mackey, 1971	Retrospective cohort	Low risk	Low risk	Low risk	Low risk	High risk	High risk	High risk	High risk	Low risk	4	Moderate risk
Madsen, 1942	Retrospective cohort	High risk	Low risk	Low risk	Low risk	Unclear	Low risk	High risk	Unclear	Low risk	4	Moderate risk
Mekaru, 2008	Cohort not spec	Low risk	Low risk	Unclear	Low risk	Low risk	Low risk	Low risk	High risk	Low risk	2	Low risk
Nordio, 1938	Retrospective, cohort	Unclear	Unclear	Unclear	Low risk	Unclear	Low risk	High risk	Unclear	Low risk	6	Moderate risk
Norris, 1956	Cohort not spec	Low risk	Low risk	Low risk	Low risk	Unclear	Low risk	High risk	Unclear	Low risk	3	Low risk

Supplementary Table 6. *(Continued)*

First author, year of publication	Study type	1. Was the study's target population a close representation of the national population in relation to relevant variables	2. Was the sampling frame a true or close representation of the target population?	3. Was some form of random selection used to select the sample, OR, was a census undertaken?	4. Was the likelihood of non-response bias minimal?	5. Were data collected directly from the subjects (as opposed to a proxy)?	6. Was an acceptable case definition used in the study?	7. Was the study instrument that measured the parameter of interest shown to have reliability and validity (if necessary)?	8. Was the same mode of data collection used for all subjects?	9. Were the numerator(s) and denominator(s) for the parameter of interest appropriate?	10. Summary of the overall risk of study bias:	0-3 Low risk, 4-6 moderate risk, 7-9 High risk.
Nugent, 2002	RCT	Low risk	Low risk	Low risk	Low risk	Unclear	High risk	High risk	Unclear	Low risk	4	Moderate risk
Nunley, 1987	Cohort not spec	Low risk	High risk	Low risk	Low risk	Unclear	Unclear	High risk	Unclear	Low risk	5	Moderate risk
Palmer, 1960	Cohort not spec	Low risk	Unclear	Unclear	Low risk	Unclear	Low risk	High risk	Unclear	Low risk	5	Moderate risk
Rasmussen, 1987	Cohort not spec	Low risk	Low risk	High risk	Low risk	Unclear	High risk	High risk	Unclear	Low risk	5	Moderate risk
Riche, 1931	Retrospective cohort	High risk	Low risk	Low risk	Low risk	Unclear	Low risk	High risk	Unclear	Low risk	4	Moderate risk
Robins, 1951	Cohort not spec	Unclear	Unclear	Low risk	Low risk	Unclear	Low risk	High risk	Unclear	Low risk	5	Moderate risk
Rubin, 1928	Cohort not spec	Unclear	High risk	Unclear	Low risk	Unclear	High risk	High risk	Unclear	Unclear	8	High risk
Rutherford, 1948	Cohort not spec	Low risk	High risk	Low risk	Low risk	Unclear	Low risk	High risk	Unclear	Low risk	4	Moderate risk
Satoh, 2015	Retrospective cohort	Unclear	Unclear	Low risk	High risk	Low risk	Low risk	Low risk	Low risk	Low risk	3	Low risk
Schultze, 1932	Retrospective cohort	Unclear	Low risk	Low risk	Low risk	Unclear	High risk	High risk	Unclear	Low risk	5	Moderate risk
Schwabe, 1983	RCT	Low risk	Unclear	Low risk	Low risk	Unclear	High risk	High risk	Unclear	Low risk	5	Moderate risk
Shuhei So, 2017	Cohort not spec	Low risk	Unclear	Unclear	High risk	Low risk	Low risk	Low risk	Low risk	Low risk	3	Low risk
Slater, 1959	Prospective cohort	High risk	Unclear	Unclear	Low risk	Low risk	Unclear	Unclear	Low risk	High risk	6	Moderate risk

Supplementary Table 6. *(Continued)*

First author, year of publication	Study type	1. Was the study's target population a close representation of the national population in relation to relevant variables	2. Was the sampling frame a true or close representation of the target population?	3. Was some form of random selection used to select the sample, OR, was a census undertaken?	4. Was the likelihood of non-response bias minimal?	5. Were data collected directly from the subjects (as opposed to a proxy)?	6. Was an acceptable case definition used in the study?	7. Was the study instrument that measured the parameter of interest shown to have reliability and validity (if necessary)?	8. Was the same mode of data collection used for all subjects?	9. Were the numerator(s) and denominator(s) for the parameter of interest appropriate?	10. Summary of the overall risk of study bias:	0-3 Low risk, 4-6 moderate risk, 7-9 High risk.
Steiner, 2003	RCT	Low risk	Low risk	Low risk	Low risk	Unclear	High risk	High risk	Unclear	Low risk	4	Moderate risk
Stoll, 1956	Retrospective cohort	Unclear	Unclear	Low risk	Low risk	Unclear	Low risk	High risk	Unclear	Low risk	5	Moderate risk
Tan, 2019	Prospective cohort	Low risk	Low risk	Moderate risk	Low risk	Low risk	Low risk	High risk	Low risk	Low risk	2	Low risk
Vara, 1950	Retrospective cohort	Low risk	Unclear	Unclear	Low risk	Unclear	Low risk	High risk	Unclear	Low risk	5	Moderate risk
Volk, 1936	Retrospective cohort	High risk	Low risk	Low risk	Low risk	Unclear	High risk	High risk	Unclear	Unclear	6	Moderate risk
Welie, 2020	Retrospective cohort	Low risk	High risk	Low risk	High risk	Low risk	Low risk	Low risk	Low risk	Low risk	2	Low risk
Witwer, 1930	Retrospective cohort	Unclear	Unclear	Unclear	Low risk	Unclear	Low risk	High risk	Unclear	Low risk	6	Moderate risk
Woltz, 1958	Cohort not spec	Unclear	High risk	Low risk	Low risk	Unclear	Low risk	High risk	Unclear	Low risk	5	Moderate risk
Zachariae, 1955	Cohort not spec	High risk	Low risk	Low risk	Low risk	Unclear	Low risk	High risk	Unclear	Low risk	4	Moderate risk

CHAPTER 3

COMPLICATIONS AFTER HYSTEROSALPINGOGRAPHY WITH OIL- OR WATER-BASED CONTRAST: RESULTS OF A NATIONWIDE SURVEY

I. Roest, N. van Welie, V. Mijatovic,
K. Dreyer, M. Bongers, C. Koks, B.W. Mol.

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ABSTRACT

Study question

What is the incidence of complications after hysterosalpingography (HSG) using oil-based contrast versus water-based contrast?

Summary answer

Among 5165 women undergoing HSG, the most frequently reported complication after HSG with oil- and water-based contrast was intravasation of the contrast medium (4.8% versus 1.3%, respectively), which was without further consequences, and pulmonary embolization or death did not occur.

What is known already

An HSG with oil-based contrast increases pregnancy rates in women with unexplained infertility. However, there have been some concerns regarding complications, including the risks of intravasation of contrast medium, oil-embolism and infection. Here, we present the incidence of complications after HSG with different types of contrast media used in the Netherlands in the year 2017.

Study design, size, duration

In January 2018, an electronic survey was sent to all 73 clinics in the Netherlands that perform HSG. The survey consisted of 12 questions addressing the number of HSGs performed in 2017, the amount and type of contrast medium used, the occurrence of post-procedural complications and what their clinical consequences were. Non-responding clinics were sent multiple reminders.

Participants/materials, setting and methods

We calculated the incidence of the complications and reported on their clinical consequences. Furthermore, we examined the average amount of contrast used as well as the administration of prophylactic antibiotics.

Main results and the role of chance

The response rate was 96% (67/70) (during the study, one site closed and was not included, while two clinics no longer performed HSGs). In the 67 clinics, 3289 HSGs with oil-based contrast and 1876 HSGs with water-based contrast were performed in 2017. The median amount of contrast used was 8.0 mL (interquartile range [IQR] 7.0-10.0) for oil-based contrast and 10.0 mL for water-based contrast (IQR 10.0-10.0). Antibiotic prophylaxis was administered in 61% (41/67) of the clinics. Intravasation occurred in 4.8% of the HSGs performed with oil-based contrast and in 1.3% of the HSGs with water-based contrast (Relative Risk [RR] 3.6, CI 2.4-5.4). Pulmonary embolism or death was not reported. Pelvic inflammatory disease (PID) occurred in 0.3% of the HSGs performed with oil-based contrast versus 0.4% with water-based contrast. PID occurred in 0.3% of the HSGs in clinics using antibiotic prophylaxis and 0.2% in clinics not using antibiotic prophylaxis. Allergic reactions were reported in one HSG performed with oil-based contrast (0.03%) compared with two HSGs performed with water-based contrast (0.1%). Anaphylactic reactions did not occur. The overall complication rate was 5.1% in the clinics that used oil-based contrast versus 1.8% in the clinics that used water-based contrast (RR 2.8, CI 1.9-4.0, P-value <0.0001).

Limitations, reasons for caution

Half of the clinics did not routinely register complications, and the incidence of the complications in their clinic was based on the recall of the clinician. Estimated complication rates in the clinics with and without systematic registration did not significantly differ. The survey asked about the frequency of intravasation but no classification system is being used in daily practice, which may create differences in reporting. There was no standard screening of post-HSG thyroid function for the mother and the foetus.

Wider implications of the findings

In this nationwide cohort study, the complication rates after HSG were low. Intravasation occurred more frequently with the use of oil-based contrast compared to water-based contrast but did not lead to any problems or symptoms in any of the women. We therefore conclude that safety concerns should not be a reason to deny the use of oil-based contrast in women with unexplained infertility. The data also support that fluoroscopy appears to be an essential safety measure during HSG.

INTRODUCTION

Knowledge of tubal patency during the fertility workup is essential for the choice of treatment. Hysterosalpingography (HSG) is the most commonly used diagnostic method to test tubal patency in patients suffering from infertility (National Institute Care Excellence (NICE), 2017). Over the years, both water and oil-soluble contrast media have been used. In 2017, a large randomized controlled trial (RCT) showed that an HSG with the use of oil-based contrast medium (Lipiodol® Ultra-Fluid) results in a 10% higher ongoing pregnancy rate, within 6 months after the procedure, compared to an HSG with water-based contrast (39.7% versus 29.1%, relative risk 1.4, 95% CI 1.2–1.6, P<0.001) (Dreyer *et al.*, 2017). Afterwards, two systematic reviews with meta-analyses confirmed these favourable effects of oil-based contrast media on pregnancy and live birth rates (Fang, et al., 2018; Wang et al., 2019).

Given these favourable results on fertility, HSG with oil-based contrast is preferred. However, there is concern about the risk of complications from using oil-based contrast media during HSG. The most frequently mentioned concerns are the possible risks of venous intravasation and, as a result of that, embolism, the risk of a pelvic infection and maternal/fetal risks of thyroid dysfunction (Uzun *et al.*, 2004; Kaneshige *et al.*, 2015; Satoh *et al.*, 2015; So *et al.*, 2017).

It is known that the risk of intravasation is higher with the use of oil-based contrast as compared to water-based contrast. A recent meta-analysis reporting on 793 women found an odds ratio of 5.1 (95% CI, 2.3–11.2), respectively) for the risk of intravasation with the use of oil-based contrast compared to water-based contrast, with all intravasations being clinically asymptomatic (Wang *et al.*, 2019). Another meta-analysis of 1179 HSGs performed with oil-based contrast, similarly reported no cases of embolism, granulomas or allergic reactions (Fang *et al.*, 2018). Since sample sizes of these meta-analyses were relatively low, concern regarding complications of oil-based contrast media remains. Therefore, we evaluated the incidence of complications after HSG and their possible consequences through a nationwide survey.

MATERIALS AND METHODS

An electronic survey was sent to all 73 clinics in the Netherlands that perform HSG, addressed to gynaecologists specialized in reproductive medicine. The clinics that did not respond on the first attempt were approached again by telephone or e-mail.

The questionnaire (Supplementary Data) consisted of 12 questions regarding the number of HSGs performed in 2017, the type and average amount of contrast medium used, and the occurrence of post-procedural complications as well as their clinical consequences. Fluoroscopy screening is routinely used during HSGs in the Netherlands. Questions were asked on the frequencies of the following complications; allergic reactions, anaphylactic reactions, intravasation of the contrast medium, embolisms and pelvic inflammatory disease (PID) as well as other complications that had occurred post-HSG. No description of the terms was included in the survey. Furthermore, information on the standard registration of complications, the use of antibiotic prophylaxis and standard safety precautions was requested. If a clinic had no standardized complication registry, the respondent was asked to provide an estimated number of complications based on their recall. The study was approved by the Institutional Review Board of the Máxima MC (reference number N19.056).

Categorical data were reported as absolute numbers and percentages. Relative risks (RR) and 95% CI were calculated for binary outcome measurements. The Pearson chi-square-test or Fisher's exact-test were used as appropriate. For normally distributed continuous variables, means with SDs were summarized, non-normally distributed continuous variables were represented as medians with interquartile ranges (IQRs). Continuous outcomes were analysed with the use of an independent t-test or the Mann–Whitney U-test, as appropriate. A P-value of <0.05 was considered to indicate statistical significance. The data were analysed by IBM SPSS Statistics, version 24 (IBM-corporation, Armonk, NY, USA).

RESULTS

From January 2018, 73 questionnaires were sent out. During the study, one site was closed and was not further included in the study, while two clinics responded that they did not perform HSGs. The response rate was 96% (67/70 clinics). In the 67 clinics, a total of 5165 HSGs had been performed in 2017. The number of HSGs per clinic varied between 3 and 328.

In 44 clinics an oil-based contrast (Lipiodol® Ultra-Fluid) was used, of which 29 clinics used Lipiodol® Ultra-Fluid for the whole year, while 15 clinics had started using Lipiodol® Ultra-Fluid since mid-2017. The total amount of HSGs performed with Lipiodol® Ultra-Fluid in 2017 was 3289. The years of experience with Lipiodol® Ultra-Fluid ranged from 0.5 year to more than 20 years, with a median of 3 years (IQR 0.5–10.0). Twenty-one clinics responded that they only used water-based contrast during HSG. The following water-based contrast media were used; Omnipaque® (six clinics), Ultravist® (four

clinics), Visipaque® (three clinics), Iomeron® (two clinics), Hexabrix®, Telebrix®, Xenetix®, Omnipaque®/Visipaque® (one clinic), while the remaining two clinics did not specify the type of water-based contrast media used. The total amount of HSGs performed with water-based contrast medium in 2017 was 1876. The remaining two clinics responded that they used a combination of the two contrast media: they perform an extra flushing with Lipiodol® Ultra-Fluid after diagnosing tubal patency with the use of water-based contrast.

The median amount of contrast used was 8.0 mL (IQR 7.0–10.0) in the clinics that used oil-based contrast and 10.0 mL (IQR 10.0–10.0) in the clinics that used water-based contrast (P-value 0.09). One clinic used an infusion pump, while the other 66 clinics performed a manual instillation of the contrast medium.

In 54% of the clinics (36/67) complications were registered in the electronic patient file. A total of 37% of the clinics (25/67) did not register the complications that occurred during HSGs. Six clinics did not respond to this question. The reported complications that occurred after HSGs with oil and water-based contrast are displayed in Table 1. The overall complication rate was 5.1% in the clinics that used oil-based contrast versus 1.8% in the clinics that used water-based contrast (RR 2.8, CI 1.9–4.0, P-value <0.0001). The most reported complication was intravasation, which occurred in 4.8% of the HSGs performed with oil-based contrast and 1.3% of the HSGs) performed with water-based contrast (RR 3.6, CI 2.4–5.4, P-value <0.0001). All cases with intravasation were reported as asymptomatic, without leading to pulmonary embolism or death. In 0.3% of the HSGs with oil-based contrast and in 0.4% of the HSGs with water-based contrast, a PID occurred (RR 0.7, CI 0.3–2.0, P-value 0.54). In these cases, antibiotic treatment and/or hospital admission took place. Anaphylactic reactions were not reported at all. Allergic reactions occurred in one of the HSGs with the use of oil-based contrast and two with water-based contrast (RR 0.3, CI 0.03–3.1, P-value 0.30). One clinic reported vasovagal reactions as other complications (8.7%, 15/172 women).

The complication rate was not significantly different between clinics with and without a standard registration of complications. The incidence of intravasation in 36 clinics with standard registration was 4.4% (62/1412) with oil-based contrast and 1.3% (15/1131) with water-based contrast, compared to 5.6 % (88/1579) and 1.3% (10/745) in 25 clinics without standard registration, respectively. The relative risk of intravasation when comparing clinics with standard registration to clinics without registration was 0.8 (CI 0.6–1.1, P-value 0.14) with oil-based contrast and 1.0 (CI 0.5–2.2, P-value 1.00) with water-based contrast.

Table 1. *The incidence of the reported complications for HSGs with oil- and water-based contrast, as reported in a nationwide survey of clinics.*

	Oil-based contrast (N = 3,289)	**Water-based contrast (N= 1,876)**	**Relative Risk (95% CI)**	**P-value***
Intravasations	157 (4.8%)	25 (1.3%)	3.6 (2.4 – 5.4)	<0.0001
Embolism	0 (0%)	0 (0%)	-	-
Allergic reactions	1 (0.03%)	2 (0.1%)	0.3 (0.03 – 3.1)	0.30
Anaphylactic reactions	0 (0%)	0 (0%)	-	-
Pelvic Inflammatory Disease	9 (0.3%)	7 (0.4%)	0.7 (0.3 – 2.0)	0.54
Sum of complications	167 (5.1%)	34 (1.8%)	2.8 (1.9 – 4.0)	<0.0001
Women without any complication	3,122 (94.9%)	1,842 (98.2%)	1.0 (0.96 – 0.98)	<0.0001

*Pearson chi-square-test or Fisher's exact test as appropriate.

Antibiotic prophylaxis

In 61% of the clinics (41/67) antibiotic prophylaxis was prescribed, while 39% of the clinics (26/67) did not use prophylactic antibiotics. Among the clinics that prescribe antibiotics there were different indications for prophylactic antibiotics.

In 15% of the clinics (6/41, 444 women) all patients received antibiotic prophylaxis (risk of infection 0.0% [0/444]), while in 61% of the clinics (25/41, 2533 women) patients with a high risk of tubal pathology (based on their medical history, a positive chlamydia antibody titre [CAT], or a positive sexual transmitted disease [STD] Polymerase chain reaction [PCR]) received antibiotic prophylaxis (risk of infection 0.3% [6/2389]). In 12% of the clinics (5/41, 308 women) also patients with an unknown STD PCR outcome or unknown CAT received antibiotic prophylaxis (risk of infection 0.6% [2/308]. In 7% of the clinics (3/41, 259 women) antibiotics were prescribed after the procedure, in case of tubal pathology diagnosed on HSG (risk of infection 0.4% (1/259). Two clinics (2/41) did not specify the indication for antibiotic prophylaxis, and in these clinics the risk of infection was 1.8% (3/171). The overall incidence of PID in the clinics who used prophylactic antibiotics was 0.3% (12/3571). The incidence of PID in the clinics that did not routinely prescribe antibiotic prophylaxis was 0.2% (4/1949).

Safety precautions

In 40% of the clinics (27/67), a crash cart was available in the HSG room to provide first aid in case of, for example, an allergic reaction, while 55% (37/67) did not have such

a facility available during the performance of HSGs. Three clinics did not respond to this question.

DISCUSSION

In this nationwide retrospective analysis of 5165 women undergoing HSG, the overall complication rate was 5.1% after HSG performed with oil-based contrast and 1.8% with water-based contrast. The most frequently reported complication was intravasation in 4.8% of the HSGs performed with oil-based contrast and 1.3% of the HSGs performed with water-based contrast (OR, 3.6; 95% CI, 2.4–5.4, P-value <0.0001). All cases of intravasation were asymptomatic. In this analysis of 5165 women undergoing HSGs, oil-embolisms or other clinical consequences of intravasation were not observed.

Limitations

The main limitation of our study is its retrospective design. Because half of the clinics have no standard complication registration, the incidence of the HSG-related complications presented here is partly based on the recall of the clinician. However, the estimated rates in the clinics with and without systematic registration did not significantly differ. The incidence of thyroid dysfunction after HSG is not reported because no standard screening of post-HSG thyroid function is performed in the Netherlands. The survey asked about the frequency of intravasation; however, no classification system is being used in daily practice, which may create differences in reporting. Intravasation is defined as the passage of contrast media into the veins or the lymphatics, and an intravasation severity score was proposed in 2013 by (Dusak *et al.*, 2013), however this system has not been implemented in daily practice.

Clinical implications

A higher incidence of intravasation in HSGs with oil-based contrast media in comparison to water-based contrast media, as shown in our results, is in line with recent evidence. A recent meta-analysis of three RCTs, reporting on 793 HSGs, compared the risk of intravasation with oil-based contrast to the risk with water-based contrast. Pooling the data of these three studies showed that, compared with water-based contrast, oil-based contrast was associated with higher odds of intravasation (OR, 5.1; 95% CI, 2.3–11.2). All reported intravasations in this meta-analysis were asymptomatic without clinical consequences of an oil embolism (Wang *et al.*, 2019).

