Medicine
Case Reports and Study Protocols

Study Protocol Clinical Trial

OPEN

The effect of acute and chronic iodine excess on thyroid profile and reproductive function of women using Lipiodol during hysterosalpingography and the potential impact on thyroid function of their offspring: The SELFI study protocol

Divya M. Mathews, MD[a,b,*], Jane M. Peart, FRANZCR[c], Robert G. Sim, FRANZCR[c], Neil P. Johnson, FRANZCOG[d,e,f], Susannah O'Sullivan, FRACP, PhD[g], José G.B. Derraik, PhD[a,h,i,j], Paul L. Hofman, FRACP[a,b]

Abstract

Background: Hysterosalpingography (HSG) is a radiological procedure using iodinated contrast media to assess tubal patency in women with infertility. HSG using Lipiodol, an oil-soluble contrast medium (OSCM) has been shown to improve pregnancy rates, so its therapeutic use has increased. However, OSCM can cause marked and prolonged iodine excess, potentially impacting thyroid function. If pregnancy occurs, there is also concern regarding possible neonatal hypothyroidism resulting from maternal iodine excess. This study aims to improve knowledge on the safety profile of OSCM HSG in the context of iodine excess.

Methods: This is a prospective longitudinal study of 200 consecutively consenting women undergoing an OSCM HSG in Auckland, New Zealand. After informed consent, participants will undergo baseline thyroid function tests and measurement of urine iodine-to-creatinine ratio (UI/Cr) and anti-thyroid antibodies. During the HSG, the volume of OSCM used will be recorded, and a delayed radiograph obtained to check for further spill of the contrast and for a semi-quantitative assessment of peritoneal retention of OSCM. Thyroid function tests, UI/Cr and reproductive hormones will be serially monitored over the next 6 months. If the woman conceives within the study period, the offspring's thyroid function will be tested at 7 days of age, in addition to the routine newborn screening. The primary outcome will be development of subclinical hypothyroidism (SCH) in these women. Secondary outcomes will include the

Ethics approval and consent to participate: The study protocol (version no.2; dated 26/03/2019) was approved by the Northern B Health and Disability Ethics Committee (Ministry of Health, New Zealand; 19/NTB/52), and was registered with the Australian New Zealand Clinical Trials Registry (ACTRN 12620000738921). Any major amendments to the protocol will be submitted to the Health and Disability Ethics Committee. Written informed consent will be obtained from all participants prior to the enrollment into the study, by the coordinating investigator. A new consent will be also obtained from those who get pregnant during the study period, regarding newborn's day 7 thyroid test, and this will be done by contacting them closer to delivery.

Availability of data and materials: Not applicable as this is a study protocol and it does not contain any new data. The study protocol, model participant information sheets and consent forms, and deidentified datasets generated from the current study will be available from the corresponding author on reasonable request.

Competing interests: The study is funded by an unrestricted institutional research grant to the Liggins institute (University of Auckland) by Guerbet pharmaceuticals, the manufacturer of Lipiodol. NPJ is involved in projects at the University of Auckland and the University of Adelaide that are funded by Guerbet, and has also undertaken paid consultancies for Guerbet. DMM and PLH are involved with University of Auckland research on OSCM safety (SELFI study), through an unrestricted independent grant to the Liggins institute (University of Auckland) by Guerbet. PLH has received fees for speaking in two webinars sponsored by Guerbet. RGS and JMP have been paid for presentations to Guerbet and for being advisory board members to that company. RGS, JMP, and NPJ regularly undertake Lipiodol HSG as a part of their professions. JGBD has no conflicts of interest to disclose.

The study is funded by a research grant awarded by Guerbet pharmaceuticals to Liggins institute. This is an investigator-initiated research and the fund is administered independently by Liggins Institute (Contact address: Private Bag 92019. Victoria Street West Auckland 1142. New Zealand). Guerbet have no role in any aspects of the study including study design, recruitment or delivery of the study, manuscript preparation, data interpretation or decision to publish it.

[a] Liggins Institute, University of Auckland, Auckland, New Zealand, [b] Starship Children's Hospital, Auckland, New Zealand, [c] Auckland Radiology Group, Auckland, New Zealand, [d] Robinson Research Institute, University of Adelaide, Australia, [e] Department of Obstetrics and Gynaecology, University of Auckland, Auckland, New Zealand, [f] Repromed Auckland, Auckland, New Zealand, [g] Endocrinology, Greenlane Clinical Centre, Auckland District Health Board, Auckland, New Zealand, [h] Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden, [i] NCD Center of Excellence, Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, Thailand, [j] Department of Endocrinology, Children's Hospital, Zhejiang University School of Medicine, Hangzhou, China.

[*] Correspondence: Divya M. Mathews, Liggins Institute, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand (e-mail: d.mathews@auckland.ac.nz).

How to cite this article: Mathews DM, Peart JM, Sim RG, Johnson NP, O'Sullivan S, Derraik JGB, Hofman PL. The effect of acute and chronic iodine excess on thyroid profile and reproductive function of women using Lipiodol during hysterosalpingography and the potential impact on thyroid function of their offspring: The SELFI study protocol. Med Case Rep Study Protoc 2021;2:8(e0148).

Received: 13 July 2021 / Accepted: 13 July 2021

http://dx.doi.org/10.1097/MD9.0000000000000148

incidence of elevated iodine levels in these women, and a transient or permanent thyroid dysfunction in the neonates conceived within the study period. In addition, mixed-model analyses will attempt to identify 'high-risk' groups for thyroid dysfunction.

Discussion: This study will explore the acute and chronic effects of iodine excess on thyroid function in women who undergo an OSCM HSG and in their offspring conceived in the immediate cycles following HSG. Further, this study will provide information on the profile of thyroid function abnormalities following an OSCM HSG, and help guide the establishment of international protocols for thyroid monitoring and management in women undergoing this procedure.

Trial registration: Trial acronym–SELFI (Safety and Efficacy of Lipiodol in Fertility Investigations)

ACTRN: ACTRN12620000738921, retrospectively registered on 14/07/2020

Abbreviations: AMH = anti-mullerian hormone, ANZCTR = Australian New Zealand Clinical Trials Registry, FSH = follicle stimulating hormone, HSG = hysterosalpingogram, IQ = intelligence quotient, IUI = intrauterine insemination, LH = luteinising hormone, OSCM = oil-soluble contrast medium, RDA = recommended daily allowance, SCH = subclinical hypothyroidism, SGA = small-for-gestational age, T3 = triiodo thyronine, T4 = thyroxine, TSH = thyroid-stimulating hormone, UI/Cr = urine iodine-to-creatinine ratio, WSCM = water-soluble contrast medium.

