The Significance of Hysteroscopy Screening prior to Assisted Reproduction

Jenneke C. Kasius

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The Significance of Hysteroscopy Screening prior to Assisted Reproduction

De waarde van hysteroscopie screening voorafgaande aan geassisteerde reproductie (met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 27 september 2011 des middags te 4.15 uur

door

Jenneke Cornelia Kasius
geboren op 12 september 1985 te Driebruggen

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Aan Kajemathir
Bedankt voor jullie ‘warme nest’

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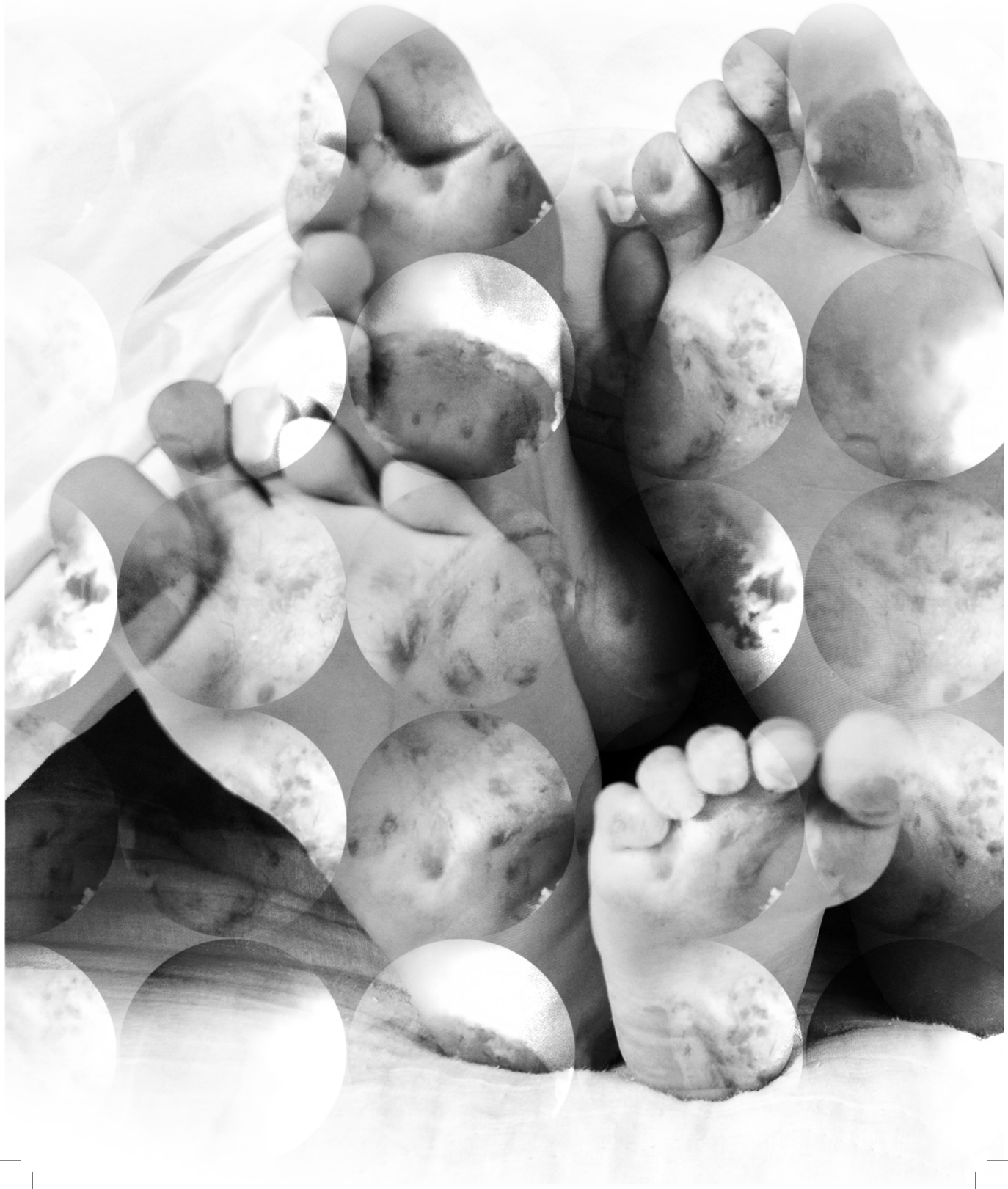


Chapter 1

General Introduction

"...the womb is the field of generation; and if this field be corrupted it is in vain to expect any fruit though it be ever so well sown."

Aristotle

The uterus is the reproductive organ in the genital tract that connects the cervix, which opens into the vagina, with the fallopian tubes that lead to the ovaries. It provides a unique entry for the male semen to get to the female oocyte. The remarkable mucosa of the uterus allows sperm to pass trough without compromising its immune surveillance against pathogens, like bacteria or viruses. Moreover, it is able to retain the allogeneic blastocyst and create the ultimate environment for implantation and development (Lea and Sandra, 2007). The uterus is ´the field of generation´ and therefore, it is inevitably related to fertility.

Subfertility

Subfertility is the failure to conceive within a period of 12 months, despite unprotected intercourse. In the Netherlands, one in every five couples visits their general practitioner because of fertility problems. Ten percent finally will end up at the gynaecologist office and meet the criteria for infertility. Almost half of these couples, about 7.000 couples yearly, will rely on in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatment as a tool to enhance their chances for a live birth (Kremer, 2009).
Despite the numerous advances in the field of IVF/ICSI, there still exists a maximum implantation rate per embryo transferred of about 30% (Andersen et al., 2008). Even if both ovum pick-up and fertilization occur successfully in the process of IVF, there is a large unexplained drop between embryo transfer and the occurrence of pregnancy. Implantation failure could be related to suboptimal embryo quality, uterine environment, or a combination of both. Non-implantation represents a major clinical challenge and is a cause of considerable stress to patients and the medical and paramedical crew in assisted reproductive technology. If progress is to be made in improving implantation rates, a greater understanding of the factors determining successful implantation is required.

The uterine environment

Six to seven days after fertilization of the oocyte, implantation of the blastocysts occurs. The three phases of implantation: apposition, adhesion and invasion, are established by continuous interchange of hormones and cytokines between the blastocyst and the endometrium (Singh et al., 2011). Major and minor uterine cavity abnormalities are assumed to interfere with the factors that regulate this interplay, precluding the occurrence of pregnancy. Still, a thorough understanding of both the degree and effect mechanism of abnormalities like endometrial polyps, leiomyoma, adhesions or septa, is absent to date.

Macroscopic uterine cavity abnormalities

Macroscopic intrauterine pathology usually consists of excessive presence of physiologic uterine tissue. The most common acquired abnormality is the endometrial polyp. Per definition, this endometrial stalk like mass protrudes into the uterine cavity and has its own vascular supply. Depending on the patient population and applied diagnostic test, endometrial polyps are present in 1% - 41% of the infertile patients (Silberstein et al., 2006). Excessive tissue growth originating from the muscular tissue of the uterus, leiomyoma, is found in 2.4% of infertile patients, without any other obvious cause of infertility (Donnez and Jadoul, 2002). Exclusively the submucous leiomyoma that deforms the uterine cavity is thought to interfere with fertility. Adhesions, fibrous tissue strings connecting parts of the uterine wall, may be present at birth or result from inflammation or (iatrogenic) tissue damage. In an infertile population, the prevalence of adhesions lies between 0.3% - 14% (Tur-Kaspa et al., 2006; Hinckley and Milki, 2004; Fatemi et al., 2010). Finally, the uterine septum may impair fertility. This is one of the congenital Müllerian duct malformations, in which the longitudinal fibroid tissue band, separating the left and right Müllerian ducts, has not entirely been resorbed. The uterus septum is present in 1% - 3.6% in otherwise unexplained infertile patients (Saravelos et al., 2008).

Microscopic intrauterine pathology

Also less distinct endometrial pathology is thought to cause impaired endometrial receptivity resulting in infertility. During the monthly menstrual cycle, the endometrium undergoes structural changes under influence of steroid-hormones. Estrogen dependent proliferation is followed by the secretion or luteal phase, when progestagen levels dominate. Maximum receptivity for embryo implantation lies between day 20 and 23 of the menstrual cycle, in the mid-luteal cycle phase. Altered or insufficient hormonal status may interfere with the synchronic changes of blastocyst and endometrium and therefore with implantation (Lessey, 2010). Moreover, acute as well as chronic endometritis induce an inflammatory reaction, thereby creating a hostile environment, possibly resulting in implantation failure (Kamiyama et al., 2004). With the lack of systematic screening and a lack of clinical criteria, the prevalence of acute endometritis is in fact unknown. Diagnosing chronic endometritis may also be complicated as it is rarely clinically suspected. Depending on the patient population and biopsy method, the prevalence of chronic endometritis has been reported to be between 0.2% - 46% in infertile women (Polisseni et al. 2003; Féghali et al., 2003; Cicinelli et al., 2005; Johnston-Macananny et al., 2010; Sahmay et al., 1995; Wild et al., 1986). Last, atypical polypoid adenomyoma and hyperplasia are less distinct abnormalities of the endometrium. Both

are rarely present and hard to diagnose except under direct visualization and endometrial biopsy.

Diagnosis and treatment of intrauterine pathology

In reproductive medicine, a trend is developing towards diagnosis and treatment of all uterine cavity abnormalities prior to fertility treatment. However, for most intrauterine pathology, the true benefit of that working method has not yet been assessed. For endometrial polyps, leiomyoma, adhesions and septa, cohort studies have shown clear improvement in spontaneous pregnancy rate after removal of the abnormality (Taylor and Gomel, 2008). Several retrospective case-control studies also found a major positive effect of metroplasty on fertility in women with a uterine septum (Mollo et al., 2008; Tomasevic et al., 2010; Shokeir et al., 2011). Resection of the septum increased the live birth rate after transfer of two to three embryo's in an IVF/ICSI program from 2% to 39%. Only for endometrial polyps and leiomyoma the effect of treatment on fertility has been confirmed in randomized controlled trials. Polypectomy in women with a polyp between 3 and 24mm on transvaginal sonography (TVS) improved the pregnancy rate after intrauterine insemination by 35% (28% vs. 63%, RR 2.3: 95% CI 2 - 5) (Perez-Medina et al., 2005). Also, a 35% increase in spontaneous pregnancy occurred after hysteroscopic resection of a submucous leiomyoma in infertile women (28% vs. 63%, RR 2.1: 95% CI 1.5 - 2.9) (Shokeir et al., 2010). Although the available literature is sparse and frequently of low quality, due to the positive results it is increasingly advised to screen the uterine cavity with the most accurate diagnostic test.

Evaluation of the uterine cavity

At present, the basic work-up for evaluation of the uterine cavity prior to IVF consists of TVS, possibly followed by saline infusion/gel instillation sonography (SIS/GIS), hysterosalpingography (HSG) or hysteroscopy.

Transvaginal sonography

TVS is a universally accepted, routine non-invasive diagnostic test to screen for intrauterine pathology. Making use of a vaginal ultrasound transducer, the cervix, uterine endometrium and myometrium and adnexa can be visualized. In an infertile patient population, TVS is found to be rather accurate in diagnosing intracavitary pathology, such as polyps, leiomyoma, septa and adhesions. The sensitivity and specificity of TVS compared to hysteroscopy or endo-

metrial histology has been reported to be between 0.00 - 0.95 and 0.80 - 1.00, respectively (Soares et al., 2000; Loverro et al., 2001; Ragni et al., 2005; Bingol et al., 2011; El-Sherbiny and Nasr, 2011). Most of the variation in the accuracy of TVS is related to variation in sensitivity in detection of intrauterine adhesions (Soares et al., 2000; Loverro et al., 2001).

Saline infusion or gel instillation sonography

In SIS/GIS, a distension media is inserted into the uterine cavity via a transcervical catheter, during transvaginal ultrasound scanning. This provides a better view of the endometrial lining and the presence and position of abnormalities in the fluid filled cavity. Moreover, developments of applying this technique to test for tubal patency have shown promising results (Luciano et al., 2011). Due to more detailed information of the endometrium, the accuracy of a sonography increases remarkably by using distension media. Some studies have described it to be as accurate as hysteroscopy (Bingol et al., 2011). Overall, the sensitivity and specificity of SIS compared to hysteroscopy or histology for diagnosing intrauterine pathology is described to be between 0.70 - 1.00 and 0.93 - 0.98, respectively (Soares et al., 2000; Ragni et al., 2005; Bingol et al., 2011; El-Sherbiny and Nasr, 2011). Three-dimensional ultrasound is an upcoming technique in which 3 planes are registered simultaneously, creating a 3D image. The accuracy of the 3D sonohysterography are even more encouraging, with a sensitivity and specificity of both 1.00 (El-Sherbiny and Nasr, 2011).

Hysterosalpingography

A HSG is an X-ray of the uterus and fallopian tubes after injection of radiographic contrast media through the cervix. The contrast will fill up the uterine cavity, the fallopian tubes and, in case of open tubes, spill into the abdominal cavity. This relatively invasive and painful method is mainly used for detection of tubal patency or obstruction.

Results on the accuracy of HSG in assessment of the uterine cavity integrity in infertile patients vary widely. The sensitivity and specificity in infertile women compared to hysteroscopy has been described to be between 0.44 - 0.81 and 0.15 - 0.96 (Golan et al., 1996; Soares et al., 2000; Roma Dalfó et al., 2004).

Hysteroscopy

Hysteroscopy detects uterine cavity pathology by direct visualization of the endometrial lining making use of a vaginally inserted endoscope. Due to the development of thinner working channels and the vaginoscopic insertion technique, it has become easy to perform in an outpatient setting without anaesthesia. Moreover, hysteroscopy enables instant treatment of small uterine pathology. Therefore, it is frequently referred to as the golden standard (Bosteels

et al., 2010; Bettocchi et al., 2011).
Hysteroscopy has been shown to be almost as accurate as histopathology examination itself in patients who underwent hysterectomy after hysteroscopy (Ceci et al., 2002). Also in infertile patients, the sensitivity and specificity compared to histology are significant; 1.00 and between 0.98 and 1.00, respectively (Bingol et al., 2011).
Despite all its advantages, also the hysteroscopy examination has its flaws. As it is impossible to judge the outer contour of the uterus and the myometrium, it can be difficult to differentiate congenital malformations and diagnose leiomyoma. Also, the exact distinction between polypoid endometrium and a polyp, or atypical tincture; the difference between a normal and abnormal cavity has not been well defined yet. An additional hysteroscopy guided endometrial biopsy could be of use to confirm the diagnosis.

Table 1 Overview of the specificity and sensitivity of diagnostic tests for uterine cavity evaluation compared to hysteroscopy or endometrial histology in infertile patient populations

Diagnostic test	Sensitivity	Specificity	References
TVS[a]	0.00 - 0.95	0.80 - 1.00	Soares et al., 2000; Loverro et al., 2001; Ragni et al., 2005; Bingol et al., 2011; El-Sherbiny and Nasr, 2011
SIS[b]	0.70 - 1.00	0.93 - 0.98	Soares et al., 2000; Ragni et al., 2005; Bingol et al., 2011; El-Sherbiny and Nasr, 2011
HSG[c]	0.44 - 0.81	0.15 - 0.96	Golan et al., 1996; Soares et al., 2000; Roma Dalfó et al., 2004
Hysteroscopy	1.00	0.98 - 1.00	Bingol et al., 2011

[a]: Transvaginal sonography, [b]: Saline infusion sonography, [c]: Hysterosalpingography

Considerations for the daily fertility work-up

In spite of the suspicion for intrauterine abnormalities to negatively affect the chance to conceive combined the low discomfort and high accuracy of screening tests like SIS/GIS and hysteroscopy, national and international guidelines do not recommend standard additional diagnostic testing for uterine cavity evaluation prior to infertility treatment (Crosignani and Rubin, 2000; NVOG, 2004; RCOG, 2004). It has been argued that the significance of treating unsuspected intrauterine abnormalities has not yet been fully proven.

Two issues need to be addressed here. First, the prevalence of intrauterine abnormalities in patients without symptoms suspect for uterine pathology is found to be rather high, and also, considerably variable. In asymptomatic patients with a normal TVS and/or HSG, hysteroscopy detected minor abnormalities (endometrial polyps, submucous leiomyoma, adhesions and septa) in 20% - 40% (Balmaceda and Ciuffardi, 1995; La Sala et al., 1998; Hinckley and Milki, 2004). This questions the reliability of these tools in asymptomatic infertile populations. Second, the true value of diagnosing and treating these abnormalities in order to optimize the condition of the uterine environment and thereby the outcome of IVF/ICSI treatment has not been demonstrated in a scientifically sound fashion. In a retrospective cohort analysis, the pregnancy rate after operative hysteroscopy of patients with intrauterine abnormalities diagnosed by SIS was compared to the pregnancy rate of patients with a normal uterine cavity. A 31.6% increase in pregnancy rate was observed after treatment of detected abnormalities (Gera et al., 2008). Furthermore, two randomized trials reported exceptional improvements in pregnancy rates after office hysteroscopy and instant treatment of detected pathology in patients with a normal HSG after two failed IVF attempts. Intervention resulted in a 9% - 13% increase in clinical pregnancy rate after the subsequent IVF cycle (from 21.6% - 30.4% and from 26.2% - 39.6%) (Demirol and Gurgan, 2004; Rama Raju et al., 2006). These results endorsed the findings of other, previously published observational studies (Shamma et al., 1992; Oliveira et al., 2003). Despite some relevant study weaknesses, the results of these studies do indicate a trend towards a beneficial effect of screening hysteroscopy on IVF outcome.

The combination of the high prevalence of unsuspected minor intrauterine abnormalities and indications of a positive effect of hysteroscopic treatment on fertility, has led to the suggestions for routine hysteroscopy in the pre-IVF workup. On the other hand, due to paucity of multicenter randomized controlled trials to provide high quality evidence on the exact impact of unsuspected intrauterine abnormalities on IVF/ICSI outcome in asymptomatic infertile patients, there is the threat of widespread introduction of hysteroscopy and other imaging techniques prior to IVF/ICSI, without the certainty that this policy is truly effective.

From a societal point of view, next to the true effects of hysteroscopy on infertility, also the effect on the costs of fertility treatment should be considered. IVF and ICSI both are expensive procedures, while the costs of one hysteroscopy procedures decline, through new inventions (Bouwmans et al., 2008). As the number of couples seeking infertility treatment increase, optimization of all conditions that influence IVF/ICSI success rates is warranted.