Despite this reassuring evidence, case reports are published on serious consequences of intravasation, including a pulmonary and cerebral oil embolus after an HSG with oil-based contrast: a remarkable fact is that no fluoroscopy guidance was used during

this HSG (Uzun *et al.*, 2004). This is of importance as real time fluoroscopy guidance might prevent oil embolism and its clinical consequences, when discontinuation of the HSG procedure is accomplished, in case of intravasation. This is confirmed in our study, which evaluates 5165 HSGs performed with fluoroscopy and shows no symptomatic intravasations. Therefore, fluoroscopy appears to be an essential safety measure during HSG.

Older publications suggest an increased incidence of symptomatic intravasation if large volumes of contrast medium are used. An amount of 4 to 6 mL with a maximum pressure of 180 to 200 mmHg was suggested (Eisen & Goldstein, 1945). Our study shows that the median amount of contrast used is 8.0 mL for the oil-based contrast (IQR 7.0–10.0) and 10.0 mL for the water-based contrast (IQR 10.0–10.0), compared to 9.0 mL (IQR 5.7–15.0) and 8.0 mL (IQR 5.9–13.0) for oil and water-based contrast, respectively, in the H2Oil study (Dreyer *et al.*, 2017). Hypothetically a higher amount of contrast could increase the risk of intravasation due to elevated pressure in the uterus. However, both in our study and the H2Oil study embolisms were not reported.

In none of the more than 5000 HSGs performed did an anaphylactic reaction occur. The incidence of PID was low and did not differ between clinics that routinely prescribe antibiotic prophylaxis or not, and was also not different after HSGs with oil-based or water-based contrast. In the literature, a PID incidence of 0.5% after an HSG with antibiotic prophylaxis and 1.4% without prophylaxis is described (Li *et al.*, 2018). In addition, two RCTs (Lindequist et al., 1994; Rasmussen et al., 1991) reported on PID after HSG using water-based compared to oil-based contrast. Pooled analysis showed that there was insufficient evidence of differences in PID incidence between these two contrast media (OR 0.23; 95% CI, 0.04–1.27) (Wang *et al.*, 2019).

Both types of contrast contain iodine, which can affect thyroid function after HSG (Mekaru et al., 2008). The concentration of iodine is higher in oil-based contrast (480 mg Iodine/mL) as compared to water-based contrasts (240 to 300 mg Iodine/mL, depending on the pharmaceutical company). A cohort study demonstrated that women who had a subclinical hypothyroidism prior to HSG have a higher risk of developing hypothyroidism after an HSG, compared with euthyroid women; 35.7% versus 2.2%, respectively (Mekaru et al., 2008). Subclinical hypothyroidism has been associated with different pregnancy complications, including an increased risk of (recurrent) miscarriage (van den Boogaard *et al.*, 2011). However, two meta-analysis comparing oil-based with water-based contrast did not show an increased risk of miscarriage with the use of oil-based contrast (Fang *et al.*, 2018; Wang *et al.*, 2019).

Fifteen of the 44 clinics that use oil-based contrast, started using oil-based contrast in mid-2017, which is shortly after the publication of the H2Oil study results (Dreyer *et al.*, 2017). We can conclude that this publication has significantly changed the daily practice of fertility clinics in the Netherlands.

Despite the fact that there were no consequences of intravasation in our study, we advise to use the minimum amount of contrast needed for the diagnosis of tubal patency. Furthermore, we suggest additional research in the form of prospective studies on thyroid function after HSG in a western population and studies on the mechanism of action of oil-based contrast (Lipiodol® Ultra-Fluid).

CONCLUSION

This retrospective analysis of 5165 HSGs performed in the Netherlands during a single book year, 2017, shows that serious complications after HSGs using oil-based contrast media and water-based contrast media are rare. The most frequent complication was intravasation (4.8% with oil-based contrast, 1.3% with water-based contrast), without any clinical consequences. Presumably, the use of fluoroscopy during HSGs may contribute to the prevention of embolism and death due to intravasation. The incidence of maternal and neonatal thyroid dysfunction is not reported. Further research, especially on maternal and neonatal thyroid function after HSG in a western population, is needed.

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SUPPLEMENTARY DATA

Translated questionnaire

1. What is your position?
   ☐ Gynaecologist
   ☐ Gynaecologist in training (in Dutch: AIOS)
   ☐ Doctor not in training for gynaecologist (in Dutch: ANIOS)
   ☐ Fertility doctor
   ☐ Other: _____

2. How many HSGs did your clinic perform in 2017? _____ HSGs

3. Who described the HSG images?
   ☐ Gynaecologist
   ☐ Radiologist
   ☐ Gynaecologist and radiologist
   ☐ Other: _____

4. Does your clinic use an infusion pump for the HSG procedure?
   ☐ Yes
   ☐ No, the contrast is infused manually

5. Which contrast medium does your clinic use standardly during HSGs?
   ☐ Oil-based contrast
   ☐ Water-based contrast, namely: _____

6. How long has your clinic been using oil-based contrast during HSGs?
   Since _____ months/years

7. What is the average amount of contrast used per HSG procedure _____ mL.
   (1 ampoule Lipiodol contains 10 mL)

8. Which maternal complications have occurred after HSGs? And how often did they occur?

Complication:	**Frequency in 2017:** *In case no exact number of complications of 2017 is known, make an estimation of the frequency.*
☐ Allergic reactions	_____ times/year
☐ Anaphylactic shock	_____ times/year
☐ Intravasation	_____ times/year
☐ Embolisation	_____ times/year
☐ Pelvic Inflammatory Disease (PID)	_____ times/year
☐ Thyroid dysfunction	_____ times/year
☐ Other:_____	_____ times/year
☐ No complications	_____ times/year

9. Is there a standardized registration of the maternal complications?
   ☐ Yes, they are registered on: _____
   ☐ No

10. What were the consequences of these complication(s)?
    ☐ Antibiotic treatment (per os / intravenous)
    ☐ Follow-up as an outpatient
    ☐ Hospital admission, amount of days: _____

11. Do you provide antibiotic prophylaxis before or after the HSG, and for which indications?
    ☐ Yes, indications: _____
    ☐ No

12. Is a crash cart available in the room where the HSGs are performed?
    ☐ Yes, this crash cart contains: _____
    ☐ No

Thank you for your cooperation!

For questions and remarks mail to: Inez.roest@mmc.nl

CHAPTER 4

THYROID FUNCTION IN NEONATES CONCEIVED AFTER HYSTEROSALPINGOGRAPHY WITH IODINATED CONTRAST

N. van Welie, I. Roest, M. Portela, J. van Rijswijk,
C. Koks, C.B. Lambalk, K. Dreyer, B.W.J. Mol, M.J.J. Finken,
V. Mijatovic and the H2Oil Study Group

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ABSTRACT

Study question

Does exposure to preconceptional hysterosalpingography (HSG) with iodinated oil-based contrast affect neonatal thyroid function as compared to iodinated water-based contrast?

Summary answer

Preconceptional HSG with iodinated contrast did not influence the neonatal thyroid function.

What is known already

HSG is a commonly applied tubal patency test during fertility workup in which either oil- or water-based contrast is used. Oil-based contrast contains more iodine compared to water-based contrast. A previous study in an East-Asian population found an increased risk of congenital hypothyroidism (CH) in neonates whose mothers were exposed to high amounts of oil-based contrast during HSG.

Study design, size, duration

This is a retrospective data-analysis of the H2Oil study, a randomised controlled trial (RCT) comparing HSG with the use of oil- versus water-based contrast during fertility workup. After an HSG with oil-based contrast, 214 women had an ongoing pregnancy within 6 months leading to a live birth compared to 155 women after HSG with water-based contrast.

Participants/materials, setting, methods

Of the 369 women who had a live born infant, 208 consented to be approached for future research and 138 provided informed consent to collect data on the thyroid function tests of their offspring (n = 140). Thyroid function tests of these children were retrieved from the Dutch neonatal screening program, which includes the assessment of total thyroxine (T4) in all newborns, followed by thyroid-stimulating hormone (TSH) only in those with a T4 level of ≤-0.8 SD score. Furthermore, amount of used contrast medium used and time between HSG and conception were compared between the two study groups.

Main results and the role of chance

Data were collected from 140 neonates conceived after HSG with oil-based (n = 76) or water-based contrast (n = 64). The median T4 concentration was 87.0 nmol/l (interquartile range [IQR] 76.0-96.0) in the oil-group versus 90.0 nmol/l (IQR 78.0-106.0) in the water-group (P-value 0.13). None of the neonates had a positive screening result for congenital hypothyroidism.

The median amount of used contrast was 9.0 mL (IQR 6.0 -11.8) in the oil-group and 10.0 mL (IQR 7.5–14.0) in the water-group (P-value 0.43). No influence of the amount of contrast on the effect of contrast group on T4 concentrations was found (P-value for interaction 0.37).

Limitations, reasons for caution

A relatively small sample size, and possible attrition at follow-up are limitations of this study. Although our results suggest that the use of iodinated contrast media for HSG is safe for the offspring, the impact of a decrease in maternal thyroid function on offspring neurodevelopment could not be excluded, as data on maternal thyroid function after HSG and during conception were lacking.

Wider implications of the findings

As HSG with oil-based contrast does not affect thyroid function of the offspring, there is no reason to withhold this contrast to infertile women undergoing HSG. Future studies should investigate whether HSG with iodinated contrast influences the periconceptional maternal thyroid function and, consequently, offspring neurodevelopment.

INTRODUCTION

Hysterosalpingography (HSG), to assess tubal patency, is a standard test during female fertility workup. Although it was introduced as a diagnostic tubal patency test, it recently became clear that HSG increases ongoing pregnancy rates, especially after the use of oil-based contrast (Fang*, et al.*, 2018, Wang*, et al.*, 2019). All contrast media used for HSG are rich in iodine, with oil-based contrast containing more iodine (480 mg Iodine/mL) than water-based contrasts (ranging from 240 to 300 mg Iodine/mL, depending on the manufacturer). In addition, the clearance of oil-based contrast in the abdomen is slower than that of water-based contrast (Brown*, et al.*, 1949, Miyamoto*, et al.*, 1995).

Previous studies found that HSG resulted in a long-lasting suppression of thyroid hormone synthesis in euthyroid women, and, even more profoundly, in women with subclinical hypothyroidism (Mekaru*, et al.*, 2008, Kaneshige*, et al.*, 2015, So*, et al.*, 2017). Subclinical hypothyroidism has been associated with an increased risk of pregnancy complications, including pre-eclampsia, perinatal mortality and (recurrent) miscarriage (van den Boogaard*, et al.*, 2011, Korevaar*, et al.*, 2019). Up till now two subsequent systematic reviews showed no increased risk of miscarriage or stillbirth in women exposed to oil-based contrast at HSG, which is reassuring (Fang*, et al.*, 2018, Wang*, et al.*, 2019).

There is some evidence showing that maternal iodine excess due to high dietary iodine intake or iodine-containing antiseptics may put offspring at risk of congenital hypothyroidism (CH) (l'Allemand*, et al.*, 1983, Nishiyama*, et al.*, 2004, Connelly*, et al.*, 2012, Hamby*, et al.*, 2018). To date, surprisingly few studies have focussed on the impact of oil-based contrast during HSG on the neonatal thyroid function; all of them were conducted in Asian populations, who are known to consume diets rich in iodine. One study from Japan reported a high risk of CH (of 2.4%, as compared to 0.7% in the norm population) in neonates whose mothers were exposed to high amounts of oil-based contrast medium during HSG (Satoh*, et al.*, 2015). The other studies described associations between HSG and the presence of fetal goiter or transient thyroid dysfunction at birth, but not with permanent thyroid dysfunction (Omoto*, et al.*, 2013, Sasaki*, et al.*, 2017). Indeed, neonates born to mothers exposed to HSG had a higher urinary excretion of iodine (Li*, et al.*, 2018).

We recently published the results of a large RCT (under the acronym H2Oil study) investigating the effects of oil- versus water-based contrast in women undergoing HSG as part of fertility workup on live birth rates, indicating that the first was superior (Dreyer*, et al.*, 2017). The present study investigated the thyroid function in their offspring at birth.

METHODS

This is a retrospective data analysis of neonatal screening results for CH in the offspring of mothers participating in the H2Oil study who conceived within 6 months after HSG (NTR 3270). For this purpose, neonatal screening results were retrieved from the Dutch National Institute for Public Health and Environment (in Dutch: Rijksinstituut voor Volksgezondheid en Milieu [RIVM]). This specific study (NTR 7526) was approved by the Institutional Review Board of the Amsterdam University Medical Centre – VU University Medical Centre, the Netherlands (reference 2018.463, dated 7 September 2018).

Participants

The H2Oil study is a multicenter RCT comparing oil-based contrast (Lipiodol® Ultra Fluid, Guerbet France, containing 480 mg Iodine/mL) with water-based contrast (Telebrix Hystero®, Guerbet France, containing 250 mg Iodine/mL) in women undergoing HSG during fertility workup. Details of the H2Oil study have been published elsewhere (Dreyer, *et al.*, 2017). Here, we only briefly describe the trial essentials. Infertile women between 18 and 39 years of age with spontaneous menstrual cycles were included in the H2Oil study. Known endocrine disorders (e.g. hyperthyroidism) were among the exclusion criteria. No routine screening of the thyroid function was performed.

A total of 1119 women were randomised to receive HSG with oil-based contrast (n = 557) or water-based contrast (n = 562) (Supplementary Figure 1). After HSG with oil-based contrast, within 6 months 214 women had an ongoing pregnancy leading to a live birth compared to 155 women after HSG water-based contrast (Dreyer, *et al.*, 2017). Of these women, 208 (56%) had given permission to be approached for future research.

Parents were approached by postal mail, containing information on this study. For the retrieval of the neonatal screening results, both parents or legal guardians had to give written informed consent. Additionally, they were also asked to provide additional information of the medical history of their child, including previous or current thyroid hormone supplementation. Parents who did not respond within two weeks were sent a reminder.

Study outcomes

The main outcome was the neonatal total thyroxine (T4) concentration (nmol/l). Other outcomes were, if available, concentrations of thyroid-stimulating hormone (TSH) (mU/L) and thyroxine-binding globulin (TBG) (nmol/l).

Statistical analysis

Demographic characteristics of the study population were compared between the two study groups using the appropriate descriptive statistics. Categorical data were reported as absolute numbers with percentages (%), and continuous variables as medians with interquartile ranges (IQRs). Dichotomous outcomes were compared using the chi-square test, and continuous outcomes using the independent t-test or Mann–Whitney U-test as appropriate. We tested whether amount of contrast or time between HSG and conception modified the effect of contrast medium on neonatal T4 concentration. Effect modification by amount of contrast was tested using a linear regression model with T4 concentration as the dependent variable and amount of contrast, type of contrast (oil versus water) and their two-way interaction as independent variables. Effect modification by time between HSG and conception was tested using analysis of variance (ANOVA) with T4 concentrations as the dependent variable and time between HSG and conception, type of contrast (oil versus water) and their two-way interaction as independent variables. A P-value less than 0.05 was considered statistically significant. Box- and scatterplots were used to visualize the investigated associations. The IBM Statistical Package for Social Sciences (SPSS) version 26.0 was used for all statistical analyses (IBM Corp., USA).

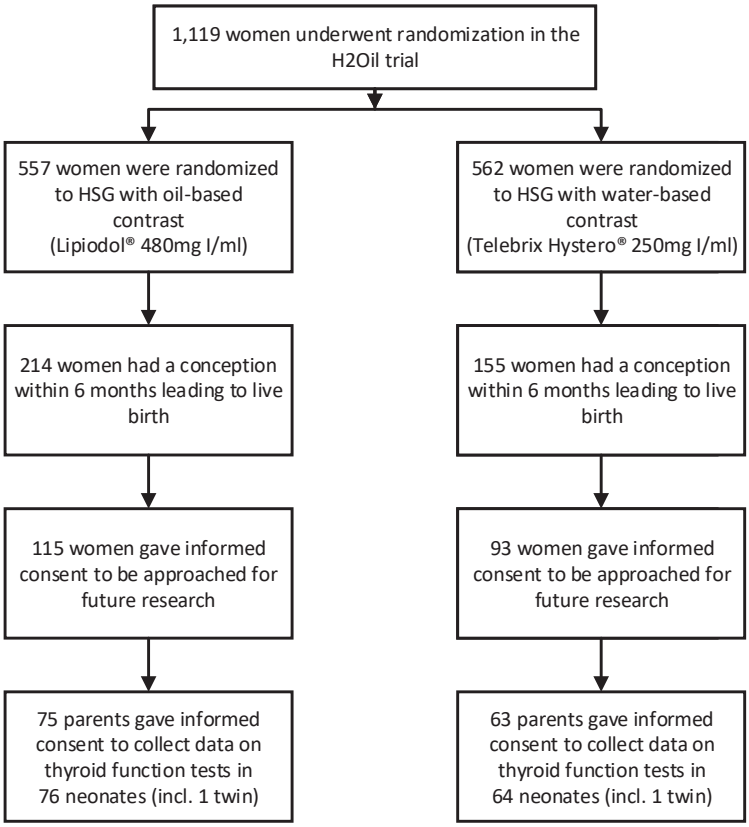
Neonatal CH screening

The Dutch neonatal screening for CH is primarily based on T4 measurement in filter paper blood spots obtained during the heel prick at 4 to 7 days after birth. Details of the Dutch CH screening program have been described by (Kempers*, et al.*, 2006). In summary, T4 concentrations are expressed as standard deviation score (SDS) from the daily mean. This is the standard screening procedure for CH in the Netherlands (Verkerk*, et al.*, 2014). The daily means are used instead of population reference means, to account for fluctuation in laboratory measurements. If the T4 level is -0.8 SDS or less, the TSH concentration is measured as well. This is accompanied by TBG concentration when T4 is -1.6 SDS or less. Newborns with abnormal screening results are immediately referred to a paediatrician. In case of a dubious result, a second heel prick is performed, after which the child is referred if the result is dubious again or abnormal.

RESULTS

In the oil group, 75 (65.2%) of the 115 parents gave informed consent to collect data on the thyroid function tests of their children (n = 76). In the water group, 63 (67.7%) of the 93 parents gave informed consent to collect these data (n = 64) (Figure 1). The baseline characteristics were comparable between the two groups (Supplementary Table 1). Non-responders were not different from responders in baseline characteristics.

Figure 1. *Flowchart of the study*



None of the neonates conceived after HSG with oil- or water-based contrast had a positive screening result for CH. Their data are presented in Table 1. T4 concentrations and T4 SDSs were comparable between the two groups. None of the children were currently on thyroid hormone supplementation.

The amount of contrast used for HSG was reported in 44 women in the oil group versus 25 women in the water group. The median amount of contrast was 9.0 mL (IQR 6.0–11.8) in the oil group and 10.0 mL (IQR 7.5–14.0) in the water group (P-value 0.43). Linear regression showed no influence of the amount of contrast on the effect of the contrast group on T4 concentrations (P-value for interaction 0.37). Figure 2a and 2b depict the association of neonatal T4 concentrations with the amounts of oil-based or water-based contrast used during HSG.

There was a significant difference in iodine content between the two contrast media used (4.3 grams [IQR 2.9–5.7] versus 2.5 [IQR 1.9–3.5], P-value 0.001).

Time between HSG and conception was comparable between the oil and water groups (2.3 months [IQR 1.1–4.3] and 2.1 months [IQR 1.1–4.0]: P-value 0.83). ANOVA showed no influence of time between HSG and conception on the effect of the contrast group on T4 concentrations (P-value for interaction 0.47).

Consequently, time between HSG and delivery did not differ between the two groups (11.1 months [IQR 9.6–13.0] in the oil group versus 10.7 months [IQR 9.8–12.9] in the water group, P-value 0.73).

