SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Effectiveness of minimally invasive interventions in the assessment and treatment of infertility

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Background and objectives of the doctoral dissertation

Infertility - according to the definition of the World Health Organization (WHO) - is when a couple (where the female member of the couple is younger than 35 years old) lives a regular, unprotected sex life, despite this, the desired pregnancy does not occur within a year. Based on WHO data, more than 15% of couples worldwide suffer from infertility, while according to the data provided by the Central Statistical Office (KSH), approximately 20% of married couples in Hungary face the difficulty of conceiving, which may be due to several factors. On the one hand, the demographic composition of mothers and women of childbearing age has changed significantly. The average age of primiparas is increasing year by year: while in the 1980s the average age of women giving birth to their first child was 22.86, in 2020 this number in Hungary rose to 28.92 years. Infertility has become a significant health policy issue, as diagnosis, examinations, and therapy impose a high financial burden on both the patient and the health insurance system.

The reason for the increase in the number of infertile couples is very complex, approx. 40% female, 40% male, and 20% common causes. The cause of female infertility can be of functional or organic origin. Infertility is a serious challenge for gynecologists all over the world. Establishing a diagnosis is often a multidisciplinary task, during the examination, in addition to the gynecological examination, endocrinological, genetic, hematological, immunological, psychological, and urological examinations may also become necessary.

The causes of infertility are largely discoverable and can be successfully treated. Assisted reproduction techniques, including in vitro fertilization (IVF), are a solution for many infertile couples thanks to their development in recent decades. These technologies are still largely inaccessible, and unaffordable in many parts of the world, especially in low- and middle-income countries. On the female side, the main indication for IVF and the most frequent and definitive cause of infertility is fallopian tube occlusion. We speak of fallopian tube-induced infertility in the case of obstruction or complete absence of both tubes. With a positive result of the patency test, if the blockage of the Fallopian Tubes on both sides is confirmed, in addition to IVF, reconstructive reproductive surgery can be considered as a solution. However, the effectiveness of operative interventions does not exceed the success rate of IVF, and they are also more burdensome.

However, it is important to emphasize that the indication of IVF also entails great responsibility, given the perinatal characteristics of pregnancies conceived through new assisted reproduction techniques and the material impact of the methods, which are of particular importance from the point of view of the national economy. This is why it is extremely important to reduce false-positive results.

In 1561, Gabriele Fallopius was the first to describe and compare the Fallopian tubes and their different sections with the oviducts of other mammals.

The approx. 10-12 cm long, mobile, seromuscular organ in the abdomen: the paired uterine horn (salpinx, uterine tube, Fallopian tube) is a derivative of the Müller tube, which is functionally divided into intrauterine (intramural), narrower isthmic and wider infundibular, ampullary sections. Knowledge of anatomical and histological formulas is essential for examining the tubes and the uterine cavity, for which we have more and more safe and effective examination methods.

Fertilization of the ovum usually takes place in the distal part of the ampulla, from where the embryo moves in a proximal direction through the peristaltic movement of the ciliated epithelial cells and the well-developed muscle layer lying under the mucous membrane. The mucus layer on the mucous membrane of the uterine horn is in continuous flow in the direction of the uterine cavity. This, as well as the peristaltic contractions of the smooth muscle layers in the wall of the Fallopian tube, ensure the migration of the released egg towards the uterine cavity. The flow guides the movement of the spermatozoa, which travel up the Fallopian tube in an active whip-like motion, moving against the flow. The flow of the mucus layer also has a barrier function, preventing the ascending spread of pathogens. The muscle layer consists of smooth muscle, which forms a thick inner circular, a middle circular, and a thinner outer longitudinal layer. The amount of muscle tissue decreases towards the ampulla. The smooth muscle ensures the progressive contractions of the Fallopian tube in the direction of the uterus.

The blockage of the Fallopian tubes can be the result of inflammatory processes: previous fever, miscarriage, Chlamydia infection, Gonorrhea, tuberculosis. Damage to the ciliary hairs can lead to a decrease in ovarian function. Fallopian tube blockage can be caused by adhesions after surgical interventions or pelvic surgery. In case of blocked Fallopian tubes, the transport of sperm and blastocysts is impaired.

In 1849, William Tyler Smith assumed that the cause of infertility could be the blockage of the Fallopian tubes, and to solve this, he used a J-shaped silver cannula containing a whalebone needle inserted through the vagina.

During the procedures used today, contrast material is introduced into the uterine cavity through the cervix, and then the material entering the horns is visualized with the help of an imaging method.

Permeability tests can be divided into two large groups: direct and indirect methods.

Indirect methods:

- hysterosalpingography (HSG) with X-ray imaging
- ultrasound imaging hysterosalpingography (SHSG) with contrast solution (hystero-contrast-salpingography, HyCoSy) or physiological salt solution (saline infusion sonography, SIS)
- radionuclide isotope hysterosalpingo scintigraphy (HSSG)