Keywords: HSG, hysterosalpingogram, infertility, iodine, lipiodol, OSCM, thyroid

1. Introduction

Hysterosalpingography (HSG) with iodine-containing contrast has been used for assessment of fallopian tubal patency for many decades, with extensive use of both oil-soluble (OSCM) and water-soluble (WSCM) contrast media.[1,2] The serendipitous observation that pregnancy rates improved following HSG with OSCM was confirmed in a series of studies[3–5] and a Cochrane review, which found tubal flushing with OSCM HSG to be better than 'no intervention' in subfertile women (ongoing pregnancy rate of 29%–55% vs 17%).[6] In the most recent, large multi-center trial, OSCM was shown to improve pregnancy rates by 39.7% versus 29.1% when compared with a water-soluble contrast (WSCM).[7] While the precise mechanism underlying the increase in pregnancy rate remains uncertain, this observation has proven reproducible and robust.[8] The side-effect profile is promising as the recognized complications due to OSCM HSG are extremely rare (i.e., fat embolism, symptomatic intravasation in the uterine myometrium, granuloma formation in the peritoneum, and post-procedure bleeding or infection).[9,10] Unsurprisingly, there has been a dramatic rise in requests for diagnostic HSG with OSCM.[9] However, the increased use of OSCM for HSG has highlighted potential risks to the woman and the fetus in the case of a successful pregnancy. Of particular concern is the markedly high iodine content of the most commonly used OSCM (Lipiodol has 480 mg iodine in one millilitre (ml) vs 150 μg iodine in one standard iodine supplement tablet), and it's very long half-life in tissue (50 days).[11]

Tubal patency is established during HSG by visualising passage of the contrast through one or both fallopian tubes into the peritoneal cavity. Following a Lipiodol HSG, there is a variable amount of contrast retained in the peritoneal cavity, depending upon tubal patency and the volume of Lipiodol instilled.[10] As up to 10 ml of Lipiodol are typically used in HSG studies, it is conceivable that some women may retain several ml of Lipiodol in the peritoneum after the procedure. Given the half-life of Lipiodol is approximately 50 days,[11] and assuming it takes 5 half-lives to result in complete excretion from the system, a single procedure may result in exposure to excess iodine for 250 days or approximately 35 weeks. For instance, if only 2 ml of OCSM is retained, this would result in iodine release of approximately 9600 μg daily in the initial 50 days after Lipiodol HSG. This equates to more than 60 times the recommended daily allowance (RDA) for iodine intake established by Australian National Health and Medical Research Council, which is 150 μg/d in adults and 220 μg/day in pregnant women.[12] Even after 4 half-lives or 200 days, the estimated iodine released would be approximately 600 μg daily, or four times the recommended daily intake.

A small study from Japan of 22 women who underwent OSCM HSG demonstrated that some had iodine levels elevated by 100-fold, which was maintained up to 24 weeks post HSG.[13] Urine and serum iodine levels normalised by 40 weeks post HSG, consistent with the known half-life of OSCM. However, the increase in iodine levels was variable and not a universal phenomenon. Some women had no elevation in iodine levels post HSG, while others had a marked rise in iodine levels, possibly reflecting variation in the amount of retained OSCM following the HSG.

The most obvious risk from markedly elevated iodine levels is altered thyroid function. In particular, a sudden increase in iodine levels results in acute inhibition of both thyroid hormone release and organification, which is termed the Wolff-Chaikoff effect.[14,15] This effect is generally transient, lasting less than 2 weeks until 'adaptation' or 'escape' from the Wolf-Chaikoff effect occurs. However, in some situations the 'adaptation' or 'escape' mechanism fails, resulting in prolonged hypothyroidism. Such situations include underlying autoimmune thyroid disease, non-thyroidal illness, and iodine exposure in early life (fetus and newborn).[15–17] Paradoxically, iodine excess can occasionally induce hyperthyroidism, especially in those with pre-existing Graves disease or multinodular goitre.[18,19] This effect labelled the Jod-Basedow phenomenon induces hyperthyroidism over varying periods following the iodine load, with cases presenting 3 to 6 months following the contrast use.[20] Most of these cases with hyperthyroidism are self-limited and often clinically silent.[21] Thyroid dysfunction (both hypo- and hyperthyroidism) is known to affect fertility and pregnancy.[22] Severe hypothyroidism can cause diminished libido and failed ovulation.[23] While pregnancy can occur with milder hypothyroidism, there is increased risk of fetal loss in the absence of adequate levothyroxine replacement.[24]

Previous studies have suggested occurrence of subclinical hypothyroidism (SCH) following OSCM HSG.[13,25,26] Subclinical hypothyroidism (SCH) is defined as an elevated thyroid stimulating hormone (TSH) level, with normal free thyroxine (T4) and free triiodothyronine (T3) levels.[27] Importantly, even

relatively subtle thyroid dysfunction, as seen in SCH, can adversely impact pregnancy. A metanalysis of 38 studies found that SCH was associated with 1.7-fold increase in pre-eclampsia and 2.7-fold increase in perinatal mortality.[28] SCH during first trimester may also be associated with adverse pregnancy outcomes such as preterm birth, miscarriages, or having a baby born small for gestational age (SGA).[29] Furthermore, maternal SCH during pregnancy can affect the offspring's neurocognitive development and was associated with a 5-point reduction in Intelligence quotient (IQ) for children at 7 to 8 years of age in a case-control study (full scale IQ composite score 103.9 in cases vs 109.1 in controls).[30] Another metanalysis also suggested lower intellectual and motor development in children born to mothers who had SCH during pregnancy.[31]

Aside from the thyroid function abnormalities due to iodine excess and subsequent effects on fertility and pregnancy, there could be other direct pathways by which iodine excess can affect fertility.[32] Potential mechanisms include a direct effect of iodine on the uterus and ovaries, as well as alterations in local and systemic immunological milieu.[33–35] Some earlier animal studies pointed to the negative impact of excess iodine on ovulation and fertility index.[33,34] However, to date, no human studies have examined the potential effects of iodinated contrast on ovarian function or its markers, such as AMH (anti-mullerian hormone), LH (luteinizing hormone), or FSH (follicle stimulating hormone).

