Performance of outpatient transvaginal hydrolaparoscopy


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STUDY QUESTION: What is the feasibility of performing transvaginal hydrolaparoscopy (THL) in an outpatient setting?

SUMMARY ANSWER: It is feasible to perform THL in an outpatient setting, reflected by a low complication and failure rate and a high patients’ satisfaction.

WHAT IS KNOWN ALREADY: THL is a safe method to investigate tubal patency and exploring the pelvis in subfertile women.

STUDY DESIGN, SIZE, DURATION: Retrospective cohort study of 1127 subfertile women who underwent THL as primary diagnostic method for testing tubal patency in an outpatient setting.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We studied all THL procedures performed as a primary diagnostic tubal patency test in an outpatient setting in subfertile women starting from the initial THL in four large hospitals. Baseline characteristics were obtained, as well as the outcome of the procedures in terms of success, complications and findings by examining medical records. We used a uniform visual analogue scale (VAS) score document to collect data on pain and acceptability prospectively and compared two methods of pain relief.

MAIN RESULTS AND THE ROLE OF Chance: We studied a total of 1103 women who underwent THL. Successful access to the pouch of Douglas was achieved in 1028 women (93.2%), and 1017 women had a complete evaluation (92.2%). Double-sided tubal patency was found in 844 women (83%), unilateral tubal patency in 127 women (12.5%), while in 46 women (4.5%) bilateral occluded tubes were diagnosed. Endometriosis alone was seen in 64 women (6.3%), adhesions alone in 87 women (8.6%) and both endometriosis and adhesions in 42 women (4.1%).

Complications occurred in 29 (2.6%) women, including 10 perforations of the rectum (0.9%), 8 perforations of the posterior uterine wall (0.7%) and 5 infections/pelvic inflammatory diseases (PIDs) (0.5%). Bleeding of the vaginal wall requiring intervention and hospital admissions due to pain was seen in 4 (0.4%) and 2 women, respectively (0.2%). The average pain score was rated 4.0 (± 2.4 SD) on a VAS from 0 to 10 with 0 meaning no pain at all and with no difference in different types of pain relief. Acceptability was rated 1.5 (± 2.1 SD).

LIMITATIONS, REASONS FOR CAUTION: The main limitation of the study is its retrospective character and the fact that only a fourth of the women were asked for pain and acceptability scores.

WIDER IMPLICATIONS OF THE FINDINGS: THL can be used as a primary method for tubal assessment in an outpatient setting. Further randomized studies are needed to assess whether THL is superior to other methods and strategies for tubal assessment in terms of prognostic capacity and cost-effectiveness.

STUDY FUNDING/COMPETING INTEREST: No external funding was either sought or obtained for this study. The authors have no competing interests to declare.

Key words: transvaginal hydrolaparoscopy / tubal pathology / fallopian tubes / fertility / patency tests
Introduction

Subfertility, i.e. the failure to conceive within 1 year of unprotected regular sexual intercourse, occurs in ~10% of couples who wish to conceive. Tubal pathology is an underlying cause in 10–30% of the couples with subfertility (Evers, 2002). As a consequence, a basic fertility workup includes assessment of tubal patency. The feasibility and cost-effectiveness of the different techniques to investigate tubal patency, however, is subject of debate.

Diagnostic laparoscopy is considered to be the reference standard, but poses a risk of major gastrointestinal and vascular complications next to the need for general anaesthesia and hospitalization (Chapron et al., 1999; Tarik and Fehmi, 2004). Hysterosalpingography (HSG), often used as the primary method, has a limited sensitivity of 65% and a specificity of 83% (Swart et al., 1995). In women without risk factors for tubal occlusion, the sensitivity is even lower (Broeze et al., 2011). Furthermore, HSG is known to have limitations in diagnosing peritubal adhesions and has the disadvantage of exposing the women to radiation.

An alternative for diagnostic laparoscopy and HSG, is transvaginal hydrolaparoscopy (THL), which was first described by Gordts et al. (1998). THL is a technique that uses hydroflotation as mechanism to explore the pelvic abdomen via the transvaginal route. It has been shown to be a safe procedure with a learning curve of 50 procedures (Gordts et al., 2001; Verhoeven et al., 2004). THL can be carried out in an outpatient setting under local anaesthesia (Gordts et al., 2000; Cicinelli et al., 2001; Van Tetering et al., 2007), allowing to explain the findings directly to the woman.