Aims of the thesis

The aim of this thesis is to assess the true value of screening hysteroscopy in infertile, IVF indicated patients, who are not suspected of intrauterine pathology. Focus was on the following topics:

- The prevalence of unsuspected intrauterine pathology
- The reliability of screening hysteroscopy as a diagnostic tool for unsuspected pathology
- The clinical significance and reliability of diagnosing endometritis by hysteroscopy guided endometrial biopsy
- The cost-effectiveness of screening hysteroscopy in assisted reproductive technology

Outline of the thesis

Chapter 2 describes the prevalence of detected intrauterine abnormalities (endometrial polyps, submucous leiomyoma, septa or adhesions) by screening hysteroscopy in asymptomatic patients with a normal TVS prior to a first IVF/ICSI cycle.

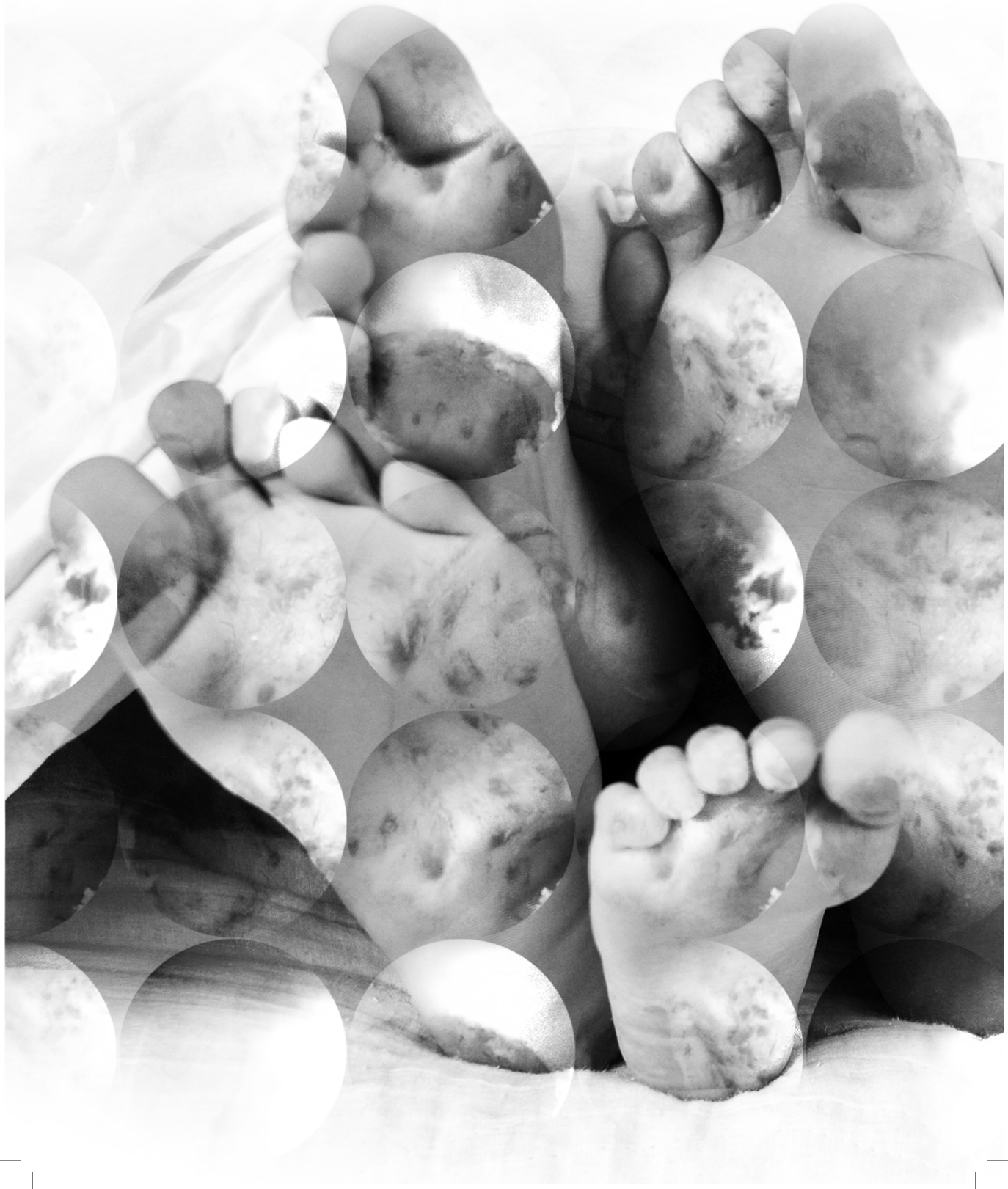
Chapter 3 investigates the observer agreement in diagnosing intrauterine abnormalities at screening hysteroscopy. The intra- and interobserver agreement was evaluated.

Chapter 4 evaluates the importance of the hysteroscopy guided endometrial biopsy regarding chronic endometritis. The prevalence as well as the impact of chronic endometritis on reproductive outcome was assessed.

Chapter 5 assesses the interobserver reliability on the histological diagnosis chronic endometritis, which currently is exclusively based on the presence of plasma cells in the endometrial stroma.

Chapter 6 compares the cost-effectiveness of screening hysteroscopy, hysteroscopy after two failed IVF cycles and no hysteroscopy prior to IVF treatment.

Chapter 7 contains a summery of the study results and discusses the clinical implications and future research.



Chapter 2

Prevalence of unsuspected uterine cavity abnormalities diagnosed by office hysteroscopy prior to in vitro fertilization

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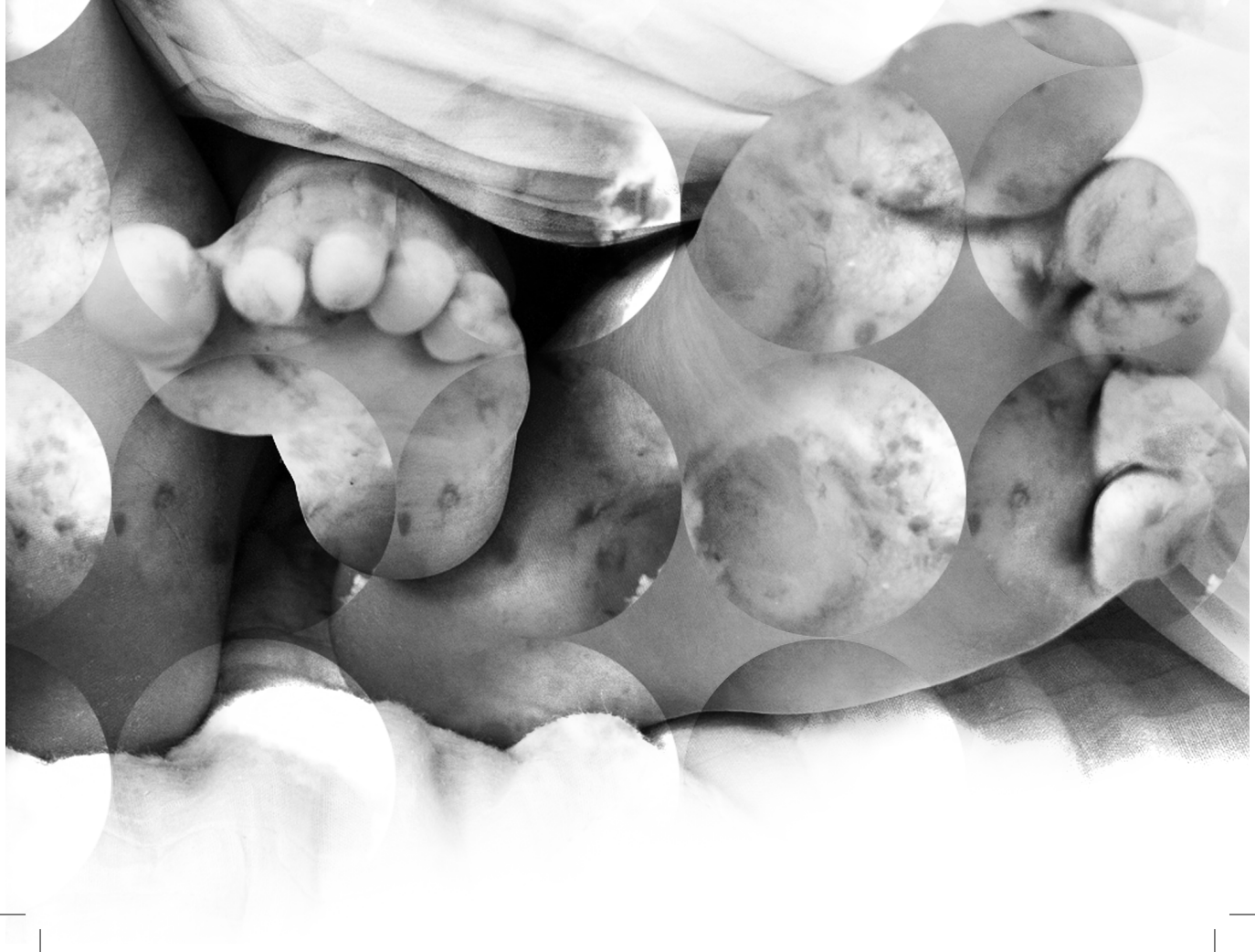


Abstract

Background Whether implantation occurs after in vitro fertilization (IVF) depends on the embryo, uterine receptivity or a combination of both. The prevalence of minor intrauterine abnormalities identified at hysteroscopy in cases with a normal transvaginal sonography (TVS) has been recorded to be as high as 20% - 45%. Diagnosing and treating such pathology prior to initiating IVF or intracytoplasmic sperm injection (ICSI), has been widely advocated without high quality evidence of a beneficial effect. The objective of the current study was to assess, by screening office hysteroscopy, the prevalence of unsuspected intrauterine abnormalities in an asymptomatic population of IVF patients, in whom TVS had not revealed any pathology.

Methods The prevalence of unsuspected intrauterine abnormalities in patients allocated for a randomized controlled trial was prospectively assessed at two tertiary infertility care units: Academic Hospital at the Dutch-speaking Brussels Free University and University Medical Center Utrecht. A total of 678 unselected, asymptomatic, infertile women with a regular indication for a first IVF/ICSI treatment underwent office hysteroscopy. Only asymptomatic patients, aged ≤42 years, with a normal TVS and no previous hysteroscopy were included. The presence of predefined intrauterine abnormalities was recorded and described in a standardized manner.

Results Endometrial polyps were identified in 41 (6%) women and submucous myomas in 6 women (1%). Some women were also diagnosed with intrauterine adhesions (2%) or septa (2%). The overall prevalence of any predefined intrauterine abnormality in this IVF/ICSI population was 11%.

Conclusions The observed prevalence of unsuspected intrauterine abnormalities in asymptomatic patients indicated for their first IVF/ICSI treatment appeared to be clearly lower than previously reported (11% versus 20% - 45%). This may have implications for the significance of these abnormalities regarding prospects in IVF/ICSI treatment cycles.

Introduction

Despite the numerous advances in the field of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), the implantation rate per embryo transferred usually does not exceed 30%, although higher rates with the use of blastocysts have been reported, depending on female age (Andersen et al., 2008; Stillman et al., 2009). Embryo quality, good conditions of the uterine environment, a skilful IVF laboratory and embryo transfer are essential in order to achieve a pregnancy in IVF. Unsuspected uterine cavity abnormalities, such as endometrial polyps, small submucous myomas, adhesions and septa are considered to have a negative impact on the chances to conceive through IVF (Rogers et al., 1986). The prevalence of such unsuspected intrauterine abnormalities, diagnosed by hysteroscopy prior to IVF, has been described to be between 20 and 45% (Balmaceda and Ciuffardi, 1995; La Sala et al., 1998; Hinckley and Milki, 2004). Moreover, diagnosing and treating these abnormalities are advocated in order to optimize the condition of the uterine environment and thereby the outcome of IVF treatment. However, this recommendation is not based on high quality evidence (Shamma et al., 1992; Oliveira et al., 2003; Demirol and Gurgan, 2004; Rama Raju et al., 2006).

For evaluation of the uterine cavity, the basic work-up consists of transvaginal sonography (TVS) with or without the use of saline or gel as contrast media, possibly followed by either hysterosalpingography (HSG) or hysteroscopy to directly assess the uterine cavity. Both TVS, as well as saline infusion sonography infusion (SIS) and gel instillation sonography (GIS) are inexpensive, non-invasive and have been shown to be excellent diagnostic tools to detect subtle intrauterine abnormalities (Ayida et al., 1997; Fabres et al., 1998; Shalev et al., 2000; Ziegler de, 2009). Office hysteroscopy has increasingly been recommended as a routine procedure in the infertility work-up (La Sala et al., 1998; Oliveira et al., 2003; Demirol and Gurgan, 2004; Hinckley and Milki, 2004; Doldi et al., 2005; Rama Raju et al., 2006; Lorusso et al., 2008). It has become easy to perform in an outpatient setting without anaesthesia. Moreover, it offers direct visualization and enables clinicians to diagnose and treat intrauterine pathology during the same session (Bettocchi et al., 2004).

The aim of this study was to verify the prevalence of unsuspected uterine cavity abnormalities, diagnosed by office hysteroscopy screening in a group of unselected asymptomatic women, indicated for a first IVF cycle.

Materials and Methods

The present study was conducted as a part of the TEA-trial (Treatment Efficacy of uterine Abnormalities, register number ClinicalTrial.gov: NCT00830401), a randomized controlled trial to assess the benefits of diagnosing and treating unsuspected intrauterine abnormalities for subsequent outcome in IVF. The study was approved by the Institutional Review Board of the two participating centers. Informed consent was obtained.

Participants

The screening hysteroscopies for the TEA-trial were conducted in the period from June 2007 until September 2008 at the Academic Hospital at the Dutch-speaking Brussels Free University (AZVUB, Belgium) and the University Medical Center Utrecht (UMCU, The Netherlands). All couples indicated for a first IVF/ICSI treatment underwent the standard infertility work-up, consisting of the medical history, physical examination, TVS, hormone status, chlamydia antibody testing (CAT), and semen analysis. In case of a positive CAT and/or the presence of risk factors for tubal pathology in the medical history, a HSG or diagnostic laparoscopy was performed, to test for tubal patency. In CAT negative cases without risk factors, no further assessment of tubal pathology was carried out. The TVS was performed by an experienced gynaecologist, evaluating the uterine cavity for the presence of features suspected for any of the predefined abnormalities (i.e. polyps, submucous leiomyoma, adhesions or septa), by looking at the regularity of the endometrial lining and measuring the double layer of the endometrium both in the sagittal and transversal planes. The findings, corresponding cause of infertility and TVS result were reported in the patient's record. In case no menorrhagia or metrorrhagia was present and TVS did not show abnormalities, women were indicated for a screening hysteroscopy on an outpatient basis. Inclusion into this screening was limited to women under the age of 43 years with no prior hysteroscopy examination nor prior IVF/ICSI attempt to conceive (Belgian statute book, 2003). Women with any of the predefined abnormalities at TVS followed the regular routine and underwent a therapeutic hysteroscopy to resolve the uterine cavity pathology prior to starting the infertility treatment.

Hysteroscopy procedure

The hysteroscopies were scheduled in the follicular phase of the menstrual cycle (days 3–15), 1–3 months prior to starting the IVF/ICSI treatment. All hysteroscopy examinations were performed by a team of three experienced gynaecologists (HF, AT, FB), using a 5-mm outer-diameter continuous flow Bettocchi hysteroscope with 30° direction of view (Karl Storz Endoscopy, Stöpler

Medical Instruments, Utrecht, The Netherlands & Olympus Belgium N.V., Aartselaar, Belgium). Normal saline solution was used for distension of the uterine cavity at the pressure of 20–50 mmHg. The hysteroscopy procedures were performed in an outpatient setting, using the vaginoscopic approach without anaesthesia or dilatation. In case, the examination could not be accomplished due to patient intolerance, the procedure was continued under paracervical block, using a lidocaine injection (2 x 2 ml, 2%) (AstraZeneca B.V., Zoetermeer, The Netherlands).

Uterine cavity assessment

Also hysteroscopically detected intrauterine abnormalities were defined as endometrial polyps, submucous myomas, intrauterine adhesions or uterine septa. According to the TEA-trial protocol, different routings were possible if any of these predefined abnormalities were identified. In cases where prior informed consent had been obtained, randomization for treatment or no treatment was applied. Without informed consent, most cases with abnormalities adhered to the prior advice of treatment. In three cases, treatment was refused by the patient in view of the specific nature of the abnormality, or could not be accomplished due to patient intolerance. Treatment consisted of removal of polyps, myomas, adhesions or septa using scissors, grasping forceps or Versapoint® (Johnson & Johnson, Dilbeek, Belgium & Hoofddorp, The Netherlands). Interventions were performed in the same outpatient setting without additional anaesthesia. After the intervention, a detailed record was completed, concerning information about patient tolerance, diagnostic findings and treatment. In the course of the study period, the prevalence of intrauterine abnormalities appeared to be too low for a valid assessment of the treatment efficacy of the predefined uterine abnormalities according to the design and power calculation of the TEA-trial. Therefore, the randomization of patients to treat or not to treat in case of abnormalities observed was abandoned.

Statistical analysis

Chi-square test and Student's t-test were used to analyze different subgroups. Uni- and multivariate logistic regression were applied in order to identify factors that could predict the presence of unsuspected uterine cavity abnormalities. A P-value of <0.05 was considered statistically significant. All statistical analyses were performed in SPSS version 15.1 (SPSS Inc., Chicago, IL, USA).