Table 1. *Clinical data of neonates conceived after hysterosalpingography (HSG) with the use of oil- or water-based contrast.*

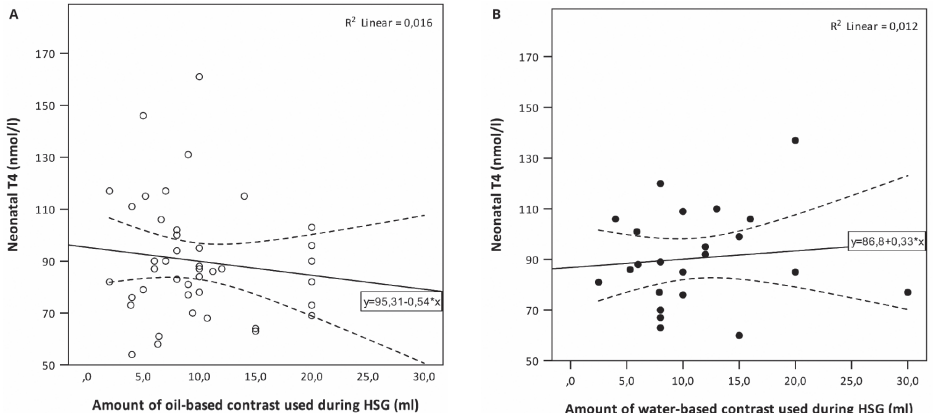
	Neonates born after HSG with		
	Oil contrast (n=76)	**Water contrast (n=64)**	**P-value**
Gestational age (weeks)	39.7 [39.0–40.9]	39.6 [38.6–40.7]	0.27
Birthweight[a] (grams)	3470 [3115–3855]	3460 [3065–3721]	0.67
Sex			
Male	38 (50)	30 (47)	0.71
Female	38 (50)	34 (53)	-
Current use of thyroid hormones	0 (0)	0 (0)	-
Neonatal screening[b]			
T4 (nmol/l)	87.0 [76.0–96.0]	90.0 [78.0–106.0]	0.13
T4 SDS[c]	-0.05 [-0.5–0.5]	0.2 [-0.3–0.9]	0.12
Amount of contrast (mL)[d]	9.0 [6.0–11.8]	10.0 [7.5–14.0]	0.43
Iodine dose (grams)[e]	4.3 [2.9-5.7]	2.5 [1.9-3.5]	0.001
Duration between HSG and conception (months)	2.3 [1.1–4.3]	2.1 [1.1–4.0]	0.83
Duration between HSG and delivery (months)	11.1 [9.6–13.0]	10.7 [9.8–12.9]	0.73

Data presented as median [quartiles] or number of women (%). [a]Birth weight was missing in one neonate in the water group. [b]Neonatal screening result was missing in one neonate in the water group, due to neonatal screening abroad. [c]The concentration of T4 is expressed as standard deviation score (SDS) and is compared with the daily mean. [d]Amount of contrast was missing in 32 in the oil group versus 39 women in the water group. [e]The calculated iodine dose is strictly correlated to the amount of contrast medium used (Lipiodol® 480 mg Iodine/mL and Telebrix Hystero® 250 mg Iodine/mL).

However, in 13 neonates in the oil group and 7 neonates in the water group T4 SDSs were ≤-0.8, and, therefore, TSH was measured (Relative Risk [RR], 1.5; 95% CI 0.7–3.6; P-value 0.32). TSH concentrations were within normal limits for all 20 neonates with T4 SDSs ≤-0.8. In one neonate in the oil group, TBG was additionally measured. Both TBG and T4/TBG ratio were within normal limits. Table 2 shows no differences in the oil group in amount of contrast or duration between HSG and conception among neonates with normal screening results and those with T4 values ≤-0.8 SD, low enough to trigger TSH testing. We found comparable results for the water group (Table 2).

Furthermore, no differences were seen in amount of contrast or duration between HSG and conception within the neonates with T4 ≤-0.8 SDS in the oil group versus the water group.

Figure 2. *Association of neonatal T4 level with the amount of contrast used for oil-based contrast (A) and water-based contrast (B)*



The scatterplots show the association of neonatal T4 concentrations with the amount of oil-based contrast (A) versus water-based contrast (B) used during HSG. Both scatterplots show the linear regression lines (solid lines) with their uncertainty (dotted lines).

Table 2. *Clinical data of neonates with normal T4 or T4 ≤−0.8 SD in the oil (n = 76) and water (n = 64) group.*

	Neonates in the oil group			*Neonates in the water group*		
	Normal T4 >-0.8 SD (n=63)	**T4 ≤-0.8 SD (n=13)**	**P-value**	**Normal T4 >-0.8 SD (n=56)**	**T4 ≤-0.8 SD (n=7)**	**P-value**
Neonatal screening						
- T4 (nmol/l)	88.0 [81.0–102.0]	61.0 [54.5–70.5]	-	92.0 [84.0–106.0]	68.0 [63.0–71.0]	-
- T4 SDS[a]	0.2 [-0.2–0.7]	-1.1 [-1.4–-0.9]	-	0.3 [-0.2–1.0]	-0.9 [-1.1– -0.8]	-
- TSH[b]*(mU/L)*	-	2.0 [1.0-2.5]	-	-	1.0 [1.0-2.0]	-
Amount of contrast (mL)[c]	9.0 [6.0–11.0]	10.0 [6.3–20.0]	0.53	10.0 [7.0–14.0]	8.0 [8.0–11.5]	1.00
Iodine dose (grams)[d]	4.3 [2.9–5.3]	4.8 [3.0–9.6]	0.53	2.5 [1.7–3.5]	2.0 [2.0–2.9]	1.00
Duration between HSG and conception (months)	2.4 [1.1–4.5]	1.9 [0.7–3.5]	0.36	2.2 [1.1–4.1]	1.9 [1.2–2.9]	0.58
Duration between HSG and delivery (months)	11.2 [9.6–13.3]	10.8 [9.1–12.1]	0.16	10.9 [9.8–13.0]	10.6 [9.7–11.6]	0.56

Data presented as median [quartiles]. [a]The concentration of T4 is expressed as SDS and is compared with the daily mean. [b]T4 values ≤−0.8 SD, low enough to trigger TSH testing. [c]Amount of contrast was missing in 26 versus 6 women in the oil-group and 35 versus 4 women in the water-group. [d]The calculated iodine dose is strictly correlated to the amount of contrast medium used (Lipiodol® 480 mg Iodine/mL and Telebrix Hystero® 250 mg Iodine/mL).

DISCUSSION

In this study, we found that preconceptional exposure to an HSG with oil-based or water-based contrast did not result in decreased thyroid function in the offspring. In addition, we did not find an impact of the amount of contrast used or the duration between HSG and conception on neonatal T4 concentration between the treatment arms. Our results are not in line with previous studies in East-Asian populations.

A Japanese study found a higher frequency of thyroid dysfunction in newborns conceived after HSG compared to normative data (2.4% versus 0.7%) (Satoh*, et al.*, 2015). In this study, mothers giving birth to offspring with thyroid dysfunction had been exposed to a higher amount of contrast during HSG (median of 20 mL versus 8 mL), although used amount was only available for 112 out of 212 neonates with normal thyroid function, and for 3 out of 5 neonates with thyroid dysfunction (Satoh*, et al.*, 2015). To the best of our knowledge, only two Japanese cases with fetal goiter after maternal HSG were reported (Omoto*, et al.*, 2013, Sasaki*, et al.*, 2017), although according to Omoto et al. "at least 17 cases of transient hypothyroidism in a fetus after

HSG have been reported in Japanese literature since 1990" (Omoto*, et al.*, 2013). In one of these fetuses the goiter resolved during pregnancy, and the thyroid function tests were normal at birth (Sasaki*, et al.*, 2017). In the other the fetal goiter persisted, and overt hypothyroidism was noted at birth. This was followed by a spontaneous resolution of the goiter by 4 weeks post-partum along with normalization of thyroid function tests in the preceding weeks (Omoto*, et al.*, 2013). None of the children in our sample were diagnosed with goiter as newborns.

As stated earlier, all studies conducted thus far were limited to East-Asian populations. There is a striking difference in background risk for CH between Japan and the Netherlands, i.e., 0.7% in Japan versus 0.05% in the Netherlands (Tokyo-Health-Service-Association, 2010, Dutch-National-Institute-for-Public-Health-and-Environment, 2014). Among the possible explanations for this difference is the high consumption of iodine-rich foods (i.e., seaweed) in Japan. It has been estimated that the iodine intake of pregnant women in Japan is approximately 3-4 times as high as the World health Organization recommendation (World Health Organization, 2007, Fuse*, et al.*, 2013).

Strengths and limitations

The current study has several strengths and limitations. The major strengths are that this study was based on a large multicenter RCT, and had included a large majority of Caucasian women. Additionally, in contrast to other countries, which generally have TSH-based screening programs, the Dutch neonatal screening program for CH is T4-TSH-TBG based, being able to detect CH of both central and thyroidal origin. The amount of contrast used during HSG was reported, instead of the calculated iodine dose, as the amount of contrast is relevant for clinicians in daily practice and iodine dose is strictly correlated to the amount of contrast used.

Limitations of our study are the relatively small sample size and attrition at follow-up. This might obscure a possible relation between the type of contrast medium and the presence of CH if women with excessive iodine exposure selectively declined to participate. However, a non-response analysis showed that responders and non-responders did not differ in a number of the baseline characteristics, implicating that non-response bias is unlikely to have materially influenced our observations, although the amount of contrast used was not known from all participants. The H2Oil RCT was not powered to study the safety in neonates of the different types of iodinated contrast media during HSG. Nonetheless, data regarding the neonatal thyroid function are reliable even though they were collected retrospectively.

Furthermore, only offspring conceived within 6 months after HSG were included (Dreyer, *et al.*, 2017). Offspring conceived between 6 months and 5 years after HSG, who also took part in the H2Oil follow-up study, were not contacted for this specific study (Van Rijswijk, *et al.*, 2018). It is unlikely that iodinated contrast could still affect the offspring's thyroid function when more time than 6 months has elapsed between HSG and conception (Kaneshige, *et al.*, 2015, So, *et al.*, 2017).

Our study did not include an assessment of the maternal thyroid function after HSG or during conception. Consequently, it was impossible to study the impact of a decrease in the maternal thyroid function on offspring neurodevelopment. Studies in East-Asian populations demonstrated that HSG could result in a long-lasting suppression in thyroid hormone synthesis in euthyroid women, and, even more profoundly, in women with subclinical hypothyroidism (Mekaru, *et al.*, 2008, Kaneshige, *et al.*, 2015, So, *et al.*, 2017). Furthermore, the use of iodine-rich products preconceptionally, during pregnancy or after delivery was not registered, which could potentially influence our findings.

Implications

Overexposure to iodine may result in a sudden cessation of thyroid hormone synthesis, a phenomenon called the Wolff-Chaikoff effect (Eng, *et al.*, 1999). This protective mechanism works for a couple of days, after which thyroid hormone synthesis is resumed. However, during prolonged exposure to excess iodine, the thyroid gland is unable to escape from the Wolff-Chaikoff effect, resulting in a long-lasting suppression of thyroid hormone synthesis (Wolff, *et al.*, 1949, Markou, *et al.*, 2001, Leung and Braverman, 2012).

From the second trimester of pregnancy the fetal thyroid gland starts to produce thyroid hormones. Therefore, during early embryonic development the fetal brain depends entirely on the supply of maternal thyroid hormones (Contempre, *et al.*, 1993, Burrow, *et al.*, 1994). Consequently, overexposure to iodine may not only disrupt fetal brain development through inhibition of fetal thyroid hormone synthesis but also through its effects on the maternal thyroid gland. This is suggested by an increasing body of evidence demonstrating that children born to mothers with decreased thyroid function during the first half of pregnancy had reductions in the achievement of developmental milestones, IQ score, reaction time, scholastic performance, and attention, although the evidence was not unequivocal (Haddow, *et al.*, 1999, Pop, *et al.*, 1999, Smit, *et al.*, 2000, Pop, *et al.*, 2003, Oken, *et al.*, 2009, Henrichs, *et al.*, 2010, Li, *et al.*, 2010, Craig, *et al.*, 2012, Noten, *et al.*, 2015, Oostenbroek, *et al.*, 2017, Thompson, *et al.*, 2018). Therefore, monitoring of the maternal thyroid function after HSG might seem warranted, but at this point no definite conclusion can be drawn.

CONCLUSION

In contrast to previous research in East-Asian populations, we found that preconceptional HSG with iodinated contrast did not influence the neonatal thyroid function. Although this suggests that iodinated contrast media are safe for the offspring, indirect effects on neurodevelopment (through inhibition of maternal thyroid hormone synthesis) could not be excluded and warrant further investigation. In the meanwhile, there is no reason to withhold HSG with oil-based contrast to infertile women. We recommend keeping the amount of contrast used as low as possible.

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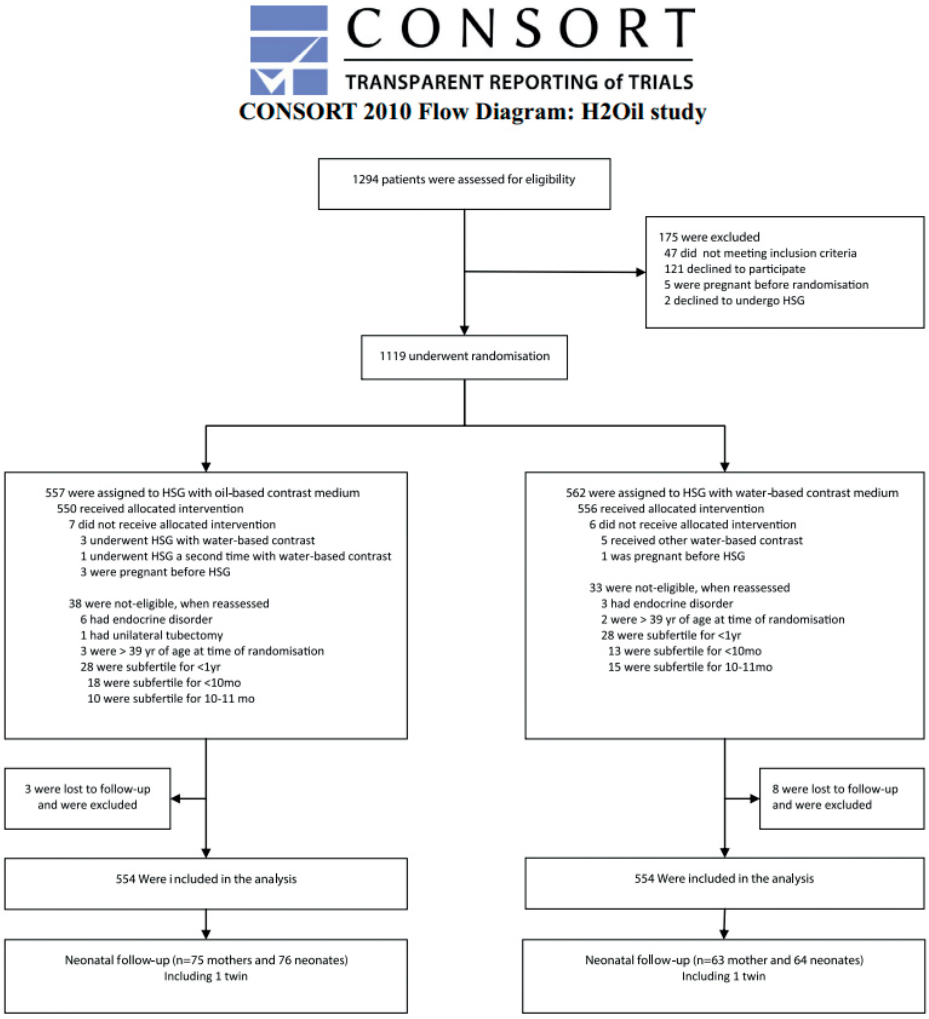
SUPPLEMENTARY DATA

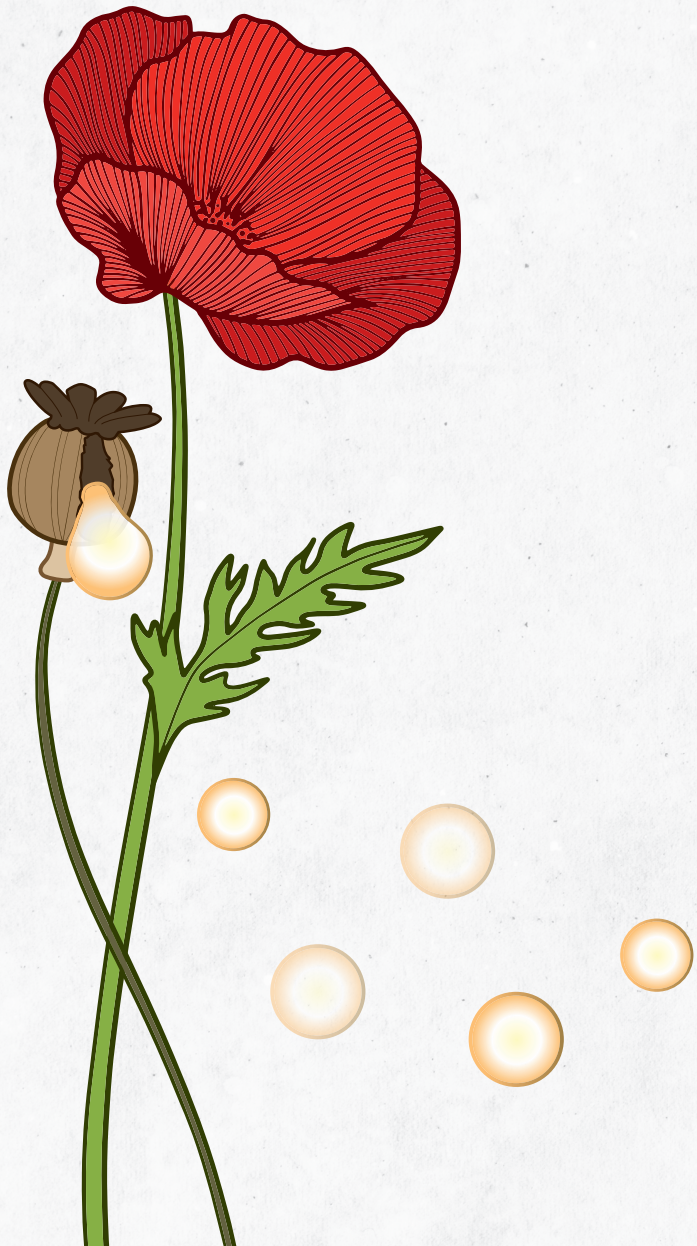
Supplementary Figure 1. *Flow diagram for the H2Oil study.*



Supplementary Table 1. *Baseline characteristics of women from the H2Oil study with a live birth after hysterosalpingography (HSG) who gave informed consent to collect data on thyroid function tests of their offspring.*

	Oil contrast (n=75)	**Water contrast (n=63)**
Age at randomization (years)	32.5 [28.9–35.3]	32.7 [30.3–35.2]
BMI[j] (kg/m^2)	22.2 [20.2–24.9]	23.0 [20.9–25.8]
Ethnicity[k]		
- *Caucasian*	65 (87)	50 (79)
- *Non-Caucasian*	1 (1)	4 (6)
- *Unknown*	9 (12)	9 (14)
Smoking[l]	8 (11)	5 (8)
Cycle duration (days)	28.0 [27.0–30.0]	28.0 [28.0–30.0]
Duration of infertility (months)	18.1 [15.8–21.4]	18.3 [15.0–27.0]
Primary infertility	54 (72)	44 (70)

The use of oil- or water-based contrast medium during HSG is compared. Data presented as median [quartiles] or number of women (%). [j]Data on BMI was missing for three women in the oil group versus seven women in the water group. [k]Ethnicity was reported by the clinicians. [l]Data on maternal smoking was missing for three women in the oil group and for four women in the water group.



CHAPTER 5

WHAT IS THE FERTILITY-ENHANCING EFFECT OF TUBAL FLUSHING?

A HYPOTHESIS ARTICLE

I. Roest, A.M. Hajiyavand, M.Y. Bongers,
V. Mijatovic, B.W.J. Mol, C.A.M. Koks, K.D. Dearn

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ABSTRACT

Hysterosalpingographies (HSGs) have formed an essential part of the fertility workup for more than a century. More recently, tubal flushing, especially with oil-based contrast, has been shown to significantly improve the natural conception rates. Critically, the mechanism of this fertility-enhancing effect during tubal flushing is still unclear. This article postulates hypotheses, based on published and own research, on the potential mechanisms and root cause of tubal flushing fertility-enhancement. Possible explanations for the increased fertility rates, especially with oil-based contrast, are divided into the biochemical and interfacial effects derived from the contrast properties. The biochemical effects may include the immunological response of the endometrium or peritoneum, the impact on the endometrial opioid receptors or the iodine content. The interfacial effects may include improvement of interfacial factors due to the lubricant effect or dislodgement of mucus debris within the Fallopian tubes.

IMPACT STATEMENT

What is already known on this subject?

Tubal flushing during HSGs increases natural conception rates, and using oil-based over water-based contrast increases that effect even further. However, the underlying mechanism of the observed fertility-enhancing effect is still poorly understood.

What the results of this study add?

This article postulates different hypotheses on the potential mechanisms and root cause of the fertility-enhancement from tubal flushing.

What the implications are of these findings for clinical practice and/or further research?

We suggest additional research on the different hypotheses, intending to determine which subfertile women will benefit most from tubal flushing using oil-based contrast and at which stage of their subfertility. Furthermore, we suggest research on administering tubal flushing with oil-based contrast, besides in HSG.

INTRODUCTION

Rindfleisch introduced Hysterosalpingography (HSG) in 1910 but only evaluated the uterine cavity by injecting a bismuth solution (Rindfleisch, 1910). In 1914, Cary was the first to describe tubal patency assessment using HSG with Collargol, a silver solution, as the contrast medium (Cary, 1914). It is, however, HSG's therapeutic potential, first suggested by William *et al.* that continues to gather interest (William *et al.*, 1951).

HSGs still have an essential role in daily practice and are advised for tubal patency testing in many countries (NICE Clinical guidelines [CG156] (NICE, 2017). Different contrasts are available for this diagnostic procedure, based on either water or oil-based medium.