Over the last decade, THL has been the method of first choice for tubal testing in the fertility work-up in four teaching hospitals in the Netherlands. Before this procedure is implemented in other hospitals, it is essential to know the feasibility in our daily practice as well as the drawbacks. Therefore, we report on the feasibility of THL as primary diagnostic tool for tubal testing in an outpatient setting in terms of success rate, findings, complications and woman’s tolerability in terms of pain scores and acceptability.

Materials and Methods

Ethical approval

During the first attendance at the outpatient clinic, patients completed a general questionnaire in which they could consent that their findings were anonymously used for research. In accordance with the code of conduct in health, 2004, and under Dutch law, further ethical approval was not required.

Participants and setting

We performed a descriptive retrospective study among subfertile women who underwent THL as part of their basic fertility work-up between January 2000 and December 2011. The study was performed in four large teaching hospitals in the Netherlands (Maxima Medisch Centrum, Veldhoven; Medisch Spectrum Twente, Enschede; Isala Klinieken, Zwolle and St. Antonius Ziekenhuis, Nieuwegein). All these four hospitals use THL as the primary diagnostic procedure for tubal patency testing in subfertile women. In Veldhoven, the gynaecologists started to perform THL in 2000, in Nieuwegein in 2008, in Enschede in 2009 and in Zwolle in 2010.

Prior to THL, women had a detailed history taken, underwent gynaecological examination including transvaginal ultrasound, Chlamydia antibody titers (CAT) and Chlamydia PCR when CAT was positive. Chlamydia PCR positive patients were treated prior to the procedure. THL was performed irrespective of the outcome of CAT. In women with a fixed retroverted uterus, ovarian cysts or suspicion of endometriosis in the pouch of Douglas, the THL was not performed and these women were excluded, as they either underwent a HSG or conventional diagnostic laparoscopy. All women undergoing a THL at the outpatient clinic received oral and written information about the procedure.

Procedure

The THL was performed by gynaecologists with a special interest in reproductive medicine and/or minimal invasive surgery. The first procedures were carried out under general anaesthesia in order to acquire the necessary skills. In the present study, only women undergoing the THL under local anaesthesia in an outpatient setting were included, starting from the first procedure of the gynaecologist under these circumstances.

THL was performed as described by Gordts et al. (1998). We used the Storz re-usable system in three hospitals (Maxima Medisch Centrum, St. Antonius Ziekenhuis and Isala Klinieken), while in the fourth hospital we used a specially designed fertiloscope (Medisch Spectrum Twente). In Veldhoven, for the initial 272 procedures, the circon-system (Circon ACMI, Stanford, CA, USA) was used.

The procedure was scheduled in the proliferative phase of the menstrual cycle. Women were premedicated with 500 mg Naproxen (Centrafarm B.V., the Netherlands). In Medisch Spectrum Twente, 2 ml of alfentanil (Janssen-Cilag B.V., the Netherlands), 0.5 mg/ml was given intravenously just before the Veress needle was inserted.

Women with a positive CAT had a prophylactic dose of 1000 mg azithromycin (Pfizer B.V., the Netherlands). In case of a positive PCR for Chlamydia, women were treated first with antibiotics and the THL was rescheduled after a negative PCR swab.

The procedure was performed with the woman in the dorsal gynaecological position positioned. The women and their partners could follow the procedure on a video screen. After insertion of a trepat speculum, the vagina was disinfected with aqueous chlorhexidine solution. The central part of the posterior cervix was infiltrated with 1–2 ml of ultracan D-S (Sanofi Aventis B.V., the Netherlands). A tenaculum was placed on the posterior cervix and a balloon catheter was put in the uterine cavity and the balloon inflated with 1–2 ml of air for the chromopertubation. Local anaesthesia with 2–3 ml of ultracan was performed in the vaginal vault, 1–2 cm below the cervix. A small incision was made at this place.

The following steps were performed depending of using the specially designed Storz re-usable system or the fertiloscope.

For the Storz reusable system at the place of the incision, the trocar system is introduced. The system consists of an adapted needle, a dilatation device and a trocar 3, 9 mm in outside diameter. All three parts fit together but the needle is longer than the dilatation device. The Veress-like needle is inserted by a special needle loading system. Progressively, the dilators and trocar are inserted into the pouch of Douglas after which the needle and dilators are removed and replaced by a rigid 2, 7 mm wide-angel 30 optical system. Continuous infusion with warmed saline solution is then started.