Table 1 Patient characteristics of the IVF/ICSI cases that underwent screening hysteroscopy, listed according to the presence or absence of predefined unsuspected uterine cavity abnormalities

Variables	Group with abnormalities N = 74		Group without abnormalities N = 596		Significance	Overall N=670[b]	
Age	34.47 ± 4.22		32.58 ± 4.61		P = 0.00[a,f]	32.84 ± 4.61	
Duration of subfertility (yr)[c]	3.05 ± 2.55		2.94 ± 2.40		NS[f]	3.00 ± 2.54	
Body Mass Index	24.74 ± 4.31		23.65 ± 4.21		P = 0.04[a,f]	23.82 ± 4.26	
VCM	82.85 ± 137.93		63.43 ± 82.96		NS[f]	65.58 ± 90.00	
Cycle day	9.51 ± 3.84		9.46 ± 4.44		NS[f]	9.45 ± 4.36	
Cause infertility					NS[g]		
- Idiopathic	31	(41.9%)	238	(39.9%)		269	(30.1%)
- Andrologic factor[d]	32	(43.2%)	274	(46.0%)		306	(45.7%)
- Subfertile female[e]	11	(14.9%)	84	(14.1%)		95	(14.2%)
Infertility woman					P = 0.05[g]		
- Primary	40	(54.1%)	391	(65.6%)		431	(64.3%)
- Secondary	34	(45.9%)	205	(34.4%)		239	(35.7%)
Race					NS[g]		
- Caucasian	50	(67.6%)	458	(76.8%)		508	(75.8%)
- African	2	(2.7%)	15	(2.5%)		17	(2.5%)
- Asian	2	(2.7%)	18	(3.0%)		20	(3.0%)
- Mediterranean	18	(24.3%)	100	(16.8%)		118	(17.6%)
- Latin American	2	(2.7%)	4	(0.7%)		6	(0.9%)
- Mix	0	(0.0%)	1	(0.2%)		1	(0.1%)

Note Values are expressed as mean ± Standard deviation NS = Not significant

[a]: P-value of univariate analysis, [b]: Failed procedures excluded, [c]: Duration of subfertility: duration in years of attempts to become pregnant, in cases of secondary infertility calculated from the last ongoing pregnancy, [d]: Defined as VCM (sperm volume (mL) * concentration sperm cells (*10^9/mL) * grade A and B sperm cell motility(%)) $<20 \cdot 10^6$, [e]: Due to tuba pathology (incl. endometriosis grade III and IV), anovulation or cervix factor, [f]: Significance, Student t-test, [g]: Significance, Chi-square test

Results

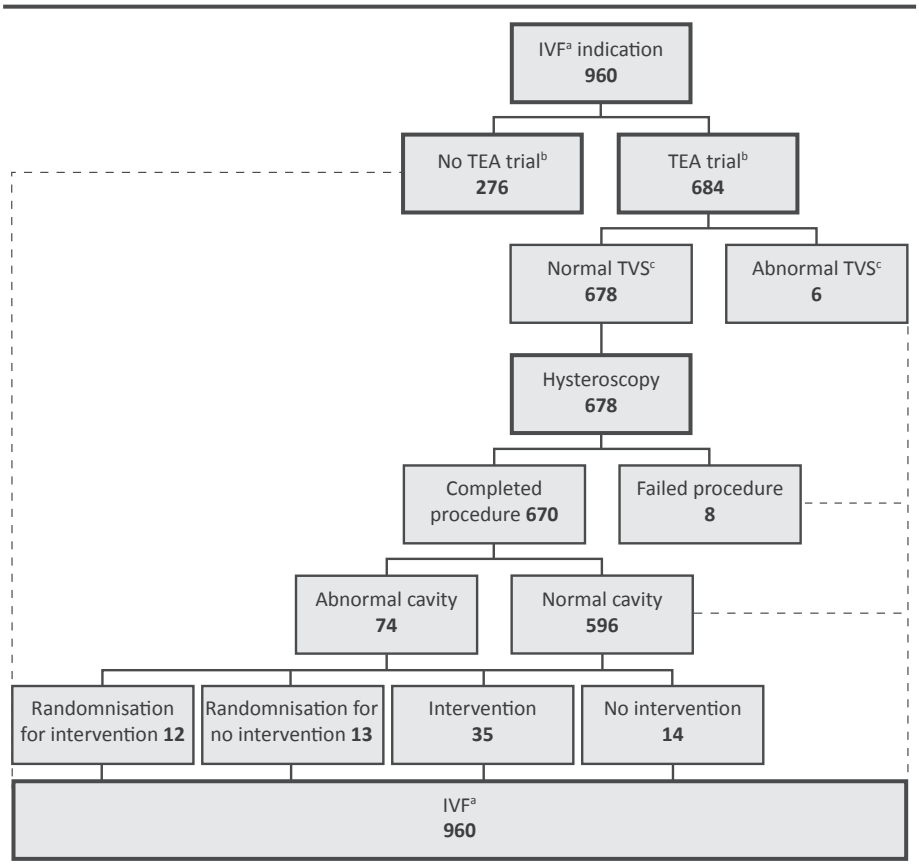
From June 2007 until September 2008, a total of 960 women presented with an indication for a first IVF/ICSI treatment cycle at one of the two research hospitals. Owing to limited capacity of the hysteroscopy facilities in the initial phases of the trial, ultimately 684 women were scheduled for hysteroscopy. However, the patients who were scheduled for hysteroscopy were a random sample of all patients indicated for a first IVF/ICSI cycle. No significant differences were found, regarding female age, body mass index (BMI), smoking behaviour or race, between the group with and without hysteroscopy examination. In the 684 patients who were scheduled for hysteroscopy, TVS did not reveal any of the predefined abnormalities in 678 of the included patients. In the remaining six patients, a cavity deforming leiomyoma was detected. Detailed information on the patient population and selection is shown in Table 1 and Figure 1. The most frequent cause of infertility was a poor semen quality, with severe male factor infertility in 26% of all cases (amount of grade A and B motile sperm cells <2.0 x 10^9). Female subfertility was present in 14.2% of the couples, mostly due to tubal pathology.

Out of all the hysteroscopies, 94% was scheduled in the follicular phase of a cycle (days 3–15). Most of them were performed on day 9 of the menstrual cycle, with an overall range of days 1–59, due to inclusion of women with oligomenorrhoea. Investigation of the uterine cavity could be adequately completed in 670 cases (99%). The main reason for failure of office hysteroscopy was patient intolerance (six cases, 0.9%). Other causes were an unclear view or inability to achieve passage of the internal ostium. Local anaesthesia was used during eight of the procedures and resulted in a successful hysteroscopy examination in six cases. An infection after the hysteroscopy occurred in one case and was treated on an outpatient basis with rapid recovery. No other complications occurred.

Hysteroscopy findings

The frequency of one or more abnormalities per patient was 11% (Figure 2). Endometrial polyps were identified in 41 cases (6%). Most detected polyps (63%) were smaller than 0.6 cm, in only three cases it concerned a polyp >1.0 cm. Submucous myomas were found in six cases (1%), all with an estimated diameter between 0.5 and 2.0 cm. Also 15 cases with intrauterine adhesions (2%) and 14 cases with a septum (2%) were diagnosed. In two cases more than one abnormality was identified. The overall abnormality rate did not significantly differ between the participating university hospitals: 12% in the AZVUB versus 10% in the UMCU.

Figure 1 Flow chart

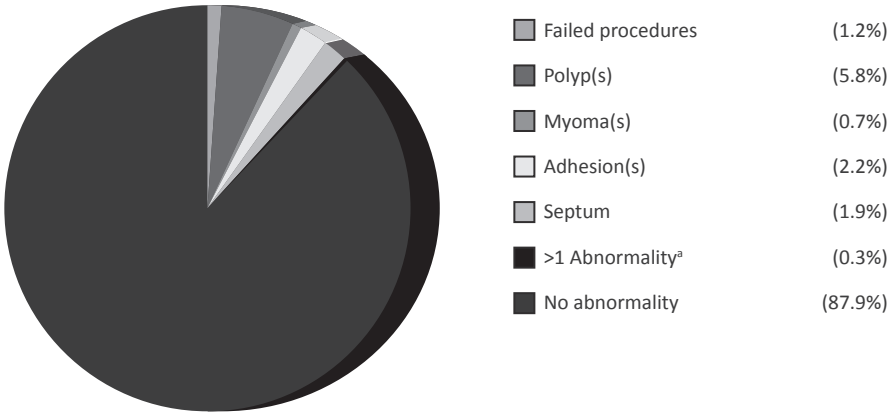


[a]: In vitro fertilization, [b]: Due to logistic factors not all patients were included in the TEA-trial (Treatment Efficacy of uterine Abnormalities), [c]: Transvaginal sonography

Predictive factors

Female age and BMI were associated with the occurrence of unsuspected intrauterine abnormalities in a univariate analysis (Table 1). Multivariate logistic regression analysis, to assess the independency of these two variables as predictors of the presence of intrauterine abnormalities, demonstrated a significant correlation only between female age and abnormalities (P-value 0.002, OR: 1.09, 95% CI: 1.03 - 1.16).

Figure 2 Findings at office hysteroscopy



[a]: In one case both an endometrial polyp and a submucous myoma were diagnosed. In another case both an endometrial polyp and a septate uterus were diagnosed

Discussion

Hysteroscopy is generally considered to be the golden standard in the diagnosis of intrauterine pathology, including endometrial polyps, submucous myomas, intrauterine adhesions and uterine septa (Bozdag et al., 2008). However, there is paucity of data on the impact of unsuspected intrauterine pathology prior to commencing IVF that will duly be discovered by routine hysteroscopy. In the present study, the prevalence of one or more intrauterine abnormalities in asymptomatic women, who underwent office hysteroscopy prior to a first IVF cycle, was found to be only 11%.

The reported results were based on a study, which was designed to be a randomized trial. Randomization was applied for instant treatment versus no treatment of unsuspected intrauterine pathology detected by hysteroscopy screening. As all detected abnormalities were not diagnosed by a prior TVS, all hysteroscopy performers knew to expect mainly subtle abnormalities. A standard, detailed scoring form guaranteed reports of all abnormalities, regardless of the size or possibility of performing hysteroscopy intervention in case an abnormality was identified. Thus, even though the data were obtained in the frame of a randomized trial instead of a pure observational study, the reported results are a reliable representative of our daily infertility practice.

Another possible study limitation has been the timing of the hysteroscopy examinations. In the publication by Hinckley and Milki, all hysteroscopies were performed during the hormonal contraceptive phase prior to controlled ovarian hyperstimulation for IVF (Hinckley and Milki, 2004). In the current study, the hysteroscopies were performed in the follicular phase of a natural cycle, since the use of oral contraceptive pills prior to starting IVF treatment with GnRH antagonist co-treatment is questionable (Kolibianakis et al., 2004). Moreover, the investigators aimed to avoid any bias by the administration of oral contraceptives during the hysteroscopy. As hysteroscopies were also performed in the late follicular phase, when endometrial proliferation may have become abundant, this may have affected the accuracy of diagnosing small abnormalities. It is unlikely that this has been of major influence on the study results though, as additional analysis did not reveal any difference in timing of hysteroscopy between the group with or without abnormalities. The prevalence of unsuspected intrauterine abnormalities has been recorded to be between 20% and 45% (Shamma et al., 1992; Balmaceda and Ciuffardi, 1995; La Sala et al., 1998; Oliveira et al., 2003; Demirol and Gurgan, 2004; Hinckley and Milki, 2004; Doldi et al., 2005; Rama Raju et al., 2006). Thus, the overall prevalence of abnormalities described by the present study considerably differs from the prevalence reported in previously published articles. This difference could be explained by factors related to study design; patient inclusion criteria and types of intrauterine pathologies included. Unfortunately, most authors solely reported that they investigated women with a normal TVS or HSG, but do not provide a thorough description of the patient characteristics. The main differences in patient population that have been recorded are the number of IVF attempts and female age. A number of studies have evaluated hysteroscopy findings in patients after two or more unsuccessful IVF cycles (Oliveira et al., 2003; Demirol and Gurgan, 2004; Rama Raju et al., 2006). If the presence of intrauterine abnormalities would have a negative impact on the chance to conceive, the abnormality prevalence is expected to be higher in such patient populations.

In the current study, the chance of finding an unsuspected abnormality appeared to increase with female age. The effect of female age on the presence of intrauterine abnormalities has not been previously described by other studies using a similar setting. Still, studies focusing on the incidence of uterine leiomyoma, did observe an increasing prevalence with age in a pre-menopausal fertile patient population (Marshall et al., 1997). The same was found for the prevalence of endometrial polyps: the prevalence rate was significantly lower in the age group 25 - 35 years and showed a peak in the group 46 - 55 years (Hileeto et al., 2005). Some investigators studied patient groups with an older mean age than in the current study (Oliveira et al., 2003; Demirol and Gurgan,

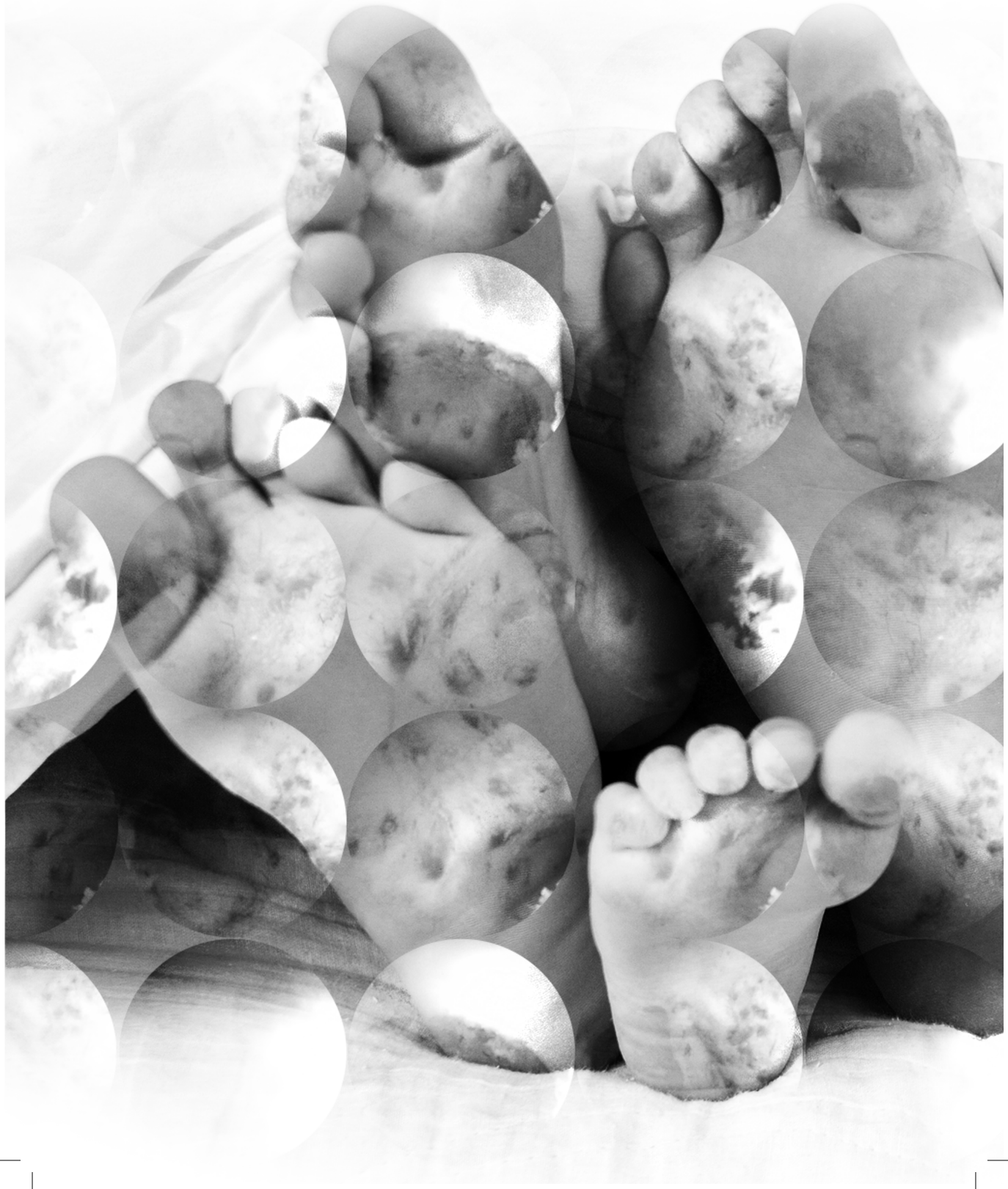
2004; Doldi et al., 2005). This particular difference in selected patient populations may also be interpreted as an explanation for the variation in overall prevalence of abnormalities.
Finally, not all investigators screened the uterine cavity for the same type of pathology. Some did not include all four of our predefined abnormalities, whereas others added certain abnormalities, like uterine hypoplasia and endometrial hyperplasia (Shamma et al., 1992; Demirol and Gurgan, 2004; Doldi et al., 2005).
Nevertheless, studies with a set-up comparable to that of the present study also reported a clearly higher prevalence (Hinckley and Milki, 2004). Variation in the interpretation of hysteroscopy findings may also explain the observed differences in abnormality prevalence. Research on the inter-observer variation in hysteroscopy visual diagnosis is minimal (Dueholm et al., 2002[a]). As such, the possibility of systematic observer bias cannot be ruled out. Future research on inter-observer agreement would be desirable.
In the current study, the most frequently found abnormality was an endometrial polyp of only small diameter. Next to the low prevalence, this notion may also question the relevance of subtle intrauterine pathologies for IVF outcome in asymptomatic women. Moreover, no studies have so far investigated the relationship between the size of the abnormalities and infertility. The current literature mostly studied patients with recurrent IVF failure, or used clinical pregnancy rate as the main outcome measure instead of ongoing pregnancy rate. Shamma et al. investigated 28 infertile patients on IVF treatment outcome after diagnostic hysteroscopy (Shamma et al., 1992). The difference in clinical pregnancy rate between patients with and without abnormalities was found to be significant (37.5% versus 8.3%). Two randomized controlled trials reported exceptional improvements in pregnancy rates after hysteroscopy screening and instant treatment of detected pathology in patients after at least two failed IVF attempts. Intervention resulted in a 9% - 13% increase in clinical pregnancy rate after the subsequent IVF cycle (from 21.6% to 30.4% and from 26.2% to 39.6%, respectively) (Demirol and Gurgan, 2004; Rama Raju et al., 2006). Moreover, Rama Raju et al. (2006) described an 8% increase in live birth rate (from 16.6% to 25%). Oliveira et al. investigated a group of 55 patients with a normal HSG, but possibly abnormal TVS. After hysteroscopy screening and instant treatment of pathology, the clinical pregnancy rate and embryo implantation rate increased significantly. However, the ongoing pregnancy rate did not significantly differ (20% with abnormalities versus 10% without abnormalities) (Oliveira et al., 2003). Doldi et al. performed a prospective analysis of 300 patients with hysteroscopic examination and treatment of pathology prior to a first IVF treatment cycle. The pregnancy rate in this group was compared with the pregnancy rate in a retrospectively analyzed group without

hysteroscopy screening. In patients that did undergo a pre-IVF hysteroscopy, the pregnancy rate was 38% compared with 18% in patients without hysteroscopy (Doldi et al., 2005). Despite some relevant study weaknesses, these results do indicate a trend towards a beneficial effect of searching for, and treatment of, unsuspected intrauterine abnormalities through hysteroscopy on IVF outcome. However, high quality evidence on its value prior to a first IVF cycle still is lacking (Cohen et al., 2007).