High iodine levels may also affect fetal thyroid development and newborn thyroid function. A Japanese study suggested an increased incidence of newborn thyroid dysfunction in offspring conceived immediately following a Lipiodol HSG.[36] Five out of 212 (2.4%) newborns had TSH elevation at birth, four of whom required thyroxine replacement, including two with persistent permanent congenital hypothyroidism.[36] Thus, the incidence of permanent congenital hypothyroidism after maternal OSCM HSG in that study was 0.94% (two out of 212 screened), compared to a background rate of 0.07% (6.8:10000 live births) in Japan.[37] Although the sample size was relatively small, this study raised the possibility that the incidence of permanent congenital hypothyroidism may be increased after an OSCM HSG. However, this pattern of increased thyroid dysfunction in newborns was not replicated in a later study in the Netherlands,[38] where post-hoc analyses on the offspring from the H2Oil trial (n=76) showed no changes in the rates of hypothyroidism among babies conceived following OSCM HSG.

Overall, the available evidence suggests that iodine excess from OSCM HSG could adversely affect maternal thyroid function as well as fetal thyroid development and neonatal thyroid function. However, most were retrospective studies or post-hoc analyses. There is a lack of comprehensive prospective studies assessing acute and long-term safety of OSCM. The SELFI study will address this gap in current knowledge, with a particular focus on thyroid safety in the woman and her offspring.

1.1. Aims and objectives

The aim of this study is to assess the safety of OSCM HSG in both the women and their offspring, in particular, regarding the potential impacts of iodine excess. The gold standard for measuring iodine status in women of reproductive age or during pregnancy is UI/Cr.[39] The UI/Cr minimizes variations caused by differences in urine volume and dilution among subjects in a well-nourished population,[40] and is considered a superior measure than alternatives when adjusted for age and gender.[41] In addition to monitoring UI/Cr, we plan to perform a semi quantitative assessment of OSCM retention in the peritoneum following the HSG, on a delayed post-HSG radiograph, and check its utility as a surrogate marker for the woman's iodine status post-HSG. A delayed radiograph of the pelvis is usually obtained in Lipiodol HSG patients after 45 minutes post procedure to confirm the presence and distribution of peritoneal spill.[10]

The primary objective of SELFI study is to determine:

1. The incidence of SCH in women undergoing OSCM HSG

The secondary objectives are to determine:

1. The types and patterns of thyroid dysfunction in women undergoing OSCM HSG
2. The baseline iodine status in women of reproductive age who seek fertility enhancement treatment
3. The incidence and pattern of iodine excess in women undergoing OSCM HSG
4. If the volume of OSCM used is proportional to iodine excess
5. If a semiquantitative assessment of peritoneal Lipiodol retention in the delayed post HSG radiograph can predict iodine excess in these women
6. If there is increased predisposition to developing thyroid dysfunction following OSCM HSG in certain populations (i.e those with autoimmune thyroiditis or subclinical hypothyroidism on the baseline investigation)
7. If there is an effect of OSCM on reproductive function assessed by LH, FSH or AMH over time
8. The incidence of transient and permanent thyroid function abnormalities in newborns conceived in the immediate 6 cycles following OSCM HSG

2. Methods

The Safety and Efficacy of Lipiodol in Fertility Investigations (SELFI) study is a prospective longitudinal cohort study of women undergoing OSCM HSG in the Auckland region (New Zealand).

2.1. Ethics approval

The study was approved by the Northern B Health and Disability Ethics Committee (Ministry of Health, New Zealand; 19/NTB/52), and is registered with the Australian New Zealand Clinical Trials Registry (ANZCTR: 12620000738921).

2.2. Participants

Potential participants will be women referred for an OSCM HSG in the Auckland region. We aim to recruit 200 consecutive candidates who meet the eligibility criteria and provide informed consent. The first participant was recruited in June 2019 and the last participant expected by the end of August 2021. The first offspring was recruited in April 2020 and the last offspring expected before June 2022.

2.3. Recruitment

The main inclusion criterion is that women have a referral for an OSCM HSG in the Auckland region. Fertility specialists in the region will identify women who would likely benefit from OSCM

HSG. Some specialists prefer that women have proven tubal patency confirmed with WSCM HSG prior to using OSCM HSG, while others use OSCM HSG as a first line for diagnostic purposes and fertility enhancement in the likely absence of tubal pathology.[9] OSCM HSG may be used for patients with either primary infertility (defined as infertility of at least one-year duration) or secondary infertility (defined as greater than one-year infertility with a previous pregnancy). OSCM HSG may be used in those with unexplained infertility (lack of conception after one year of regular unprotected sex in the absence of an identifiable cause for infertility in either of the partners) or endometriosis. Also, OSCM HSG may be offered to augment spontaneous conception or to assist with planned intrauterine insemination (IUI).

Recruitment will be from two private fertility clinics and a tertiary hospital. The research team has been in contact with fertility and radiology centers in the region to promote recruitment. Potential participants will be referred to the coordinating investigator by the fertility specialists or the radiology centers (upon receival of the referral form to perform the HSG). The coordinating investigator will contact the potential candidate by email and phone and apply the inclusion and exclusion criteria to determine eligibility to participate in the SELFI study.

The women meeting below inclusion criteria will be considered:

1. Age above 18 years; AND
2. Having primary or secondary infertility; AND
3. Referred for OSCM HSG in the Auckland region; AND
4. a. Have probable tubal patency, based on the criterion of "low risk for pre-existing tubal damage" as per Dreyer et al[7]; OR
   b. If known to have damage to one or both fallopian tubes, having a referral for an OSCM HSG advised by the fertility specialist (for fertility enhancement from uterine bathing effect of Lipiodol [42]); AND
5. a. Have normal thyroid function (i.e., normal non-pregnant free T4 and TSH), OR
   b. Have subclinical hypothyroidism (defined as a TSH > 4mIU/L and normal free T4 levels) and not be on treatment with levothyroxine

In addition, potential participants will be excluded if they:

1. Have any known contraindication for OSCM use (poppy seed or poppy seed oil allergy, or history of reactions to iodine); OR
2. Have active thyroid disease, such as overt hypothyroidism, hyperthyroidism or thyroid cancer; OR
3. Currently receive treatment with levothyroxine or antithyroid medications; OR
4. On medications known to affect thyroid function or iodine metabolism (e.g. Lithium, Amiodarone etc.)
5. Have used water-soluble contrast within 3 months prior to the OSCM HSG procedure; OR
6. Have used OSCM or any other oil-soluble contrast medium within 6 months before the procedure.