For the fertiloscope, a Veress needle was inserted at the place of the incision. After infusion of 100–200 ml pre-warmed saline solution, the Veress needle was removed. At the same puncture site, the fertiloscope was inserted. The fertiloscope is designed with a balloon to keep the trocar in place. The 30° endoscope was inserted in the trocar, and after confirming correct placement, the saline infusion was connected to the fertiloscope to give the opportunity to infused more saline when necessary. Finally, the balloon was inflated with 5 ml of air. After correct insertion, the speculum was removed in order to avoid discomfort for the women and allowing free movement of the scope. After infusion of saline and some orientation,
the investigation started at the posterior uterine wall. Then the scope was moved laterally to identify the tubo-ovarian structures on the right and the left side consecutively. The ovarian surface was inspected, consecutively the ovarian ligament, the fossa ovarica and the dorsal part of the ovary. Subsequently, both the fimbral part of the Fallopian tubes and the tubo-ovarian contact were inspected. Then a dye test was performed to test the patency of the tube. Throughout the whole procedure, continuous irrigation with warm saline kept the bowel and the tubo-ovarian structures afloat enabling clear vision.

After the procedure, the fluid was allowed to drain from the pouch of Douglas. The puncture site in the fornix posterior was not sutured unless active bleeding was noted. An additional hysteroscopy was only performed in case of suspected uterine anomaly or intraperitoneal pathology.

Women were informed that some vaginal leakage or bleeding could occur, and were advised not to use tampons. The women left the outpatient clinic within 1 h after the procedure, except for those whom had been given alfentanil (Janssen-Cilag B.V.), i.v.

Outcome measures

In this study, we studied performance of the THL in terms of four categories:

(i) Complete evaluation, defined as visualization of the entire pelvis meaning the tubo-ovarian structures, pelvic sidewalls and the pouch of Douglas together with a blue dye test.

(ii) Incomplete evaluation procedure. This meant that there was an inability for complete evaluation due to pelvic abnormalities like endometriosis or adhesions.

(iii) Incomplete diagnostic procedure. The procedure was classified as incomplete non-diagnostic when the pouch of Douglas was reached and seen, but complete visualization could not be achieved due to, for example, technical problems, blurred vision or pain. This meant that no diagnosis could be made and that the woman had to undergo another procedure for testing tubal patency, for example, a HSG or diagnostic laparoscopy with tubal testing.

(iv) Failure, defined as the inability to reach the pouch of Douglas due to tenting of the peritoneum, masses in the pouch of Douglas, obesity or technical problems.

Furthermore, we recorded the findings of the THL. These were classified as normal or abnormal. Abnormal findings were defined as tubal occlusion, endometriosis and/or adhesions. Next to this, complications and woman’s pain scores and acceptability were analysed.

Complications were defined as an unintended and undesirable event or condition during or following THL with a negative effect on the patients’ health with the need for an intervention, hospital admission or another medical treatment.

Pain scores and acceptability of the THL in an outpatient setting were investigated in Maxima Medisch Centrum during the period of January 2000 and December 2004. After which they stopped because of an acceptable score. Furthermore, in Medisch Spectrum Twente and Isala kliniek pain scores and acceptability were investigated during the period of March 2010 and December 2011. Hereby, women were asked to rate their pain directly after the procedure on a visual analogue scale (VAS) ruler, which were then read by trained nurses. VAS score 0 for pain meant no pain at all and 10 meant the worst pain one could imagine. For acceptability, a VAS score of 0 meant total willingness to undergo the procedure again under same circumstances if necessary and 10 meant no acceptability at all.

Statistical analysis

Data were analysed using the software package SPSS for Windows version 22 (IBM Corp., USA). Nominal variables are reported as numbers and frequencies; continuous variables as mean ± standard deviation. The Mann–Whitney U test was used for analysing the VAS scores. P < 0.05 was considered statistically significant.

Results

From January 2000 till December 2011, a total of 1127 women were scheduled for THL in an outpatient setting. Of these women, 24 had abnormal findings during vaginal examination before starting the THL procedure, such as masses in the pouch of Douglas, a suspicion of extensive endometriosis or a fixed retroverted uterus. They were therefore rescheduled for HSG or diagnostic laparoscopy. Consequently, 1103 women underwent THL at the outpatient clinic. The characteristics of the women are shown in Table I.