Tubal flushing with oil-based contrast compared to no flushing significantly increases the live-birth rate, with an odds ratio of 3.3 (95% CI 1.6–6.9). Tubal flushing with water-based contrast may also increase the live birth rate compared to no flushing, with an odds ratio of 1.1 (95% CI 0.67–1.9)(Wang *et al.*, 2020). Additionally, a long term follow-up study of the largest RCT on the comparison of water-based versus oil-based contrast, showed that there were significantly more naturally conceived pregnancies in women who underwent an HSG with oil-based contrast compared to water-based contrast (van Rijswijk *et al.*, 2020).

Over the years, scientific reports have focused on differences in pregnancy outcomes between HSG contrasts, but the observed fertility-enhancing effect's underlying mechanism is not well understood. Most studies focus on women with unexplained subfertility and a smaller number on subfertile women with endometriosis or repeat implantation failure undergoing IVF (Johnson *et al.*, 2004; Brent *et al.*, 2006; Reilly *et al.*, 2019).

Knowledge of the mechanism of action of tubal flushing is essential, as it can help us understand which women, and at which stage of their subfertility, can benefit from tubal flushing. This paper aims to summarise the different hypotheses on the mechanisms of the fertility-enhancing effect of tubal flushing. In doing so, we also aim to clarify the knowledge gaps and to suggest topics for further research.

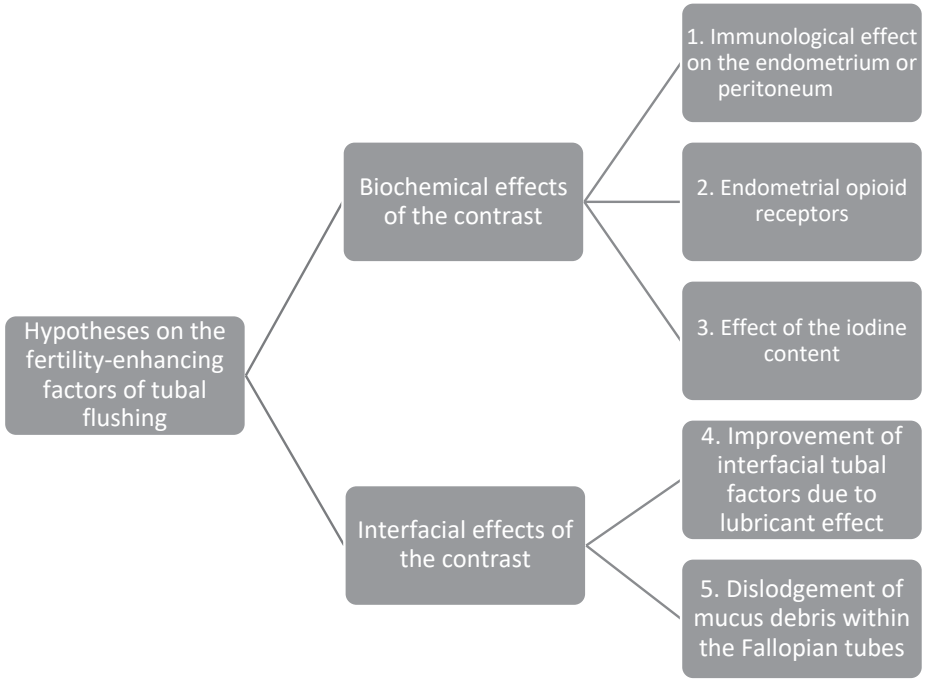
METHODS

Electronic databases including PubMed®, Scopus and Web of Science were searched for relevant publications. The key search items included "hysterosalpingography", "oil contrast", "ethiodized oil", "ethiodol", "lipiodol", "therapeutic", "peritoneum", "endometrium", "immune cells", "phagocytosis", "sperm", "iodine", "endometriosis" "ciliary motion", "sperm transport", "sperm capacitation", "isthmic plugs", "tubal obstruction"," cilia", "tubal flushing", "shear stress" and "vascular mechanobiology".

Hypotheses

The different hypotheses focus on the possible fertility-enhancing effect of tubal flushing with relation to; the biochemical effects of the contrast and the interfacial effects of the contrast properties (Figure 1). We will discuss each hypothesis, and its available evidence, separately.

Figure 1. *The hypotheses on the fertility-enhancing factors of tubal flushing*



Hypothesis 1. Immunological effect on the endometrium and peritoneum.
The first hypothesis is that oil-based contrast positively affects fertilisation and implantation due to altering the immune response to spermatozoa and the conceptus.

Uterine bathing, the effect of oil-based contrast on the endometrium
A 1987 rodent study showed the induction of decidua formation after intrauterine oil injection in appropriately sensitised uteri (Milligan, 1987). Later research in a mouse model, showed that CD205+ dendritic cells decrease after the instillation of oil-based contrast, compared with sham and saline infusion treatments. Dendritic cells present antigens and thereby stimulate the T-cells of the immune system. The decrease in these cells reduces antigen sampling and the immune system's presenting capability in the uterus. The fertility-enhancing mechanism of oil-based contrast may be via damping the immune response to antigens, including a conceptus. Two hypotheses for the decrease in dendritic cells after oil-based contrast injection are; a toxic effect on the dendritic cells or stimulation of dendritic cells's migration to lymphoid organs (Johnson *et al.*, 2005).

However, in a randomised trial in women with endometriosis or repeated implantation failure, no evidence was found that additional flushing with oil-based contrast increases the success of IVF compared to no additional flushing. A limitation of this study is that it was underpowered (including only 70 of the planned 350 women) (Reilly *et al.*, 2019).

Peritoneal bathing, the effect of oil-based contrast on the peritoneum
Clinical studies have shown that the positive effect of oil-based contrast, on pregnancy rate, is higher in women with endometriosis-related subfertility than unexplained subfertility. The relative risk to become pregnant after flushing with oil-based contrast compared to no flushing was 4.4 (1.61-12.21) in women with endometriosis compared to 1.6 (0.81–3.16) in women with unexplained subfertility (Johnson *et al.*, 2004). The following possible mechanisms may explain these results.

Inhibiting effect of spermatozoa phagocytosis
Peritoneal macrophages from women with endometriosis exhibit higher levels of phagocytosis capacity of spermatozoa in-vitro. This effect suggests that the peritoneal macrophages might negatively influence fertilisation by phagocytising spermatozoa (Muscato, Haney and Weinberg, 1982). In an in-vitro study, exposure of oil-based contrast (Ethiodol) to the peritoneal fluid of six women with unexplained subfertility or endometriosis inhibited phagocytosis of the pelvic macrophages. The proposed mechanism is that the lipids form a layer on the macrophages' cytoplasmic membrane, which hinders the contact between the macrophages and the spermatozoa (Mikulska,

Kurzawa and Rozewicka, 1994). By inhibiting macrophages' activity, oil-based contrast may positively affect fertilisation (Boyer *et al.*, 1986).

Stimulating effect on dendritic cells in peritoneal fluid
An *in-vitro* study shows that human dendritic cells that incorporate oil-based contrast show greater cellular complexity, indicating maturity compared to cells cultured without oil-based contrast. The same phenotype of dendritic cells is present in the peritoneal fluid of women who previously underwent an HSG with oil-based contrast. It may be that the dendritic cells phagocytose oil-based contrast, consequently initiating an immune response and then become mature. Mature dendritic cells may create a more favourable peritoneal environment for conceiving, presumably because dendritic cells' maturation reduces their phagocytosis activity. This process may be critical in women with immunological abnormalities in the peritoneal cavity, such as endometriosis (Izumi *et al.*, 2017).

Hypothesis 2. Endometrial opioid receptors
The second hypothesis is that oil-based contrast increases endometrial receptivity due to acting on endometrial opioid receptors.

The commercial available oil-based contrast, Lipiodol Ultra Fluid®, is derived from the seeds from the Papaver somniferum plantopium, which contain natural opium (Shenoy and Lui, 2019). Research has demonstrated that opioid receptors express in human endometrial cells and that their expression changes during the menstrual cycle. mRNA expression increases during the proliferative phase and decreases during the menstrual cycles secretory phase with maximum values around ovulation time. This cyclic upregulation of opioid receptors, especially during the mid-proliferative to the mid-secretory phase, suggests a role in endometrial receptivity and consequently implantation. Therefore, we hypothesize that oil-based contrast may act through endometrial opioid receptors increasing endometrial receptivity, forming part of explaining the aforementioned increased pregnancy rates after using oil-based contrast (Totorikaguena *et al.*, 2017).

Hypothesis 3. Effect of the iodine content
The third hypothesis is that the iodine content of the contrast positively affect ovulation and implantation.

The commercially available oil-based contrast media (Lipiodol Ultra Fluid®) contains more iodine than the most common water-based contrast media, 480mg I/mL vs 250 to 300 mg I/mL respectively (depending on the type of water-based contrast media

selected). Additionally water-based contrast, and its iodine content, is more rapidly excreted from the body than oil-based contrast (Miyamoto *et al.*, 1995). Worldwide, there has been a drastic decrease in urinary-iodine-concentration (UIC) in pregnant women. In 1971 the National Health and Nutrition Examination Surveys (NHANES) began measuring the UIC levels within the United States. The median UIC was 327 µg/L for pregnant women in 1971–1974. The second survey (1988-1994) showed a median UIC of 141 µg/L for pregnant women (Hollowell *et al.*, 1998).

Further studies from the United States published have shown a median UIC of 100.5 µg/L, in women wishing to conceive (Stagnaro-Green *et al.*, 2015). The reference values for an adequate mean UIC are 100–199 µg/L for adults and 150–249 µg/L for pregnant women, according to the World Health Organization (WHO)(WHO, 2013). An inadequate iodine intake may lead to a decrease in the production of thyroid hormones. 11% of women in Washington, D.C., in 2015 displayed subclinical hypothyroidism at pre-conceptional consultations (Stagnaro-Green *et al.*, 2015).

An inadequate iodine intake can lead to subclinical hypothyroidism with a further negative effect on ovulation (Fairley and Taylor, 2003). Furthermore, in ovulating women with low UIC, the fecundability is significantly decreased (Mills *et al.*, 2018). This reduction may be because thyroxine increases progesterone secretion, and, in a lesser amount, the estradiol secretion of granulosa cells (Wakim, Polizotto and Burholt, 1995). Additionally, there may be an effect on the endometrium/myometrium or both, containing thyroid hormone receptors (Kirkland *et al.*, 1983). Interestingly, a study in ewes and rams with a low UIC showed a significant increase in pregnancy rate after a subcutaneous injection of iodised oils (Lipiodol), from 37% to 100% (P value 0.007) (Ferri *et al.*, 2003).

We hypothesize that the fertility-enhancing effect of oil-based contrast during HSGs may be partially due to the iodine content of the contrast influencing ovulation and implantation.

Hypothesis 4. Improvement of interfacial factors due to the lubricant effect of the contrast medium

This hypothesis states that the lubricating properties of the oil-based contrast have a fertility-enhancing effect, due to improvement in cilia's action and gametes transport within the oviduct.

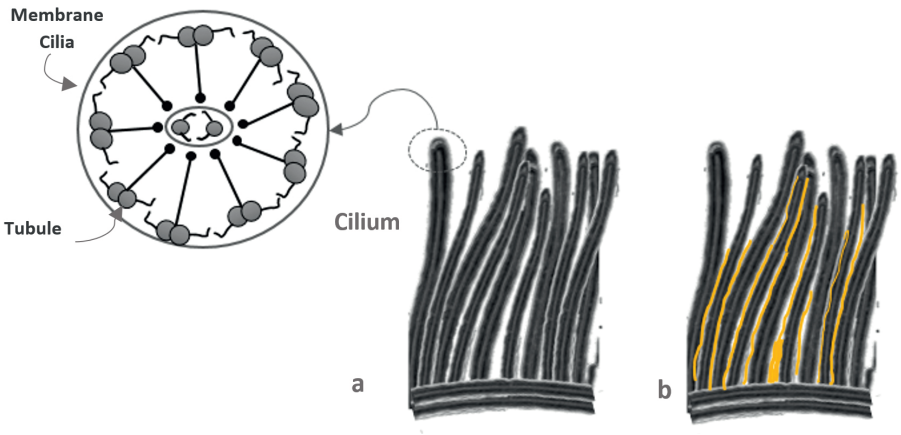
Cilia factors

Ciliary activity within the Fallopian tubes influences tubal secretions flow and the gamete transport (Jansen, 1984). The cilia structure consists of a central pack containing two single microtubules-axoneme, surrounded by nine other microtubules arranged in doublets (See Figure 2). The doublet microtubules sliding drives the rhythmical beating movement of the cilia, which generates intratubal flow (Satir and Matsuoka, 1989; Shi *et al.*, 2011).

Oil-based contrast media reduces the friction between the cilia themselves, due to its lubricating effect and by filling the cilium's irregular surface and the inner surface of the Fallopian tube. By reducing the friction, the cilia's beating movement may be enhanced, which may positively affect gametes and embryo transportation through the Fallopian tube.

Additionally, the cilia movement's improvement increases mixing of tubal secretions and gametes (Muglia and Motta, 2001).

Figure 2. *The cilia and a schematic image of the axoneme structure and its microtubules*



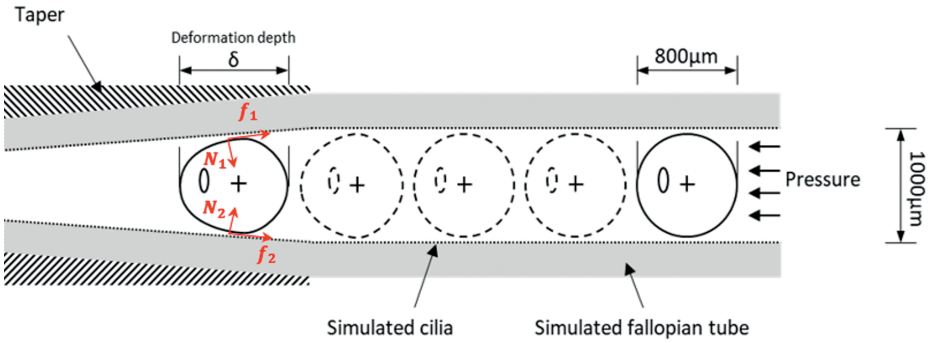
a. Cilia before flushing, b. Cilia after lubrication with oil-based contrast.

Oocyte transit

Oil-based contrast may function as a lubricant to enhance the cilia movement, reducing friction between individual cilia and between cilia and gametes, easing the transition through the Fallopian tube. Well-lubricated, low friction conditions will improve spermatozoa and oocyte transportation by reducing tangential resistance.

A mathematical model can describe friction between the oocyte and the Fallopian tube walls, where the friction coefficient dependents on the physical and geometric properties of the oocyte and the physical and chemical properties of the contrast (See Supplementary data 1). High friction restricts the movement of the oocyte, and a low friction coefficient facilitates movement. Figure 3 illustrates the oocyte transit within a microchannel, which provides insight into the influence of the tribological properties of the HSG contrasts. We hypothesize that an oil-based contrast reduces friction offering less resistance to cilial movement and oocyte transport than water-based contrast media.

Figure 3. *A schematic of oocyte transit within microchannel and exerted forces on it.*



The tangential force (f1 and f2) acts as resistance to cells' movement with the flow, and the normal force (N1 and N2) is the support force exerted on the cell from the channel's wall

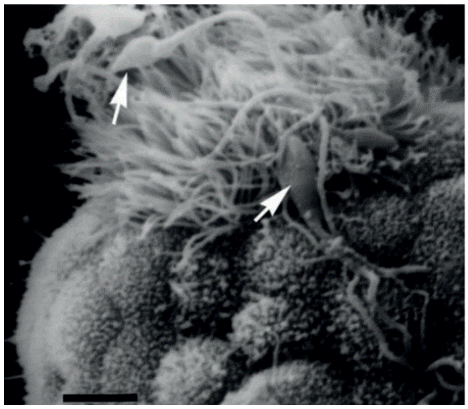
Motility effect of spermatozoa

The female genital tract contains oviductal fluid, produced by the transudate fluid from the systematic circulation and secretory ciliated epithelial cells within the oviduct (Li and Winuthayanon, 2017). When the spermatozoa come into contact with the oviductal fluid, they undergo essential changes, starting with the capacitation process. As a result of this, the motility of the spermatozoa increases through hyperactivation. This process provides a vital force to overcome the attraction between the spermatozoa and the tubal-epithelium (see Figure 4). The epithelium itself also plays a crucial role in the spermatozoa's detachment; however, this process's mechanisms are unknown (Suarez and Pacey, 2006). Oil-based contrast may reduce interfacial bonding during capacitation and hyperactivation, and therefore enhance the fertility outcomes. There are no studies available that tested this hypothesis.

An in-vitro study demonstrated that tubal fluid conditioned by cultured endosalpingeal cells, heparin, or both improve bull sperm capacitation, reducing binding to

endosalpingeal epithelium (Chian, Lapointe and Sirard, 1995; Mahmoud and Parrish, 1996). An oil-based contrast may also reduce interfacial bonding to improve fertility outcomes.

Figure 4. *A sample of Scanning electron micrograph illustrating the human spermatozoa attached to the cilia (Suarez and Pacey, 2006)*



In summary, the fertility-enhancing effect of oil-based contrast may be partially due to its lubricating properties, either through reducing resistance during gamete transport or reducing interfacial bonding providing free movement of the cilia or during capacitation and hyperactivation of spermatozoa.

Hypothesis 5. Dislodgement of mucus debris within the Fallopian tubes.
The fifth and final hypothesis states that oil-based contrast leads to more effective dislodgement of tubal debris, which improves tubal transport.

Amorphous castes of unknown aetiology can form within the Fallopian tube's proximal region (Sulak *et al.*, 1987). These 'debris plugs' consist of mucus and aggregates of histocytelike cells, originating, potentially from endometrial stromal or mesothelial cells (Gillespie, 1965; Kerin *et al.*, 1991). Such debris may cause partial or total tubal occlusions in otherwise anatomically normal Fallopian tubes (Sulak *et al.*, 1987). This debris's existence may inhibit the complete operation of the cilia within the Fallopian tube by negatively influencing the cilia's beating pattern (Jackson-Bey *et al.*, 2020), and therefore prevent natural transportation of the oocyte and spermatozoa. Tubal flushing during HSG could provide a mechanical means of removing debris (Gillespie, 1965), and may increase natural pregnancy and live birth rates (Wang *et al.*, 2019). From a technical perspective, the contrast's flow creates intratubular pressure and generates

shear forces acting against the debris that may contribute to the dislodgement, similar to that found in occluded blood vessels (Lu and Kassab, 2011).

An in-vitro fluid dynamics model incorporating both shear stress and contrast resistance (see Supplementary data 2) shows that higher viscosity fluids cause higher resistance within an artificial tube during the flow. This mechanical loading may assist in detaching the mucus plugs within the Fallopian tube. The contrast's viscosity will be essential and is directly proportional to the shear stresses and tubal resistance. However, this is not the only physical property contributing to the pressure build-up and mucus dislodgment within the Fallopian tube. Other contrasts' characteristics, such as density and surface tension, may also play a role in the dislodgement.

Perceived pain levels may also help support the pressure build-up hypothesis. Van Welie et al. report that women who experience moderate to severe pain (Visual Analogue Score ≥6) during oil-based contrast HSG benefit from higher ongoing pregnancy rates than the procedure conducted with water-based media (30 to 50%, RR 1.7; 95% CI, 1.1–2.5). Below this pain threshold, there is no significant effect on pregnancy outcomes between oil-based and water-based contrast (van Welie *et al.*, 2019). Some women's pain levels during HSG may be indicative of tubal pressure build-up followed by dislodgement of the intra-tubal debris.

It may be that tubal flushing with oil-based contrasts dislodge the debris plugs and clear occlusions more efficiently and leave less debris residue, resulting in improved fertility outcomes by restoring the cilia operation. Contrast specific properties may determine the effectiveness of the debris dislodgement.

DISCUSSION

The different hypotheses for the fertility-enhancing effect of tubal flushing, especially with oil-based contrast presented here, are; the immunological effect on the endometrium or peritoneum, the effect on the endometrial opioid receptors, the impact of the iodine content on ovulation and implantation, the improvement of interfacial factors due to the lubricant effect or the dislodgement of mucus debris within the Fallopian tubes.

Strengths and limitations

This article is the first to provide an overview of the published data on the potential mechanisms, the hypotheses, of the fertility-enhancing effect from tubal flushing. However, the amount of evidence for each hypothesis is limited and the current

knowledge behind the hypotheses is partly based on *in-vitro* studies and/or animal studies. Nevertheless, summarizing the insights from animal studies is an important first step in determining subsequent human studies. Our hypotheses are drawn from critical analysis of medical and clinical engineering research sources.

Clinical implications

The ultimate goal is to understand which women, and at which stage of their subfertility, can benefit from tubal flushing, especially with the use of oil-based contrast. However, with the published studies until now this is not yet possible to determine. We suggest further research to obtain more data to test each hypothesis. Additionally, we advise research on the route of administration of tubal flushing, other than the traditional HSG, for example, hysterosalpingo-foam/contrast sonography (HyCoSy/HyFoSy), transvaginal hydrolaparoscopy (THL) or laparoscopy. These techniques are being used more frequently in daily practice because they do not require radiation and may, in the future, even replace traditional HSG. However, there is no knowledge on tubal flushing's effect and safety with oil-based contrast in these alternative tubal patency testing methods.