HSG - hysterosalpingography

Hysterosalpingography (HSG) is an X-ray contrast procedure that provides an accurate picture of the abnormalities of the shape of the uterine cavity and the condition of the fallopian tubes.
In 1909, Novenov was the first to use Lugol's solution to outline oviduct. Rindfleish tried a bismuth emulsion in 1910, in 1922 Sicard Fureshier started using Lipidol, which contained 40% iodine mixed with poppy seed oil. Water-soluble media began to be used only after 1950, as it was noticed that the previous hyperosmolar solutions caused great pain both during and after the procedure. The HSG test can be performed under ultrashort anesthesia. After placing the vaginal speculum, 10-20 ml of water-soluble iodinated contrast material is gradually injected into the uterine cavity. Previously, a rigid Schultze device was used for this, and more recently, a thin balloon Foley catheter is used. Under normal conditions, the uterus fills up, and the contrast material quickly appears in the free abdominal cavity after passing through the healthy tubes. The examination is performed with the use of transillumination equipment under eye control, and anteroposterior images are taken. The amount of irradiation to the ovary during the examination is around 10 mGy, which is an acceptable value.
Even though the fertilized egg is radiosensitive, the ovum before fertilization is relatively radioresistant.
Complications can be expected in 1% of HSG examinations, which can be pain, allergic reactions, venous intravasation, bleeding or uterine perforation.
Today, hysterosalpingography is still a routinely performed procedure in many places during the investigation of infertility. Contraindications are pelvic inflammation, sepsis, STD, severe kidney and heart failure, contrast material allergy, recent tubal dilation and curettage, pregnancy. The optimal time for the examination is between the 6th and 12th day of the cycle. There are often therapeutic consequences, so-called the "tubal flushing" effect: injection with a contrast agent can expose the cavity system of the fallopian tube. The disadvantage of the

method is that it does not provide information about pelvic conditions, requires anesthesia, and involves a small amount of X-ray exposure. Based on a meta-analysis of seven studies, in which 4,521 women were analyzed, compared to laparoscopy, which is considered the gold standard, the specificity of the method is 86%, and its sensitivity is 53% for the detection of changes involving the fallopian tube. In another work-up, HSG has a specificity of 83% and a sensitivity of 65% for horn patency.

HyCoSy- Hystero-contrast salpingography or SHSG - Sono-hysterosalipngography

In 1989, Deichert and Schlief first used contrast-enhanced transvaginal ultrasound to assess fallopian tube patency. With the hystero-contrast-sonography (HyCoSy) procedure, the uterine cavity and the patency of the fallopian tubes can be examined relatively quickly and easily. The essence of the procedure is that a contrast material is injected through a thin catheter into the uterine cavity, and with an ultrasound examination, a real-time image of the uterine cavity, the fallopian tubes, and the ovaries is obtained. The contrast material is emptied from the cavity and passes through the fallopian tubes, so we can make sure of the free flow, or about the localization of its blockage.
The contrast agent can be a galactose solution (e.g.: Echovist, Schering AG, Berlin, Germany) or even a sterile physiological saline solution (saline infusion sonography, SIS). However, in the latter case, the passage of the tubes is rarely visible, but the echo-free fluid appearing around the uterus proves that at least one of the fallopian tubes is passable. Premedication is not necessary, the examination is performed on an outpatient basis with an empty bladder, on a gynecological examination table with a vaginal ultrasound device. First, we check the state and position of the uterus, cervix, endometrium, and ovaries with a comprehensive ultrasound examination. After disinfecting the cervix, a small catheter (e.g., 8Ch balloon bladder catheter) is inserted into the uterine cavity, then, under transvaginal ultrasound control, saline is slowly injected into the uterine cavity through the catheter, thus distending it. The contrast medium has a hyperechoic appearance, and the saline solution has an anechoic appearance. In combination with color or power Doppler examination, the slow flow of the liquid can be made visible. The optimal time for the examination is the first half of the cycle, within the 10th day after menstruation. Although mild lower abdominal discomfort like menstrual cramps may occur, anesthesia is not required. Rarely, stronger pain, quickly passing nausea, possibly vomiting, and a drop in blood pressure occur. Another rare complication can be irritation of the

peritoneum and the accompanying spasmodic pain. However, peritonitis associated with the procedure practically does not occur.

When comparing HSG and HyCoSy/SHSG, in most cases the latter procedure proves to be more favorable, as it does not involve radiation exposure and can be performed on an outpatient basis. SHSG is a procedure with excellent sensitivity and specificity in the diagnosis of uterine abnormalities, such as endometrial polyps and submucosal myomas. Regarding fallopian tube patency, in the examination of infertile patients who underwent both HSG and HyCoSy examination, the two methods proved to be equivalent in terms of specificity and sensitivity. However, it is interesting that the detectability of the patency on the left side is lower than that on the right side.

Hysterosalpingo scintigraphy (HSSG)

Hysterosalpingo radionuclide scintigraphy (HSSG) has been used in gynecology since the second half of the 1900s.

György Hevesy (1885-1966) used radioactive isotopes for the first time in 1923 to monitor the processes taking place in living organisms. In recognition of his work, he was awarded the Nobel Prize in 1943. In 1957 H.O. Anger invented the gamma camera. Hysterosalpingo scintigraphy was first described by Iturralde and Venter in 1979, and the procedure was modified several times until 1991. Following the migration of technetium-99m human albumin (ppmTc-HAM) radioactive particles, they visualized the patency of the pathways leading from the fornix to the peritoneal cavity in the female reproductive system.