2.4. Assessments

Women meeting the eligibility criteria who provide written informed consent will be enrolled into the study. Their demographic data will be collected from the OSCM HSG referral form from the fertility specialist. Participants will not undergo any anthropometric measurements or physical (clinical) examinations in the SELFI study. However, participants will undergo baseline non-fasting blood tests at a community laboratory (Labtests NZ) in the Auckland region, carried out by phlebotomist at these local laboratories. Blood parameters measured will include TSH, free T4, free T3, as well as reproductive hormones (LH and FSH), anti-thyroid antibodies, and AMH (Table 1). A first morning urine sample will also be collected to measure UI/Cr.

Each participant will undergo an OSCM HSG procedure at one of the two private radiology clinics performing these in the Auckland region. The OSCM HSG will be booked between days 5 and 12 of the woman's cycle and will be performed by one of three experienced radiologists and a fertility specialist following the SELFI protocol. This will include the standard HSG, recording of the exact volume of OSCM used in the procedure, and a post-procedure delayed radiograph after 45 minutes. The steps followed are detailed further in the next section under the heading "OSCM HSG procedure". Note that we do not expect any differences in performance or outcomes between practitioners as they are all experienced and will closely follow the SELFI protocol; however, we aim to record the practitioner's ID to examine possible inter-observer variability. A semiquantitative assessment of peritoneal retention of OSCM will be recorded for each delayed radiograph by a single radiologist.

Following the OSCM HSG, the participant will undergo serial blood and urine tests over the next 6 months as detailed in

Table 1

Assessments of Blood and Urine parameters and their Timing in the SELFI study.

			Time elapsed since OSCM HSG						
Investigation	**Baseline**		**Day 7**	**wk 4**	**wk 8**	**wk 12**	**wk 16**	**wk 20**	**wk 24**
TSH	✓	OSCM HSG procedure	✓	✓	✓	✓	✓	✓	✓
Free T4	✓		✓	✓	✓	✓	✓	✓	✓
Free T3	✓		✓	✓	✓	✓	✓	✓	✓
Urine iodine/creatinine	✓		✓	✓		✓			✓
Thyroid antibodies	✓								
LH	✓		✓	✓	✓	✓	✓	✓	✓
FSH	✓		✓	✓	✓	✓	✓	✓	✓
AMH	✓					✓			

AMH = anti-mullerian hormone, FSH = follicular stimulating hormone, LH = luteinising hormone, T3 = triiodothyronine, T4 = thyroxine, TSH = thyroid stimulating hormone.

Table 1. In particular, the UI/Cr will be assessed at 1, 4, 12, and 24 weeks following the Lipiodol HSG. TSH, FreeT4 and FreeT3 will be assessed at 1, 4, 8, 12, 16, 20, and 24 weeks following HSG. To ensure adherence to the scheduled tests, participants will be reminded by a text message or email by the coordinating investigator on the previous day. If any thyroid function abnormality is identified during the serial monitoring, this will be discussed with the reproductive endocrinologist in the research team, and recommendations will be given to the primary fertility specialist, who has the clinical override.

If the woman conceives during the study period, a repeat consent will be obtained in later part of pregnancy to include the newborn in the study. Following the written informed consent, the newborn will undergo a heel prick test on the seventh day of life at a community lab (Labtests NZ). The blood parameters tested on the neonate will include TSH, free T4, and free T3.

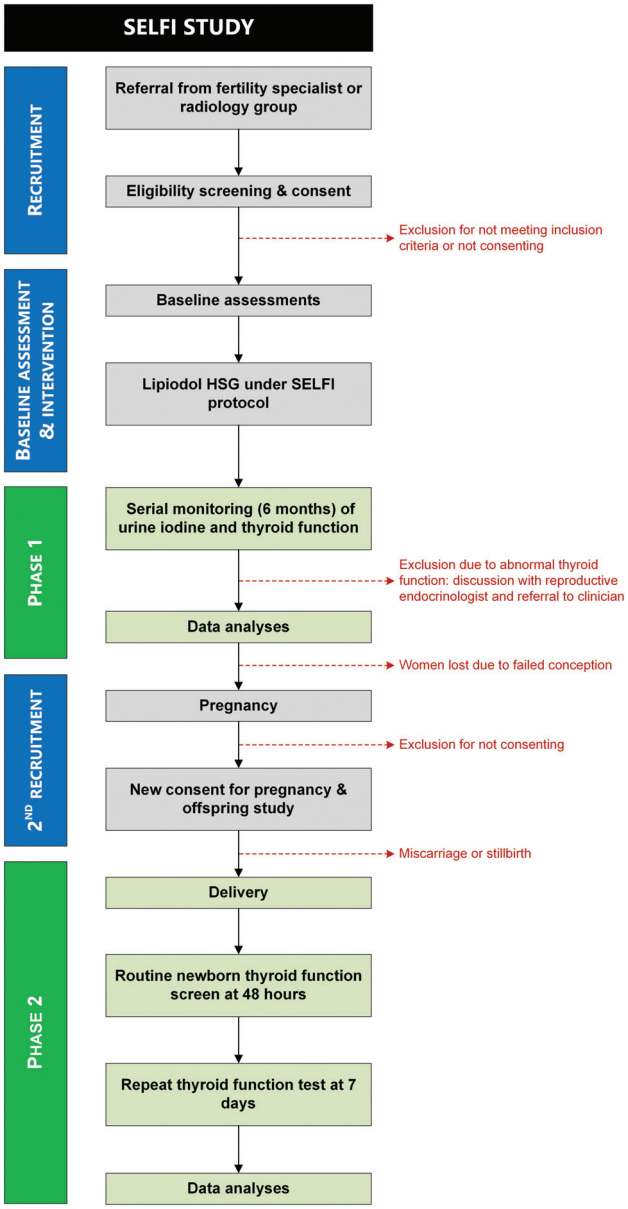

Figure 1. Flow diagram showing the flow of participants in the SELFI study.

These tests will be in addition to the routine 48-hour newborn screening for all neonates born in New Zealand.

All the blood tests will be analysed in the private laboratory upon collection of the sample by standard protocols, and results sent to the researchers. All test results will also be made available to the participant's clinical team, including GP and fertility specialists. Figure 1 and Table 1 outline the flow of participants through the study and the timing of blood and urine investigations with respect to the OSCM HSG.