Which diagnostic tests for evaluation of the uterine cavity should be part of the standard infertility evaluation in daily practice is still a point of debate. Usually, the basic work-up consists of at least TVS, with or without saline or gel infusion, possibly followed by either HSG or hysteroscopy. The accuracy of HSG in assessment of the uterine cavity integrity in infertile patients has been reported to be rather disappointing. The sensitivity and specificity are described to be 79% - 98% and 15% - 82%, respectively (Gaglione et al., 1996; Golan et al., 1996). TVS on the other hand, especially when performed during the late follicular phase, provides excellent imaging of the uterus and endometrial abnormalities. When hysteroscopy is considered to be the gold standard, the sensitivity, specificity, positive and negative predictive value for TVS in detecting cavity abnormalities were all reported to be between 81% and 100% (Ayida et al., 1997; Shalev et al., 2000). Out of the wide variety of possible intrauterine abnormalities, intrauterine adhesions may be the most difficult to diagnose at TVS. In the present study, the prevalence of intrauterine adhesions was 2%. The question arises whether this prevalence endorses the use of an office hysteroscopy as a routine examination prior to IVF/ICSI treatment. The use of contrast media such as saline with TVS is increasingly used to improve the delineation of uterine cavity abnormalities (Ayida et al., 1997). According to a review of the literature, SIS is a very accurate method for evaluation of the uterine cavity in pre- and post-menopausal women with abnormal uterine bleeding (Kroon et al., 2003). The pooled sensitivity and specificity in such patients were 95% and 88%, respectively. Moreover, the rate of correct predictions for the SIS in pre-menopausal women amounted to 95%. Also in infertility patients, SIS has shown to be rather promising. Performing operative hysteroscopy in patients with abnormalities at SIS resulted in an increase in pregnancy rate from 54% to 86% compared with patients with a normal uterine cavity (Gera et al., 2008). Recently, GIS has shown to be a promising alternative for the saline infusion with possibly improved patient comfort (Ziegler de, 2009). These new ultrasound methods might become an easy, safe and well-tolerated alternative to diagnostic hysteroscopy in the initial evaluation of the uterine cavity. Therefore, it would be of great importance to compare the accuracy of hysteroscopy to SIS and GIS as a screening tool to diagnose intracavitary abnormalities, especially in asymptomatic IVF patients.

Prior to implementation of a medical test into daily practice, next to its accuracy and usefulness, also the costs and tolerability of the test and all alternative options need to be reconsidered. Therefore, the following simple scenario analysis was performed. In a patient population with recurrent IVF failure, treatment resulted in a 9% - 13% increase in pregnancy rate after the subsequent IVF cycle (from 21.6% to 30.4% and from 26.2% to 39.6%) (Demirol and Gurgan, 2004; Rama Raju et al., 2006). Assuming that treatment of unsuspected intrauterine abnormalities would result in a 5% increase in pregnancy rate after a first IVF treatment cycle (from 20% to 25%), an abnormality prevalence of 11% implicates a number needed to screen by hysteroscopy of 184 in order to obtain one additional pregnancy. If screening is not implemented, a total of 28 extra IVF cycles would be needed after the first IVF attempt to obtain the same number of pregnancies after two attempts for a group of 1000 infertile patients, compared with a similar group of patients, who would undergo screening hysteroscopy and be treated for the detected pathology. It needs to be established whether the costs of 28 extra IVF cycles would compensate for the costs raised by routinely performing 1000 hysteroscopies. This scenario analyses would only be complete by implementation of alternative strategies, like performing a SIS prior to the hysteroscopy. SIS is a highly accurate and relatively inexpensive test for diagnosing intrauterine abnormalities and could therefore reduce the number of hysteroscopies needed to be performed and reduce the costs (Gera et al., 2008). Moreover, to assess the ultimate usefulness of hysteroscopy in infertile patients, the strategy in which hysteroscopy is performed after (repeated) IVF failure should also be evaluated. However, such scenario analysis would exceed the available data and aim of the present study. Nevertheless, cost-effectiveness analyses do not account for patients' tolerance of the procedures of the infertility workup. Making use of the vaginoscopic approach, hysteroscopy was found to be a patient-friendly technique by both Dutch as well as international investigators (Garbin et al., 2006; van Dongen et al., 2007). IVF itself is accompanied by a psychological and physical burden, which naturally increases with every failed IVF treatment cycle (Eugster and Vingerhoets, 1999). Therefore, investment in high quality evidence on the usefulness of hysteroscopy as a screening tool prior to IVF is essential. A thorough cost-effectiveness analysis should determine the strategy with the best profits on all items. Ultimately, a patient's preference study would elucidate the balance between the tolerability of office hysteroscopy and the burdens that accompany IVF/ICSI. Despite the low prevalence of intrauterine pathology detected in patients with a normal TVS and the imaginable accompanying high costs, hysteroscopy still cannot be ruled out as a routine procedure prior to IVF. Recently, renewed initiatives for a randomized trial have been published that may provide the

final clues (El-Toukhy et al., 2009). In conclusion, the observed prevalence of unsuspected intrauterine abnormalities in an asymptomatic infertile population is clearly lower than previously reported. This may have implications for the assessment of the significance of these abnormalities regarding prospects in IVF/ICSI treatment. Further research on the value of hysteroscopy as a routine procedure to evaluate the uterine cavity prior to IVF is urgently needed.



Chapter 3

Observer agreement in the evaluation of the uterine cavity by hysteroscopy prior to in vitro fertilization

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Abstract

Background Hysteroscopy is known as the most accurate test for diagnosing intrauterine pathology. To optimize fertility treatment, it is increasingly common to perform hysteroscopy as a routine procedure prior to in vitro fertilization (IVF). However, literature on the reproducibility of screening hysteroscopy is lacking. Therefore, the aim of the study was to assess the intra- and inter-observer agreement in the individual evaluation of the uterine cavity using video recordings of hysteroscopy procedures in asymptomatic patients prior to IVF.

Methods Screening hysteroscopies of 123 unselected, asymptomatic, infertile women with an indication for IVF or intracytoplasmic sperm injection (ICSI) treatment were recorded on DVD. After editing, the hysteroscopy performer and three other experienced gynaecologists independently assessed all recordings, focusing on the appearance of predefined intrauterine abnormalities (i.e. polyps, myomas, adhesions or septa). The intra- and inter-observer agreement was calculated and expressed as perfect agreement and kappa coefficient or intraclass correlation coefficient.

Results In total, 123 hysteroscopy procedures were recorded. After editing and selection, based on the record quality, 107 remained for assessment and analysis. The intraobserver agreement on the appearance of any of the predefined intrauterine abnormalities was substantial (kappa = 0.707), whereas the inter-observer agreement was moderate (kappa = 0.491). Perfect agreement occurred only in 77.6% of the cases.

Conclusions Interobserver agreement among experienced gynaecologists appeared to be rather disappointing. The latter may have implications for the diagnostic accuracy of screening hysteroscopy prior to IVF, as well as for its clinical significance in IVF programs.

Introduction

Evaluation of the uterine cavity is a basic step in the investigation of infertile women (Collins and Crosignani, 1992; Van Voorhis, 2008). Both the condition of the endometrium as well as the uterine cavity are thought to be important factors in determining receptivity for embryo implantation (Margalioth et al., 2006; Taylor and Gomel, 2008). It has been suggested that unsuspected intrauterine abnormalities may negatively affect the uterine environment and thereby the likelihood of achieving an ongoing pregnancy (Rogers et al., 1986). Hence, it is recommended to diagnose and treat these abnormalities, in order to optimize the uterine conditions and subsequent in vitro fertilization (IVF) success rates. Still, high quality evidence of a beneficial effect is lacking (Oliveira et al., 2003; Demirol and Gurgan, 2004; Doldi et al., 2005; Rama Raju et al., 2006).

Transvaginal ultrasonography (TVS) is the standard method applied to screen for possible endometrium or uterine cavity abnormalities in the work up of infertility patients. When indicated, this evaluation of the uterine cavity lining can be expanded with saline infusion/gel instillation sonography (SIS/GIS), hysterosalpingography (HSG) or hysteroscopy (Nederlandse Vereniging voor Obstetrie en Gynaecologie, clinical guideline, 2004). Hysteroscopy is known as the gold standard procedure for uterine cavity assessment. It enables diagnosis and treatment of intrauterine pathology in the same, outpatient setting. Hysteroscopy is quick, safe and well-tolerated (Bettocchi et al., 2004). Therefore, it has become an excellent tool for the diagnostic and therapeutic infertility work-up. It has been frequently advised to perform hysteroscopy as a routine procedure prior to IVF or intracytoplasmic sperm injection (ICSI) treatment (La Sala et al., 1998; Demirol and Gurgan, 2004; Hinckley and Milki, 2004; Doldi et al., 2005; Rama Raju et al., 2006).

Compared with histopathology or hysterectomy findings, hysteroscopy is regarded as very accurate for diagnosing intrauterine abnormalities (Widrich et al., 1996; Fabres et al., 1998; Ceci et al., 2002; Dueholm et al., 2002[b]; Karageyim Karsidag et al., 2010). Nevertheless, the reported prevalence of minor intrauterine abnormalities detected by hysteroscopy prior to IVF/ICSI differs considerably between studies applying a comparable set up (prevalence 11% - 40%) (La Sala et al., 1998; Demirol and Gurgan, 2004; Hinckley and Milki, 2004; Doldi et al., 2005; Rama Raju et al., 2006; Fatemi et al., 2010). This inconsistency may be related to the validity of the hysteroscopic examination in diagnosing abnormalities that have not been identified at TVS, where both accuracy and reproducibility play a role. To the best of our knowledge, so far only a single study has reported on the reproducibility of diagnosing intrauterine abnormalities through hysteroscopy by different gynaecologists. In patients who

underwent hysterectomy for symptomatic benign uterine diseases, two observers were compared in assessing the uterine cavity (Dueholm et al., 2002[a]). Interobserver agreement for the exclusion of uterine abnormalities by video-recorded hysteroscopies was found to be substantial (kappa = 0.63), yet only moderate for diagnosing polyps (kappa = 0.50).
So far, the reproducibility of hysteroscopy screening prior to IVF/ICSI has not been studied and the question can be posed, whether the variation in the observed prevalence of intrauterine abnormalities in asymptomatic infertile patients is caused by observer bias. The aim of the current study was to clarify the reproducibility of screening office hysteroscopy. For that reason, the intra- and inter-observer agreement for diagnosing intrauterine abnormalities was calculated, making use of video recordings of hysteroscopy procedures performed in asymptomatic IVF patients.

Materials and Methods

Hysteroscopy recordings were obtained in the context of a trial on the 'Treatment Efficacy of unsuspected uterine Abnormalities' on subsequent IVF treatment (TEA-trial, register number: NCT00830401) (Fatemi et al., 2010). For this purpose, 678 office hysteroscopies were performed in a group of asymptomatic infertile patients, indicated for IVF/ICSI treatment at the University Medical Center Utrecht (UMCU) and the Academic Hospital at the Dutch-speaking, Free University of Brussels (AZVUB). The study was approved by the Institutional Review Board of the two participating centers and written informed consent was obtained.

Hysteroscopy recordings
Hysteroscopy procedures were scheduled in the early-mid follicular phase of a cycle (day 3–15), 1–3 months before starting the IVF/ICSI treatment. From February to October 2008, all hysteroscopy examinations, performed under the supervision of one gynaecologist at the UMCU, were recorded on DVD. These office hysteroscopies were carried out in a standardized manner, using a 5-mm outer-diameter continuous flow Bettocchi hysteroscope with 30° direction of view (Karl Storz Endoscopy, Stöpler medical instruments, Utrecht, The Netherlands). Normal sterile, isotonic saline solution was used for distension of the uterine cavity. Hysteroscopy recordings were deleted if patient identification was missing, or only a part of the standard procedure was captured. The remaining recordings were edited in such a way that every recording started at the entrance into the uterine cavity and ended just before leaving the outer ostium of the cervix. This resulted in a total of 118 recordings, which -on aver-

age- lasted 5 min. The recordings were written on a total number of 13 DVDs, each with a total recording time between 22 and 54 min. All recordings were made anonymous by replacing the patient identification with a serial number.

Observers

The DVDs were distributed among four gynaecologists; all senior investigators in four different hospitals. Their years of clinical experience in endoscopy ranged from 5 to 19 years, with an average of 13. The observers independently evaluated all 13 DVDs. One of the four observers was the gynaecologist who also performed the real-time hysteroscopy. The time period between the real-time hysteroscopy and the evaluation of the DVD recordings was at least 2 months. During the evaluation of the recorded hysteroscopies, all four observers were informed about the study design, though blinded for the medical history of the patients.

Evaluation

Primary evaluation of the DVD recordings as well as the real-time hysteroscopy was conducted using a scoring form. This standardized form contained questions about the quality of the recording and the appearance of the uterine cavity. The uterine cavity was assessed on its shape (normal, arcuate or septate) and the presence or absence of predefined abnormalities (endometrial polyps, myomas, adhesions and septa). Records that were scored to be of too poor quality by one of the observers were not further used for statistical analysis.
Five months after the initial evaluation, a meeting between the four observers was organized. The purpose of this meeting was to clarify the cause of possible differences in their judgments and to assess the impact of discussion on observer agreement. Therefore, the records with the highest observer variance were selected. During the meeting, each of the selected recordings was assessed twice: once before and once after discussion on the presence and relevance of observed abnormalities. A scoring form was used, similar to the form administered during the initial evaluation, added with questions about the reason for altering opinions, if applicable.

Statistical analysis

For assessment of the intraobserver agreement, the findings of the performing gynaecologist at real-time hysteroscopy were compared with his assessment of the hysteroscopy recordings. Interobserver agreement was calculated, making use of the assessment of solely the hysteroscopy recordings by the three other gynaecologists. Intra- and inter-observer agreement was expressed in a kappa coefficient. Kappa is a measure of agreement above or below what is expected to be the agreement by chance [κ = (observed agreement - agreement

Table 1 Patient characteristics of the IVF/ICSI cases, in whom screening hysteroscopy was recorded and statistically analyzed

Variables	(N=107)	
Age	34.16	± 4.55
Duration of subfertility (years)[a]	3.06	± 2.40
CD[b]	7.05	±0.17
BMI	23.24	± 3.47
VCM	59.24	± 74.17
Cause infertility		
- Idiopathic	41	(38.3%)
- Andrologic factor[c]	45	(42.1%)
- Subfertile female[d]	21	(19.6%)
Infertility woman		
- Primary	55	(51.4%)
- Secondary	52	(48.6%)
Race		
- Caucasian	88	(82.2%)
- African	1	(0.9%)
- Asian	9	(8.4%)
- Mediterranean	7	(6.5%)
- Mix	2	(1.9%)

Note Values are expressed as mean ± standard deviation

[a]: Duration of subfertility: duration of attempts to become pregnant, in cases of secondary infertility calculated from the last ongoing pregnancy,[b]: Day of menstrual cycle on which the hysteroscopy was performed,[c]: Defined as VCM (sperm volume (mL) * concentration sperm cells (*10^9/mL) * grade A and B sperm cell motility(%)) < $20 \cdot 10^6$, [d]: Due to tubal pathology (incl. endometriosis grade III and IV), anovulation or cervix factor

by chance)/(1 - agreement by chance)]. A kappa-value of <0.20 represents slight agreement, a value between 0.21 and 0.40 fair agreement, a value between 0.41 and 0.60 moderate agreement, a value between 0.61 and 0.80 substantial agreement and a value of 0.81 - 1.00 indicates almost perfect agreement (Landis and Koch, 1977). The equivalent of the overall weighted kappa, the intraclass correlation coefficient (ICC) was applied to calculate the mean kappa-values (Fleiss and Cohen, 1973).

To ensure that observer agreement would not be influenced by the quality of a record, a linear mixed model was used. The estimated variance of the recordings of the highest quality was compared with the estimated variance of the recordings of the lowest quality, making use of the z-test. A higher estimated variance was associated with a higher ICC and therefore higher observer agreement. A P-value of <0.05 was considered statistically significant. The same method was used to analyze whether observer agreement was affected by the day of the menstrual cycle (CD) on which the hysteroscopy was performed. Furthermore, linear mixed models were also used to assess whether the observer agreement, expressed as ICC, would significantly differ between the primary and secondary evaluations, before and after discussion at the expert evaluation meeting. For that reason, comparisons were made between linear mixed models with and without evaluation occasion specified as random effects parameter, using a likelihood ratio test. All statistical analyses were performed in SPSS version 15.1 (SPSS Inc., Chicago, IL, USA).

Table 2 Findings of the hysteroscopy performer at real time hysteroscopy

Findings	Prevalence	%
Normal cavity	94	87.9%
Abnormal cavity	13[a]	12.1%
- Polyp	12	11.2%
- Myoma	1	0.9%
- Adhesion	0	0.0%
- Septum	2	1.9%
Total	107[a]	100%

[a]: In two cases more than one abnormality was detected

Results

In total, 678 asymptomatic, infertile women were included in the TEA-trial and scheduled for office hysteroscopy. From February to October 2008, 123 hysteroscopy examinations were performed by one gynaecologist at the UMCU, and therefore recorded. No significant differences were found between the groups with and without hysteroscopy recording, regarding female age, duration of infertility, BMI, total motile sperm count, fertility cause, primary/

secondary infertility or race. After editing, 118 hysteroscopy recordings remained. Of those, 11 were scored to be of poor quality, resulting in a total of 107 hysteroscopy recordings that was used for the observer agreement analysis (Table 1).

Primary evaluation

The intra- and inter-observer agreement was calculated for evaluation of the uterine cavity to be with or without abnormalities (abnormal versus normal) and for the presence or absence of each of the predefined abnormalities (endometrial polyps, leiomyomas, adhesions and septa) separately.

At real-time hysteroscopy, 12% of the patients was diagnosed to have one or more predefined intrauterine abnormalities (Table 2). Most frequently it concerned endometrial polyps (11%). Also, in one case, a septate uterus was detected. In two cases, more than one abnormality was observed: a septate uterus in combination with a polyp, and a submucous leiomyoma in combination with a polyp. In the recording sample, the prevalence of abnormalities was identical, compared with the overall sample of 678 cases.

The intraobserver agreement of the one hysteroscopy performer for the assessment of a normal versus abnormal uterine cavity was substantial (Table 3). The kappa-value was 0.71 and perfect agreement was found in 93.5% of the cases. For diagnosing each of the predefined abnormalities separately (polyps, leiomyoma, adhesions and septa), kappa-values were 0.68, 0.66, -0.01 and 1.00, respectively (Table 3). These findings indicate a substantial agreement for polyps, as only this abnormality had a sufficient prevalence to have a meaningful kappa-value calculated.

The interobserver agreement between three gynaecologists for the assessment of the cavity to be normal or abnormal was found to be moderate, with an ICC (as equivalent of the overall kappa-value) of 0.49 (Table 3). Perfect agreement on this issue occurred in 82.2% between Obs2 and Obs3, in 75.7% between observer Obs3 and Obs4, and in 74.8% between Obs4 and Obs2 (Table 4).

The reproducibility of the detection of each of the predefined abnormalities separately was best for diagnosing polyps and septa. The ICCs (as equivalent of the mean kappa) were 0.51 [95% confidence interval (CI): 0.40 - 0.62] and 0.48 (95% CI: 0.36 - 0.58), respectively (Table 3). Interobserver agreement for diagnosing leiomyoma, expressed in an ICC, was 0.28 (95% CI: 0.12 - 0.40). The poorest agreement was found for the detection of adhesions (ICC: 0.02, 95% CI: -0.12 - 0.10). Due to the low prevalence of leiomyoma, adhesions and septa, these results mainly designate a moderate agreement for the detection of polyps.