CONCLUSIONS

We summarised the different hypotheses on the fertility-enhancing mechanism of tubal flushing, especially with oil-based contrast, during the fertility workup. More research is needed to determine which subfertile women will benefit from tubal flushing using oil-based contrast, at which stage of their subfertility and after which route of administration.

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SUPPLEMENTARY DATA

Supplement 1:

Equation 1 is used to calculate the coefficient of friction.

$$\mu_k = \frac{3[F_D - \rho_{cell} V_{cell} a]}{(\rho_{oocyte} - \rho_{contrast}) \times \pi d^3 g.cos\theta}$$

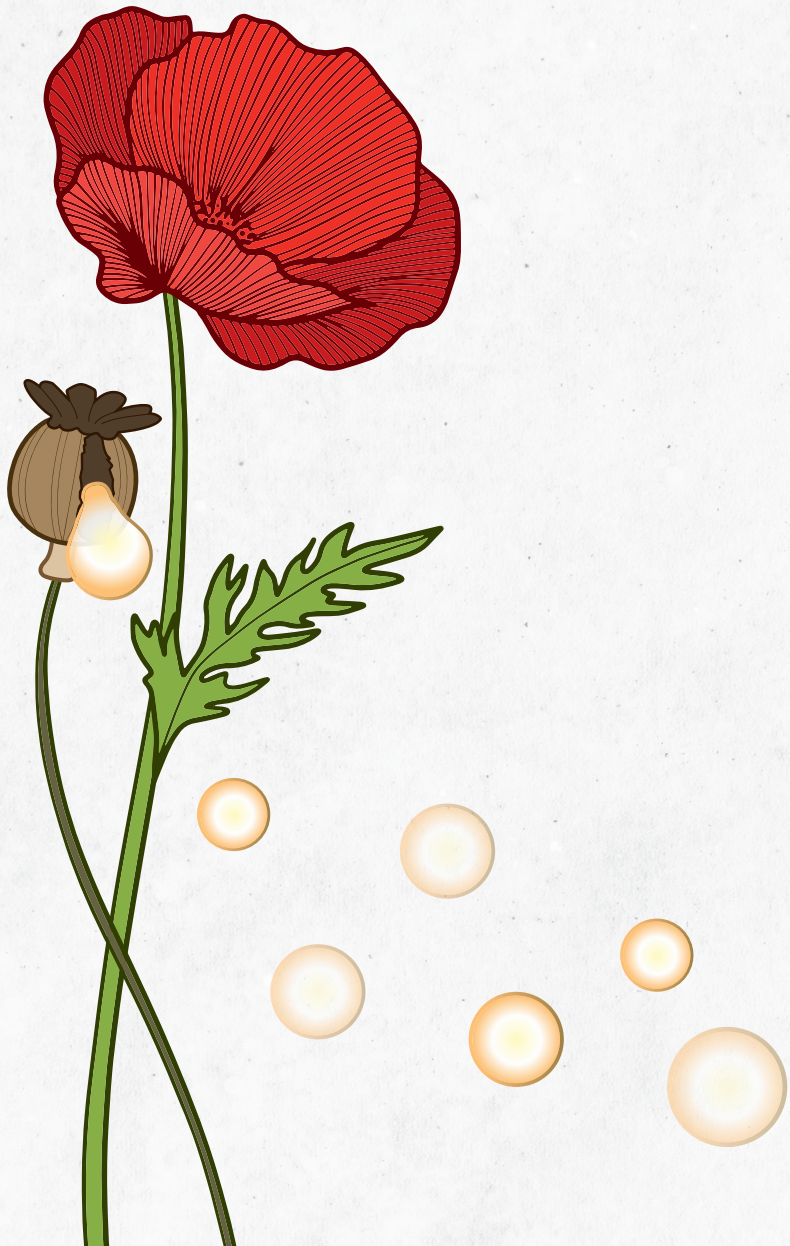
Where ρ represents the densities of the oocyte and the contrast, d and V indicate the oocyte diameter and volume respectively. The friction coefficient μ_k confirms the sole contribution of the contrasts on the tribology properties between the cell and surface as the results will be comparative and not absolute.

Supplement 2:

In a way similar to blood vessels, Fallopian tubes are under mechanical loading from the pressure of the flow which causes internal shear and circumferential wall stresses. Based on shear stress and contrast resistance equations (Eq.2 and Eq.3), it is demonstrated that the higher viscosity fluids cause higher resistance to the wall during the flow. Based on this modelling, the viscosity has a direct proportional relationship with shear stresses and resistance within the tube.

$$\tau_S = \frac{4\mu Q}{\pi R^3} \quad \text{Eq.2} \qquad \text{and} \qquad R = \frac{8\mu L}{\pi R^4} \quad \text{Eq.3}$$

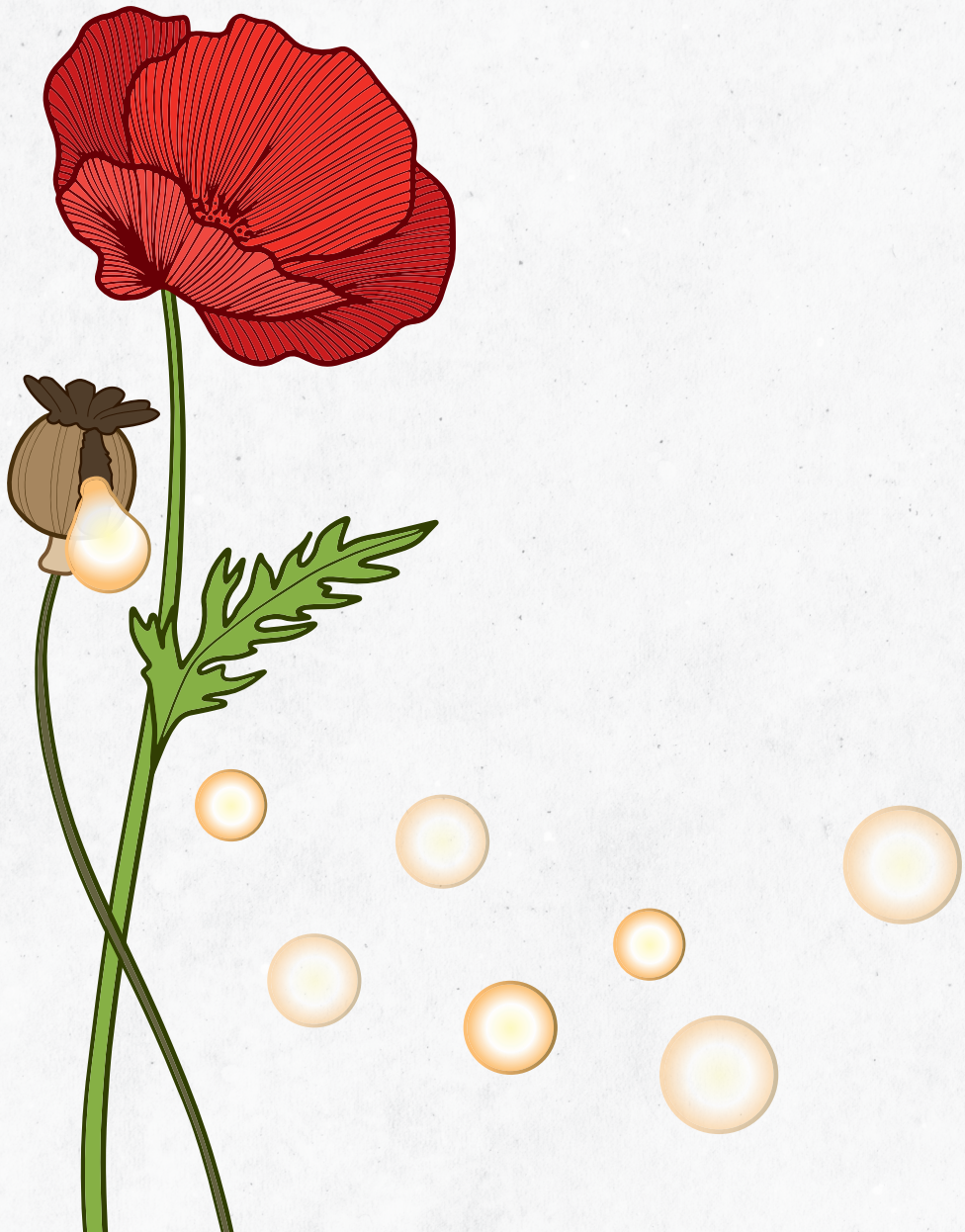
Where μ indicates the viscosity of the contrast, L and R are the length and radius of the fallopian tube respectively and Q is the flowrate of the contrasts.



CHAPTER 8

IN-VIVO PRESSURE BUILD-UP WITHIN THE REPRODUCTIVE TRACT DURING HYSTEROSALPINGOGRAPHY: A PILOT STUDY

I. Roest, A.M. Hajiyavand, V. Mijatovic,
M.Y. Bongers, B.W.J. Mol, C.A.M. Koks, K.D. Dearn



CHAPTER 7

TUBAL FLUSHING WITH OIL-BASED CONTRAST DURING TRANSVAGINAL HYDROLAPAROSCOPY: A CASE REPORT

I. Roest, A.M. Hajiyavand, K.D. Dearn,
M.Y. Bongers, V. Mijatovic, B.W.J. Mol, C.A.M. Koks

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ABSTRACT

Background

Oil-based contrast has been shown to have a fertility-enhancing effect during hysterosalpingography (HSG) but is not yet used during transvaginal hydrolaparoscopy (THL).

Objective

To asses if additional tubal flushing with oil-based contrast during THL is feasible.

Materials and methods

Case report with video assessment. A healthy 29-year-old woman with primary unexplained subfertility, underwent a THL under local anaesthesia. First, chromopertubation was performed by methylene blue. Afterwards, tubal flushing with 3 mL oil-based contrast (Lipiodol® UltraFluid, Guerbet) was performed.

Main outcome measures

In this case report we evaluated the feasibility of additional tubal flushing with oil-based contrast during THL, in terms of; the visibility of the oil-based contrast at the tubal fimbriae, the pain and acceptability scores.

Results

Both Fallopian tubes were patent to methylene blue as well as to oil-based contrast. Interestingly, the oil-based contrast came out of the Fallopian tube in the form of free droplets with strong internal bonding. Furthermore, some residue of the droplets was visible on the surface of the peritoneal wall in the form of oily micro-droplets.

Conclusions

We present the first subfertile woman, in which additional tubal flushing with oil-based contrast during THL was performed. It is likely, that the residue of oily micro-droplets is also present inside the Fallopian tube, where it may enhance the cilia movement by introducing lubrication. These lubricating characteristics of the oil-based contrast may be important for its fertility-enhancing effect. More research is necessary to confirm this hypothesis and the feasibility of tubal flushing with oil-based contrast during THL in more women.

Learning objective

Oil-based contrast is frequently used during HSG (HSG) in subfertile women, because of its fertility-enhancing effect. THL is an alternative procedure, which explores the tubo-ovarian structures and the pouch of Douglas, in addition to tubal patency. However, so far, only water-based media are used during THL. The aim of this case report was to determine if additional tubal flushing with oil-based contrast was feasible during THL. We observed that oil-based contrast forms micro-droplets which may enhance the tubal cilia movement due to lubrication, this is one of the hypotheses for the fertility-enhancing effect of oil-based contrast.

INTRODUCTION

Subfertility is defined as the lack of conception after 12 months of timed unprotected intercourse (Zegers-Hochschild et al., 2009). Unfortunately, around 15% of couples suffer from subfertility (Thoma et al., 2013). During the fertility workup in these couples, tubal patency testing, traditionally in the form of hysterosalpingography (HSG), is often performed. A previous study showed that an HSG with an oil-based contrast has a positive effect on the natural conception rate, compared to an HSG with water-based contrast (van Rijswijk et al., 2020). Transvaginal hydrolaparoscopy (THL) is an alternative procedure to test tubal patency. The benefit of THL is the possibility to explore the tubo-ovarian structures and the pouch of Douglas, neither evaluated during an HSG (Gordts et al., 1998; Gordts et al., 2021). However, during a THL procedure, a water-based medium (methylene blue) is used instead of an oil-based medium, of which no fertility-enhancing effect has been shown. This case report is the first direct observation of oil-based contrast spill from the tubal fimbriae in-vivo.

PATIENT AND METHOD

A 29-year-old woman (nullipara) was referred to the fertility department of our hospital, because of primary subfertility with her current partner for almost 3 years. She had no history of sexually transmitted diseases or abdominal surgery. Aside from prenatal multivitamins, including folic acid and vitamin D, she did not take any medication. Her menstrual cycle was regular at an interval of 30 days. At presentation, she smoked around ten cigarettes per day. Her body mass index was 26.7 kg/m2. Transvaginal ultrasonography did not show any abnormalities of the uterus and both ovaries had a multifollicular aspect. Blood examination was negative for chlamydia antibody titre and showed a normal thyroid-stimulating hormone (TSH) level (1.7 mU/L). The semen analysis of her partner showed 240 million motile spermatozoa.

Because of prolonged subfertility, tubal testing was performed. The woman underwent a THL, which is the first line tubal testing method at our clinic. The THL procedure was performed at the outpatient department with local anaesthesia, as is standard at our clinic for both THL and HSG, using the reusable trocar system (Storz), by a specialized gynaecologist (CK). Details of the procedure have been previously described (Coenders-Tros et al., 2016). First, chromopertubation was performed by the use of 6 mL methylene blue. After the diagnosis of tubal patency, an additional tubal flushing with 3 mL oil-based contrast (Lipiodol® Ultra Fluid, Guerbet Netherlands, Gorinchem) was performed. The infusion of the contrast was stopped when spill from both Fallopian tubes had been observed, in this case the amount of contrast used was relatively low.

Ethical approval

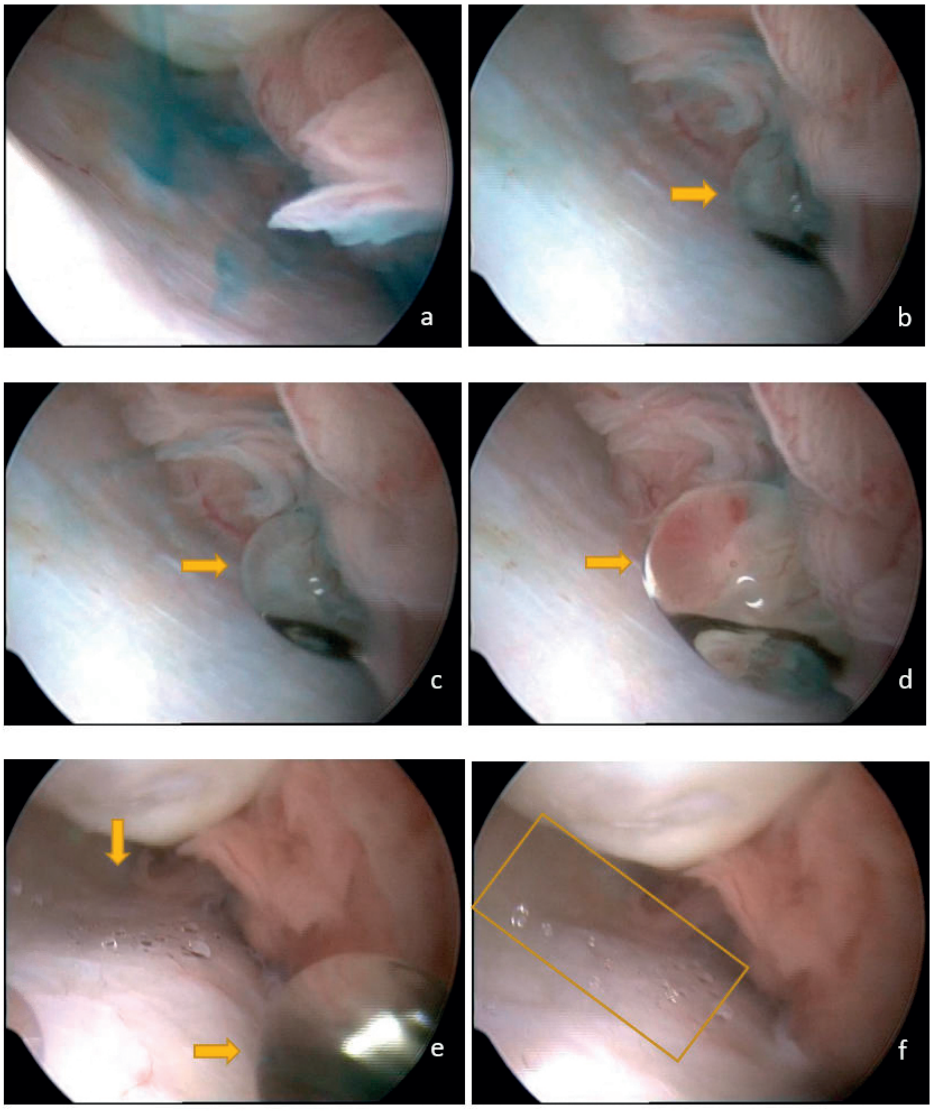
Written patient consent was received from the patient before performing the THL-procedure with additional tubal flushing with oil-based contrast and before reporting her case.

RESULTS

Inspection at the start of the THL procedure showed no abnormalities of the Fallopian tubes or the abdominal cavity. The video (see the QR-code) starts when the camera is positioned at the fimbrial end of the Fallopian tube. The abdominal cavity is infused with pre-warmed saline water (NaCl 0.9%, 37 °C). As soon as the methylene blue exits the Fallopian tube, it immediately disperses in the saline solution, without forming a droplet (See Figure 1a). Afterwards, the oil-based contrast (Lipiodol® Ultra Fluid) comes out of the Fallopian tube in the form of free droplets (See Figure 1b, c, d). When the first droplet moves out of the scope of the camera, almost directly a second droplet forms, some residue of the droplet of oil-based contrast is visible on the surface of the peritoneal wall in the form of micro-droplets (See Figure 1e, f). Additionally, Figure 1d shows that the droplet of oil-based contrast has strong internal bonding (surface tension) because it does not disperse when it comes in contact with the tubal fimbriae.

The pain score, reported on the Visual Analogue Scale by the patient, was 50 mm during the installation of methylene blue and 70mm during the instillation of the oil-based contrast (0 mm no pain, 100 mm worst possible pain). The procedure, including the additional flushing with oil-based contrast, was moderately acceptable to the patient (5 out of 10, 10 being completely acceptable, 0 completely unacceptable). Furthermore, she would advise friends or family to undergo the same procedure (10 out of 10, 10 being the highest score, 0 the lowest). Blood examination four weeks after the procedure showed a TSH of 3.1 mU/L. The couple was counselled for expectant management, with timed intercourse, for the next few months.

Figure 1.



a) Dispersion of methylene blue from the tubal fimbriae. b, c, d) Oil-based contrast exits the tubal fimbriae as a droplet. e, f) A trace of oil-based contrast in the form of micro-droplets is left on the peritoneal wall.

DISCUSSION

To the best of our knowledge, this is the first patient in which tubal flushing with oil-based contrast has been directly visualised during a THL-procedure. In this case, tubal flushing with an oil-based contrast during THL was feasible and acceptable to the patient; however, further studies need to be performed to confirm these observations.

The oil-based contrast used in this case, Lipiodol® Ultra Fluid, is a combination of iodine and fatty acid ethyl esters of poppyseed oil (Guerbet, Netherlands). The oil-based contrast is non-water soluble, hence it doesn't mix with saline water, and it forms an emulsion in water. Due to the high density of this oil-based contrast compared to saline water (1.28 g/cm3 for Lipiodol® Ultra Fluid and 1.00 g/cm3 for 0.9% saline water at 37 °C), the oil-based contrast moves in the form of a droplet (See Figure 1b). The droplets of oil-based contrast have a high surface tension, therefore they have a strong internal bonding and the droplets do not break when they come into contact with the tubal fimbriae. Furthermore, the video as well as the images show that the droplets of oil-based contrast leave a trace (a residue) on the surfaces of the peritoneal wall once it moves away (See Figure 1 e f). This residue is in the form of micro-droplets, which is also referred to as the wetting properties of the oil-based contrast. It is likely that this sequence of events also happens inside the Fallopian tube. The residue of oil-based contrast inside the Fallopian tube and in between the cilia may enhance the cilia movement, by introducing lubrication in between the cilia of the tubal epithelium. This lubrication effect of oil-based contrast is one of the hypotheses for the fertility-enhancing effect of tubal flushing (Roest et al., 2022). It is also possible to attempt salpingoscopy during a THL, which may visualise the aspect of the residue of oil-based contrast inside the Fallopian tube (Suzuki et al., 2005). However, salpingoscopy was not performed in this case.

The main concern against the standard use of oil-based contrast in tubal patency testing, without fluoroscopic guidance, is the risk of intravasation of the contrast media and the subsequent risk of developing an oil-embolism (Roest et al., 2021). With the use of fluoroscopy guidance, the instillation of the contrast media can be directly halted if intravasation occurs, without fluoroscopy guidance intravasation may not be detected. Therefore, we propose to only use oil-based contrast during THL as an additional flushing medium, after the diagnosis of at least unilateral tubal patency by the use of water-based contrast. Additionally, we advise to use a limited amount of oil-based contrast, in this case 3 mL was used. We propose the same restrictions when using oil-based contrast during laparoscopic tubal patency testing.