Direct methods:

- laparoscopic fallopian tube patency test, laparoscopic chromohydrotubation (CHT)
- transvaginal hydrolaparoscopy (TVHL)
- office hysteroscopy - selective pertubation (OHSC - SPT)

Laparoscopy - chromohydrotubation

In 1940, a revolutionary new examination method was launched, culdoscopy, whose name comes from the French word cul de sac. During the intervention, a light-optical device was introduced into the abdominal cavity (through the posterior vaginal vault), and it was possible to examine the pelvic organs, including the fallopian tubes. Culdoscopy evolved into laparoscopy, which today is one of the most important diagnostic and operative techniques in gynecological surgery. Hydrolaparoscopy was introduced only after that.

The chromopertubation (LSC-CHT) performed during the laparoscopic examination, which examines the patency of the fallopian tube, (combined with or without hysteroscopy) is the "gold standard" of infertility investigation. During the procedure, methylene blue dye is injected into the uterine cavity through a device placed in the cervix, which fills the uterine cavity, then the fallopian tubes, and finally flows out towards the abdominal cavity through their orifices. Blue dye appearing in the abdominal cavity is a sure sign of fallopian tube patency. During laparoscopy, it is also possible to recognize other pelvic lesions and to solve them immediately. Although the high accuracy of LSC-CHT and its extensive diagnostic possibilities can be considered the most reliable diagnostic method, we have to take into account the invasiveness, the greater health burden and risks for the patient (general anesthesia, possible vascular and gastrointestinal injuries), therefore having other methods to examine the fallopian tubes, laparoscopy in first place is used less and less. However, the advantage is that pathological conditions in the pelvis that cannot be accurately diagnosed with other imaging methods can be recognized and, if possible, eliminated. In their study, Corson et al. described that out of 100 cases where the HSG was negative and the fallopian tubes were permeable, an unexpected abnormality in the pelvis was found in 68 cases during laparoscopy. According to Al-Badawi et al., during laparoscopy performed after a negative HSG test, 49% of the patients had some pathology (endometriosis, adhesion), although 93% of these were only mild abnormalities.

TVHL – transvaginal hydrolaparoscopy

In 1998, Gordts used transvaginal hydrolaparoscopy for the first time in Leuven, which is an examination method created from the combination of culdoscopy, laparoscopy and hysteroscopic procedure. During the examination, the endoscope guided into the abdominal cavity through an incision made on the posterior vault of the vagina, is immersed in the physiological saline solution delivered to the pelvis, so that the fallopian tubes and ovaries can be observed floating freely. The Douglas pouch, sacrouterine ligaments, posterior latum plates, ovaries (which are predilection sites for endometriosis) and fallopian tubes are excellent for observation with appropriate viewing angle optics. In addition, chromopertubation can also be performed, and the appearance of the blue dye in the abdominal cavity can also be confirmed. During the procedure, it was possible to observe for the first time the fimbrial uptake of the ovum released in connection with ovulation into the fallopian tube.

TVHL surgery is performed under local anesthesia, unlike laparoscopy, general anesthesia is not required. If there is a negative palpation finding and a pelvic ultrasound, the procedure can be performed, the first step of which is a diagnostic hysteroscopy, with a view to increasing the diagnostic efficiency. A small-diameter balloon catheter (8 Ch) is inserted into the uterine cavity for chromohydrotubation. After that, penetration with a special tool - developed for transvaginal endoscopy - takes place through an incision made on the posterior vault of the vagina, which minimizes the risk of intestinal injury. The pelvis is filled with 300 ml of physiological salt solution. TVHL is a fast, safe, and less burdensome diagnostic procedure for patients compared to diagnostic laparoscopy. In addition to examining the patency of the Fallopian tubes, with the development of the toolkit, it became possible to perform several operative interventions (adhesiolysis, cystapunctio, ovarian drilling), also under local anesthesia, in ambulatory conditions.

Office hysteroscopy - selective pertubation (SPT)

The appearance of video endoscopy also made it possible for hysteroscopy to gain ground in the 90s. More recently, the so-called the "no-touch" office hysteroscopic technique, with the use of thin optics, there is no need to explore, grasp, probe, or dilate the cervix. The test can also be performed painlessly on an outpatient basis without anesthesia.

During hysteroscopy, we naturally get a more complete picture of the uterine cavity than with HSG or SHSG examinations. The discovered changes (intrauterine polyps, myomas, septum) can even be removed based on the "see and treat" principles. The excellent hysteroscopic image quality and the observation of the exact anatomy inside the uterus raised the possibility of examining the fallopian tubes.

To test the patency of the Fallopian tubes, we introduce a plastic catheter to the oral cavity of the salpinx with the help of an optical sleeve with a working channel. Methylene blue dye, routinely used during laparoscopic chromohydrotubation, is injected separately into the horns on both sides. The blue dye appearing in the transparent catheter can be easily followed in the uterine cavity saturated with saline. If the tube is permeable, the uterine cavity remains transparent, but if the tube is closed, the colored salt solution flows back into the uterine cavity and colors it blue. One of the advantages of this method compared to other methods is its selectivity. Hence its name: selective pertubation during office hysteroscopy (OHSC-SPT). The specificity of the method is 100%, and its sensitivity is 87.5%.

Objectives

Our goal during the research that forms the basis of this dissertation was to describe the new aspects of modern infertility testing, their importance, and actuality within the discipline, to compare them, and possibly to develop new procedures with the involvement of some frontier areas, or to modernize the existing techniques.

In the literature dealing with this, many areas have been described that are only assumptions or not detailed enough (adhesions, uterine and tubal contractions, etc.), but they greatly influence the effectiveness of the methods.