For evaluation of the uterine cavity to be normal or abnormal and for diagnosing polyps, the observer agreement, based on the recordings of optimal

Table 3 Level of overall perfect observer agreement expressed in kappa coefficients/ICC[a]

Finding	*Intra-observer* agreement	Kappa	(95% CI)
Normal cavity	93.5%	0.707	(0.517 - 0.897)
Polyp	93.5%	0.683	(0.463 - 0.903)
Myoma[b]	99.1%	0.662	(0.043 - 1.281)
Adhesions[b]	99.1%	-0.009	(-0.198 – 0.180)
Septum[b]	100%	1.000	[c]
Finding	*Inter-observer* agreement	ICC[a]	(95% CI)
Normal cavity	77.6%	0.491	(0.378 - 0.598)
Polyp	83.2%	0.511	(0.399 - 0.616)
Myoma[b]	95.6%	0.281	(0.161 - 0.406)
Adhesions[b]	96.3%	-0.018	(-0.116 - 0.100)
Septum[b]	93.8%	0.475	(0.360 - 0.584)

[a]: ICC: intraclass correlation coefficient (equivalent of the overall weighted kappa) (Fleiss and Cohen, 1973), [b]: The discrepancy between the perfect agreement and mean kappa value is caused by the low prevalence of these abnormalities (Feinstein and Cicchetti, 1990), [c]: Impossible to compute with ordinary statistics, as also used by SPSS version 15.1

quality did not (significantly) improve the ICC (P-value: 0.9 and 0.8, respectively). Hence, the observer agreement did not seem to be influenced by the quality of the hysteroscopy recordings. Due to the low prevalence of leiomyoma, adhesions and septa, ordinary statistics could only execute this analysis for the other two variables.

All recorded hysteroscopy procedures were performed between day 4 and 11 of the menstrual cycle, most on CD 7. To analyze the effect of CD on observer agreement, the recordings of the hysteroscopies performed on CD ≤6 were compared with those performed on CD >6. Again, this could solely be computed for the appearance of all abnormalities together and for diagnosing polyps. For both findings, the ICC did not significantly differ between recordings performed up till or after CD 6 (P-value: 0.9 and 1.0, respectively).

Table 4 Level of inter observer agreement and corresponding kappa values for the assessment of different features of the uterine cavity

Diagnosing the cavity to be normal or abnormal

		Obs3	
		Yes	No
Obs2	Yes	68	3
	No	16	20
Agreement		*82.2%*	
Kappa		*0.563*	
95% CI		*(0.394-0.732)*	

		Obs4	
		Yes	No
Obs3	Yes	63	21
	No	5	18
Agreement		*75.7%*	
Kappa		*0.425*	
95% CI		*(0.249-0.601)*	

		Obs2	
		Yes	No
Obs4	Yes	56	12
	No	15	24
Agreement		*74.8%*	
Kappa		*0.446*	
95% CI		*(0.270-0.622)*	

Detecting polyps

		Obs3	
		Yes	No
Obs2	Yes	13	5
	No	5	84
Agreement		*90.7%*	
Kappa		*0.666*	
95% CI		*(0.473-0.858)*	

		Obs4	
		Yes	No
Obs3	Yes	13	5
	No	18	71
Agreement		*78.5%*	
Kappa		*0.404*	
95% CI		*(0.210-0.598)*	

		Obs2	
		Yes	No
Obs4	Yes	14	17
	No	4	72
Agreement		*80.4%*	
Kappa		*0.456*	
95% CI		*(0.266-0.646)*	

Detecting septa

		Obs3	
		Yes	No
Obs2	Yes	3	10
	No	0	94
Agreement		*90.7%*	
Kappa		*0.345*	
95% CI		*(0.053-0.637)*	

		Obs4	
		Yes	No
Obs3	Yes	3	0
	No	0	104
Agreement		*100%*	
Kappa		*1.000*	
95% CI		*(1.000-1.000)*	

		Obs2	
		Yes	No
Obs4	Yes	3	0
	No	10	94
Agreement		*90.7%*	
Kappa		*0.345*	
95% CI		*(0.053-0.637)*	

Secondary evaluation

The 16 recordings with the highest observer disagreement were selected for re-evaluation. As expected, the revision of the recordings resulted in low kappa-values for observer agreement before interaction between the four observers (Figure 1). As a result of the subsequent discussion, the initial judgment of an observer changed in 33% of all cases. In 95% of cases, the observer declared to be convinced by the arguments or instructions of the other observers as the reason for altering opinions. Only once an observer changed because he had not noticed the particular abnormality during the primary evaluation. Consequently, significantly more consensuses were obtained in the evaluation of the cavity to be normal or abnormal and the presence or absence of polyps and adhesions. The ICC increased from 0.074, 0.296 and 0.356 to 0.399, 0.643 and 0.580, respectively (Figure 1). The observer agreement in diagnosing myomas

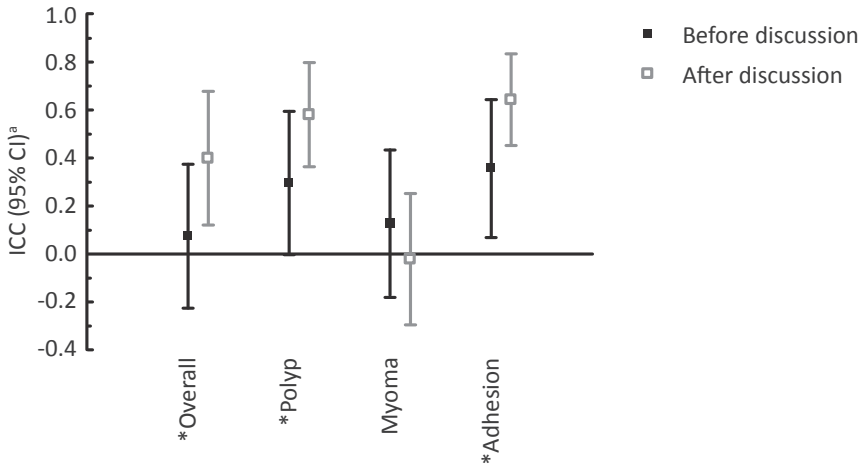
was not affected by the dialog between the observers. The effect on detecting septa could not be analyzed by ordinary statistics, due to its low prevalence.

Discussion

The present prospective study demonstrates that the reproducibility of office hysteroscopy for diagnosing unsuspected intrauterine abnormalities in an asymptomatic infertile patient population is not satisfying. By making use of video-recorded hysteroscopies, the intraobserver agreement of the one hysteroscopy performer in the evaluation of the uterine cavity was found to be substantial. However, the interobserver agreement among three gynaecologists, experienced with performing hysteroscopy, was shown to be only moderate. Subsequent discussion -after revision of the recordings- regarding the classification and significance of the observed findings generally improved the observer agreement.

Observer variability is usually expressed in a kappa coefficient. The advantage of kappa statistics is the 'adjustment' for the agreement expected to occur by chance, yet the disadvantage is that kappa is influenced by prevalence (Feinstein and Cicchetti, 1990). This explains the discrepancy between the low kappa-values and the percentage of perfect agreement for abnormalities with a low prevalence (leiomyoma, adhesions and septa). Therefore, interpretation of the results of the present study should and will be limited to the intra- and inter-observer agreement on the evaluation of the uterine cavity to be normal or abnormal and the detection of polyps. With that, one should also realize that the interpretation of kappa-values is just based on the opinion of researchers and that objective criteria for such classification are absent (Landis and Koch, 1977). Another study limitation is that the assessment of video recordings is subject to the skills of the primary hysteroscopy performer. However, the quality of the recordings seemed not to have influenced the level of agreement among the observers. Also, the number of observers could be considered as a disadvantage of the current study. This number was chosen because it has been shown that the use of more than three observers (or replicates) will hardly further improve the reliability of agreement studies using the kappa-value (Giraudeau and Mary, 2001). The blinding of the observers for the patient's additional medical history could also be interpreted as a disadvantage. Different aspects, for example age, could change the a priori chance of intrauterine abnormalities and could therefore influence the recognition of an abnormality by a physician. Although the period between the real-time hysteroscopy and evaluation of the recording was at least 2 months, the hysteroscopy performer could have remembered some of the patient characteris-

Figure 1 Level of interobserver agreement expressed as ICC[a] before and after discussion between observers



Note Impossible to compute ICC for diagnosing septa with ordinary statistics, as also used by SPSS version 15.1

[a]: ICC: intraclass correlation coefficient (equivalent of the overall weighted kappa) (Fleiss and Cohen, 1973), *: For diagnosing the uterine cavity to be normal or abnormal, diagnosing polyps and adhesions, the interobserver agreement significantly increased through discussion ($p<0.01$)

tics. To prevent the interobserver agreement being biased by his knowledge, the scorings of the hysteroscopy performer were not used for the interobserver analysis. However, since the chance of remembering patient information while watching a hysteroscopy recording with an interval of 2 months is slight and due to its importance, the intra-observer analysis was also performed. The chosen study design is the only possible way to investigate the impact of observer bias on routine hysteroscopy prior to IVF/ICSI. Nevertheless, the small sample size and other study limitations of the current study should be considered whilst interpreting the results.

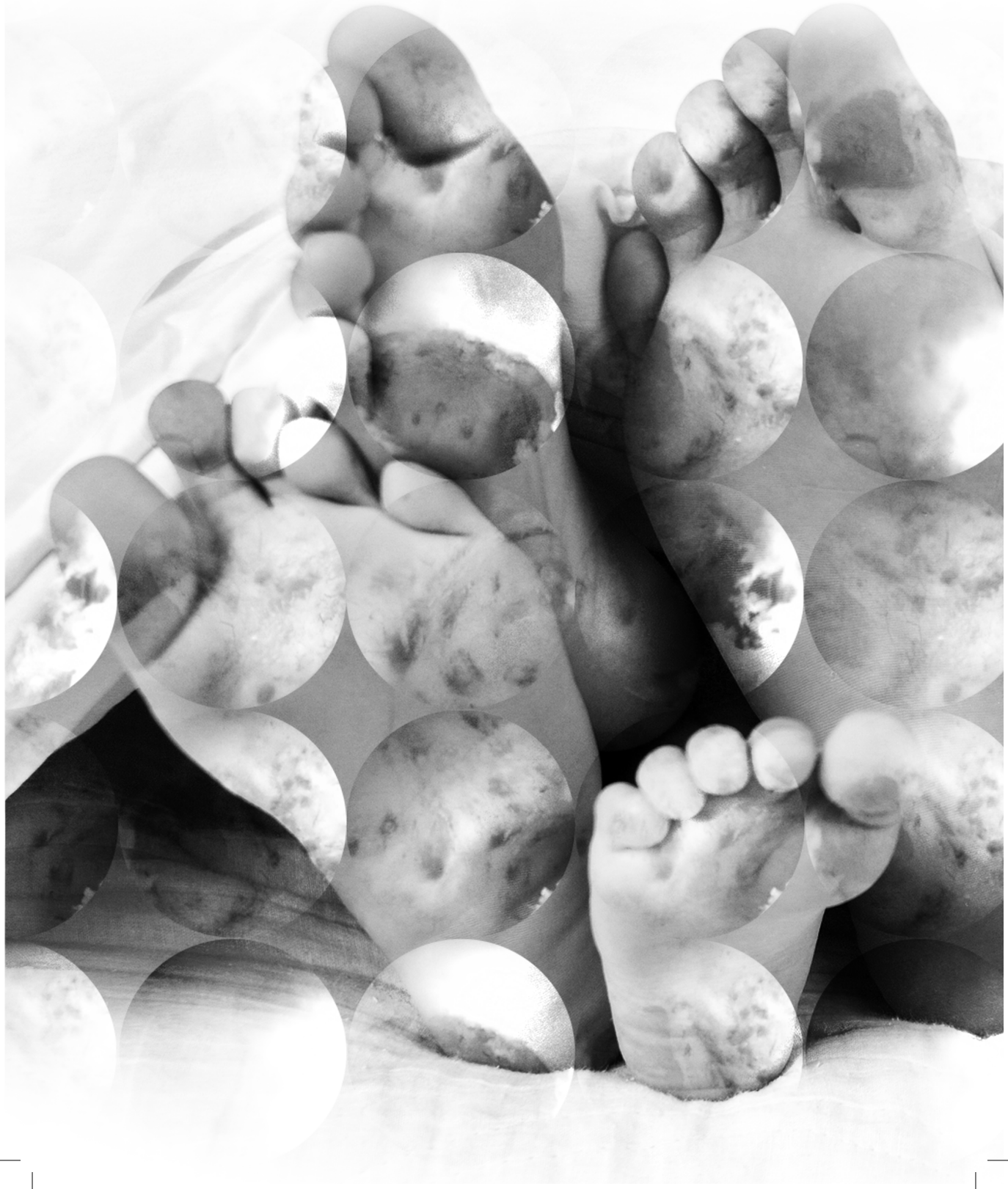
The current study showed that, in asymptomatic patients, the interobserver agreement on the evaluation of the uterine cavity for abnormalities in general, or polyps, in particular, was only moderate (kappa-values: 0.49 and 0.51, respectively). In contrast to the current study, all reproducibility studies on uterine cavity evaluation so far investigated populations of symptomatic patients, or did not clearly describe the patient characteristics. For the interpretation of video recordings of TVS in women with abnormal uterine

bleeding, the interobserver agreement was found to be substantial (mean kappa-value: 0.75) (Emanuel et al., 1996). In another symptomatic patient population, the kappa-values expressing the intra- and inter-observer agreement for evaluating the uterine cavity by recorded SISs were calculated to be 0.66 and 0.48, respectively (Beemsterboer et al., 2008). For HSGs performed in infertile women, the observer variation for the assessment of the uterine status was disappointing, with a kappa-value of 0.35 (Glatstein et al., 1997). Although some studies have shown that these imaging techniques are nearly as accurate as hysteroscopy in the assessment of the uterine cavity integrity in infertility, one would expect a higher observer reproducibility for a diagnostic test that is known as a gold standard procedure (Narayan and Goswamy, 1993; Ayida et al., 1997; Fabres et al., 1998; Shalev et al., 2000). This hypothesis could be confirmed for the three-dimensional ultrasound, which is postulated as a new gold standard (Puscheck and Cohen, 2008). The reproducibility of this diagnostic test for diagnosing congenital malformations of the uterus, in women with an abnormal TVS, was found to be almost perfect, with a kappa coefficient of 0.97 (Salim et al., 2003). Still, the one study that also investigated the reproducibility of hysteroscopy has demonstrated results comparable to the findings of the current study. In women, who underwent hysterectomy for benign diseases, video recorded pre-surgery hysteroscopy was evaluated by two separate investigators (Dueholm et al., 2002[b]). The kappa-values expressing the agreement for assessment of the uterine cavity to be normal/abnormal and for diagnosing polyps in this study were 0.63 and 0.50, respectively. A low reproducibility, as observed in the present study on hysteroscopy screening in infertile women with normal ultrasound, does negatively affect the validity of this diagnostic test. The results of the observer meeting implicate that the agreement between observers on hysteroscopy findings generally improved by discussion, yet always remained less than perfect. Polyps and septa appeared to be the most frequently discussed abnormalities. One observer detected polyps more often compared with the other observers, whereas another observer scored relatively more septa (Table 4). Concerning polyps, the difference between a well-circumscribed polyp and irregular, bumpy endometrium was found to be unclear. Moreover, the difference between a septate uterus, which usually requires treatment, and an arcuate uterus, which is known as a variation of a normal uterine cavity, was repeatedly being discussed. Thorough debate between four experts could not resolve this observer disagreement, even for those alleged to be obvious, like septa.

Persistent observer disagreement may affect the number of unsuspected uterine cavity abnormalities detected at office hysteroscopy. Consequently, observer bias could be a plausible explanation for the difference in abnormality prevalence in IVF indicated patients, reported by studies with a comparable design.

Since consensuses on the definition of abnormal hysteroscopy findings between experts are currently lacking, the relevance of these abnormalities regarding the chances for success in the subsequent IVF may be questioned. Screening hysteroscopy for detecting intrauterine abnormalities in an asymptomatic infertile patient population may be less useful than currently assumed. Clearly defined standards for which hysteroscopy findings are seen as an abnormality, and possibly require intervention, are absent. Improvement of the observer agreement on the presence or absence of minor intrauterine abnormalities probably will be accomplished by developing these standards and implementing education. Therefore, one of the main priorities in future research should become the development of a guideline with exact definitions of what should be judged as an intrauterine abnormality. Hysteroscopy-guided biopsies of polypous-like structures might clarify the definition of a polyp. Moreover, hysteroscopy features of a septate uterus might be compared with a HSG or three-dimensional ultrasound image to make its hysteroscopic diagnosis clear. Through such research, the exact difference between intrauterine pathology and physiological variations of a normal uterine cavity should become universal.

In conclusion, the interobserver agreement on assessment of the uterine cavity for abnormalities diagnosed by video-recorded hysteroscopies in asymptomatic infertile women was found to be moderate. This unsatisfying result might be an explanation for the variation in prevalence of subtle intrauterine pathology in existing literature on infertility patients. Moreover, it may have implications for the diagnostic validity of hysteroscopy and therefore the clinical significance of screening hysteroscopy in asymptomatic patients prior to IVF/ICSI.



Chapter 4

The impact of chronic endometritis on reproductive outcome

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Submitted

Abstract

Background Chronic endometritis is known as a subtle condition, which is hard to diagnose. Moreover, the influence of endometritis on fertility has not been fully assessed. The aim of this study was to assess the prevalence of chronic endometritis and the impact on the reproductive outcome of asymptomatic patients indicated for in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatment.

Methods In the context of a randomized controlled trial, a group of 678 patients, unsuspected of intrauterine pathology, underwent hysteroscopy guided endometrial biopsy prior to IVF/ICSI. The endometrial samples obtained, were histological examined by two pathologists for the diagnosis chronic endometritis. The live birth rate (including spontaneous pregnancies) of patients diagnosed with chronic endometritis within 3 years after the start of the trial was compared to the live birth rate of a randomly selected matched control group of patients without endometritis. Also, the cumulative live birth rate per embryo transfer was assessed.

Results The prevalence of chronic endometritis of the 606 patients with an adequate biopsy was 2.8%. The live birth rate did not significantly differ between patients with or without endometritis, 76% versus 54% (P-value: 0.11). Also, the cumulative live birth rate per embryo transfer was not significantly different (Hazard Ratio 1.456, 95% CI: 0.770 - 2.750, P-value: 0.2).

Conclusions Chronic endometritis is rarely diagnosed in a population of asymptomatic, infertile patients with a normal TVS, prior to a first IVF/ICSI treatment. Moreover, the reproductive outcome was not found to be negatively affected by chronic endometritis. Therefore, the clinical implication of chronic endometritis in infertility seems minimal.