CONCLUSIONS

Concluding, additional tubal flushing with oil-based contrast is possible during THL and probably laparoscopy, without the use of fluoroscopic guidance. However, additional research is needed to determine the feasibility and safety in more patients. Our research group has started a prospective cohort study to examine the feasibility of additional tubal flushing with an oil-based contrast during THL in 50 women. Furthermore, the characteristics of an oil-based contrast, especially the lubricating effect, may be the reason for the fertility-enhancing effect of tubal flushing with oil-based contrast compared to water-based contrast. The direct observation of the oil-based contrast residue, micro-droplets, on the peritoneal wall is an important step in gaining more knowledge on the characteristics of oil-based contrast.

VIDEO SCAN (READ QR-CODE)

https://vimeo.com/esge/review/665957218/0c3d5e917d

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CHAPTER 8

FEASIBILITY OF ADDITIONAL TUBAL FLUSHING WITH ETHIODIZED OIL AT TRANSVAGINAL HYDROLAPAROSCOPY: A PILOT STUDY

I. Roest, A.M. M.Y. Bongers,
V. Mijatovic, B.W.J. Mol, C.A.M. Koks.

CHAPTER 9

GENERAL DISCUSSION

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Subfertility is a worldwide problem, affecting almost one out of six couples (Thoma *et al.*, 2013). From 1948 onwards, the therapeutic value of using ethiodized oils during hysterosalpingography (HSG) on fertility has been speculated (Rutherford, 1948). Decennia later, in 2017 and 2022, two well-powered randomized controlled trials (RCTs) have shown a 10% and 9% higher ongoing pregnancy rate after an HSG with ethiodized oil compared to various types of water-based contrast (Dreyer *et al.*, 2017; Zhang *et al.*, 2022). Additionally, this fertility-enhancing effect of ethiodized oil during HSG is confirmed in two recent meta-analyses (Fang *et al.*, 2018; Wang *et al.*, 2019) and a Cochrane systematic review (Wang *et al.*, 2020). However, the mechanism behind this beneficial effect of ethiodized oil remains unclear, and questions regarding safety are still being raised.

This thesis has shown that the use of ethiodized oil during HSG with fluoroscopic guidance is safe (Chapter 2 and 3). Additionally, we have shown that a preconceptional HSG with ethiodized oil does not influence the neonatal thyroid function (Chapter 4). Furthermore, we have elucidated different hypotheses on the fertility-enhancing effect of ethiodized oil (Chapter 5). Chapter 6 shows the feasibility of the analysis of the in-vivo pressure build-up within the reproductive tract during HSG, which is essential to further investigate the interfacial effects of tubal flushing. Moreover, in a video case report of tubal flushing with ethiodized oil during transvaginal hydrolaparoscopy (THL), we have provided in-vivo observation of the interaction between ethiodized oil and human tissue, and its possibly lubricating characteristics (Chapter 7). Finally, we have demonstrated that a maximum of 10 mL ethiodized oil can be safely used during THL, without fluoroscopic guidance, if it is used as an additional flushing medium after the diagnosis of at least unilateral tubal patency during chromopertubation (Chapter 8). The upcoming sections will discuss what this new information adds to the care for our future subfertile patients.

To which subfertile women should tubal flushing with ethiodized oil be offered?
As previously mentioned in this thesis, studies have shown that there is a positive effect of an HSG with ethiodized oil, compared to water-based contrast, on the pregnancy rates of women with unexplained subfertility (Dreyer *et al.*, 2017; Zhang *et al.*, 2022). However, the diagnosis "unexplained subfertility" covers a widely heterogeneous group of subfertile couples. Unfortunately, a multivariate model based on a secondary analysis of the H2Oil trial (Dreyer *et al.*, 2017), previously discussed in the introduction (Chapter 1), was unable to identify patients in which the fertility-enhancing effect is most distinct. However, their univariable analyses did show that the treatment effect of ethiodized

oil, compared to water-based contrast, was significantly stronger in women with a BMI < 30 kg/m2 and in women whose partner had a semen volume >3 mL (van Rijswijk *et al.*, 2019).

Another secondary analysis of the H2Oil trial showed that, with the use of ethiodized oil, there are significantly more ongoing pregnancies in women with a higher pain score (Visual Analogue Scale, VAS ≥6) compared to women with a VAS ≤5. Interestingly, with the use of water-based contrast this difference was not present. The authors postulate that the pain is caused by increased intrauterine pressure, due to the presence of pregnancy hindering debris and/or mucus plugs (van Welie *et al.*, 2019). Dislodgment of such debris and/or mucus may restore the tubal epithelium function and can be considered the root cause of the increased pregnancy rates after tubal flushing. The difference between ethiodized oil and water-based media may be explained by a more efficient pressure build-up caused by the characteristics of ethiodized oil. In Chapter 5 we describe this process in more detail as one of the hypotheses for the fertility-enhancing effect of ethiodized oil. In Chapter 8 we describe the observation that cell and/or mucus debris appears from the tubal fimbriae, during tubal flushing, in almost one-third of the women participating in the THL-oil pilot study. Our research group is currently working on the follow-up of this THL-oil pilot study to investigate the pregnancy rates 6 months after a THL with ethiodized oil. In Chapter 6 we describe a pilot study that investigates a method to measure the in-vivo pressure build-up within the reproductive tract during an HSG. This is an important start in further examining the hypothesis of the pressure build-up and the dislodgment of debris and/or mucus from the proximal part of the otherwise anatomically normal Fallopian tubes.

However, the question remains, which women are more likely to have this possibly pregnancy hindering debris and/or mucus plugs, and could therefore benefit from tubal flushing with ethiodized oil? A limited amount of studies has been performed so far to answer this question. Reduced cilia function inside the Fallopian tubes may be the source of accumulation of mucus and/or cell debris (Lyons *et al.*, 2006). This accumulation of cell debris may further block the flow of tubal secretion and the ciliary beating capacity, leading into a downwards spiral. An *in-vitro* study has shown that exposure to elevated testosterone levels, which are present in women with Polycystic Ovarian Syndrome (PCOS), leads to impaired tubal ciliary function (Jackson-Bey *et al.*, 2020). Furthermore, the ciliary beating frequency is regulated by a wide range of different factors, among which endometriosis and smoking of cigarettes (Lyons *et al.*, 2002; Ezzati *et al.*, 2014).

In summary, it is plausible that there is a specific group of women with unexplained subfertility who have yet undiagnosed reduced cilia function. This group might benefit most from tubal flushing with ethiodized oil to dislodge the accumulated mucus/cell debris and/or to reduce the friction between individual cilia, and between cilia and gametes via lubrication (Chapter 5). More, preferably in-vivo, research is necessary to determine which women are at-risk for cilia dysfunction and consequently the formation of pregnancy hindering debris and/or mucus plugs. Additionally, it is plausible that, if the root cause of the ciliary dysfunction cannot be removed, the effect of tubal flushing is only temporary. This has also been shown in a secondary analysis of the H2Oil trial (van Welie *et al.*, 2020b), which revealed that the hazard ratio for ongoing pregnancy after an HSG with ethiodized oil compared to water-based contrast is the highest in the months following an HSG, and gradually decreases to no effect after two years. While it is understandable that women with secondary subfertility, who have previously conceived after tubal flushing, request this same procedure again from their physicians, further research is still needed to justify this practice.

On the other hand, it is likely that women with advanced age (38 years or older) and women with a high risk of tubal pathology (history of a pelvic inflammatory disease [PID], peritonitis, and/or pelvic surgery) may not benefit from tubal flushing. The mechanism of subfertility in these women is expected to be based on the diminished ovarian reserve and unchangeable tubal/uterine factors, respectively. Currently, an ongoing randomized clinical trial (H2Oil2, NTR7925) (Rosielle *et al.*, 2022) is comparing the pregnancy outcomes after an HSG with ethiodized oil or water-based contrast in the aforementioned groups of women. Additionally, this RCT also includes women with ovulation disorders, which will likely contain many women with PCOS. It will be interesting to see if there is a treatment effect of ethiodized oil, compared to water-based contrast in this group of women.

Regardless of the fact that tubal flushing has a beneficial effect on fertility, it is important to realize that an HSG is experienced as a highly stressful procedure by women, which causes anxiety and fear of pain (Handelzalts *et al.*, 2016). In a comparable study on the fertility-related quality of life (FertiQoL) after an HSG and THL-procedure, the only patient characteristic associated with a decreased FertiQoL was lower age. No differences in the FertiQoL were seen between tubal patency testing by HSG and THL (van Kessel *et al.*, 2022). To assists women and their partners in making a personalized choice for a tubal patency testing strategy, a discrete choice experiment is currently being performed by our research group (Máxima MC, L20.005).

At which moment should tubal flushing be offered to subfertile women?
One of the current knowledge gaps is the preferred timing to perform tubal flushing. The benefit of tubal flushing with ethiodized oil during HSG has been shown in women with unexplained subfertility for 18 months (Dreyer *et al.*, 2017). Reasonably, the question has risen if tubal flushing with ethiodized oil could also be beneficial if performed at an earlier stage of subfertility. At the moment the H2Oil-timing study is being performed to answer this question in subfertile women with a > 30% prognosis for natural conception in the next 12 months based on the model of Hunault. Women are randomized for an HSG with ethiodized oil (Lipiodol Ultra Fluid, Guerbet) directly after the initial fertility workup investigations or after six months of expective management (H2Oil-timing, NTR7926, (Kamphuis *et al.*, submitted)). After completion of this trial, we will have more information on the preferred timing of tubal flushing with ethiodized oil regarding the pregnancy rates and its cost-effectivity.

Aside from that, it is crucial to be aware of the global differences in fertility care. As has become increasingly apparent in recent years with an aging population, healthcare resources are limited, which is why several countries with universal health coverage, such as the Netherlands and the United Kingdom (UK), use general practitioners (GP) in the process of deciding which patients should be referred to secondary care. Moreover, GPs also perform some first-line investigations in subfertile couples. The extent of this varies between countries, and is illustrated here with three examples. According to the NICE guideline, UK GPs are advised to offer their subfertile patients, with a low risk of tubal pathology, an HSG as part of the fertility workup (NICE, 2017). In the Netherlands all tubal patency testing is performed after referral to a gynaecologist (NHG richtlijn subfertiliteit, 2010). Furthermore, in Australia there is no clinical guideline for the management of infertility by GPs in primary care (Chambers *et al.*, 2019). Nonetheless, Australian GPs may perform initial assessment, including tubal patency testing by HSG or Hysterosalpingo-contrast/foam-sonography (HyCoSy/HyFoSy) (Hunt and Vollenhoven, 2020). Awareness of these global healthcare differences is important, especially when aiming to implement updated evidence-based subfertility management strategies in the future, in particular when considering the timing of tubal flushing and its fertility-enhancing properties, when using ethiodized oil.

How should tubal flushing be performed and under which conditions?
In addition to the common use of ethiodized oil during HSGs, this thesis has shown the feasibility of additional tubal flushing with ethiodized oil during THL procedures (Chapter 8). For safety reasons we advise tubal flushing with ethiodized oil (max 10 mL) only after the diagnosis of at least unilateral tubal patency to water-based blue dye, in procedures without fluoroscopy guidance such as THL, HyFoSy and diagnostic laparoscopy (DLS).

This advice is based on the assumption that in the case of bilaterally blocked tubes there will be a higher intra-uterine pressure build-up, which occurs proximal to the tubal obstruction. This may increase the risk of massive intravasation of ethiodized oil with the possible, yet unlikely, consequences of an oil-embolism. During an HSG, the risk of an oil-embolism is low, presumably because the procedure is halted as soon as intravasation is seen at fluoroscopy. Our systematic review (See Chapter 2) showed that oil-embolisms occurred in 0.1% of the HSGs with ethiodized oil performed in cohort studies and RCTs. With the use of fluoroscopy guidance, no serious consequences of these oil-embolisms occurred.

Water-based contrast contains 240-320 mg Iodine/mL (based on the type of contrast), which is lower than the 480 mg Iodine/mL that ethiodized oil (Lipiodol Ultra Fluid® or generic variant) contains. Furthermore, water-based contrast is excreted into the urine after two days, while the clearance of ethiodized oil is much slower with a half-life time of 50 days (Miyamoto *et al.*, 1995). An iodine excess in our body, leads to increased transport of iodine into the thyroid gland. Due to a negative feedback loop, known as the Wolff-Chaikoff effect, a decline in thyroid hormone production is caused. Most people are able to escape from this Wolff-Chaikoff effect after a few days and the normal thyroid hormone production is restored. Especially people with pre-existing thyroid dysfunction have been described to be unable to escape this negative feedback loop (Wolff and Chaikoff, 1948). However, the THL-oil pilot study (Chapter 8) showed subclinical hypothyroidism in 14% (6/42), overt hypothyroidism in 2% (1/42), isolated hypothyroxinemia in 2% (1/42) and no subclinical/overt hyperthyroidism four weeks after the use of ethiodized oil in previously euthyroid women, even though the median amount of ethiodized oil used was only 5.0 mL (IQR 3.4-6.3). Additionally, in a prospective cohort study of 196 euthyroid New Zealand women undergoing an HSG with ethiodized oil, subclinical hypothyroidism occurred in 38%, overt hypothyroidism in none, subclinical hyperthyroidism in 2.0% and overt hyperthyroidism in 2.6%. The subclinical/overt hyperthyroidism mostly did not develop until 16 weeks after the use of ethiodized oil during HSG (Mathews *et al.*, 2022). A prolonged subclinical hypothyroidism after iodine exposure cannot be explained with the Wolff-Chaikoff effect. Based on animal research, iodine excess may lead to prolonged elevated thyroid stimulating hormone (TSH) release via increased expression of type 2 deiodinase (Dio2) in the hypothalamus, which plays a vital role in thyroid function (Sun *et al.*, 2022). The development of hyperthyroidism may be due to the Jod-Basedow phenomenon (iodine-induced thyrotoxicosis), where there is gradual hypersecretion of thyroid hormones 2-12 weeks after an iodine excess, mostly occurring in patients with underlying nodular thyroid disease (Dunne *et al.*, 2013).

While the percentage of abnormal thyroid function of previously euthyroid women in the THL-oil pilot study can be perceived as high, subclinical hypothyroidism has an overall prevalence of up till 15% during pregnancy (Blatt *et al.*, 2012). Nevertheless, routine thyroid screening is at the moment not advised as part of subfertility investigations or standard antenatal care by the NICE and ACOG guidelines (NICE, 2017, 2021; ACOG, 2021). However, this has become a topic of debate in recent years. Clinicians that argue against routine screening mainly base their opinion on two different RCT's that have shown that levothyroxine treatment for subclinical hypothyroidism does not have a significant effect on childhood IQ or neurodevelopmental outcomes (Lazarus *et al.*, 2012; Casey *et al.*, 2017). However, a limitation of both studies is that the levothyroxine therapy was started at a median gestation of 13 weeks and 3 days in the study by Lazarus et al. and 16 weeks and 6 days for Casey et al. This can be an important limitation, because the foetus fully relies on the maternal thyroid hormone during the first trimester, as its own thyroid hormone production has not yet started. Moreover, the first trimester is especially important for the early foetal brain development (Burrow *et al.*, 1994). It is therefore possible that there would have been significant neurodevelopmental improvement if the levothyroxine therapy had been started earlier. On the other hand, meta-analyses have shown that levothyroxine treatment for subclinical hypothyroidism decreases the risk of pregnancy loss (OR 0.55; 95% CI, 0.43–0.71) and might also decrease the risk of gestational hypertension (OR 0.78; 95% CI, 0.63–0.97) and preterm birth (OR 0.63; 95% CI, 0.41–0.98) compared to placebo. However, the last two outcomes both have a very wide confidence interval (Ding *et al.*, 2021). These meta-analyses have only included studies which diagnosed subclinical hypothyroidism according to the new 2017 American Thyroid Association (ATA) criteria: TSH above 4.0 mU/L or above the upper limit of pregnancy-specific reference range (Ding *et al.*, 2021). A cohort study of women with recurrent miscarriages showed that the live birth rate was not significantly different in women with euthyroidism or subclinical hypothyroidism (live birth rate 52% and 45% respectively, OR 0.69, 95% CI 0.28–1.71) (van Dijk *et al.*, 2016). However, these results from a cohort of women with recurrent miscarriages cannot directly be extrapolated to women with unexplained subfertility.

In contrast to the NICE and ACOG guidelines, the ATA guideline does advise thyroid function testing in newly pregnant women, or women seeking pregnancy, if one risk factors of an elaborate list is present, including e.g. everyone from the age of 30 years old (Alexander *et al.*, 2017). The guideline of the European Thyroid Association (ETA) does even recommend that all women seeking medical advice for subfertility should be screened for TSH, however this same guideline also states that the evidence of subfertile women with subclinical hypothyroidism is scarce (Poppe *et al.*, 2021).

Studies on the effect of a preconceptional HSG with ethiodized oil on the thyroid function of the neonate show contradicting results. Both a Dutch (Chapter 4) and New Zealand preconceptional HSG cohort study showed no abnormal thyroid function within their respective populations of neonates, while a Japanese cohort study showed abnormal thyroid function in 2% of the neonates (Satoh et al., 2015; van Welie et al., 2020a; Mathews et al., 2022). In the study of Satoh et al., one of the five neonates with abnormal congenital thyroid screening had a mother who had received thyroid hormone replacement therapy, because of a hypothyroidism during the pregnancy. Comparison of the studies is complex, as a relatively high volume of ethiodized oil had been used in the study of Satoh et al (median of 20 mL, compared to 9 mL in the study of Welie et al., not reported for the study of Mathews et al.). Moreover, the background risk of developing congenital hypothyroidism is higher in Japan, possibly due to an iodine rich diet (0.7%, compared to 0.04% in the Netherlands) (Activity Report Tokyo Health Service Association, 2010; Verkerk *et al.*, 2014; Satoh *et al.*, 2015; van Welie *et al.*, 2020a).

In conclusion, the use of ethiodized oil during THL does seem to temporarily impact the thyroid function of previously euthyroid women. However, the debate on the effect of subclinical hypothyroidism, and its suppletion with levothyroxine, on foetal neurodevelopment is not yet concluded. Therefore, there is a necessity for long-term research on the effect of ethiodized oil during fertility investigations on the offspring. Currently, the neuro-H2Oil study (NCT05168228) investigates the neurodevelopment of offspring, born after a preconceptional HSG with ethiodized oil, at the age of 6-8 years. We advise routine thyroid function testing before the use of ethiodized oil for tubal flushing, and to postpone the procedure in case of thyroid dysfunction. Furthermore, we suggest using no more ethiodized oil than necessary (up to a maximum of 15 mL for HSG and 10 mL during THL) to flush the tubes. Finally, even in euthyroid women the thyroid function may be monitored after the use of ethiodized oil. In case of overt hypothyroidism levothyroxine treatment should be started, alternatively, women may be counselled for active monitoring and should be discouraged to attempt pregnancy in the meantime. If subclinical hypothyroidism is found, expectant management with adequate follow-up can be advised. In case of pregnancy and subclinical hypothyroidism the start of levothyroxine treatment could be offered, but based on the current evidence no definite conclusion can be drawn on the effect on the neurodevelopment of the offspring. Even several months after the use of ethiodized oil clinicians should be aware of the possibility of a developed hyperthyroidism (Mathews et al., 2022).

What could the future of tubal flushing look like?

Within the field of radiology the ALARA-principle, an abbreviation for as low as reasonably achievable, has been in use for decennia to justify the amount of radiation-

dose used per investigation. Nowadays, this acronym has been slightly modified to ALADA, as low as diagnostically acceptable (NCRP, 2014). At the moment there are different reliable tubal patency testing methods at hand, which do not require any form of radiation. Following the ALARA/ALADA-principle research should therefore focus on a future of tubal testing, without the use of radiation.

Currently, the following tubal tests without the need of radiation are available, from most to least invasive: laparoscopy, THL and HyFoSy. To stay in line of principles, the latin expression; *primum non nocere*, first do no harm, guides us toward the least invasive procedure, HyFoSy. Moreover, a HyFoSy or THL procedure may be preferred above HSG by women, because of the lower mean pain scores reported in previous studies (HyFoSy vs HSG: VAS 3.1 vs 5.4, P<0.001 (Welie *et al.*, 2022b), THL vs HSG: VAS 4.7 vs 5.4, P-value 0.038 (Tros *et al.*, 2019)).