2.5. The OSCM HSG procedure

For all OSCM procedures, Lipiodol (Guerbet, France) will be used. The SELFI protocol for the Lipiodol HSG consists of the following:

1. Fluoroscopic HSG images as per radiologist standard practice.
2. Preliminary and post-procedure/delayed pelvic radiographs.
   a. The post-procedure radiograph is obtained at least 45 minutes after completion of the HSG. An unopened ampoule of Lipiodol is taped over the right iliac bone with its long axis transverse, which acts as a reference density.
   b. Both pre- and post-procedure radiographs are obtained on the same x-ray unit under the same conditions (e.g., height of tube and exposure factors), while attempting to match them as closely as possible, including in relation to bony landmarks. The preliminary radiograph will be digitally subtracted from the delayed radiograph, and an experienced radiologist (JMP) will derive a semi-quantitative assessment of the amount of retained OSCM in the pelvis after HSG (i.e. mild, moderate, or excessive).

The radiologist documents:

a. Volume of Lipiodol used – excluding the dead space in the tubing and catheter.
b. Tubal spill – classified as none, unilateral, or bilateral.
c. Length of delay (in minutes) for the post procedure radiograph.
d. Presence or absence of intravasation, graded into 4 levels: 0 – no intravasation; 1 – myometrial/lymphatic intravasation; 2 – intravasation into the adnexal veins; or 3 – intravasation into the pelvic/gonadal veins.

Examples of the pre- and post-HSG radiographs are shown in Figure 2.

2.6. Data collection and management

The coordinating investigator will organise the tests with the community laboratory and receive the results of hormone tests and iodine levels. The radiologist will provide the necessary data from OSCM HSG and post-HSG radiograph to the coordinating investigator. All data collected will remain confidential. Each participant will be assigned a unique code and all data and information used for analyses will be deidentified. Only the clinicians in the study team will be able to match a code with a subject. All collected information in hard copy will be stored in locked cabinets, and electronic data in password-protected hardware or servers, accessible only by the study investigators. Hard copies of assessments and personal information will be stored in locked cabinets at the Liggins Institute (University of Auckland) for a minimum of 10 years, while electronic data will be stored indefinitely on password-protected hardware and/or

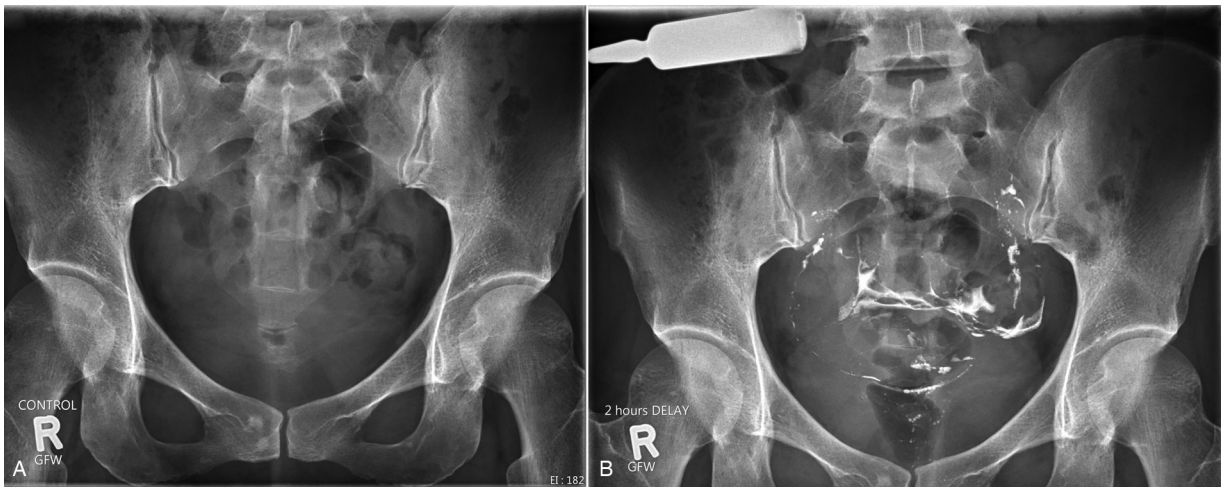

Figure 2. Pelvic radiographs: A) preliminary image obtained prior to OSCM hysterosalpingogram (HSG); B) after HSG, with lipiodol ampoule.

servers. Anonymised findings from the study (without any identifiable information) will be reported in peer-reviewed publications and as part of a PhD thesis, and presented at reputable scientific meetings.

2.7. Safety monitoring process

The OSCM HSG report will be sent within two days to the fertility specialist who referred the patient for the HSG, ensuring appropriate clinical management from the fertility perspective. Any abnormal thyroid result will be discussed with the research team reproductive endocrinologist, participant's fertility specialist, and their primary physician as needed. The participant will be also be informed of all their results.

Of note, the HSG procedures will be occurring independent of the this study as they are the established current standard of practice. This study, irrespective of the results, will have no impact on use of HSGs, but rather will provide data that can be used to ensure that thyroid function is appropriately monitored following the HSGs and treatment initiated appropriately. Thus, as we are prospectively collecting safety data only, a data monitoring committee was not considered necessary.

2.8. Primary outcome

The primary outcome in this study will be the development of SCH in the women following the HSG.

Note: The reference range used are those for the community lab (Lab tests), where the thyroid assay (3rd generations Siemens) is performed and analysed.

Development of SCH defined as[43]

a. TSH $\leq$4 mIU/L at baseline; AND
b. TSH >4 mIU/L; AND free T4 $\geq$11 but $\leq$22 pmol/L at any assessment post-HSG