Introduction

The implantation rate per embryo transfer in IVF (in vitro fertilization) generally does not exceed 30% per cycle (Andersen et al., 2008). Factors that affect chances of embryo implantation are either related to the embryo itself or to the receptivity of the endometrium. Endometrial polyps, submucous myomas, adhesions and septa all are believed to have a negative impact on the uterine environment and therefore interfere with fertility (Taylor and Gomel, 2008; Bosteels et al., 2010). Also less distinct endometrial pathology (such as altered hormonal status or inflammation) are thought to cause impaired endometrial receptivity resulting in infertility (Romero et al., 2004).

Chronic endometritis is a subtle condition and therefore difficult to diagnose. Clinically, the condition is rarely suspected, as chronic endometritis is usually asymptomatic. Also at hysteroscopy with direct visibility of the endometrial surface, the diagnosis often remains doubtful or unnoticed (Polisseni et al., 2003). A hysteroscopy guided endometrial biopsy is assumed to be the method of choice to ensure the integrity of the uterine cavity prior to IVF (Eede van den, 1995). Although infiltration of the endometrium by lymfocytes and eosinophiles is associated with chronic endometritis, the diagnosis is ultimately based on the presence of plasma cells in the endometrial stroma (Greenwood and Moran, 1981; Dechaud et al., 1998; Matteo et al., 2009; Adeboyega et al., 2010,). The search for plasma cells may be hampered by many factors such as inadequate staining, preservation of the endometrial tissue or mimicking of plasma cells by plasmacytoid stroma cells (Greenwood and Moran, 1981; Crum et al., 1983; Adeboyega et al., 2010). Therefore, also the histological diagnosis is difficult to make.

The significance of chronic endometritis in infertility has not been fully assessed yet. First of all, the prevalence reported in previous published articles varies greatly. Depending on the patient population and biopsy method, the prevalence of chronic endometritis has been reported to be between 0.2% - 46% (Wild et al., 1986; Feghali et al., 2003; Polisseni et al., 2003; Cicinelli et al., 2005; Johnston-Macananny et al., 2009). Moreover, literature regarding the possible impact of chronic endometritis on fertility is scarce (Fatemi et al., 2009). To the best of our knowledge, the only available evidence concerns a prospective study among patients with symptomatic endometritis and a retrospective chart review among patients with recurrent IVF failure (Haggerty, 2003; Johnston-Macananny et al., 2009). Both studies failed to detect a clear association between chronic endometritis and reproductive outcome.

Therefore, the aim of this study was to identify the prevalence of chronic endometritis in a population of asymptomatic patients with normal findings at transvaginal ultrasound (TVS), prior to a first IVF or intracytoplasmic sperm in-

jection (ICSI) cycle, based on the histological diagnosis of a hysteroscopy guided biopsy. In addition, the impact of chronic endometritis on fertility was assessed by comparing the reproductive outcome between the group of patients with and without chronic endometritis.

Materials and Methods

The data of the current study were collected in the context of the TEA-trial (Treatment Efficacy of uterine Abnormalities, register number ClinicalTrial.gov: NCT00830401) (Fatemi et al., 2010). The aim of this randomized controlled trial (RCT) was to clarify whether diagnosis and treatment of unsuspected intrauterine abnormalities by office hysteroscopy would affect the reproductive outcome of the subsequent IVF/ICSI treatment cycles. The Institutional Review Board of the two participating centers approved this study. Informed consent was obtained.

Participants

The investigated population consisted of infertile patients, who visited the Academic Hospital at the Dutch-speaking Brussels Free University (AZVUB) and the University Medical Center Utrecht (UMCU). From June 2007 until September 2008, subsequent, asymptomatic patients <43 year were allocated for the TEA-trial and scheduled for office hysteroscopy prior to a first IVF/ICSI treatment. Inclusion was limited to patients without abnormalities at transvaginal ultrasonography and no prior hysteroscopy examination.

Hysteroscopy & endometrial biopsy

The hysteroscopies were scheduled in the follicular phase of the menstrual cycle (day 3-15). On the day of the examination, prior to the hysteroscopy, a single dose of antibiotic prophylaxis was prescribed at the UMCU; 625 mg of amoxicillin/clavulanate potassium p.o. (Augmentin®, GlaxoSmithKline b.v., Zeist, The Netherlands) and 200 mg of doxycyclin p.o. (Vibramycin®, Pfizer b.v., Rotterdam, The Netherlands). The hysteroscopies were performed on an outpatient basis, making use of a 5-mm outer-diameter continuous flow Bettocchi hysteroscope (Stöpler Medical Instruments, Utrecht, The Netherlands & Olympus Belgium N.V., Aartselaar, Belgium). A vaginoscopic approach was used, generally without cervical dilatation or anesthesia. The endocervical canal, uterine cavity, tubal orifices and endometrium were inspected methodically and the findings recorded. In case predefined abnormalities were detected (i.e. endometrial polyps, myoma, adhesions or septa), randomization for treatment versus no treatment was performed. Despite previous permission, some patients with-

drew from randomization and applied for definite treatment. During the procedure, an endometrial biopsy was obtained from the uterine fundus with the use of grasping forceps (UMCU) (Karl Storz Endoscopie, Nieuwegein, The Netherlands) or a Pipelle de Cornier under local anesthesia (AZVUB) (Laboratoire CCD, Paris, France).

Histology examination

The endometrial biopsies were placed in a fixative of 4% phosphate buffered formaldehyde and processed routinely. At each research hospital, one pathologist specialized in endometrial pathology, examined the endometrial samples, initially making use of solely hematoxylin-eosin-stained (HE) tissue specimens. Additional staining by immunohistochemical markers CD20, CD79a and/or CD138 only was provided in case the diagnosis was doubtful. For plasma cells the CD138, the Clone B-B4, batch 605, was used applying dilution 1:1000, an antibody from Serotec. For plasma cells and B-lymphocytes the CD79a, the DAKO, Clone JCB117, batch 2791 (dilution 1:200) antibody was used. For B-lymphocytes CD20, the DAKO, Clone L26, batch 083 (dilution 1:400) antibody was used. For all antibodies, antigen retrieval in citrate buffer was applied and staining was done with the Bond-Max autostainer (Leica).

The histological examination consisted of classification based on the Kurman criteria and the presence or absence of inflammatory cells, such as plasmacells, lymfocytes, neutrofils etc. (Mazur and Kurman, 2005). Finally, the examining pathologists of each research hospital reported the corresponding diagnosis: no inflammation, possibly endometritis, evident (sub)acute endometritis or evident chronic endometritis.

Fertility treatment

Besides the antibiotic prophylaxis, at the UMCU no antibiotic treatment was administered. At the AZVUB the gynaecologist decided whether antibiotic treatment was indicated in patients diagnosed with endometritis. Treatment consisted of Ofloxacin 400mg/day p.o., during five days for the patient and her partner. One to three months after the hysteroscopy and biopsy, standard IVF/ICSI treatment was started. Rec-FSH or HMG, long (day 21) agonist protocol (Leuprolide/Triptorelin 100µg) or day 6 GnRH antagonist protocol (Ganirelix/Cetrorelix 0.25mg/d) was used. Final oocyte maturation was obtained by administration of 10.000 IU of HCG (Pregnyl®) as soon as ≥3 follicles of >16 mm were present. Oocyte retrieval was carried out 36 hours after HCG administration. A maximum of two good quality embryos were transferred, generally 3-4 days after oocyte retrieval. Luteal phase supplementation consisting of 600mg natural micronized progesterone in three separate doses (Utrogestan®/Progestan® 100mg 3x2 per day) was started one day after oocyte retrieval

and continued until 7 weeks gestation if pregnancy is achieved. Unused, good quality embryo's could be cryopreserved and transferred in the next treatment cycle. The ongoing pregnancies resulting in a live birth after treatment cycles with fresh or cryopreserved embryos performed within 3 years after the start of the TEA-trial were used for analysis. Also, the spontaneous pregnancies resulting in a live birth were taken into account.

Statistical analysis

The Chi-square test, Fisher's exact test, Student's t-test and multinomial logistic regression were used to analyze the differences in patients' characteristics of the group with and without endometritis. To analyze the impact of the histological diagnoses on the chance to conceive, the group of patients with chronic endometritis (case group) was compared to a control group. For each case, 4 controls out of the patients without endometritis were randomly selected, matched for the research hospital and day of menstrual cycle on which the biopsy was performed. Survival analysis (Kaplan Meier) was performed to evaluate the difference in pregnancy rate between the cases and controls over time, expressed as number of embryo transfers. Cox regression was used to analyze the size of the effect. A value of <0.05 was considered statistically significant. All statistical analyses were performed in SPSS version 15.1.

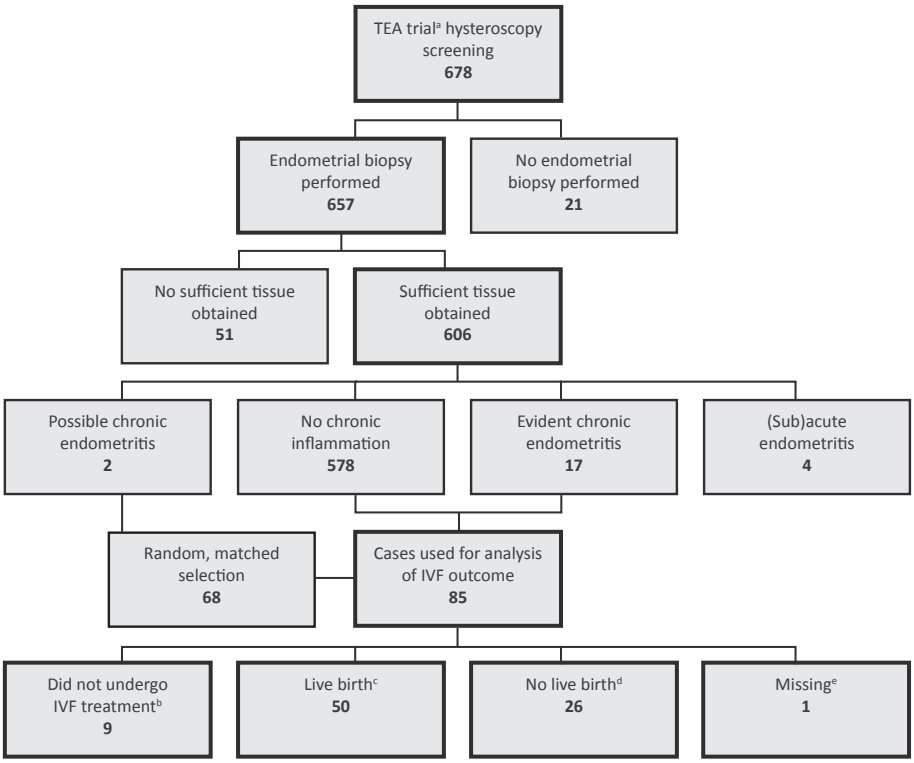
Results

From June 2007 until September 2008, a total of 678 patients underwent office hysteroscopy. Adequate histological examination could be performed on the endometrial samples of 606 patients. Causes for inadequate histological examination are shown in Figure 1. No significant clinical differences were found between the group with or without histological examination.

Histology findings

Based on solely the hematoxylin-eosin-stained endometrial samples evident chronic endometritis was diagnosed in 15 patients and focal (sub)acute endometritis in 4 patients. Additional staining with a CD marker confirmed the diagnosis chronic endometritis in 3 patients. Moreover, it revealed chronic endometritis in 2 additional patients. The diagnosis remained doubtful in two cases. Overall, in the group of 606 patients with adequate histopathological examination, the observed prevalence of possibly endometritis, evident (sub)acute endometritis or evident chronic endometritis was 0.3%, 0.7% and 2.8%, respectively (Figure 1).

Figure 1 Flowchart illustrating the selection of cases used for the analysis of the IVF treatment outcome. Cases diagnosed with chronic endometritis were replenished by a sample of randomly selected patients not diagnosed with chronic endometritis. Matching was performed for research center and day of menstrual cycle on which the endometrial biopsy was obtained.



[a]: The trial "Treatment Efficacy of unsuspected uterine Abnormalities" on subsequent in vitro fertilization (IVF) or intra cytoplasmic sperm injection (ICSI) treatment (register number: NCT00830401) [b]: Most patients did not start IVF/ICSI treatment due to personal reasons. One patient did not start because of spontaneous pregnancy resulting in a live birth. [c]: Including 3 patients who had a spontaneous pregnancy resulting in a live birth (of whom one did not undergo IVF/ICSI treatment) and 4 patients, who had an early miscarriage before the live birth [d]: Including 11 patients who had an early miscarriage and did not become pregnant afterwards and one patient, who had a preterm delivery at 22 weeks gestation [e]: The final outcome after IVF/ICSI remained unknown. After a sonography at 8 weeks gestation -which showed an intact intrauterine twin pregnancy- no record was kept

Table 1 Patient characteristics of the IVF/ICSI cases that underwent hysteroscopy guided endometrial biopsy which was histological examined. The data are listed according to the presence or absence of chronic endometritis.

Variables	Group with chronic endometritis N = 17	Group without chronic endometritis N = 589	Significance *Univariate analysis* p-value	Significance *Multivariate analysis* p-value
Age	34.6 ± 2.7	32.6 ± 4.6	0.01[d]	0.195
Duration of subfertility (yr)[a]	3.1 ± 1.7	2.9 ± 2.4	0.81[d]	
Body Mass Index	24.2 ± 5.1	23.7 ± 4.1	0.68[d]	
TCM[b]	93.3 ± 229.0	64.0 ± 82.3	0.63[d]	
Cycle day	9.0 ± 3.8	9.6 ± 4.4	0.57[d]	
Other uterine cavity abnormalities present at screening hysteroscopy	6 (35%)	64 (11%)	0.007[e]	0.009[g,h]
Cause infertility			0.88[f]	
- Idiopathic	8 (47%)	241 (41%)		
- Andrologic factor[b]	7 (41%)	265 (45%)		
- Subfertile female[c]	2 (12%)	83 (14%)		
- Tubal pathology	1 (6%)	65 (11%)	1.00[e]	
Infertility woman			0.99[f]	
- Primary	11 (65%)	382 (65%)		
- Secondary	6 (35%)	206 (35%)		

Note Values are expressed as mean ± Standard deviation NS = Not significant

[a]: Duration of subfertility: Duration of attempts to conceive, in cases of secondary infertility calculated from the last ongoing pregnancy, [b]: Defined as TMC (total motile count, semen volume (mL) * concentration spermatozoa (*10^9/mL) * grade A and B spermatozoa motility(%)) < 20·106, [c]: Due to tuba pathology (incl. endometriosis grade III and IV), anovulation or cervix factor, [d]: Significance, Student t-test, [e]: Significance, Fisher's Exact test, [f]: Significance, Chi-square test, [g]: Significance, corrected for age using multinomial logistic regression, [h]: Odds ratio 4.03, 95% CI: 1.41-11.51

Patient characteristics

Between the 17 cases with evident chronic endometritis (2.8%) and the rest of the 606 patients with adequate histological examination, univariate analysis showed significant differences for female age and other intrauterine abnormalities found at hysteroscopy (Table 1). Yet, multivariate analysis solely confirmed the significant difference for presence of uterine abnormalities. More intrauterine abnormalities were detected in the group with chronic endometritis (35% versus 11%), of which mostly endometrial polyps (p=0.009, OR 4.03, 95% CI: 1.41 - 11.51).

Reproductive outcome

The group investigated for reproductive outcome, consisted of 17 cases with and 68 controls without chronic endometritis. Details of the IVF/ICSI treatment are shown in Table 2. Of those 85 patients, 9 patients (11%) did not start treatment, one because of spontaneous pregnancy resulting in a live birth. A live birth after IVF/ICSI treatment occurred in 47 patients (58%). Two patients had a spontaneous pregnancy during IVF/ICSI treatment. Twentysix patients (31%) did not become pregnant. Thus, the live birth rate (including spontaneous pregnancies) was 50 out of 85 patients (59%).

The live birth rate (including spontaneous pregnancies) of the cases compared to the controls was 76% (13/17) versus 54% (37/68). This difference was not significantly different (P-value: 0.11). Taking into account the number of IVF/ICSI embryo transfers to obtain pregnancy, also no significant difference was found between the group with and without chronic endometritis (Kaplan Meier curve, Figure 2). Cox regression showed that the difference in cumulative pregnancy rate in consecutive embryo transfers was not significantly different (Hazard Ratio 1.456, 95% CI: 0.770 - 2.750, P-value: 0.2).

Discussion

Chronic endometritis is known as a subtle condition, which is hard to diagnose. Moreover, the influence of endometritis on fertility has not been fully assessed. In the present study, histological examination of hysteroscopy guided endometrial biopsies of infertile patients prior to a first IVF/ICSI treatment cycle revealed chronic endometritis in 2.8%. In the rather small group of patients diagnosed with endometritis, the live birth rate (including spontaneous pregnancies) within 3 years after initiation of IVF/ICSI treatment was not significantly different compared to patients without endometritis.

One of the possible study limitations that should be considered is the use of an enriched sample. For each of the 17 cases with evident chronic endometritis,

Table 2 IVF/ICSI results of the patients with chronic endometritis (cases) compared to those of the control group (controls). The control group consisted of a randomly selected sample of patients without endometritis, matched for the research hospital and day of the menstrual cycle on which the biopsy was performed.

Variables	Chronic endometritis N = 17	No chronic endometrits N = 68	Significance p-value
Number of started cycles[a]	2.5 ± 2.1	2.5 ± 1.8	0.91[b]
- Fresh cycles	2.2 ± 1.9	1.8 ± 1.2	0.33[b]
- Cryocycles	0.3 ± 0.8	0.7 ± 1.3	0.09[b]
Number of embryotransfers	2.0 ± 1.6	2.2 ± 1.7	0.70[b]
Number of embryo's transferred per cycle	1.4 ± 0.5	1.4 ± 0.4	0.45[b]
Live birth rate	13 (76%)	37 (54%)	0.11[c]

Note Values are expressed as mean ± Standard deviation NS = Not significant

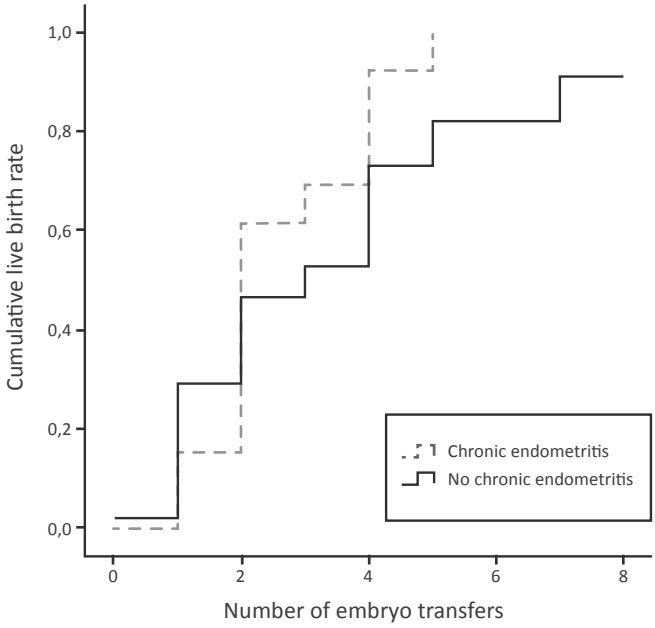
[a]: Number of started cycles within three years after the start of the initial trial (TEA-trial, register number: NCT00830401), or until a live birth or spontaneous pregnancy was obtained or treatment was stopped [b]: Significance, Student t-test [c]: Significance, Chi-square test

4 controls without chronic endometritis were randomly selected. This may have affected the study results. However, it concerned a matched, random selection and additional analysis with correction for possible confounders (female age, duration of infertility and the presence of intrauterine abnormalities) did not change our study outcome.