The 1103 procedures were performed by 16 different gynaecologists. The number of procedures per gynaecologist varied between one and 296 procedures (mean 75, Table II). Each of them carried out more than 100 procedures for a total of 695, accounting for more than 60% of the THLs performed. The average procedure time was 13.7 min (± 6.7 SD). All gynaecologist performing up to 10 THL’s were supervised by an experienced gynaecologist during these procedures.

Access to the pouch of Douglas was achieved in 1028 women (93%). In 1017 women (92%), a complete evaluation (n = 989) or an incomplete evaluation procedure (n = 28) could be carried out (Table II). There were 11 incomplete diagnostic procedures (1.0%) due to technical problems, inability to find the tubo-ovarian structures or blurred vision of unknown etiology. Consequently, in 75 women (6.8%), a THL failure occurred. In 39 of these women, pre-peritoneal Veress needle- or trocar placement (3.5%) was the underlying cause. Other causes were vaginismus/pain n = 12 (1.1%), rectum perforation n = 8 (0.7%), perforation of the uterus n = 5 (0.5%), a retroverted uterus n = 5 (0.5%), cervical stenosis (occluding adhesions of the internal cervical ostium) n = 3 (0.3%) or obesity n = 3 (0.3%).

Although a retroverted uterus is a relative exclusion criterion for performing THL, 31 women with a mobile retroverted uterus underwent a THL. Access to the pouch of Douglas was achieved in 25 women (80.6%), and in 22 (71.0%) a complete evaluation or incomplete evaluation procedure could be performed. Moreover, four complications occurred in this group (12.9%), whereas only 29 complications (2.6%) occurred in all women (Table III).

Of all complications, rectum perforation was the most common one and occurred 10 times (0.9%) (Table III). In all cases, expectant management was applied with (n = 4) or without (n = 6) antibiotics. Eight times the rectum perforation was the cause of the THL failure; two times the

<table>
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<th>Table I: Characteristics of the participants.</th>
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<td><strong>Women (n)</strong></td>
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<tr>
<td><strong>Mean age (years ± SD)</strong></td>
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<tr>
<td><strong>Primary subfertility (%)</strong></td>
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<tr>
<td><strong>Mean duration of subfertility (months ± SD)</strong></td>
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<tr>
<td><strong>Ovulatory cycles (%)</strong></td>
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<td><strong>Positive Chlamydia serology (%)</strong></td>
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<td><strong>Normal semen analysis partner (%)</strong></td>
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procedure could be continued after replacing the trocar. One major complication occurred in this series: a case of bleeding of the vaginal wall which required suturing under general anaesthesia with 500 ml blood loss in total. No blood transfusion was needed and the woman recovered uneventfully.

When looking at the performance of the four gynaecologists exceeding 100 procedures, the THL failure rate ($n = 44$ out of 695) was 6.3%. Gynaecologists, who performed between 50 and 100 procedures, had a failure rate of 8.1% ($n = 22$ out of 270) and gynaecologists between 11 and 50 procedures of 7.8% ($n = 9$ out of 116). The complication rate of the four gynaecologists exceeding 100 procedures was 2.3% ($n = 16$ out of 695) with 10 complications in the 50 first procedures (5%) performed, 4 between 50 and 100 procedures (2%) and 2 above 100 procedures performed (0.7%).

Table IV shows the findings of the THL. In the 1017 women with a complete evaluation or incomplete evaluation procedure 729 (71.7%) showed bilateral tubal patency without other abnormalities. In the other 288 women (28.3%), tubal occlusion or abnormalities were detected. Bilateral tubal occlusion was seen in 46 (4.5%) women, while 127 women (17.0%) had unilateral tubal occlusion. There were 115 (11.3%) women with bilateral tubal patency, who had endometriosis ($n = 49$; 4.8%), adhesions ($n = 41$; 4.0%) or both ($n = 25$; 2.5%).