A direct and two indirect methods investigated by our working group, which are the main subject of my dissertation:

1. New minimally invasive procedures in the examination of fallopian tubes

- predictive value of bubble signal during hysteroscopy: the indirect bubble signal detected during HSC-LSC performed within the framework of infertility investigation has already been described in the literature. This phenomenon, observed as a side effect, may indirectly indicate the patency of the fallopian tubes.

2. Modification of known methods to achieve better results

- HSSG (hystero-salpingoscintigraphy) supplemented with SPECT-CT: one of the indirect examination methods of fallopian tube patency is the isotopic Fallopian tube patency test (scintigraphy, HSSG), which was used in practice supplemented with a SPECT CT examination.

3. Evaluation of the effectiveness of selective pertubation during office hysteroscopy setting

New scientific results of the thesis

Methodologies (material and method)

Examination of the indirect bubble signal during hysteroscopy

This prevalence study was conducted between December 1, 2015, and December 31, 2016 at the Obstetrics and Gynecology Clinic of the Faculty of General Medicine, University of Debrecen. 61 female patients participated in the study.

The study was approved by the Local Ethics Committee and was based on the STROBE study and the Declaration of Helsinki. Before the interventions, written consent was obtained from all participants after detailed information. All patients were women between the ages of 18 and 45, who applied for the examination after at least 1 year of infertile period (6 months of infertile period for those over 35). Following the protocol, according to the selection criteria, the patient who had negative STD, functional and andrological results was suitable for the study. Existing uterine pathology, e.g., endometrial polyps, submucosal myoma, adhesions were exclusion factors. As part of the infertility examination, hystero-laparoscopy was performed everytime to examine the pelvis, the patency of the fallopian tubes and the uterine cavity. The interventions were carried out under sterile operating room conditions, under general anesthesia. As a first step, a diagnostic hysteroscopy was performed: with a 2.9 mm rigid-wall optic, a 3.7 mm sleeve (EMD-Hungary). In all cases, the surgery was performed in the follicular phase between days 5 and 12 of the cycle. After mapping the uterine cavity, viewing the endometrium, and identifying the fallopian tubes, air bubbles were introduced into the uterine cavity, and the movement of the bubbles was monitored for at least 30 seconds and followed towards both fallopian tubes. The movement of the bubbles towards the fallopian tube through the fallopian tubes was recorded. After removing the hysteroscope, an 8 Fr catheter was placed in the uterine cavity and inflated with 2 ml of saline.

After that, we performed a diagnostic laparoscopy, during which we performed chromohydrotubation and, if necessary, other operative interventions. Fallopian tube patency was tested with 20 ml of dye (1 ml of methylene blue (Patent Blue) diluted in 1000 ml of saline). The result of the pertubation was also recorded in all cases. The sample number was 122, since we examined 1-1 tube as a separate event in all 61 patients.

The test subjects were divided into subgroups based on the type of patency tests and the patency of the Fallopian tubes, i.e. based on the patency observed during bubble sign and chromohydrotubation. This applies to both sides of the fallopian tube separately and is dichotomized into subgroups "neither side can be penetrated" and "tube can be penetrated on either side". Student's T-test or Mann-Whitney test, Chi-square or Fisher's exact test were used for comparative analyses, and all statistical analyzes were performed using SPSS version 21.0 (IBM Corp., Armonk, NY). Cross-tabulations were used to calculate sensitivity, specificity, and positive and negative predictive values. The significance level was taken as $p<0.05$. Regarding clinical test results: by convention in medical practice, negative results usually indicate a healthy condition, while a positive result means an abnormal condition. However, during hysteroscopy, our working group recorded a bubble signal passing through the fallopian tube as a positive result, indicating the patency of the fallopian tube. The literature on this topic is a bit vague, so it is very important to accurately define negative and positive results.

Hysterosalpingo scintigraphy for the examination of ovarian patency

This prospective cohort study was conducted by our working group between January 2017 and January 2020 at the Institute of Nuclear Medicine of the University of Debrecen. The study was prepared with the approval of the local Ethics Committee (protocol number: DERKEB/IKEB 4738-2017). The study was conducted with the informed consent of all participants. Inclusion criteria included: primary or secondary infertility of unknown origin, exclusion factors were: abnormal karyotype (including that of the partner), thrombophilia (factor V Leiden mutation, prothrombin mutation, antiphospholipid syndrome), reduced ovarian reserve (if FSH on 3rd cycle day > 10 mUI/mL, antral follicle count between 5-7 and AMH between 0.5-1.1 ng/ml), severe endometriosis (ASRM classification stage III-IV), 30 kg/m2- a higher body mass index (BMI), positive urine pregnancy test, urinary tract complaints, malignant tumor or uterine pathologies (polyps, myoma, intrauterine adhesions, cervical stenosis), other serious conditions (e.g.: serious heart disease, systemic disease), and age above 45 years.

In all cases, the tests were performed in the late follicular phase, between day 7-14 cycle days. The patients were laid down on the table of the gamma camera, the radiation recording device, so that the gamma camera used for the examination looked at the pelvis from the anterior direction.