Also, the small patient population could be interpreted as a study weakness. As this study was performed as part of the TEA-trial, post hoc power analysis was done. Considering the number of included patients, this study would have 80% power to pick up a difference in live birth rate for patients without and with endometritis of 51% versus 88%, instead of 51% versus 76%, respectively. More important is the Hazard ratio on cumulative cycles with the 95% confidence interval of 0.770 - 2.750. The lower boundary of 0.770 indicates that the chance lowering effect of endometritis will maximally be a 23% reduction.

A good condition of the uterine cavity is essential for successful reproduction. Implantation concerns a process of physiologic inflammation, involving inflammatory mediators, such as leukocytes, cyto- and chemokines and other endometrial factors (Romero et al., 2004). The presence of high concentrations of

Figure 2 Survival curve showing the cumulative ongoing pregnancy rate (including the spontaneous pregnancies) in consecutive embryo transfer cycles for the cases with endometritis compared to the controls without endometritis



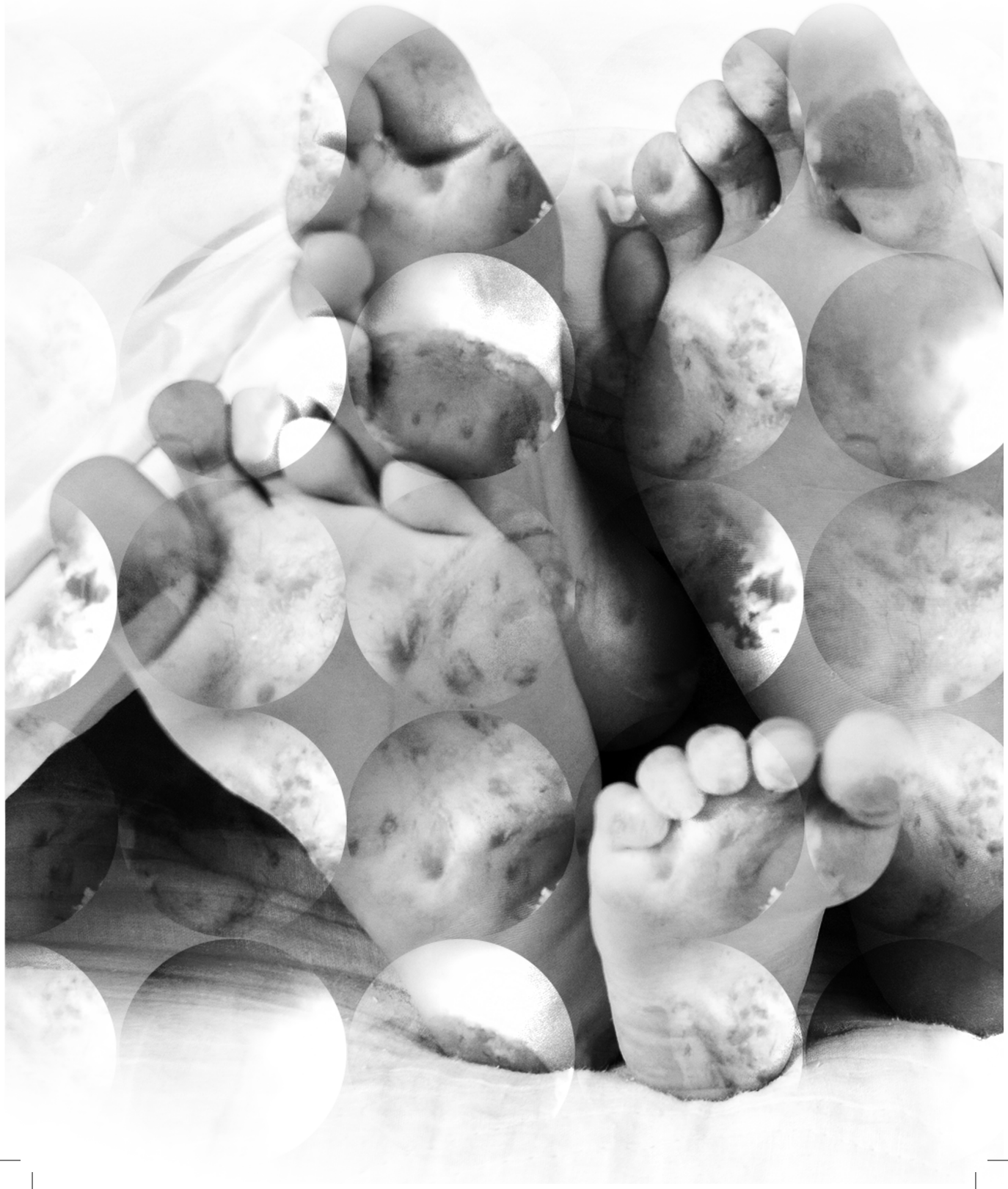
endotoxin, components of Gram negative bacteria, is said to induce a reaction of Th1 inflammatory cells. Th1 cells may predispose a hostile endometrial environment and thereby cause implantation failure, spontaneous abortion or premature labour (Kamiyama et al., 2004). Endometritis is also considered to be an inflammatory reaction. In contrast to acute endometritis, in chronic endometritis, usually no causal pathogen can be identified (Achilles et al., 2005). As well as the precise aetiology of chronic endometritis, also the impact on female fertility is unclear. The prevalence varies between 0.2% and 46% depending on the biopsy method (blind versus hysteroscopy guided) and the investigated, infertile patient population (Wild et al., 1986; Feghali et al., 2003; Polisseni et al., 2003; Cicinelli et al., 2005; Johnston-Macananny et al., 2009). The present study observed a prevalence of 2.8%, which seems to be in line with the literature, as asymptomatic patients indicated for IVF/ICSI were investigated.

The current literature on the impact of chronic endometritis on fertility consists of two studies. In a population of patients suspected of pelvic inflammatory disease (PID), the pregnancy rate and infertile status of the 356 patients with endometritis did not significantly differ from the pregnancy rate of the 258 patients without endometritis (Haggerty et al., 2003). Moreover, among patients with recurrent IVF failure, the ongoing pregnancy rate was similar between the 10 patients with and the 23 patients without endometritis (Johnston-Macananny et al., 2009).

The current study found a live birth rate (including spontaneous pregnancies) of 76% versus 54% for patients with or without chronic endometritis, respectively. The difference was not significant, however, the tendency of a benefit for patients with chronic endometritis is remarkable. A positive effect of chronic endometritis on fertility has not been described in literature. Possible explanations for the observed difference in live birth rate could be related to the variation in patient characteristics. First, patients with chronic endometritis more frequently had endometrial polyps. This is a previously reported association, which would unlikely lead to an increase in pregnancy rate (Bosteels et al., 2010; Cicinelli et al., 2004). Second, prescription of antibiotic treatment was not standardized. However, the effect of antibiotics on chronic endometritis is questionable, as usually no causal pathogen can be identified (Achilles et al., 2006). Third, additional analysis showed coincidental significant differences between the randomly selected, matched control group (n=68) and the other patients without endometritis and an adequate histopathology examination (n=521). The women of the control group were on average 2 years older and suffered 0.5 year shorter from infertility. Yet, besides the presence of intrauterine abnormalities, the patient characteristics did not significantly differ between the cases and controls. Moreover, correction for possible confounders did not change the study results. Finally, the presence of plasma cells, the diagnostic criteria for chronic endometritis, has also been reported in women with endometrial atrophy (Gilmore et al., 2007; Thurman et al., 2007). Patients with endometrial atrophy could hypothetically experience more benefit from fertility treatment compared to patients without atrophy, as follicular stimulation also increases endometrial thickness.

Still, based on the present study and published literature so far, chronic endometritis has no proven association with infertility (Haggerty et al., 2003; Johnston-Macananny et al., 2009). Moreover, the effect of antibiotic treatment on non-specific chronic endometrial inflammation is disputable. Routine performance of a hysteroscopy and guided endometrial biopsy to detect chronic endometritis therefore should not be recommended for daily clinical practice. A randomized controlled trial should be performed to find out whether detection and treatment of chronic endometritis prior to the start of fertility treatment

would indeed optimize fertility outcome. However, considering the low prevalence of chronic endometritis in asymptomatic patients, ~1500 patients should be included, making realization of such a trial very difficult. Moreover, an inter-observer study of pathology specimens would be desirable to investigate the reliability and reproducibility of the difficult diagnosis of chronic endometritis. In a population of asymptomatic patients prior to IVF/ICSI treatment, chronic endometritis was rarely diagnosed. Moreover, the reproductive outcome in patients, indicated for IVF/ICSI treatment did not seem to be negatively affected by chronic endometritis. Therefore, the significance of the subtle condition chronic endometritis for fertility remains unproven.



Chapter 5

The reliability of the histological diagnosis of endometritis in asymptomatic IVF cases: a multicenter observer study

J.C. Kasius, F.J.M. Broekmans, D.M.D.S. Sie-Go, C. Bourgain, M.J.C. Eijkemans, B.C.J.M. Fauser, P. Devroey, H.M. Fatemi

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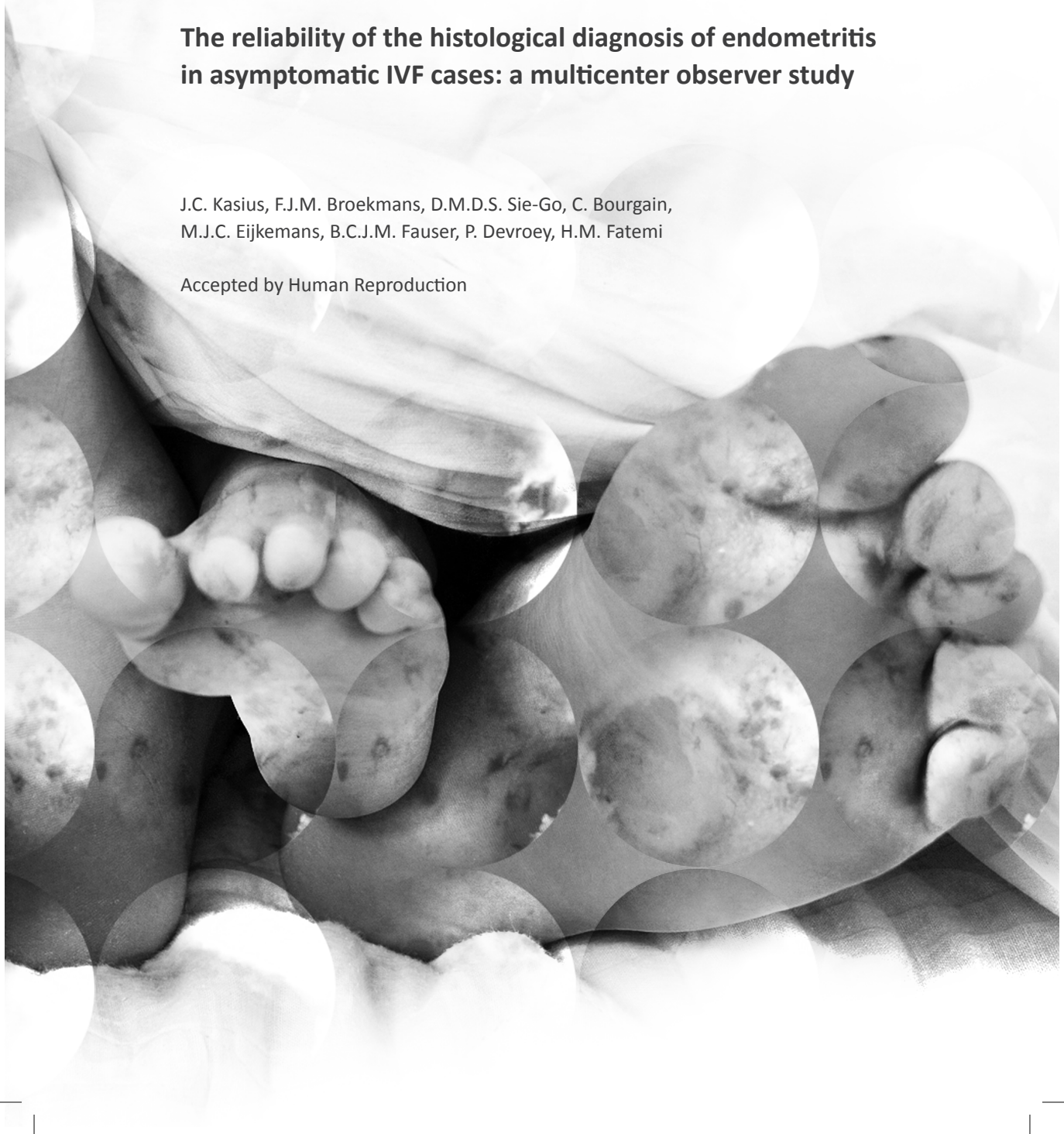


Abstract

Background Chronic endometritis is associated with abnormal uterine bleeding, recurrent abortion and infertility. It is a subtle condition, which is difficult to diagnose. The diagnosis is ultimately based on the presence of plasma cells in the endometrial stroma on histopathological examination. Literature on the reproducibility of the diagnosis chronic endometritis is lacking. Therefore, the aim of the current study was to assess the interobserver agreement of two pathologists in diagnosing chronic endometritis in asymptomatic, infertile patients.

Methods In the context of a randomized controlled trial, an endometrial biopsy was taken during a screening hysteroscopy prior to in vitro fertilization (IVF). All endometrial samples were independently examined by two pathologist. The slides, diagnosed with chronic endometritis, replenished with a random sample of the remaining slides up to a total of 100, were exchanged between the two pathologists and reassessed.

Results Of the 678 patients, who underwent hysteroscopy, 19 patients were diagnosed with (possible) chronic endometritis (2.8%). Perfect agreement between the pathologists, before and after inclusion of the 13 slides with additional immunohistochemistry staining, was found in 88% and 86%, respectively. The interobserver agreement was substantial, with kappa-values of 0.55 and 0.66, respectively.

Conclusions The interobserver agreement in diagnosing chronic endometritis in asymptomatic infertile patients was found to be substantial. Although the diagnostic reliability is sufficient with the methods in the present study, the low prevalence and unknown clinical significance warrants further study.

Introduction

Chronic endometritis is a persistent inflammation of the inner lining of the uterine cavity. It is thought to be associated with abnormal uterine bleeding, recurrent abortion and infertility (Greenwood and Moran, 1981; Polisseni et al., 2003; Romero et al., 2004). In 12% - 46% of the hysteroscopy guided endometrial biopsies in infertile patients, chronic endometritis was found to be present (Polisseni et al. 2003; Féghali et al., 2003; Cicinelli et al., 2005; Johnston-Macananny et al., 2010). Detection and treatment has been reported to significantly improve pregnancy rates (Féghali et al., 2003).

However, diagnosing chronic endometritis is known to be rather difficult. It is usually asymptomatic and hard to identify by most diagnostic tests. Although hysteroscopy enables direct visualisation of the endometrial lining, variable results have been reported on its diagnostic accuracy in the detection of chronic endometritis (Polisseni et al., 2003; Cicinelli et al., 2005). Histological examination of an endometrial biopsy is known as the golden standard. Abnormal percentages of lymfocytes, leukocytic infiltration of both glands and stroma, and the presence of eosinophils or macrophages are the histological features described to be associated with chronic inflammation (Greenwood and Moran, 1981; Dechaud et al., 1998; Adegboyega et al., 2010; Matteo et al., 2009). Nevertheless, the presence of plasma cells in the endometrial stroma is the only histological criterion that is generally accepted for the diagnosis of chronic endometritis. The search for plasma cells can be interfered or hampered by many conditions, such as mononuclear inflammatory cell infiltrates, stromal cell proliferation, the plasmacytoid appearance of stromal cells, or a pronounced predecidual reaction in a late secretory endometrium (Greenwood and Moran, 1981; Crum et al., 1983; Adegboyega et al., 2010). As the presence of only one plasma cell is sufficient to diagnose chronic endometritis, histological detection obviously is time-consuming and difficult. Despite the fact that immunohistochemical markers that specifically stain plasma cells, simplify the diagnostic decision, additional staining is not routinely provided in daily practice (Crum et al., 1983; Bayer-Garner et al., 2004).

The endometrial biopsy and histological examination has been widely used as a part of the infertility work-up in order to assess endometrium development during the luteal and/or the follicular phase. The accuracy, intra- and interobserver agreement of an endometrial biopsy for diagnosing luteal phase defects has been assessed thoroughly (Scott et al., 1993; Smith et al., 1995; Duggan et al., 2001; Myers et al., 2004). In literature, there is a lack of studies accessing the intra- or interobserver variation in diagnosing chronic endometritis. Therefore, the aim of this study was to assess the interobserver agreement in diagnosing chronic endometritis in asymptomatic, infertile patients.

Materials and methods

Patients

In the period from June 2007 until September 2008 endometrial biopsies were obtained in the context of the TEA-trial ("Treatment Efficacy of unsuspected uterine Abnormalities") (Fatemi et al., 2010). The aim of this randomized controlled trial was to assess the treatment efficacy of intrauterine abnormalities on subsequent in vitro fertilization (IVF) or intra cytoplasmic sperm injection (ICSI) treatment (trial register number: NCT00830401). Patients under the age of 43 years indicated for fertility treatment at the University Medical Center Utrecht (UMCU) or the Academic Hospital at the Dutch-speaking Brussels Free University (UZVUB) underwent office hysteroscopy prior to a first IVF/ICSI treatment cycle. Exclusion criteria were symptoms suggestive of intrauterine pathology, abnormalities at transvaginal ultrasound or a prior hysteroscopy examination. The Institutional Review Board of the two participating centers approved of the study and informed consent was obtained.