Pain scores from 356 women were obtained in three hospitals (Maxima Medisch Centrum, Isala klinieken and Medisch Spectrum Twente) with a response rate of 86%. The mean pain score was rated 4.0 ($\pm$ 2.4 SD) on a scale from 0 to 10 with 0 meaning no pain at all and 10 meaning the worst pain one can imagine (Fig. 1). It must be stated that 163 of these women (45.5%) received 2 ml of alfentanil 0.5 mg/ml intravenously prior to the procedure. No statistical significant difference in VAS scores was found between the women with and without alfentanil (VAS 4.2 (SD 2.3) and 3.8 (SD 2.5), respectively). Acceptability was valued 1.5 ($\pm$ 2.1 SD) by 233 women, with 0 meaning absolute acceptance, whereas 10 meant no acceptance (Fig. 2).

**Discussion**

This study shows that THL is a safe and reliable method for tubal patency testing as we demonstrated low complication rates (2.6%), a high acceptability and pain scores (respectively, VAS 1.5 and 4.0) at acceptable performance with a complete evaluation in 92% of the women. The major drawback of the study is the retrospective study design with lack of controls and thus possible selection and information bias. Nevertheless, it gives a good overview of tubal testing in daily practice in teaching hospitals.

The overall failure rate of THL in this study is higher than reported in some studies (Gordts et al., 2000; Verhoeven et al., 2004; Van Tetering et al., 2007; Kissler et al., 2011), but comparable to others (Darai et al., 2000; Nawroth et al., 2001). Pre-peritoneal Veress needle- or trocar placement was the main reason for failure, which is known to happen more often during the first procedures as reported by Verhoeven et al. (2004). In this study, all first procedures in four hospitals performed by 16 gynaecologists were taken into account. Only 8 of them exceeded 50 procedures, proposed to be the learning curve threshold (Gordts et al., 2001; Verhoeven et al., 2004).

Another contributor to the failure rate could be the fact that 31 women with a retroverted uterus were included. The reason for these inclusions is speculative in this retrospective cohort. Many women in
this cohort were selected and planned for THL not by the operators themselves but by fertility doctors. Poor selection of women for the THL procedure could therefore be an explanation for the higher failure rate. This is also reflected by the 15 women who were planned to undergo a THL but were excluded from the procedure after assessment by the gynaecologist due to perform the THL. The women with a retroverted uterus had a higher risk for an incomplete non-diagnostic evaluation or THL failure compared with the whole group, 29.1 versus 7.8% and a higher complication risk overall (12.9 versus 2.6%). This emphasizes that a retroverted uterus can be considered as a relative contraindication for performing a THL. Nevertheless, our overall complication rate is in the same range as known in the literature (Gordts et al., 2000, 2001; Shibahara et al., 2007; Van Tetering et al., 2007; Kissler et al., 2011). Our analysis also indicates that experience might lower the complication rate with a decrease in complications from 5% within the first 50 procedures to 0.7% after 100 procedures.

An advantage of THL is that it can discover subtle fertility problems at an early stage of the fertility workup contrary to the poor performance of HSG to diagnose these abnormalities. Cincinelli et al. described, among others, a concordance of 95% in tubal patency testing between THL and HSG, but HSG missed other pathology known to compromise fertility such as peritubal adhesions and endometrioses (Shibahara et al., 2001; Fujiwara et al., 2003). In the present cohort, endometriosis and/or adhesions were diagnosed in 115 women (11.3%) with patent tubes and these subtle fertility problems would have been missed if HSG was applied as a tubal patency testing method. The clinical implications of these problems is discussed by Van Kessel et al. (unpublished data), who showed a fecundity rate ratio of 0.42, meaning less probability of spontaneous intrauterine pregnancy per time unit for women with patent tubes but with endometriosis and adhesions, compared with those without.