2.9. Secondary outcomes (women)

- Incidence of overt hypothyroidism:[44] free T4 <11 pmol/L; AND TSH >10 mIU/L at any assessment
- Incidence of overt hyperthyroidism:[45] free T4 >22 pmol/L; AND TSH <0.3 mIU/L at any assessment
- Incidence of subclinical hyperthyroidism:[45] free T4 $\geq$11 but $\leq$22 pmol/L; AND TSH < 0.3 mIU/L at any assessment
- Change (Δ) in serum TSH levels after HSG in women with SCH i.e. those with TSH>4 mIU/L at baseline assessment
- Frequency and timing of SCH in women who did not have SCH at baseline; AND did not become pregnant during the study
- Incidence of abnormal urine iodine (UI) concentrations in non-pregnant woman[46]: Deficiency <100 μg/L; Mild deficiency $\geq$50 but <100 μg/L; Moderate deficiency $\geq$20 but <50 μg/L; Severe deficiency <20 μg/L; Excessive $\geq$300 μg/L
- Incidence of abnormal UI concentrations in pregnant woman [46]: Deficiency <150 μg/L; Excessive $\geq$500 μg/L
- Incidence of abnormal UI concentrations at baseline
- Pregnancy rates in association with abnormal UI concentration
- Visual patterns of UI and UI/Cr over time (curve shapes)
- Associations between the area-under-the-curve (AUC) for both UI and UI/Cr vs:
  a. pregnancy rates
  b. likelihood of SCH at any time point post-HSG
  c. likelihood of offspring SCH
- Associations between UI AUC and UI/Cr AUC and TSH AUC. Note: If a woman is treated for SCH or becomes pregnant, all subsequent time-points will be excluded for TSH. If pregnant, UI/Cr AUC alone will be looked for at subsequent time points as UI/Cr would not be affected by the increased urine excretion with pregnancy
- Associations between Lipiodol volume instilled and:
  a. UI AUC, UI/Cr AUC, and TSH AUC (accounting for the above-described exclusions due to treated SCH or pregnancy)
  b. pregnancy rates
  c. likelihood of SCH at any time point post-HSG
  d. likelihood of offspring SCH
- Associations between post-HSG radiograph Lipiodol peritoneal spill grading (i.e. mild, moderate, or extensive) and:
  a. UI AUC, UI/Cr AUC, and TSH AUC (accounting for the above-described exclusions due to treated SCH or pregnancy)
  b. pregnancy rates
  c. likelihood of SCH at any time point post-HSG
  d. likelihood of offspring SCH
- Visual patterns of LH, FSH, and AMH over time

- Incidence of SCH in women with autoimmune thyroiditis vs in those without autoimmune thyroiditis

2.10. Secondary outcomes (offspring)

- Incidence of SCH in newborns, defined as:
  a) mild TSH elevation during newborn screening (i.e. above the screen cut-off) from Guthrie cards;[47] OR
  b) TSH elevation (above age-appropriate lab reference range) with normal Free T4 on day 7 test
- Incidence of permanent congenital hypothyroidism, defined as persistent elevated blood TSH levels in the newborn requiring ongoing thyroxine replacement based on the stepwise complex protocol for diagnosis.[48,49]

2.10.1. Statistical considerations

2.10.1.1. Sample size. The prevalence of SCH in a large Dutch study was 10.1% among 147,390 women with TSH levels >4 mIU/L and not on any thyroid medication.[50] Assuming relatively comparable population and an equivalent incidence of SCH, a sample size of 193 women undergoing OSCM HSG would allow us to detect a statistically significant difference in SCH incidence of 50% (i.e., an SCH incidence >15.1% or <5.1%) with 98% confidence. Thus, we aim to recruit 200 participants, as in the event of an approximate loss to follow-up of ≈33%, our study (n = 135) would still be powered to detect the same difference with 95% confidence. Of note, based on the data from the Japanese study showing a post-HSG rise in iodine levels, we conservatively estimate that 50% of women undergoing OSCM HSG with Lipiodol will develop iodine excess,[13] and consequently SCH.

In this study, the TSH level will be examined in the context of iodine exposure, in particular, for the group of women with the highest and most prolonged iodine excess. Previous studies suggested that thyroid autoimmunity and subclinical hypothyroidism predispose to greater thyroid dysfunction.[51] Thus, although the primary outcome is SCH following Lipiodol HSG, the potential adverse effects of Lipiodol HSG will likely be greater in those with iodine excess and positive thyroid antibodies, and/or SCH prior to HSG on the baseline test results. While the prevalence of positive anti-thyroid antibodies is ethnicity dependent,[52] its overall prevalence in Australasia is 15% to 24%.[53,54] Therefore, from our target sample size of 200 participants, it is likely that approximately 100 women will have iodine excess, 15 to 25 of whom will also have positive anti-thyroid antibodies or baseline SCH, both of which predispose to a higher incidence of thyroid dysfunction.

Lastly, we estimate that 40% of participants will become pregnant based on data from the H2Oil study.[7] As a result, 60 to 80 successful pregnancies are anticipated from conceptions achieved within 6 months of HSG.

2.10.2. Data analyses. The data will be entered into Microsoft Excel spreadsheets with data entry rules to minimise data entry errors. All records entered will be checked by a second investigator to maximise data transcription accuracy. Descriptive statistics will be generated for primary and secondary outcomes. The incidence of SCH in our cohort (i.e., the primary outcome) will be compared to the expected background population rate of 10.1%[50] using a one-proportion *Z*-test. A range of additional analytical methods will be adopted to examine the various secondary outcomes. Data will be analysed using SAS v9.4 (SAS Institute, Cary, NC), SPSS v27 (IBM Corp, Armonk) and/or Minitab v19 (Pennsylvania State University, State College, Pennsylvania). All statistical tests will be two-tailed, with statistical significance at $P < .05$.

3. Discussion

There are compelling data suggesting that OSCM HSG enhances pregnancy rates.[7,8] Indeed, a recent Australasian consensus guideline suggested there was good quality evidence to support the use of OSCM to assess tubal patency and enhance pregnancy rates in couple with infertility.[9] However, the guidelines suggested there be counselling for rare complications and that possible thyroid dysfunction would need monitoring.

As noted in the introduction, iodine excess is well described following OSCM HSG and likely common. Abnormalities in thyroid function have also been reported,[13,25,26] most commonly SCH and rarely hyperthyroidism. Existing literature indicates SCH occurs[13,25,26] in as many as 1 in every 4 women who undergo an OSCM HSG.[26] The available evidence on thyroid safety for the offspring remains contradictory.[36,55]

Our study has been designed to address this concern about the safety profile of OSCM HSG with respect to iodine excess and the impact on thyroid function. These data will subsequently be used to establish a protocol for monitoring thyroid function following OSCM HSG, including the frequency and duration of thyroid testing. Our study will also define the characteristics of women who are more likely to develop thyroid dysfunction. In particular, we hypothesize that those with higher iodine levels, elevated thyroid antibodies, or baseline SCH are at greater risk. We will also assess the utility of a semi-quantitative assessment by the radiologist of peritoneal OSCM spill from the delayed film post-HSG, as a marker for iodine excess, and its ability to identify those who are likely to develop thyroid dysfunction. Furthermore, this research will provide information on thyroid safety for the offspring conceived following a OSCM HSG.