We used three different methods:

1. Method "A" or simple dynamic method, during which 37 MBq 99mTc nanoalbumin was injected through the cervix into the uterine cavity through a cannula, and a 20-minute (40x30 seconds) dynamic examination was performed
2. Method "B" or forced dynamic method, during which the radionuclide solution was injected into the uterine cavity through a thin catheter, over the inner cervix, to avoid the influence of cervical factors and for a better assessment of active fallopian tube transport. After that, a balloon catheter was inflated with physiological saline solution in the uterine cavity, and by injecting 5 ml of saline solution into the catheter, the passage of the radiopharmaceutical through the fallopian tubes was accelerated, and 5-minute (10x30 seconds) dynamic recordings were performed.
3. "C" method or SPECT/CT: the forced dynamic method was followed by an additional pelvic SPECT/CT examination to localize the activity of the pelvic area and to rule out contamination: to identify the radioactive material accumulating on the skin under the buttocks. SPECT creates 3D images using gamma cameras to detect internal radiation.

After detailed information, the patients signed the consent form. The patients were placed on the examination table and human serum albumin microspheres labeled with 99mTc were suspended in 1 ml of saline. After vaginal dissection, a flexible silicone catheter was inserted through the cervix into the uterine cavity, and then the solution containing microspheres labeled with radioactive isotopes was injected into the uterine cavity. After that, one of the three methods mentioned above (A, B, C) was used. The series of antero-posterior scintigrams was recorded for 20 minutes using a gamma camera (Mediso Kft. Budapest, Hungary). The relative distribution of radioactivity appeared in the form of color prints on the 2-dimensional images captured by the camera.
During the "C" method, we used a SPECT/CT AnyScan SPECT/CT FLEX hybrid gamma camera (Mediso Kft. Budapest, Hungary) with the following specifications: 128x128 matrix size, 10 seconds/frame, tube voltage 120 kV and 5 mAs, 1.5 bed speed and 90 rpm rotation speed.
Contraception was recommended to the patients in the following cycle. During the tests, the reference standard method was laparoscopic chromohydrotubation. We evaluated the test as negative if at least one of the Fallopian tubes was permeable, and as positive if both fallopian

tubes were closed. We compared the data of the dynamic base, post-rinse dynamic, and SPECT/CT examinations with the results of the reference examinations (LSC-CHT).

Selective pertubation during office hysteroscopy

This cross-sectional study was conducted at our infertility clinic in Debrecen between 2018 and 2022. The study was approved by the Local Ethics Committee and was conducted in accordance with the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines. Informed consent was obtained from all participants before the study. All patients were between 18 and 45 years of age with a history of at least one year of infertility (6 months for patients over 35 years of age). Inclusion criteria included negative STD test, negative functional test and negative andrological status of the partner.

Before and after the interventions, 3D ultrasound was used in all cases to evaluate the uterine cavity, and then, as part of the infertility investigation, an outpatient hysteroscopy was first performed to examine the patency of the fallopian tube and the uterine cavity.

All procedures were performed in the office's examination room without general anesthesia. Local anesthesia (lidocaine infiltration of the cervix or filling of the cervical canal and uterine cavity with lidocaine gel), psychological guidance, and inhaled nitrous oxide were recommended to the patients as effective pain relief. As a first step, a diagnostic hysteroscopy was performed using 2.9 mm rigid optics with a 3.7 mm sheath (EMD-Hungary). The OHSC was performed during the follicular phase, between days 5 and 12 of the cycle. After identification of normal uterine cavity, endometrial, and the os, a flexible plastic catheter (Cavafix; B. Braun Melsungen AG, Mel-sungen, Germany) was inserted through the working channel of the device. Normal physiological saline (0.9% sodium chloride) was used for distension. The hysteroscope was inserted into the cervical canal without grasping or dilating the cervix. A digital camera was connected to the optics so that the results could be objectively evaluated and documented. Then, a 1.7 mm plastic catheter was inserted through the working canal, the tip of it was placed in the tubal cavity. Through the catheter, we slowly inject 2-10 ml of methylene blue dye (Patent Blue, 2 ml in 1000 ml of saline). If the fallopian tube is open, the blue fluid flows quickly and does not appear in the uterine cavity. The endometrium is normal in color, while the catheter is blue due to the methylene blue dye flowing through it. However, if the fallopian tube is occluded, the distending media in the uterine cavity will be stained blue due to reflux of methylene blue dye. After assessing fallopian tube patency, the blue dye cleared within 3-4 seconds and the entire procedure was repeated on the other side. The total examination time took an average of about 4-8 minutes.

Results and statistical analysis

Examination of the indirect bubble signal during hysteroscopy

61 patients participated in the study. All interventions were uncomplicated. The average age of the patients was 32.0 ± 4.36 years, and their average body mass index (BMI) was 22.7 ± 4.19 kg/m2. The indication for the test was primary or secondary sterility. The average duration of the infertile period was 29.4 months. The initial step was a diagnostic hysteroscopy. Tubal fluid on both sides was identified, so the movement of air bubbles could be reliably evaluated. Based on the indirect bubble signal method, the right fallopian tube was blocked in 36 (29.5%) cases out of a total of 122 tubes of the 61 examined subjects, and the left fallopian tube was blocked in 33 (27%) cases. The reference method (LSC-CHT) confirmed 57 (46.7%) blocked fallopian tubes.

Based on this, we evaluated the specificity, sensitivity, positive (PPV) and negative predictive value (NPV) of the indirect bubble signal detected during hysteroscopy.

We found no evidence of differences in age, height, weight, and BMI between the indirect bubble sign categories; however, it was observed that in the group where Fallopian tubes proved to be patent during laparoscopic chromohydrotubation, the age was significantly younger and the BMI was lower for that group compared to where the fallopian tubes were occluded.