When discussing the optimal tubal patency test, it is important to take into account that clinical management based on the results of an HSG or HyFoSy does not change the live birth rate. This has been shown in a clinical trial in which women underwent both an HSG and a HyFoSy, in case of discordant results, women were randomized to clinical management based on the results of the HSG or HyFoSy (Welie *et al.*, 2022). Nonetheless, THL has as benefit over HyFoSy in that minimal endometriosis and adhesions can be diagnosed, which significantly reduce the non-IVF conception (fecundity rate ratio of 0.35; 95% CI 0.17–0.71) (van Kessel *et al.*, 2018). Moreover, THL can also be performed without general anaesthesia and has a relatively low overall complication rate of 0.74% (Gordts *et al.*, 2021). A recent cost-effectiveness study additionally showed that a THL as a first-line investigation for subfertile women is cost-effective compared to HSG (Van Kessel *et al.*, 2022). Therefore, THL may be offered to women, with a low-risk of tubal pathology, as a first-line investigation. However, randomized clinical trials on the effect of early treatment of minimal endometriosis and adhesions diagnosed at THL on the time to pregnancy and/or live birth rate have not yet been performed. In studies regarding laparoscopic surgery on rASRM stage I/II endometriosis, surgical intervention does however seem to increase the pregnancy rates, compared to diagnostic laparoscopy only, but the quality of this evidence is moderate and there is no data on live birth rates (Bafort *et al.*, 2020).

At the moment ethiodized oil is only being used during HyFoSy in a research setting. Currently, the randomized HYFOIL-study (NCT04379973) is undertaken to evaluate the live birth rate after an HyFoSy with or without additional tubal flushing with ethiodized oil (De Neubourg *et al.*, 2021). As for the pregnancy rates after a THL with ethiodized oil, our research group is working on the follow-up for the THL-oil pilot study, to investigate the

ongoing pregnancy rates of conceptions within 6 months after the THL. Furthermore, the preparations of a new RCT are ongoing, which will compare the cost-effectiveness and pregnancy rates of a fertility workup including an HSG with ethiodized oil vs an HyFoSy with ExEm®-foam (FOAM2-study, ZonMw grant application nr 10390012110083). Depending on the result of these clinical trials, in the near future the HSG as part of the fertility workup could be fully replaced by HyFoSy or THL with ethiodized oil.

The crucial question remains however; "Which characteristics of the ethiodized oil form the root cause to its fertility-enhancing effect?" (See also Chapter 5). After answering that question, we may develop a new tubal flushing medium, with the chemical characteristics of ethiodized oil, the same efficacy, but possibly without the (high) iodine content. However, to start answering this question more research is needed on the different hypotheses for the fertility-enhancing effect of the currently used ethiodized oil. Finally, the ultimate choice if any, and which, tubal testing method is used should be made by the women and their partners. In the upcoming years, we can hopefully obtain more data on which subtype of subfertile women are most likely to benefit from therapeutic tubal flushing. This data should be used to develop decision-making tools for subfertile couples to guide them in making their informed decision.

FUTURE PERSPECTIVES

In summary, my recommendations for future studies, which have been explained in more detail in the discussion section above, are to:

- Perform additional research on the root cause of the fertility-enhancing effect of ethiodized oil. It is yet unknown which of its characteristics are most important, e.g. we do not know if its positive effect on pregnancy rates remains with a decreased iodine content. Moreover, studies on the pressure build-up and dislodgment of debris and/or mucus from the Fallopian tubes in relation to pregnancy rates are necessary.
- Study which patients will benefit most from tubal flushing with ethiodized oil and who will not benefit from it. A start to further investigate this is currently being undertaken with the H2Oil2 trial (NTR7925).
- Define if an HSG with ethiodized oil should be a standard procedure within the fertility workup after 12 months. To determine the (cost) effectiveness of such an extension of the initial fertility workup, the H2Oil-timing trial is presently ongoing (NTR7926).
- Develop tools for subfertile couples to guide them in a personalised informed decision on tubal patency testing methods.
- Establish if there is a need for standard thyroid screening after tubal flushing with ethiodized oil, and if so, at which interval this should be performed. Moreover, identification of subgroups of patients, who may need extra attention due to an increased risk of developing thyroid dysfunction after exposure to ethiodized oil, is important.
- Determine the effect of a preconceptional HSG with ethiodized oil on the neurodevelopment of the offspring. A first study, the neuro-H2Oil study, is being performed to investigate this in a subgroup of the H2Oil population (NCT05168228). Additionally, studies in different populations, with varying background risks for thyroid dysfunction, are necessary.
- Verify the positive effect of ethiodized oil on the pregnancy rates of subfertile women, when used during other tubal patency testing methods than HSG. Our research group is working on completing the follow-up for the THL-oil pilot study to investigate the ongoing pregnancy rates after a THL with ethiodized oil. Additionally, the HYFOIL-study (NCT04379973) is exploring this for HyFoSy. Finally, the effect on diagnostic laparoscopy needs to be clarified.

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CHAPTER 10

ENGLISH SUMMARY

SUMMARY

Chapter 1 provides a general introduction to this thesis as well as its outline and objectives. The following research questions were formulated:

1. What are the possible adverse events, and their clinical consequences, during or after a hysterosalpingography (HSG) with the use of ethiodized oil (oil-based contrast) in subfertile women and their offspring?
2. What is currently the risk of adverse events in subfertile women and their offspring after an HSG using ethiodized oil compared to water-based contrast?
3. Which mechanisms form the potential root cause of the fertility-enhancing effect of tubal flushing, and how can we further investigate these mechanisms?
4. Can ethiodized oil also be used during other methods of tubal flushing than HSG?

In line with the thesis itself, its summary is also divided into two parts. The first part focuses on the safety of ethiodized oil during tubal patency testing and discusses research questions 1 and 2. The second part discusses the fertility-enhancing effect of ethiodized oil and its implementation in different methods of tubal flushing with the aim of answering research questions 3 and 4.

Part I – What is the safety of ethiodized oil during tubal patency testing?

In **chapter 2** a systematic review with meta-analysis answers the fundamental question: "what have been the risks of performing an HSG with ethiodized oil in subfertile women over the years?". This systematic review and meta-analysis includes all published studies, including case reports, without publication date or language restrictions. In total 108 studies were included, published between 1928 and 2020, which covered a total of 23,536 HSGs with the use of ethiodized oil. The most frequently reported complication of HSGs performed for subfertility was intravasation of the contrast media. This occurred in 2.7% of the 19,339 HSGs with ethiodized oil (31 studies, 95% CI 1.7–3.8), compared to 2.0% of the 1006 HSGs with the use of water-based contrast (8 studies, 95% CI 1.2–3.0) in cohort studies and randomized controlled trials (RCTs), case reports/series excluded. Oil-embolisms occurred in 0.1% of the 19,339 HSGs performed in cohort studies and RCTs, all without serious lasting consequences. Furthermore, four cases, within the case reports/series, with serious consequences (retinal oil-embolism [n=1] and cerebral complaints [n=3]) of an oil-embolism were described, but these reports did not describe the use of adequate fluoroscopy guidance during HSG. Infection occurred in 0.90% of the 11,287 HSGs with the use of ethiodized oil (20 studies, 95% CI 0.47–1.5) and in 1.9% of the 564 HSGs with water-based contrast

(4 studies, 95% CI 0.27–4.6), in cohort studies and RCTs. One case of non-infection-related mortality, most likely due to an anaphylactic reaction, after an HSG in 1947 was reported. Based on all included studies, including case reports/series, 85 cases of oil remnants (half of which were diagnosed within two weeks after the procedure) and 41 cases of lipogranuloma formation intra-abdominally post-HSG were reported. Women with subclinical hypothyroidism prior to an HSG with ethiodized oil are more likely to develop overt hypothyroidism afterwards, compared to euthyroid women (35.7% versus 0–2.2%), however, this is based on only 28 and 202 women from respectively one and three studies. We concluded that, with the use of fluoroscopy screening during HSG and limiting the volume of ethiodized oil, safety concerns should not be a reason to deny the use of ethiodized oil in euthyroid women with unexplained subfertility.

A retrospective survey in the Netherlands, **chapter 3,** describes the incidence of adverse events during HSG procedures performed outside a clinical trial setting. This nationwide retrospective survey of 5165 HSG procedures, performed in a single book year, showed an overall complication rate of 5.1% after an HSG performed with ethiodized oil and 1.8% with water-based contrast (RR, 2.8; 95% CI, 1.9-4.0, P-value <0.0001). The most frequently reported complication was intravasation, which was reported significantly more often in the HSGs performed with ethiodized oil, 4.8% compared to 1.3% of the HSGs performed with water-based contrast (RR, 3.6; 95% CI, 2.4–5.4, P-value <0.0001). However, all cases of intravasation were asymptomatic and in none of the 5165 HSG procedures an oil-embolism or other clinical consequence of intravasation was observed. Presumably, the use of fluoroscopy during HSGs contributes to the prevention of an oil-embolism, as it provides early detection of intravasation of the contrast media. In this retrospective survey the incidence of maternal and neonatal thyroid dysfunction was not reported.

In **chapter 4,** the effect of a preconceptional HSG on the offspring is shown in a Dutch population. We performed a retrospective data analysis of the neonatal screening results for congenital hypothyroidism (from the National Institute for Public Health and the Environment [RIVM]) in the offspring of mothers participating in the H2Oil study. The data of 76 and 64 neonates, respectively conceived within 6 months after an HSG with ethiodized oil or water-based contrast, were available. The results for congenital hypothyroidism were normal in all neonates. Additionally, the median total thyroxine (T4) concentration was not significantly different between the two groups (87.0 nmol/l [IQR 76.0–96.0] in the group with ethiodized oil versus 90.0 nmol/l [IQR 78.0–106.0] after water-based contrast [P-value 0.13]). These results are not in line with previous studies in East-Asian populations, probably due to the striking difference in background risk for congenital hypothyroidism between Japan and the Netherlands, i.e., 0.7% in

Japan versus 0.05% in the Netherlands. In conclusion, exposure to a preconceptional HSG with ethiodized oil in a Dutch population seems to be safe for the offspring.

Part II – What is the fertility-enhancing effect and feasibility of ethiodized oil during different methods of tubal flushing?

In **chapter 5** the different hypotheses on the fertility-enhancing effect of tubal flushing are discussed. The hypotheses are divided into the biochemical and interfacial effects derived from the contrast media properties. The possible biochemical effects are; the immunological effect on the endometrium or peritoneum, the effect on the endometrial opioid receptors, and the impact of the iodine content on ovulation and implantation. The improvement of the interfacial factors may be due to the lubricating effect of the ethiodized oil, which may improve the movement of the cilia within the Fallopian tube due to reduced friction. Moreover, the interfacial effects of contrast media may lead to the dislodgement of mucus/cell debris within the Fallopian tube, resulting in restored cilia operation. For the latter, we hypothesize that ethiodized oil provides a more effective dislodgment of mucus/cell debris compared to water-based contrasts. Currently, the evidence for each hypothesis is limited and it is yet unknown which properties of the ethiodized oil are most important for improving fertility.

To further investigate the hypothesis of dislodgment of mucus/cell debris as the underlying mechanism of the fertility-enhancing effect of ethiodized oil, it is essential to be able to measure the pressure build-up during HSG. In **chapter 6** we showed the feasibility of measuring the in-vivo pressure build-up within the reproductive tract during ten HSG procedures. We succeeded in obtaining 29 in-vivo pressure values per second, with the use of a disposable fluid syringe with an integral pressure transducer (DiamondTOUCH™). Further studies are necessary to investigate the implication of the pressure values and its build-up, to eventually determine if there is a correlation between the pressure build-up within the reproductive tract, the experienced pain by women during the procedure, and the pregnancy rates afterwards.

In **chapter 7** a video case report is presented, in which tubal flushing with ethiodized oil is performed during transvaginal hydrolaparoscopy (THL). This case report is the first to describe the in-vivo interaction between ethiodized oil and human tissue during tubal flushing. THL is an alternative method of tubal patency testing, with the additional possibility to diagnose endometriosis due to the direct visualization of the pouch of Douglas and the tubo-ovarian structures. In recent years, the use of ethiodized oil during HSG has gained interest, because of its positive effect on pregnancy rates. However, chromopertubation during THL is currently only performed with water-based

media (e.g. methylene blue) and no ethiodized oil is used. In this video case report, firstly, tubal flushing was performed with methylene blue, which appeared from both fimbrial ends of the Fallopian tubes. The methylene blue directly dispersed in the saline solution, that was infused into the pelvic cavity at the start of the THL procedure. After that, the additional tubal flushing with ethiodized oil was performed. The ethiodized oil formed an emulsion in water and was clearly visible exuding from the Fallopian tubes as oil droplets. Moreover, the droplets of ethiodized oil left a trace (a residue) of micro-droplets on the surfaces of the peritoneal wall. It is likely that this trace of micro-droplets is also present inside the Fallopian tube, where it may provide lubrication of the cilia of the tubal epithelium and hence lead to increased fertility rates. Lubrication of the cilia by ethiodized oil is one of the potential root causes of the fertility-enhancing effect of ethiodized oil as described in chapter 5.

To investigate the feasibility of additional tubal flushing with ethiodized oil in subfertile women undergoing a THL, we performed the THL-oil pilot study as shown in **chapter 8**. In this study 50 euthyroid women received additional tubal flushing with ethiodized oil after establishing at least unilateral tubal patency for methylene blue. The median volume of ethiodized oil used was 5.0 mL (Interquartile range [IQR] 3.4-6.3). The procedure was rated as highly acceptable by the women (10 out of 10, IQR 8–10). The median pain score on the Visual Analogue Scale (VAS) was 2.9 (IQR 1.0–5.0) after flushing with methylene blue and 3.0 (IQR 1.0–5.0) after ethiodized oil. In 48 women there was bilateral patency to methylene blue, in 77% (37/48) both tubes were also patent to the ethiodized oil, in 17% (8/48) this was the case for one tube, and in 6% (3/48) the ethiodized oil did not appear at the fimbrial ends. In two women there was unilateral tubal patency to methylene blue, the additional ethiodized oil appeared unilateral in one and did not appear in the other women. The ethiodized oil was visible as free oil droplets, similarly as described in the case report of chapter 7. Mucus/cell debris was visible exuding from the fimbrial ends in 32% (16/50) of the procedures. Subclinical hypothyroidism developed in 14% (6/42) four weeks after the procedure. One woman developed overt hypothyroidism (Free thyroxine [FT4] slightly decreased to 11.0 pmol/L and Thyroid Stimulating Hormone [TSH] 5.60 mU/L), and one woman had isolated hypothyroxinemia (FT4 11.0 pmol/L and TSH 3.40 mU/L). One adverse event, persistent bleeding at the trocar insertion site occurred, for which a diagnostic laparoscopy had to be performed. We concluded that a maximum of 10 mL ethiodized oil may be safely used during THL, without fluoroscopic guidance, if it is used as an additional flushing medium after establishing at least unilateral tubal patency during chromopertubation.

Chapter 9 provides a general discussion of the clinical implications of this thesis and suggestions for further research. Finally, the impact paragraph can be found in **chapter 12**.

CHAPTER 11

NEDERLANDSE SAMENVATTING

SAMENVATTING

De veiligheid en effectiviteit van het doorspoelen van de tubae met gejodeerd oliehoudend contrastmiddel

Subfertiliteit is een wereldwijd probleem waar gemiddeld een op de zes paren mee te maken krijgt. Een hysterosalpingografie (HSG) is een veelgebruikte methode om de doorgankelijkheid van de tubae te onderzoeken. Vanaf 1948 wordt er gespeculeerd over een mogelijk gunstig effect van een HSG uitgevoerd met gejodeerd oliehoudend contrastmiddel, ook wel oliehoudend contrastmiddel genoemd, op de zwangerschapskansen nadien. Decennia later, in 2017 en 2022, zijn twee kwalitatief sterke Randomized Controlled Trials (RCTs) gepubliceerd die een 10% en 9% hoger doorgaand zwangerschapspercentage laten zien, bij vrouwen met een onverklaarde subfertiliteit, na een HSG uitgevoerd met oliehoudend contrastmiddel in vergelijking met waterhoudend contrastmiddel. Dit gunstige effect bleek tevens uit twee meta-analyses en een Cochrane systematische review. Echter, het onderliggende mechanisme van dit fertiliteitsbevorderende effect van oliehoudend contrastmiddel blijft een raadsel en de discussie over de veiligheid van oliehoudend contrastmiddel is nog steeds actueel.

Hoofdstuk 1 van dit proefschrift bevat een algemene inleiding, daarnaast worden de onderzoeksvragen en de opbouw van het proefschrift besproken. De volgende onderzoeksvragen werden voor dit proefschrift geformuleerd:

1. Wat zijn de mogelijke nadelige effecten en de bijbehorende klinische consequenties tijdens, en na, een HSG uitgevoerd met oliehoudend contrastmiddel bij subfertiele vrouwen en hun nakomelingen?
2. Wat is het huidige risico op nadelige effecten van een HSG met oliehoudend contrastmiddel bij subfertiele vrouwen en hun nakomelingen?
3. Welke mechanismes vormen de grondslag van het fertiliteitsbevorderende effect van het doorspoelen van de tubae met oliehoudend contrastmiddel en hoe kunnen we deze mechanismes nader onderzoeken?
4. Kan oliehoudend contrastmiddel ook toegepast worden tijdens andere methoden van tubadiagnostiek dan het HSG?

Net zoals het proefschrift, is deze samenvatting verdeeld in twee delen. Het eerste deel richt zich op de veiligheid van het gebruik van oliehoudend contrastmiddel tijdens HSG en beantwoordt onderzoeksvraag 1 en 2. Het tweede deel bediscussieert het fertiliteitsbevorderende effect van oliehoudend contrastmiddel en de toepassing

daarvan bij andere methoden van tubadiagnostiek dan het HSG, met als doel om onderzoeksvraag 3 en 4 te beantwoorden.

Deel I – De veiligheid van tuba diagnostiek met oliehoudend contrastmiddel

In **hoofdstuk 2** beantwoordt een systematische review met meta-analyse de fundamentele vraag: "wat is het risico van een HSG met oliehoudend contrastmiddel bij subfertiele vrouwen geweest over de jaren heen?". Voor deze systematische review met meta-analyses hebben we alle gepubliceerde studies geïncludeerd, inclusief case reports en zonder enige publicatiedatum of taalrestrictie. In totaal werden 108 studies geïncludeerd, gepubliceerd tussen 1928 en 2020, die gezamenlijk 23.536 HSGs met oliehoudend contrastmiddel beschreven. De meest frequent gerapporteerde complicatie, van de HSGs uitgevoerd bij subfertiele vrouwen, was intravasatie van het contrastmiddel. Dit werd gerapporteerd in 2,7% van de 19.339 HSGs uitgevoerd met oliehoudend contrastmiddel (31 studies, 95% CI, 1,7–3,8) en in 2,0% van de 1006 HSGs met waterhoudend contrast (8 studies, 95% CI, 1,2–3,0) in cohort studies en RCTs, de case reports/series niet meegenomen. Een olie-embolie kwam voor in 0,1% van de 19.339 HSGs verricht in cohort studies en RCTs, in geen van deze gevallen was er sprake van langdurige ernstige klinische consequenties. Daarnaast zijn vier casussen beschreven, in case reports/series, van complicaties met ernstige klinische gevolgen van een olie-embolie (één in de retina en drie in het centrale zenuwstelsel), bij deze casussen is het gebruik van doorlichting tijdens de HSG procedure niet vermeld. Infecties traden op in 0,90% van de 11.287 HSGs met oliehoudend contrastmiddel (20 studies, 95% CI, 0,47–1,5) en in 1,9% van de 564 HSGs met waterhoudend contrastmiddel (4 studies, 95% CI, 0,27–4,6) in cohort studies en RCTs. Een casus van een dodelijke, niet-infectie gerelateerde, complicatie, hoogstwaarschijnlijk als gevolg van een anafylactische reactie op een HSG in 1947 werd gerapporteerd. In totaal, inclusief de case reports/series, werden er 85 casussen van intra-abdominale olie restanten (waarvan de helft gediagnosticeerd werd binnen twee weken na het HSG) en 41 casussen van lipogranuloma vorming na een HSG gerapporteerd. Vrouwen met een subklinische hypothyreoïdie vooraf aan een HSG met oliehoudend contrastmiddel hebben een hoger risico op het ontwikkelen van een hypothyreoïdie nadien, in vergelijking met euthyreote vrouwen (35,7% versus 0–2,2%), echter is deze data slechts gebaseerd op respectievelijk één studie met 28 vrouwen en drie studies met in totaal 202 vrouwen. Uit deze systematische review met meta-analyse concludeerden wij dat bij het gebruik van doorlichting tijdens HSG en het beperken van de hoeveelheid oliehoudend contrastmiddel, er vanuit veiligheidsoverwegingen geen reden is om euthyreote vrouwen, met een onverklaarde subfertiliteit, een HSG met oliehoudend contrastmiddel te ontzeggen.

Een retrospectieve vragenlijststudie uitgevoerd in Nederland, weergegeven in **hoofdstuk 3**, beschrijft de incidentie van complicaties tijdens reguliere HSG procedures, uitgevoerd buiten klinische onderzoeken om. Uit deze landelijke retrospectieve vragenlijststudie van 5165 HSG procedures, allen uitgevoerd binnen een enkel boekjaar, bleek de totale kans op complicaties van een HSG met oliehoudend contrastmiddel 5,1% en van een HSG met waterhoudend contrastmiddel 1,8% (RR, 2,8; 95% CI, 1,9-4,0, P-waarde <0,0001). De meest gemelde complicatie was intravasatie van het contrastmiddel, wat significant vaker gerapporteerd werd in HSGs uitgevoerd met oliehoudend contrastmiddel, 4,8% in vergelijking tot 1,3%, bij HSGs met waterhoudend contrastmiddel (RR, 3,6; 95% CI, 2,4–5,4, P-waarde <0,0001). Echter in alle gevallen van intravasatie verliep dit asymptomatisch en in geen van de 5165 HSG procedures werd een olie-embolie of andere klinische consequentie van intravasatie vastgesteld. Vermoedelijk draagt het gebruik van doorlichting tijdens een HSG bij aan het verlagen van het risico op een olie-embolie, doordat dit het tijdig opmerken van intravasatie van het contrastmiddel mogelijk maakt. In deze retrospectieve vragenlijst studie werd de incidentie van maternale en neonatale schildklierdysfunctie na een HSG niet gemeld.