The main limitation of the study is the lack of a control group. While the latter would have been useful, the primary aim of this study is to assess the prevalence and severity of iodine excess, and how these impact on the thyroid function in women receiving OSCM HSG.

The major strengths of the study are our relatively large planned sample size and multiple measurements of study outcomes. Although other studies have investigated thyroid function following Lipiodol HSG,[13,25,26] their sample sizes were relatively small, thyroid testing strategy heterogenous, and outcome endpoints inconsistent. Moreover, subjects with higher iodine levels were not grouped together and assessed for the extent of thyroid dysfunction.

In conclusion, we expect the SELFI study will improve the existing knowledge regarding the safety of Lipiodol HSG for the woman and their offspring. This will help infertile couples make informed choices on whether to undergo a Lipiodol HSG, and provide the supervising clinicians with valuable data to determine the appropriate frequency and duration of thyroid monitoring following a Lipiodol HSG.

Acknowledgments

The author would like to thank the team at Auckland Radiology Group, Repromed fertility clinic, and Ascot Radiology for their

support with recruitment. We would like to acknowledge Janene McMillan for the ongoing help with organising clinical assessments, Alice Wang for assistance with the SELFI accounts, and Adora Husseini for help with data entry.

Author contributions

DMM drafted the manuscript and will be coordinating the study and clinical assessments. PLH and JMP conceptualised the study. PLH, NPJ, JMP, RGS, SO'S and JGBD contributed to the study design and critically reviewed this manuscript. All authors have contributed to the manuscript and approved its final version for submission.

References

[1] Dutton WA, Stapleton JG. The use of hysterosalpingography in the diagnosis of infertility and other gynecological conditions. Can Med Assoc J 1963;89:1159–64.
[2] Girio-Fragkoulakis C, Tiwari S, Geary S. Hysterosalpingography through the ages: 1914-2014. Clin Radiol 2014;69:S5–6.
[3] Johnson N, Farquhar C, Hadden W, et al. The FLUSH trial—flushing with lipiodol for unexplained (and endometriosis-related) subfertility by hysterosalpingography: a randomized trial. Hum Reprod 2004;19: 2043–51.
[4] Brent K, Hadden WE, Weston-Webb M, et al. After the FLUSH trial: a prospective observational study of lipiodol flushing as an innovative treatment for unexplained and endometriosis-related infertility. Aust N Z J Obstet Gynaecol 2006;46:293–7.
[5] Court KA, Dare AJ, Weston-Webb M, et al. Establishment of lipiodol as a fertility treatment - prospective study of the complete innovative treatment data set. Aust N Z J Obstet Gynaecol 2014;54: 13–9.
[6] Mohiyiddeen L, Hardiman A, Fitzgerald C, et al. Tubal flushing for subfertility. Cochrane Database Syst Rev 2015;CD003718.
[7] Dreyer K, van Rijswijk J, Mijatovic V, et al. Oil-based or water-based contrast for hysterosalpingography in infertile women. N Eng J Med 2017;376:2043–52.
[8] Fang F, Bai Y, Zhang Y, et al. Oil-based versus water-based contrast for hysterosalpingography in infertile women: a systematic review and meta-analysis of randomized controlled trials. Fertil Steril 2018;110: 153–60.
[9] Glanville EJ, Venetis C, Boothroyd CA, et al. The use of oil-soluble contrast media for tubal flushing in infertility: A consensus statement from ACCEPT (Australasian CREI Consensus Expert Panel on Trial evidence). Aust N Z J Obstet Gynaecol 2020;60:667–70.
[10] Peart JM, Sim R. Lipiodol hysterosalpingogram: a modified HSG technique to minimize risks associated with lipiodol use. J Med Imaging Radiat Oncol 2020;64:516–21.
[11] Miyamoto Y, Tsujimoto T, Iwai K, et al. Safety and pharmacokinetics of iotrolan in hysterosalpingography. Retention and irritability compared with Lipiodol. Invest Radiol 1995;30:538–43.
[12] National Health and Medical Research Council. Iodine Supplementation for Pregnant and Breastfeeding Women; National Health and Medical Research Council: Canberra, Australia; 2010. Available at: https://www.nhmrc.gov.au/about-us/publications/iodine-supplementation-pregnant-and-breastfeeding-women
[13] Kaneshige T, Arata N, Harada S, et al. Changes in serum iodine concentration, urinary iodine excretion and thyroid function after hysterosalpingography using an oil-soluble iodinated contrast medium (lipiodol). J Clin Endocrinol Metabol 2015;100:E469–72.
[14] Leung AM, Braverman LE. Consequences of excess iodine. Nat Rev Endocrinol 2014;10:136–42.
[15] Markou K, Georgopoulos N, Kyriazopoulou V, et al. Iodine-induced hypothyroidism. Thyroid 2001;11:501–10.
[16] Parravicini E, Fontana C, Paterlini GL, et al. Iodine, thyroid function, and very low birth weight infants. Pediatrics 1996;98(4 Pt 1):730–4.
[17] Braverman LE, Ingbar SH, Vagenakis AG, et al. Enhanced susceptibility to iodide myxedema in patients with Hashimoto's disease. J Clin Endocrinol Metab 1971;32:515–21.
[18] Dunne P, Kaimal N, MacDonald J, et al. Iodinated contrast-induced thyrotoxicosis. CMAJ 2013;185:144–7.
[19] Rhee CM, Bhan I, Alexander EK, et al. Association between iodinated contrast media exposure and incident hyperthyroidism and hypothyroidism. Arch Intern Med 2012;172:153–9.
[20] Lee SY, Rhee CM, Leung AM, et al. A review: radiographic iodinated contrast media-induced thyroid dysfunction. J Clin Endocrinol Metab 2015;100:376–83.
[21] van der Molen AJ, Thomsen HS, Morcos SK. Effect of iodinated contrast media on thyroid function in adults. Eur Radiol 2004;14:902–7.
[22] Krassas GE. Thyroid disease and female reproduction. Fertil Steril 2000;74:1063–70.
[23] Goldsmith RE, Sturgis SH, Lerman J, et al. The menstrual pattern in thyroid disease. J Clin Endocrinol Metab 1952;12:846–55.
[24] Abalovich M, Gutierrez S, Alcaraz G, et al. Overt and subclinical hypothyroidism complicating pregnancy. Thyroid 2002;12:63–8.
[25] Mekaru K, Kamiyama S, Masamoto H, et al. Thyroid function after hysterosalpingography using an oil-soluble iodinated contrast medium. Gynecol Endocrinol 2008;24:498–501.
[26] So S, Yamaguchi W, Tajima H, et al. The effect of oil and water-soluble contrast medium in hysterosalpingography on thyroid function. Gynecol Endocrinol 2017;33:682–5.
[27] Peeters RP. Subclinical hypothyroidism. N Eng J Med 2017;376: 2556–65.
[28] van den Boogaard E, Vissenberg R, Land JA, et al. Significance of (sub) clinical thyroid dysfunction and thyroid autoimmunity before conception and in early pregnancy: a systematic review. Hum Reprod Update 2011;17:605–19.
[29] Schneuer FJ, Nassar N, Tasevski V, et al. Association and predictive accuracy of high TSH serum levels in first trimester and adverse pregnancy outcomes. J Clin Endocrinol Metab 2012;97:3115–22.
[30] Murphy NC, Diviney MM, Donnelly JC, et al. The effect of maternal subclinical hypothyroidism on IQ in 7- to 8-year-old children: A case-control review. Aust N Z J Obstet Gynaecol 2015;55:459–63.
[31] Liu Y, Chen H, Jing C, et al. The association between maternal subclinical hypothyroidism and growth, development, and childhood intelligence: a meta-analysis. J Clin Res Pediatr Endocrinol 2018;10: 153–61.
[32] Mathews DM, Johnson NP, Sim RG, et al. Iodine and fertility: do we know enough? Hum Reprod 2021;36:265–74.
[33] Mahapatra D, Chandra AK. Biphasic action of iodine in excess at different doses on ovary in adult rats. J Trace Elem Med Biol 2017;39:210–20.
[34] Mahapatra D. Studies on the effect of excessive iodine in female reproduction with reference to thyroid in rats. PhD thesis, Department of Physiology, University of Calcutta, India. 2017. Available at: http://hdl.handle.net/10603/248215.
[35] Yang X, Gao T, Shi R, et al. Effect of iodine excess on Th1, Th2, Th17, and Treg cell subpopulations in the thyroid of NOD.H-2h4 mice. Biol Trace Elem Res 2014;159:288–96.
[36] Satoh M, Aso K, Katagiri Y. Thyroid dysfunction in neonates born to mothers who have undergone hysterosalpingography involving an oil-soluble iodinated contrast medium. Horm Res Paediatr 2015;84:370–5.
[37] Gu YH, Kato T, Harada S, et al. Seasonality in the incidence of congenital hypothyroidism in Japan: gender-specific patterns and correlation with temperature. Thyroid 2007;17:869–74.
[38] van Welie N, Roest I, Portela M, et al. Thyroid function in neonates conceived after hysterosalpingography with iodinated contrast. Hum reprod 2020;35:1159–67.
[39] Li C, Peng S, Zhang X, et al. The urine iodine to creatinine as an optimal index of iodine during pregnancy in an iodine adequate area in China. J Clin Endocrinol Metab 2016;101:1290–8.
[40] Bourdoux P. Evaluation of the iodine intake: problems of the iodine/creatinine ratio–comparison with iodine excretion and daily fluctuations of iodine concentration. Exp Clin Endocrinol Diabetes 1998;106(Suppl 3):S17–20.
[41] Knudsen N, Christiansen E, Brandt-Christensen M, et al. Age- and sex-adjusted iodine/creatinine ratio. A new standard in epidemiological surveys? Evaluation of three different estimates of iodine excretion based on casual urine samples and comparison to 24 h values. Eur J Clin Nutr 2000;54:361–3.
[42] Reilly SJ, Glanville EJ, Dhorepatil B, et al. The IVF-LUBE trial - a randomized trial to assess Lipiodol® uterine bathing effect in women with endometriosis or repeat implantation failure undergoing IVF. Reprod Biomed Online 2019;38:380–6.
[43] Toloza FJK, Abedzadeh-Anaraki S, Maraka S. Subclinical hypothyroidism in pregnancy. Curr Opin Endocrinol Diabetes Obes 2019;26:225–31.