Using cross tables, we calculated the specificity, sensitivity, positive (PPV) and negative predictive value (NPV) in the case of an indirect bubble sign, taking laparoscopic chromohydrotubation as a reference. The sensitivity of the bubble sign is 73.2 % (57.1-85.8 %, 95 % CI), its specificity is 70 % (45.7-88.1 %, 95 % CI), its positive predictive value is 83.3 % (67.2-93.6 %, 95 % CI), its negative predictive value was 56 % (34.9-75.6 %, 95 % CI) in case of patency of any fallopian tube. To be able to describe the classification method of the bubble signal in more detail, the analysis was repeated for each side.

On the left side, the sensitivity is 65.6 % (46.8-81.4 %, 95 % CI), the specificity is 75.9 % (56.5-89.7 %, 95 % CI), the positive predictive value is 75 % (55.1-89.3 %, 95 % CI) the negative predictive value was 66.7 % (48.2-82.0 %, 95 % CI).

In the case of the right fallopian tube, the results were weaker: the sensitivity was 48.5 % (30.8-66.5 %, 95 % CI), the specificity was 67.9 % (47.6-84.1 %, 95 % CI), and the positive and negative predictive values were 64 % (42.5-82.0 %, 95 % CI) and 52.8 % (35.5-69.6 %, 95 % CI).

Hysterosalpingo-scintigraphy to examine the patency of the fallopian tubes.

Statistical analysis was performed using STATA version 14.1 (STATA Corp, College Station TX, USA). Continuous variables were expressed as mean ± standard deviation (SD), dichotomous data as absolute numbers and percentages. The difference in proportions between groups was analyzed using the Chi-square test. The significance level was set at $p<0.05$. Determination of sensitivity, specificity, positive predictive value, and negative predictive value 95% confidence intervals were used for the binomial distribution using Wilson's theorem. During the study, we examined 70 infertile female patients. The average age was 30.87 ± 4.24 years. The mean follow-up time was 469 days (77-1121 days). The average duration of infertility per patient was 2.09 years. We compared simple dynamic, forced dynamic and SPECT/CT results. We evaluated the individual acquisition methods based on their diagnostic performance. During the simple dynamic examination ("method A"), both fallopian tubes were found to be closed in 29 patients, in 28 cases only one side was blocked, and in 13 cases both sides were passable. Thus, the sensitivity was 87.5%, the specificity was 71.7%, and the positive and negative predictive values were 31.8% and 97.4%, respectively.

During the forced dynamic examination ("method B"), in the case of 21 patients, both fallopian tubes were occluded, in 28 female patients only one sided horn was blocked, and in 21 cases both sides were occluded, thus achieving a sensitivity of 87.5% and a specificity of 88.7%, with a positive predictive value of 53.8% and a negative predictive value of 97.9%.

The addition of SPECT/CT to the forced dynamic examination ("C" method) improved the diagnostic accuracy of hysterosalpingo scintigraphy. 22 female patients had both fallopian tubes closed, 23 patients only had one side closed, while 25 women had both fallopian tubes passable. This results in a specificity of 88.7%, the sensitivity reached 100%, while the positive and negative predictive values were 57.1% and 100%, respectively.

The difference in sensitivity and specificity by Chi-square test between forced dynamic and SPEC/CT techniques was not significant ($p>.1$). Among the three methods, the result of SPECT/CT is the most accurate. In summary, we detected 86 blocked fallopian tubes, and after the forced dynamic examination, in 16 cases (18.6%) the tubes became permeable due to the tubal flushing effect already described in previous literature.

OHSC-SPT (selective pertubation during office hysteroscopy)

Between 2018 and 2022, we performed 212 selective pertubation procedures during outpatient office hysteroscopy at our infertility clinic in Debrecen. In the studied population, the indication for outpatient hysteroscopy was primary infertility in 75%, secondary infertility in 22.17%, ultrasound abnormalities in 0.94%, and recurrent miscarriage in 1.89%. The mean age (SD) of the patients was 33.1 ± 4.77 years. The average body weight was 64.67 kg, the average BMI was 22.87 kg/m2.
During office hysteroscopic selective chromopertubation, tubal abnormalities were detected in 61.32%. In 43.08% (N=92) bilateral and in 56.92% unilateral obstruction was confirmed. During diagnostic hysteroscopy, any morphological changes in the uterine cavity or endometrium can be detected. Among the performed interventions, other pathologies were discovered during the examination of the uterine cavity in 61 cases: in 13 cases polypectomy was also necessary, in 1 patient a myomectomy was performed, and in 39 cases a biopsy was performed due to endometrial abnormalities, so chronic endometritis was detected in 8 patients. No complications occurred during the 212 diagnostic office hysteroscopies.