In **hoofdstuk 4** wordt het effect van een preconceptioneel HSG met oliehoudend contrastmiddel op de neonatale schildklierfunctie in een Nederlandse populatie beschreven. Hiervoor hebben we een retrospectieve analyse verricht van de neonatale schildklierfunctie uit het hielprik screeningsprogramma voor congenitale hypothyreoïdie (vanuit het Rijksinstituut voor Volksgezondheid en Milieu [RIVM]) van de nakomelingen van moeders die deel hebben genomen aan de H2Olie studie. Deze data was bekend van 76 en 64 neonaten, geboren na respectievelijk een HSG met oliehoudend contrastmiddel of waterhoudend contrastmiddel. In geen van deze neonaten toonde de neonatale screening voor congenitale hypothyreoïdie afwijkingen. Daarnaast verschilde de mediane T4 concentratie niet significant tussen beide groepen, 87,0 nmol/l (IQR 76,0–96,0) in de groep met oliehoudend contrastmiddel versus 90,0 nmol/l (IQR 78,0–106,0) in de groep met waterhoudend contrastmiddel (P-waarde 0,13). Deze bevindingen zijn tegenstrijdig met de resultaten van een eerdere Japanse studie, vermoedelijk komt dit door het verschil in achtergrondrisico op congenitale hypothyreoïdie in Japan en Nederland, 0,7% in Japan versus 0,05% in Nederland. Concluderend lijkt een preconceptioneel HSG met oliehoudend contrastmiddel in een Nederlandse populatie veilig voor de nakomelingen.

Deel II – Wat is het fertiliteitsbevorderende effect van oliehoudend contrastmiddel en is oliehoudend contrastmiddel ook toepasbaar tijdens andere methoden van tubadiagnostiek?

In **hoofdstuk 5** worden de verschillende hypotheses ten aanzien van het fertiliteits-bevorderende effect van het doorspoelen van de tubae bediscussieerd. Deze hypotheses worden onderverdeeld tussen de biochemische en tribiologische effecten van het contrastmiddel. De mogelijke biochemische effecten zijn: het immunologische effect op het endometrium en/of peritoneum, het effect op de endometrium opioid receptoren en de invloed van jodium op de ovulatie en embryo-implantatie. De verbetering van de tribiologie, wrijvingskunde, zou veroorzaakt kunnen worden door het lubricerende effect van oliehoudend contrastmiddel, wat de beweging van de cilia binnenin de tubae kan verbeteren door het verminderen van frictie. Daarnaast kan het oliehoudend contrastmiddel er voor zorgen dat vastzittend slijm en/of celresten weggespoeld worden uit de tubae, wat de beweeglijkheid van de cilia herstelt. Oliehoudend contrastmiddel is vermoedelijk effectiever in het wegspoelen van vastzittend slijm en/of celresten dan waterhoudend contrastmiddel. Op dit moment is de wetenschappelijke onderbouwing voor de genoemde hypotheses nog beperkt en is het nog onbekend welke eigenschappen van het oliehoudend contrastmiddel het belangrijkste zijn voor het fertiliteitsbevorderende effect.

Om de hypothese van het wegspoelen van obstruerende slijm/celresten als het onderliggende mechanisme van het fertiliteitsbevorderende effect van oliehoudend contrastmiddel nader te onderzoeken, is het essentieel om de opgebouwde druk tijdens een HSG te kunnen meten. In **hoofdstuk 6** hebben we een methode laten zien die de drukopbouw in de baarmoeder en eileiders heeft kunnen vastleggen tijdens tien HSG procedures. Deze methode, met het gebruik van een injectiespuit met een geïntegreerde drukmeter (DiamondTOUCH™), is in staat gebleken om per seconde 29 in-vivo drukwaardes te kunnen vastleggen. Vervolgstudies zijn nodig om de betekenis van de gemeten drukwaardes en het verloop hiervan te kunnen begrijpen, om vervolgens een mogelijke relatie tussen de drukopbouw gegenereerd tijdens een HSG, de ervaren pijn door vrouwen en de zwangerschapskansen nadien vast te stellen.

In **hoofdstuk 7** tonen we een video case report van het doorspoelen van de tubae met oliehoudend contrastmiddel tijdens een transvaginale hydrolaparoscopie (THL). In dit video case report wordt voor het eerst de interactie tussen oliehoudend contrastmiddel en humaan weefsel beschreven tijdens het doorspoelen van de tubae. THL is een alternatieve vorm van tubadiagnostiek waarbij ook tubo-ovariële dysfunctie en endometriose kan worden gediagnosticeerd doordat er direct zicht is op de

bekkenholte en de tubo-ovariële structuren. Het aangetoonde positieve effect van het gebruik van oliehoudend contrastmiddel tijdens een HSG op de zwangerschapskansen nadien heeft voor toegenomen interesse gezorgd in het gebruik van oliehoudend contrastmiddel tijdens tubadiagnostiek. Bij een THL wordt normaliter geen oliehoudend contrastmiddel toegepast. In de beschreven THL procedure werden de tubae eerst doorgespoeld met het standaard gebruikte methyleenblauw (een waterige blauwe kleurstof), welke beiderzijds zichtbaar was aan de fimbriële uiteindes van de tubae, beide tubae waren dus doorgankelijk. Het methyleenblauw verspreidde zich hierbij direct in de zoutoplosssing, waarmee de bekkenholte gevuld is tijdens een THL. Daarna vond de aanvullende spoeling plaats met het oliehoudend contrastmiddel. Het oliehoudend contrastmiddel vormde een emulsie in de zoutoplossing en was daardoor duidelijk zichtbaar als losse olie druppels. Daarnaast lieten de oliedruppels een spoor van minuscule druppels achter (een residu) op de oppervlaktes van de bekkenholte. Het is aannemelijk dat een soortgelijk residu ook aanwezig is binnenin de tubae, waar het door middel van lubricatie van de cilia van het tubaire epitheel mogelijk het fertiliteitsbevorderend effect kan veroorzaken. Deze lubricatie van de cilia door oliehoudend contrastmiddel is een van de hypotheses van het onderliggende mechanisme van het fertiliteitsbevorderende effect zoals te lezen is in hoofdstuk 5.

Om de toepasbaarheid van het doorspoelen van de tubae met oliehoudend contrastmiddel gedurende een THL bij subfertiele vrouwen te onderzoeken hebben we de THL-olie pilot studie uitgevoerd, zoals beschreven in **hoofdstuk 8**. In deze studie hebben 50 euthyreote vrouwen een extra spoeling van de tubae met oliehoudend contrastmiddel gehad, nadat er minimaal unilaterale doorgankelijkheid van de tubae aangetoond was voor methyleenblauw. Gemiddeld werd er 5,0 mL (IQR 3,4-6,3) oliehoudend contrastmiddel gebruikt. De deelnemende vrouwen rapporteerden een hoge tevredenheid ten aanzien van de THL procedure met aanvullende spoeling van de tubae met oliehoudend contrastmiddel (score van 10 uit 10, IQR 8–10). De gemiddelde pijnscore op de Visual Analogue Scale (VAS) was 2,9 (IQR 1,0–5,0) voor de tuba spoeling met methyleenblauw en 3,0 (IQR 1,0–5,0) voor de spoeling met oliehoudend contrastmiddel. In 48 van de vrouwen waren beide tubae doorgankelijk voor methyleenblauw, in 77% (37/48) waren beide tubae ook doorgankelijk voor het oliehoudend contrastmiddel, in 17% (8/48) was dit voor één van de tubae het geval en in 6% (3/48) werd het oliehoudend contrastmiddel niet zichtbaar bij de tubae-uiteinden. Bij twee vrouwen was er unilaterale doorgankelijkheid voor methyleenblauw, bij een van deze vrouwen was het oliehoudend contrastmiddel ook eenzijdig zichtbaar, bij de andere vrouw was er geen doorgankelijkheid voor het oliehoudend contrastmiddel. Het oliehoudend contrastmiddel was zichtbaar als olie-druppels, net zoals beschreven is in het video case report in hoofdstuk 7. Slijm en/of celresten waren zichtbaar bij de

fimbriële uiteindes van de tubae in 32% (16/50) van de THL procedures. Subklinische hypothyreoïdie trad op in 14% (6/42) van de vrouwen vier weken na de procedure. Bij één vrouw werd hypothyreoïdie vastgesteld (FT4 licht verlaagd 11,0 pmol/L en TSH verhoogd 5,60 mU/L), daarnaast was er bij één vrouw sprake van een geïsoleerde hypothyroxinemie (FT4 licht verlaagd 11,0 pmol/L en TSH binnen de normaalwaarden 3,40 mU/L). Bij één vrouw was er sprake van aanhoudend bloedverlies vanuit de insteekopening na de THL, waarvoor een laparoscopie uitgevoerd moest worden. Aan de hand van deze resultaten, concludeerden we dat oliehoudend contrastmiddel (maximaal 10 mL) veilig toegepast kan worden tijdens een THL, zonder het gebruik van doorlichting, indien dit gebruikt wordt als een aanvullende spoeling van de tubae na de diagnose van minimaal enkelzijdige doorgankelijkheid van de tubae.

Hoofdstuk 9 bevat een algemene discussie ten aanzien van de klinische implicaties van dit proefschrift en aanbevelingen voor vervolgonderzoek. Tenslotte is er een impactparagraaf toegevoegd in **hoofdstuk 12** over de wetenschappelijke en maatschappelijke impact van dit proefschrift.

CHAPTER 12

IMPACT PARAGRAPH

IMPACT PARAGRAPH

Research

This thesis has investigated the safety of ethiodized oil (oil-based contrast) during tubal patency testing in subfertile women. Furthermore, it focused on elucidating the underlying mechanism of the fertility-enhancing effect of ethiodized oil and the feasibility of its use during other methods of tubal flushing than hysterosalpingography (HSG).

The studies included in this thesis have shown that the use of ethiodized oil during HSG, with fluoroscopic guidance, in euthyroid subfertile women, is safe for the women and their offspring. During transvaginal hydrolaparoscopy (THL) additional tubal flushing with a maximum of 10 mL ethiodized oil is also safe and acceptable to patients, without the use of fluoroscopic guidance. However, we do advise to only perform tubal flushing with ethiodized oil in procedures without fluoroscopic guidance, such as during THL, after establishing at least unilateral tubal patency to water-based media. This advise is based on the assumption that in the case of bilaterally blocked tubes intravasation is more likely to occur due to a higher pressure build-up proximal to a tubal obstruction. We have proposed five different hypotheses for the fertility-enhancing effect of tubal flushing, especially with ethiodized oil. The hypotheses are divided between the biochemical effects of the contrast media and the interfacial effects. As a start of investigating the different hypotheses on the interfacial effects of ethiodized oil, we have shown a possible method of analysing the in-vivo pressure build-up within the reproductive tract during HSG, and have shown in-vivo observation of the interaction between ethiodized oil and human tissue during a THL.

Relevance

Subfertility, the lack of conception after 12 months of timed unprotected intercourse, is a global health issue, which affects almost one out of six couples. It is estimated that worldwide 48.5 million couples suffer from unwilling childlessness after having tried to conceive for 5 years (Zegers-Hochschild *et al.*, 2009; Mascarenhas *et al.*, 2012; Thoma *et al.*, 2013). The burden of subfertility on women and men should not be underestimated, 57% of women and 32% of men undergoing fertility treatment and/or investigations have significant depressive symptoms (Pasch *et al.*, 2016)

Already from 1948 onwards, the therapeutic value of ethiodized oils on fertility has been investigated (Rutherford, 1948). Decennia later, its fertility-enhancing effect could finally be confirmed by two well-powered RCTs, which showed a 10% and 9% higher ongoing pregnancy rate with the use of ethiodized oil, compared to different types of

water-based contrast (Dreyer *et al.*, 2017; Zhang *et al.*, 2022). This conclusion is also supported by two recent meta-analyses (Fang *et al.*, 2018; Wang *et al.*, 2019) and a Cochrane systematic review (Wang *et al.*, 2020). However, the root cause of the fertility-enhancing effect of ethiodized oil remained unclear after decades of speculation, and some questions regarding its safety were still unanswered.

After the clear conclusion of the recent meta-analyses on the effect of ethiodized oil on fertility rates, it was high time to have updated knowledge on its safety, the feasibility of its use during other tubal flushing methods, and finally elucidating the underlying root cause of the fertility-enhancing effect. This thesis aimed to tackle the aforementioned topics, that have been speculated about for decennia.

Target group

This thesis and its results are especially relevant to clinicians investigating and treating couples with subfertility, guidelines developers, fellow researchers, the healthcare industry, and of course the couples suffering from subfertility themselves.

It is important for clinicians in the field of subfertility to be aware of the increased clinical pregnancy rates after the use of ethiodized oil, the safety of its use, and its possible implementation during other tubal patency testing methods than HSG. To assist implementation of this new evidence into daily clinical care it is essential that guideline developers are aware of this. We are optimistic that, after finishing the multiple ongoing clinical trials on the topic of ethiodized oil and tubal patency testing, clinical guidelines will be updated to provide clinicians guidance in determining the optimal timing and method of tubal patency testing for each woman with subfertility. Furthermore, hopefully, the knowledge of the proposed hypotheses on the root cause of the fertility-enhancing effect of tubal flushing, inspires fellow (including non-medical) researchers and the healthcare industry to continue performing research on the remaining questions regarding ethiodized oil and fertility. With additional knowledge on the root cause of the fertility-enhancing effect of ethiodized oil, researchers and the healthcare industry may develop a new tubal flushing medium, with the same efficacy, but possibly without its (high) iodine content. Finally, the new insights from this thesis provide vital information for subfertile couples on the possible treatment options.

Activities

In performing the research of this thesis we have worked with an international team, consisting of both medical doctors and engineers. This interdisciplinary collaboration has widened the scope of the performed research. The results of the different studies in this thesis have been published in international scientific research journals. The

full articles of the published studies have all been made freely accessible to anyone interested through open access sources. Furthermore, most of the studies and their results have been presented and discussed at international scientific meetings. To involve patients we presented part of this research at a symposium of the Dutch patient association “Freya” for people experiencing fertility problems.

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APPENDICES

LIST OF ABBREVIATIONS

LIST OF PUBLICATIONS

DANKWOORD

CURRICULUM VITAE

LIST OF ABBREVIATIONS

ACOG	American College of Obstetricians and Gynecologists
ALARA	As low as reasonably achievable
ALADA	As low as diagnostically acceptable
ANOVA	Analysis of variance
ATA	American Thyroid Association
ATP	Adenosine Triphosphate
CAT	Chlamydia Antibody Titre
CENTRAL	Cochrane Central Register of Controlled Trials
CH	Congenital Hypothyroidism
CI	Confidence interval
CO2	Carbon dioxide
Dio2	Type 2 deiodinase
DLS	Diagnostic laparoscopy
ETA	European Thyroid Association
FertiQoL	Fertility-related quality of life
FT4	Fee thyroxine
GP	General practitioner
hCG	Human Chorionic Gonadotrophin
HSG	Hysterosalpingography
HyCoSy	Hysterosalpingo-contrast-sonography
HyFoSy	Hysterosalpingo-foam-sonography
IQR	Interquartile ranges

NICE	National Institute Care Excellence
NTR	Netherlands Trial Register
OR	Odds ratio
OSCM	Oil-based contrast media
PCOS	Polycystic Ovarian Syndrome
PCR	Polymerase Chain Reaction
PID	Pelvic inflammatory disease
PCD	Primary ciliary dyskinesia
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
RCT	Randomized Controlled Trial
RIVM	Rijksinstituut voor Volksgezondheid en Milieu
RR	Relative risk
SD	Standard deviation
SPSS	Statistical Package for Social Sciences
STI	Sexual transmitted infection
T4	Total thyroxine
TBG	Thyroid Binding Globulin
THL	Transvaginal hydrolaparoscopy
TSH	Thyroid Stimulating Hormone
UK	United Kingdom
VAS	Visual Analogue Scale
WSCM	Water-based contrast media

LIST OF PUBLICATIONS

Roest I, Bongers MY, Mijatovic V, Mol BWJ, Koks CAM. Feasibility of additional tubal flushing with ethiodized oil at transvaginal hydrolaparoscopy, a pilot study. *Submitted.*

Roest I, Hajiyavand AM, Mijatovic V, Bongers MY, Mol BWJ, Koks CAM, Dearn KD. In-vivo pressure build-up within the reproductive tract during hysterosalpingography: a feasibility study. *Submitted.*

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PRESENTATIONS

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Oral presentation at the annual congress of the “Centrum Voortplantingsgeneeskunde Brabant” 2020: “HSG en oliehoudend contrast”.

Oral presentation at the American Society for Reproductive Medicine (ASRM) 2019: *“Safety of oil-based contrast medium for hysterosalpingography: a systematic review”.*

Oral presentation at the annual congress of the "Vereniging voor Fertilieitsstudies" (VFS) 2018:
"Pijn tijdens follikelpuncties: een vergelijking van drie pijnstillingsprotocollen in een retrospectieve cohortstudie".

CONFERENCE POSTERS

Poster presentation at the European Society for Gynaecological Endoscopy (ESGE) 2022:
"In-vivo pressure build-up within the reproductive tract during hysterosalpingography: a pilot feasibility study".

Poster presentation at the Máxima MC Wetenschapsavond 2022:
"Analyse van de drukopbouw in de eileiders tijdens hysterosalpingografie: een pilotstudie".

Poster presentation at the Máxima MC Wetenschapsavond 2021:
"Extra spoeling van de eileiders met oliehoudend contrast tijdens een Transvaginale Hydro Laparoscopy, een case report".

Poster presentation at the Máxima MC Wetenschapsavond 2020:
"Veiligheid van oliehoudend contrast tijdens hysterosalpingografie: een systematische review".

Poster presentation at the European Society for Gynaecological Endoscopy (ESGE) 2019:
"Complications after hysterosalpingography with oil or water-based contrast: results of a nationwide survey".

Poster presentation at the Congress of the European Society of Human Reproduction and Embryology (ESHRE) 2018:
"Pain scores during oocyte retrieval: a retrospective cohort study comparing three different protocols".

Poster presentation at the Máxima MC Wetenschapsavond 2018:
"Pijnstilling bij follikelpuncties: wat geven we in Nederland?".

Poster presentation at the European Congres of Intrapartum Care (ECIC) 2017:
"Clinical application of non-invasive fetal ECG monitoring during labour".

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CURRICULUM VITAE

Inez Roest werd geboren op 7 juni 1995 in Veldhoven. Gedurende haar jeugdjaren woonde zij in Eindhoven. In 2013 behaalde zij haar VWO diploma cum laude aan het Sondervick College te Veldhoven. Na kortdurende twijfels over een studie Biomedische Technologie of Geneeskunde, verhuisde Inez naar Maastricht, waar zij met passie en verwondering Geneeskunde studeerde. Tijdens een buitenlands keuze coschap liep zij o.a. mee op het Manipal Assisted Reproduction Centre (MARC) en de afdeling Obstetrie en Gynaecologie van het Kasturba Hospital in Manipal, India. Al tijdens haar wetenschapsstage in het eerste jaar van de Master Geneeskunde raakte zij geïnteresseerd in onderzoek binnen de afdeling Obstetrie en Gynaecologie van het Máxima MC. Zij begon aldaar met wetenschappelijk onderzoek onder leiding van prof. dr. Guid Oei in de Fundamentele Perinatologie onderzoeksgroep (FUN). Tijdens dezelfde wetenschapsstage werd haar interesse gewekt door het onderzoek op de afdeling fertiliteit. Zij begon onder begeleiding van dr. Carolien Koks met het opzetten van o.a. de THL-olie pilot studie. Haar studie Geneeskunde sloot ze cum laude af met een semi-arts stage op de afdeling fertiliteit van het Máxima MC, aldaar startte ze in oktober 2019 als fertiliteitsarts en arts-onderzoeker. Zij maakte onderdeel uit van de Moordvrouwen onderzoeksgroep van prof. dr. Marlies Bongers binnen de afdeling gynaecologie van het Máxima MC. Vanaf februari 2021 begon zij als basisarts in de ouderenzorg bij de Vitalis WoonZorg Groep. Per maart 2022 werd ze toegelaten bij de opleiding tot huisarts aan de Maastricht Universiteit, locatie Eindhoven. Gedurende al deze jaren hield zij zich als arts-onderzoeker bezig met wetenschappelijk onderzoek binnen de afdeling fertiliteit van het Máxima MC. Inez woont samen met Stef en hun katten Kiara en Nala in Eindhoven.