[44] Garber JR, Cobin RH, Gharib H, et al. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. Endocr Pract 2012;18:988–1028.
[45] Ross DS, Burch HB, Cooper DS, et al. 2016 American thyroid association guidelines for diagnosis and management of hyperthyroidism and other causes of thyrotoxicosis. Thyroid 2016;26:1343–421.
[46] World Health Organization. Urinary iodine concentrations for determining iodine status in populations Available at: https://www.who.int/vmnis/indicators/urinaryiodine/en/
[47] West R, Hong J, Derraik JGB, et al. Newborn screening TSH values less than 15 mIU/L are not associated with long-term hypothyroidism or cognitive impairment. J Clin Endocrinol Metab 2020;105: e3329–38.
[48] Heather NL, Hofman PL, de Hora M, et al. Evaluation of the revised New Zealand national newborn screening protocol for congenital hypothyroidism. Clin Endocrinol 2017;86:431–7.
[49] Albert BB, Cutfield WS, Webster D, et al. Etiology of increasing incidence of congenital hypothyroidism in New Zealand from. J Clin Endocrinol Metab 2012;97:3155–60.
[50] Wouters H, Slagter SN, Muller Kobold AC, et al. Epidemiology of thyroid disorders in the Lifelines Cohort Study (the Netherlands). PloS One 2020;15:e0242795.
[51] Walsh JP, Bremner AP, Feddema P, et al. Thyrotropin and thyroid antibodies as predictors of hypothyroidism: a 13-year, longitudinal study of a community-based cohort using current immunoassay techniques. J Clin Endocrinol Metab 2010;95:1095–104.
[52] Alexander EK, Pearce EN, Brent GA, et al. 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum. Thyroid 2017;27:315–89.
[53] Blumenthal NJ, Byth K, Eastman CJ. Prevalence of thyroid dysfunction and thyroid antibodies in a private obstetrical practice in Sydney. Aust N Z J Obstet Gynaecol 2016;56:307–11.
[54] McElduff A, Morris J. Thyroid function tests and thyroid autoantibodies in an unselected population of women undergoing first trimester screening for aneuploidy. Aust N Z J Obstet Gynaecol 2008;48:478–80.
[55] van Welie N, Portela M, Dreyer K, et al. Iodine contrast prior to or during pregnancy and neonatal thyroid function: a systematic review. Eur J Endocrinol 2021;184:189–98.