Discussion

Examining the patency of the fallopian tubes plays an extremely important role in the investigation of infertility. The fallopian tube is an organ that plays an active role in fertilization, with specific smooth muscle function, sympathetic and parasympathetic innervation, and hormone receptors. The cause of a blocked fallopian tube is often unexplained, it can even be a temporary condition. A better understanding of the physiological functioning of the fallopian tube can lead to the further development of examination procedures. By exploring new aspects of the causes of infertility, minimally invasive examination methods can be further thought out and new techniques can be developed.
With the increase in the number of infertile couples and the spread of assisted reproduction techniques, there is an increasing demand for more cost-effective and less burdensome diagnostic methods that can be performed on an outpatient basis.
Hysterosalpingography is the oldest of the currently available patency test methods. Despite its good diagnostic indicators, it means radiation exposure for the patient. The diagnostic accuracy of ambulatory contrast enhanced SHSG (HyCoSy) using ultrasound imaging is the same as that

of HSG. Both methods prove to be good for detecting possible changes in the fallopian tubes, but HSG does not provide information about pelvic conditions, which can be obtained during SHSG. Although the gold standard laparoscopic chromohydrotubation (LSC-CHT) has extensive diagnostic and at the same time therapeutic possibilities, we have to take into account the invasiveness and it also means a greater financial burden for the care institution. Based on literature data, it can be stated that office hysteroscopy can be safely used in the investigation of infertility.

To reveal the causes of infertility, minimally invasive methods can be recommended as a first step. Newer and newer techniques are being developed worldwide. Our working group compared the indirect bubble signal observed during hysteroscopy and the use of laparoscopic chromohydrotubation for the purpose of tubal patency in the case of infertile female patients. The sensitivity of our test method was 73%, and its specificity was 70%. The sensitivity and specificity parameters of our method proved to be low, however, the positive predictive value was 83%, which is acceptable. Our aim was to evaluate the predictive value of the bubble sign during diagnostic hysteroscopy. During the method we developed, we did not modify the test conditions. Unlike Perry et al (longer observation, patient positioning) for more accurate results. During our method, after examining the uterine cavity, we observed the bubbles for 30 seconds. Changing the conditions, such as rotating the patient or using a flexible hysteroscopic device, could help the bubbles escape, but our goal was to simply assess the phenomenon in its physiological conditions, during normal diagnostic hysteroscopy.

In conclusion, the method based on visible air bubbles during hysteroscopy was not sufficiently accurate in predicting fallopian tube patency. However, a limitation of this study was the small sample size (122 horns). Another limitation of the study may be the fact that hysteroscopy can cause smooth muscle spasm in the fallopian tubes, which may affect the results of chromohydrotubation following the study. Also, hypothetically, a distal tubal occlusion may result in false negative air bubble signals, and the potential so-called tubal flushing effect can lead to negative chromohydrotubation after a positive bubble sign detected during hysteroscopy.

Even though the indirect bubble signal observed during hysteroscopy is a simple, cost-effective, minimally invasive, well-tolerated indirect method, it is most likely not accurate enough to assess fallopian tube patency. In case of infertility, perryscope or selective pertubation is recommended after diagnostic hysteroscopy.

On the other hand, the results of our observations in the field of hystero-salpingoscintigraphy (HSSG) are remarkable.

HSSG supplemented with SPECT-CT: one of the indirect examination methods of fallopian tube patency is the isotopic horn patency examination (tubal scintigraphy, HSSG), which was used in practice supplemented with a SPECT CT examination. By modifying a method that has been known for several decades, the efficiency of their examination in the field of patency of the fallopian tubes should be increased to develop its diagnostic accuracy.

This radionuclide method (HERS) was first described by Venter et al. in 1979. The method was developed to evaluate the migration of radionuclide particles: the radioactive tracer migrates from the vagina into the abdominal cavity and was used to visualize and functionally determine the patency of the fallopian tubes. Technetium-99m human albumin microspheres (99mTc-HAM) were delivered into the posterior vaginal vault of patients and used for tracking. In their study, it was written that HERS is a simple, noninvasive method for examining the patency of pathways in the female reproductive system.

Different from the method described by Venter et al., to increase the diagnostic accuracy, during our studies we delivered the radiopharmaceutical directly into the uterine cavity with the help of a thin catheter, making it possible to study the utero-tubal transport in vivo without stress or injury.

In a previous prospective study involving 26 patients, in which the effectiveness of HSSG was examined, some modifications were also introduced: a smaller amount of radiopharmaceutical was applied to the cervical mucosa, but the efficacy study was limited. McQueen et al prospectively examined 96 infertile women using the HSSG method and compared the findings with the gold standard laparoscopy. Based on their results, the radionuclide test correlated well with laparoscopy, patency or obstruction was diagnosed in 86% of cases. In a similar study, Steck et al published a radionuclide method with 86% sensitivity and 66% specificity compared to hysterosalpingography (HSG).

These previous studies have demonstrated the validity of tubal scintigraphy in infertility investigations, but the technique has never been combined with SPECT/CT before.

SPECT/CT has proven to be an excellent innovation, as it increases sensitivity (100%) and has good specificity (88.7%). However, one of the limitations of our study is the relatively low number of cases, which can be said to be high compared to the number of cases in other studies found in the literature. Second, although data were collected prospectively, we did not randomize patients to methods A, B, or C. In addition, it should also be considered that the low positive predictive value of dynamic tests can be increased if unnecessary contamination, reflex contractions of the smooth muscles of the ovary, and possible contractions caused by excitement are avoided.

Despite the limitations mentioned above, this further developed method has proven to be effective in detecting fallopian tube obstruction.
Our third study showed that selective tubal pertubation (SPT) performed by office hysteroscopy (OHSC) is a highly sensitive and cost-effective method if performed with appropriate equipment and sufficient practice. The negative predictive value of this ambulatory method is high; thus, it is the method to be chosen as the first step during the infertility test. Selective chromopertubation performed during office hysteroscopy is an effective, minimally invasive method for examining fallopian tube patency. Due to its low cost and minimal pain, it is an equally effective examination method that can also be performed on an outpatient basis without anesthesia.