Another possible advantage is that less diagnostic laparoscopies are needed. Many hospitals initially use HSG as tubal patency test but plan a diagnostic laparoscopy when no spontaneous pregnancy is achieved in 6–12 months in case of unexplained subfertility or before treatment is started or when HSG shows abnormalities. In our four hospitals, no diagnostic laparoscopy was scheduled for the 729 (71.7%) women with no abnormalities during THL. Furthermore, when abnormalities during THL were seen, the women were counselled and planned directly for fertility enhancing surgery. As an estimate, in this series over 800 women were spared undergoing diagnostic laparoscopy which gives an avoidance rate of 70%. In other studies, avoidance rates from 46.2% (Watrelot et al., 1999), 63% (Dechaud et al., 2001), 72% (Gordts et al., 1998; Campo et al., 2002) to as high as 93% (Watrelot et al., 2003) are stated. When looking at the concordance of THL with laparoscopy, several studies show that abnormal findings during THL are confirmed with laparoscopy. Sensitivity and specificity respectively ranges from 70 and 100% (Dechaud et al., 2001; Campo et al., 2002) to as high as 93% (Watrelot et al., 2003) are stated. When looking at the concordance of THL with laparoscopy, several studies show that abnormal findings during THL are confirmed with laparoscopy. Sensitivity and specificity respectively ranges from 70 and 100% (Dechaud et al., 2001), 92.3 and 100% (Darai et al., 2000) to 100 and 100% (Casa et al., 2002). In case of normal findings during THL, laparoscopy could still demonstrate endometriosis as THL cannot reach the bladder region (Darai et al., 2000; Dechaud et al., 2001; Nawroth et al., 2001). In the case of discordant results, Watrelot et al. (2003), showed that in only 1% it had clinical consequences.

When performing the THL, the gynaecologist may also consider to do a hysteroscopy in the same session. The question is if routine hysteroscopy is necessary during basic fertility screening. Recent preliminary

### Table IV  THL findings.

<table>
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<tr>
<th>Tubes</th>
<th>Abnormalities</th>
<th>Endometriosis</th>
<th>Adhesions</th>
<th>Both</th>
<th>Total</th>
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<tbody>
<tr>
<td>None</td>
<td>729</td>
<td>49</td>
<td>41</td>
<td>25</td>
<td>844 (83.0%)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>77</td>
<td>14</td>
<td>25</td>
<td>11</td>
<td>127 (12.5%)</td>
</tr>
<tr>
<td>Adhesions</td>
<td>18</td>
<td>1</td>
<td>21</td>
<td>6</td>
<td>46 (4.5%)</td>
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<tr>
<td>Both</td>
<td></td>
<td></td>
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<td></td>
<td>1017 (100%)</td>
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<tr>
<td>Total</td>
<td>824 (81.0%)</td>
<td>64 (6.3%)</td>
<td>87 (8.6%)</td>
<td>42 (4.1%)</td>
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</table>
result of the inSIGHT-study has shown that routine hysteroscopy does not improve the IVF outcome in terms of live birth rate (Smit et al., 2015). In the studied centres, a hysteroscopy was performed only when there was a suspicion of intrauterine pathology or congenital abnormality of the uterus.

Although this study shows that THL is a well-tolerated, safe and reliable method, in these days of economic decline, cost-effectiveness is an important issue when introducing a potentially new standard reference procedure. To our knowledge only Khouri (Khouri and Magos, 2005) performed a comparative study with THL in a one-stop fertility clinic to laparoscopy as an in-patient investigation. They calculated a saving of over 380 pounds sterling in favour of the one-stop fertility clinic or 28% cost-saving to the hospital.

Besides THL, other relatively new tubal patency techniques like hysterosalpingo-foam sonography are also being implemented. These ultrasound-based techniques might have advantages over HSG with similar test results (Maheux-Lacroix et al., 2014). An RCT between THL and sono-HSG has not been conducted as far as we know, although Ahinko-Hakamaa et al. (2009), verified tubal patency by THL after performing hysterosalpingo-contrast-sonography first. They showed a concordance of 77%. In our opinion, before implementation of novel techniques, these should first be compared with existing techniques. Eventually, all strategies for basic fertility work-up and fertility treatment should be compared in order to know what the best practice is.

In conclusion, our study shows that THL can be implemented as a method for tubal patency testing and exploration of the female pelvis to exclude abnormalities which may compromise conception. THL enables the surgeon to schedule fertility enhancing surgery immediately if required without performing a diagnostic laparoscopy beforehand. Whether or not THL is superior to HSG in terms of prognostic capacity and cost-effectiveness should be studied before implementing THL as a primary method. This is currently evaluated in a randomized setting in various fertility clinics in the Netherlands.

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Authors’ roles

All authors provided a substantial contribution to the conception of the paper. C.K. and B.M. designed the study. C.K., G.O., M.V. and W.K. participated in the trial. R.C. and M.K. collected the data. All authors were involved in redrafting and revising of the paper and approved the final version of the manuscript that is now being submitted for publication.

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Conflict of interest

None declared.

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