The aim of my dissertation and my work, on the one hand, to investigate the effectiveness of a new method - the indirect bubble sign - for evaluate the patency of the fallopian tube during hysteroscopy, and on the other hand, after modifying an already known method, the hysterosalpingoscintigraphy (HSSG), we determined its predective value in patency tests.
During our investigations, within infertility check-up, we compared the correlation of the indirect bubble sign observed during hysteroscopy and the patency of fallopian tubes detected during laparoscopic chromohydrotubation.
In summary, it can be said that the method based on visible air bubbles during hysteroscopy was not sufficiently accurate in predicting the patency of the fallopian tube in this form, however, following the appropriate modifications and standardizing the method, in the future it may be a safe, cost-effective alternative for testing the patency of the fallopian tubes as a direct method.
Our team also examined the predictive value of the hysterosalpingo-scintigraphy test method modified with SPECT/CT during the examination of fallopian tube patency. Based on our results, it can be said that the addition of SPECT/CT significantly increased the sensitivity and has good specificity, therefore it can be said that in the future, Fallopian tube scintigraphy can be an ambulatory tubal assessment method and can be performed without anesthesia.
Scintigraphy combined with SPECT/CT is a safe alternative as a first step for examining the patency of the fallopian tubes.
In addition, our results and observations confirm that selective pertubation, which can be performed during office hysteroscopy, is an efficient, accurate, minimally invasive method for ambulatory examination of the patency of the fallopian tube, for the selection of appropriate assisted reproduction techniques. Its application avoids the complications of general anesthesia

or techniques involving irradiation (HSG). Moreover, by examining the uterine cavity, other pathologies such as polyps, micropolyps, fibroids, and chronic endometritis can be detected and treated together, thus improving fertility.

In summary, it can be said that with the spread of modern, minimally-invasive, cost-effective outpatient methods that do not require anesthesia, the number of laparoscopic chromohydrotubations can be reduced, and it can be made into a second-stage examination, which thus results in significantly less nursing time and financial burden for both the care system and the patient.

Summary

The aim of my dissertation and my work, on the one hand, to investigate the effectiveness of a method - the indirect bubble sign - for evaluate the patency of the fallopian tube during hysteroscopy, and on the other hand, after modifying an already known method, the hysterosalpingoscintigraphy (HSSG), we determined its predective value in patency tests.

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Our team also examined the predictive value of the hysterosalpingo-scintigraphy test method modified with SPECT/CT during the examination of fallopian tube patency. Based on our results, it can be said that the addition of SPECT/CT significantly increased the sensitivity and has good specificity, therefore it can be said that in the future, Fallopian tube scintigraphy can be an ambulatory tubal assessment method and can be performed without anesthesia.

Scintigraphy combined with SPECT/CT is a safe alternative as a first step for examining the patency of the fallopian tubes.

In addition, our results and observations confirm that selective pertubation, which can be performed during office hysteroscopy, is an efficient, accurate, minimally invasive method for ambulatory examination of the patency of the fallopian tubes, for the indication of the appropriate assisted reproduction technique. OHSC-SPT avoids the complications of general anesthesia or techniques involving irradiation (HSG). Moreover, by examining the uterine cavity, other pathologies such as polyps, micropolyps, fibroids, and chronic endometritis can be detected and treated at once, thus improving fertility.

With the spread of modern ambulatory examinations that do not require anesthesia to check the patency of the Fallopian tubes, the number of diagnostic laparoscopies can be reduced.

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Registry number: DEENK/334/2023.PL
Subject: PhD Publication List

Candidate: Judit Lőrincz
Doctoral School: Doctoral School of Clinical Medicine

List of publications related to the dissertation

1. **Lőrincz, J.**, Vitale, S. G., Barna, S., Dinkó, F., Riemma, G., Herman, T., Nagyházi, O., Lampé, R., De Franciscis, P., Török, P.: Hystero-salpingo scintigraphy for fallopian tubal patency assessment: results from a prospective study.
   Minim. Invasive Ther. Allied Technol. 31 (5), 797-802, 2022.
   DOI: http://dx.doi.org/10.1080/13645706.2021.1986845
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2. **Lőrincz, J.**, Molnár, S., Herman, T., Vitale, S. G., Singh, J., Lampé, R., Kardos, L., Török, P.: Predictive value of bubble sign for tubal patency during office hysteroscopy.
   Eur. J. Obstet. Gynecol. Reprod. Biol. 253, 58-60, 2020.
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   IF: 2.435

List of other publications

3. **Lőrincz, J.**, Harangi, B., Lampé, R., Török, P.: Az endoszkpia jövője, a jövő endoszkópiája: Szövetek és szervek differenciálása endoszkópia során konvolúciós neurális hálózat segítségével.
   82, 20-22, 2020.

4. **Lőrincz, J.**, Harangi, B., Lampé, R., Török, P.: Az endoszkópia jövője, a jövő endoszkópiája: szövetek és szervek differenciálása endoszkópia során konvolúciós neurális hálózat segítségével.
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   J. Gynecol. Surg. 33 (3), 114-116, 2017.
   DOI: http://dx.doi.org/10.1089/gyn.2016.0079

Total IF of journals (all publications): 8,132
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The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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