Hysteroscopy and endometrial biopsies

The hysteroscopy procedures were scheduled in the follicular phase of the menstrual cycle (day 3-15), one to three months before starting the IVF/ICSI treatment. All procedures were performed in an outpatient setting, making use of a 5-mm outer-diameter continuous flow Bettocchi hysteroscope with 30° direction of view (Karl Storz Endoscopy, Stöpler Medical Instruments, Utrecht, The Netherlands & Olympus Belgium N.V., Aartselaar, Belgium). At the end of each procedure, an endometrial biopsy was obtained. At the UMCU, a grasping forceps (Karl Storz, Endoscopie Nederland B.V., Nieuwegein, The Netherlands) was used to perform the biopsy from the posterior wall, halfway the distance between the inner cervical os and the uterine fundus. At the UZVUB a Pipelle de Cornier under local anesthesia was used to perform a blind biopsy (Laboratoire CCD, Paris, France).

Histological examination

The endometrial biopsies were placed in a fixative of 4% phosphate buffered formaldehyde and processed routinely into one or two haematoxylin and eosin (HE) stained slides per patient. At each research hospital, one pathologist, with a special interest and expertise in gynaecological pathology, examined all endometrial samples obtained at that research hospital, independently from the other pathologist. Both pathologists were aware of the study design. However, the only clinical information provided was the day of the menstrual cycle on which the biopsy was taken and whether oral contraceptives had been used. A standard form was used to record the results of classifying the

endometrial tissue samples, the presence or absence of plasma cells and other inflammatory cells (i.e. lymphocytes, neutrophilic granulocytes, histiocystes/ macrophages and eosinophilic granulocytes) (Mazur and Kurman, 2005). Abnormal prevalence of different inflammatory cells, or lymfocytes destructing the endometrial tubuli were suggestive for chronic endometritis. However, presence of plasma cells ultimately set the diagnose chronic endometritis. According to the findings during histopathology examination, the concluding diagnosis was reported by the pathologist. The diagnostic categories were as follows: no chronic endometritis, possibly chronic endometritis, evident chronic endometritis.

In case the diagnosis remained doubtful after assessment of the HE stained tissue sections, additional immunohistochemistry for the plasma cell marker CD138, alone or in combination with staining for the B-cell markers CD20 or CD79a marker was performed. For CD138 the Clone B-B4, batch 605, was used applying dilution 1:1000, an antibody from Serotec. For plasma cells and B-lymphocytes the CD79a, the DAKO, Clone JCB117, batch 2791 (dilution 1:200) antibody was used. For B-lymphocytes CD20, the DAKO, Clone L26, batch 083 (dilution 1:400) antibody was used. For all antibodies, antigen retrieval in citrate buffer was applied and staining was done with the Bond-Max autostainer (Leica).

Histological revision for observer agreement

The slides of patients diagnosed with possible or evident chronic endometritis according to the first histological examination, were replenished by a sample of slides from the patients, who were not diagnosed with chronic endometritis, up to a total of 50 per research center. The added patients without chronic endometritis were matched to the patients with chronic endometritis for the research hospital and the day of the menstrual cycle on which the hysteroscopy was performed and the biopsy was taken. The 50 slides of the replenished sample per research center were exchanged between both pathologists for a second evaluation. The histological revision was performed similar to the original examination. Initially, only the HE stained slides were assessed. Thereafter, the pathologist could alter his or her opinion if additional immunohistochemistry appeared to be present.

Statistical analysis

The pathologists' findings were statistically analyzed, using SPSS version 15.1 and R2.9.2 . The agreement between both pathologists on the slides of the histological reassessed sample was calculated. Their agreement on the diagnostic categories was evaluated for the findings based on HE stained slides alone or in combination with additional immunohistochemical stained cases. The inter-

observer agreement was calculated as percentage of perfect agreement and corresponding weighted kappa-value. Kappa is a measure for agreement between observers corrected for the agreement expected to occur by chance (κ = (Observed agreement – Agreement by Chance)/(1 – Agreement by chance)). A kappa value of <0.20 is interpret as slight agreement, a value between 0.21 - 0.40 fair agreement, a value between 0.41 - 0.60 moderate agreement, a value between 0.61 - 0.80 substantial agreement, and a value of 0.81 - 1.00 as almost perfect agreement (Landis and Koch, 1977). The weighted kappa takes into account the ordinary scale of categorical variables, so that different levels of agreement between categories contribute to the kappa-value (Fleiss and Cohen, 1973).

Table 1 Findings at hysteroscopy examination

Findings	Prevalence	%
Normal cavity	596	87.9%
Abnormal cavity	74	11.0%
- Polyp	41	6.0%
- Myoma	6	0.9%
- Adhesion	15	2.2%
- Septum	14	1.2%
Total[a]	678	100%

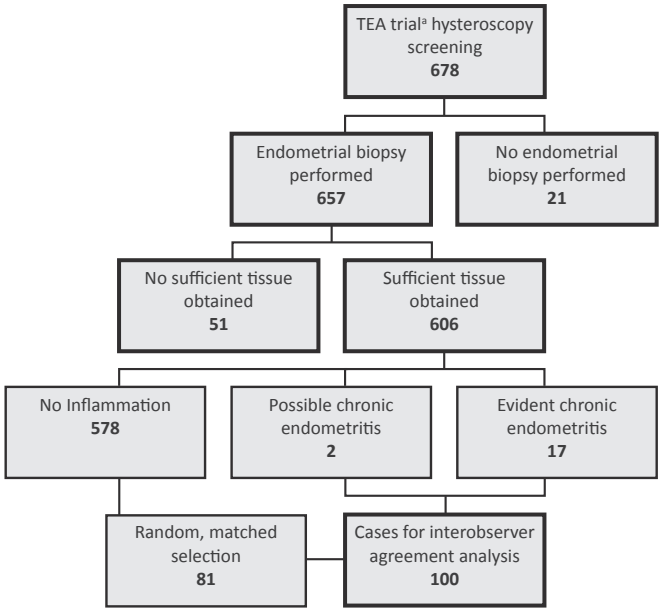
[a]: Hysteroscopy failed in 8 patients. In two cases more than one abnormality was detected

Results

A total of 678 asymptomatic, infertile patients were included in the TEA trial and underwent office hysteroscopy (Figure 1). The observed hysteroscopy findings are reported in Table 1. The endometrial tissue samples of 606 patients could be adequately examined by the two pathologists (Table 2). Histological examination failed in 11% of the participants, due to inability to complete the hysteroscopy procedure (1%), to perform the endometrial biopsy (3%) or to obtain sufficient endometrial tissue (7%).

Out of the population of 606 successfully biopsied patients, at first examination 587 were diagnosed as ‘no chronic endometritis’ (86.5%), 2 were diagnosed with ‘possible nonspecific chronic endometritis’ (0.3%) and 17 patients with ‘evident nonspecific chronic endometritis’ (2.5%).

Figure 1 Flowchart illustrating the enriched sample method. Shown is the number of patients at each step towards the group of cases in which the endometrial samples were histologically examined by both pathologists and analyzed for the interobserver agreement. Cases initially diagnosed with chronic endometritis were replenished by a sample of randomly selected patients not initially diagnosed with chronic endometritis. Matching was performed for research center and day of menstrual cycle on which the endometrial biopsy was obtained.



[a]: The trial "Treatment Efficacy of unsuspected uterine Abnormalities" on subsequent in vitro fertilization (IVF) or intra cytoplasmic sperm injection (ICSI) treatment (register number: NCT00830401)

The slides of the 19 patients in whom possible or evident chronic endometritis was detected, added up with a matched selection out of the other slides up to a total of 50 per research hospital, were revised by the pathologist from the other research center. In this histological reassessed sample of 100 slides, the slides of 13 patients were additionally stained with immunochemical markers. Between the patients contributing to the analysed, histological revision sample (n=100) and those who did not (n=506), no significant differences were found regarding day of the cycle on which the hysteroscopy was performed, age, body mass index, duration of child wish, cause for the infertility or the rate of presence of an intrauterine abnormality at hysteroscopy.

Table 2 Patient characteristics of the sample of IVF/ICSI patients used for the histological revision

Variables	N = 100	
Age	32.98	± 3.96
Duration of subfertility (years)[a]	3.05	± 2.24
Body Mass Index	24.41	± 5.20
TCM[b]	65.67	± 120.32
Day of menstrual cycle[c]	9.66	± 3.74
Infertility woman		
- Primary	63	(63%)
- Secondary	37	(37%)
Cause infertility		
- Idiopathic	43	(43%)
- Andrologic factor[b]	47	(47%)
- Subfertile female[d]	10	(10%)

Note Values are expressed as mean ± Standard deviation.

[a]: Duration of attempt to conceive, in cases of secondary infertility calculated from the last ongoing pregnancy [b]: Defined as TMC (total motile count, semen volume (mL) * concentration spermatozoa (*10^9/mL) * grade A and B spermatozoa motility (%)) < $20 \cdot 10^6$ [c]: Day of the menstrual cycle on which the endometrial biopsy was obtained [d]: Due to tubal pathology (incl. endometriosis grade III and IV), anovulation or cervix factor

Observer agreement in diagnosing chronic endometritis

The interobserver agreement was assessed in the sample of the slides of 100 patients, which were examined by both pathologists. Based on solely the HE stained tissue specimens, the pathologist at the UMCU detected evident chronic endometritis in 12 patients (Table 3). In 1 case the diagnosis remained doubtful. The pathologist at the UZVUB identified evident and possible chronic endometritis in 14 and 3 patients, respectively. They reported similar diagnostic categories in 88% of all 100 patients. The kappa value for interobserver agreement was 0.546 (95% CI: 0.351 - 0.741) which represents moderate agreement. Agreement analysis was also performed, after replacing the 13 HE stained slides by the corresponding slides with an additional immunohistochemistry

staining. The pathologist at the UMCU diagnosed evident and possible chronic endometritis in 14 and 5 patients (Table 3). The findings of the pathologist at the UZVUB were 15 and 4, respectively. Perfect agreement on the diagnostic category appeared in 86%. The kappa for interobserver agreement when additional staining using immunohistochemical markers was included was 0.659 (95% CI: 0.463 - 0.855), which is interpreted as substantial agreement.

Discussion

Chronic endometritis generally is an asymptomatic condition and therefore difficult to diagnose. Although some subtle endometrial alterations have been described to be indicative for chronic endometritis, the diagnosis ultimately relies on the presence of plasma cells at histological examination (Greenwood and Moran, 1981; Dechaud et al., 1998; Adegboyega et al., 2010; Matteo et al., 2009). The current study demonstrates that the interobserver agreement in diagnosing nonspecific chronic endometritis in asymptomatic patients prior to a first IVF/ICSI treatment is substantial.
It is rather surprising that the interobserver agreement reached the level of ´substantial', while unequivocal and easy to determine criteria for the diagnosis nonspecific chronic endometritis are currently absent. The diagnostic cri-

Table 3 Histological diagnosis by the pathologist of the UZ-VUB (horizontal) and the pathologist of the UMCU (vertical) of the analyzed, revised group of 100 patients. Also, the level of perfect interobserver agreement and corresponding kappa values of diagnosing possible or evident chronic endometritis.

	HE	UMCU		
		No[a]	Possibly[a]	Evident[a]
UZVUB	No[a]	79	0	4
	Possibly[a]	2	1	0
	Evident[a]	6	0	8
Agreement		*88%*		
Weighted κ		*0.546*		
95% CI		*(0.351 - 0.741)*		

	CD	UMCU		
		No[a]	Possibly[a]	Evident[a]
UZVUB	No[a]	76	1	4
	Possibly[a]	2	1	1
	Evident[a]	3	3	9
Agreement		*86%*		
Weighted κ		*0.659*		
95% CI		*(0.463 - 0.855)*		

Note Results split up for the findings based on the haematoxylin and eosin stained slides (HE) (on the left) and the finding if also the slides additionally stained with immunohistochemical markers (CD) are included (on the right) [a]: Referring to no chronic endometritis, possibly chronic endometritis and evident chronic endometritis

terion for the identification of plasma cells with or without additional specific staining, as applied in the present study, may come with difficulties. Plasma cells generally are present in small amounts and may be mimicked or blurred by certain conditions of the endometrium or endometrial and inflammatory cells (Greenwood and Moran, 1981; Crum et al., 1983; Adegboyega et al., 2010). In the present study, endometrial tissue was obtained by a hysteroscopy guided biopsy, which resulted in only a small sample of endometrium available for histological examination. In the UMCU a grasping forceps was used instead of a Pipelle de Cornier, which resulted in even smaller amounts of material in some cases. Moreover, the slides of the research hospitals also had a slightly different appearance due to variation in the routine processing of the endometrial tissue in the laboratories (difference in colour and amount of tissue on one slide). Another limitation in the search for plasma cells may have been the absence of a standard additional immunohistochemistry staining on all endometrial biopsies. However, in daily practice, immunochemical markers are not routinely provided either. Taking into account all factors that could have hampered the diagnosis of chronic endometritis, the substantial reproducibility between observers is rather satisfying.

The results of the present study were based on a sample of 100 patients out of the whole study population in which a hysteroscopy guided endometrial biopsy was obtained. The cases were selected on a specific criterion, namely the diagnosis (possible) chronic endometritis by a pathologist, whereas the controls were randomly selected. This may have influenced the statistic assumption underlying the calculation of the kappa-values, though it seems not plausible to have occurred in this situation. Another consequence has been the difference in the prevalence of chronic endometritis between the sample and the whole study population. Thus, the use of an enriched sample instead of the whole patient population may have affected the study results. In our setting, it was the only feasible study design. Still, most processes of diagnosing based on operator judgement include elements of information on the likelihood to find an abnormality and the specific question that has been put forward by -for instance- the clinician. As the pathologists were aware of these aspects, the approach chosen may not be really remote from daily practice and thereby serve well as a model for reliability assessment.

Among the various endometrial abnormalities that can be enlightened by endometrial biopsy, the interobserver variation on hyperplasia and the luteal phase defect has been widely assessed. The WHO classification of endometrial hyperplasia of 1994 differentiates between simple and complex hyperplasia with or without atypia. The first study evaluating the agreement between 6 gynaecologists on this WHO classification found a maximum kappa-value of 0.25 (95% CI 0.23 - 0.28) (Skov et al., 1997). Two following studies, which used

slightly different diagnostic categories, found moderate to substantial agreement between 5 pathologists, with kappa-values of 0.47 - 0.70 (Kendall et al., 1998; Bergeron et al., 1999).

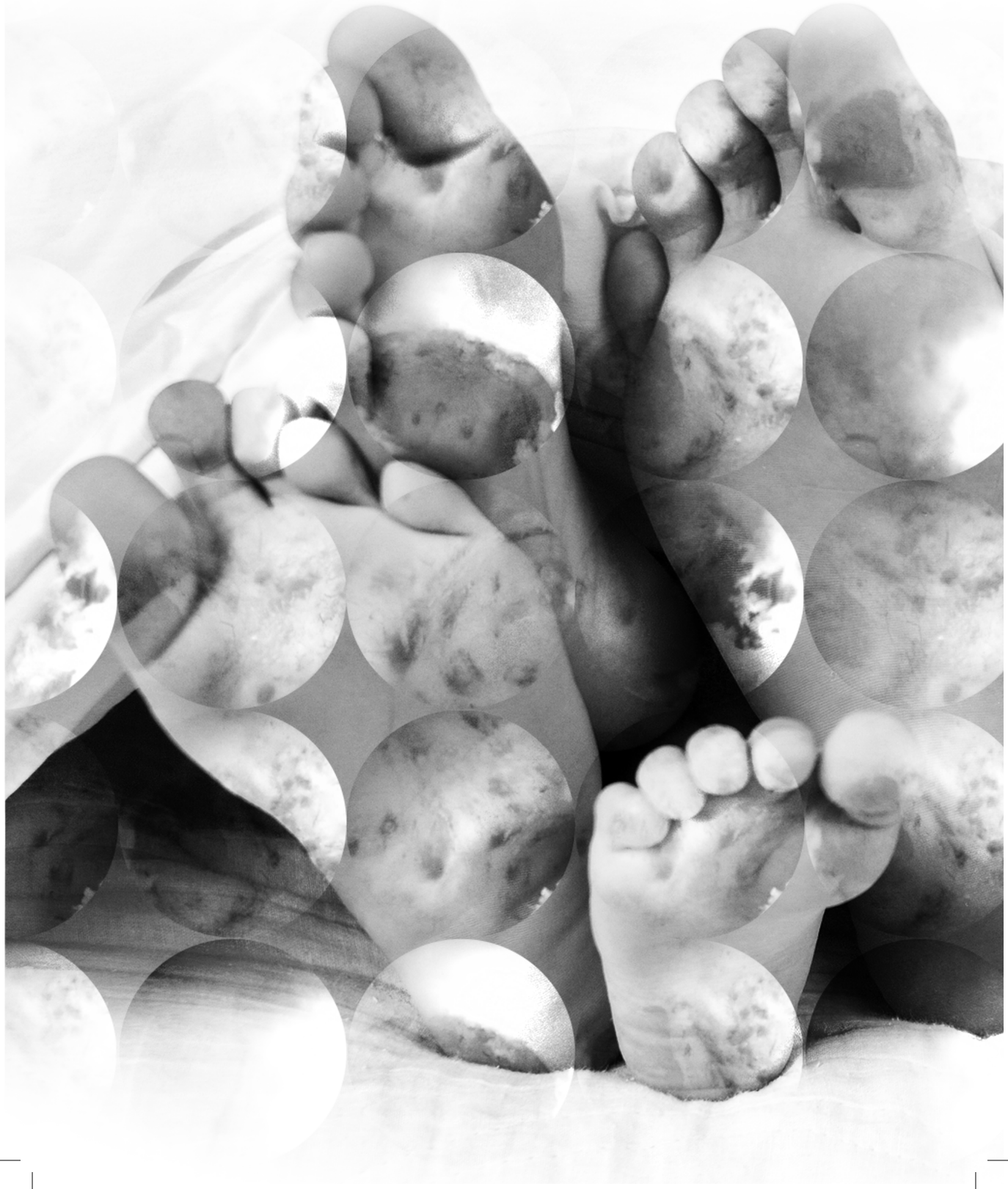
In diagnosing luteal phase defects the observer agreement was found to be somewhat disappointing. In 78 slides of infertile patients the reproducibility of endometrial dating was assessed. The agreement among 4 pathologists on the dating categories 'proliferative', 'secretory', 'menstrual' or 'undateble' was substantial (maximum kappa-value: 0.70) (Duggan et al., 2001). Furthermore, the observer agreement based on the diagnosis of 'in-phase' or 'out-of phase', defined as a 2-day difference between the histological date and the calculated luteal phase date based on the urinary LH surge was evaluated. The reproducibility was shown to be moderate, with kappa-values between 0.4 and 0.6 in biopsies of 82 fertile and 83 infertile patients (Myers et al., 2004).

To the best of our knowledge, reproducibility studies on diagnosing chronic endometritis are absent. In view of the results of the available observer studies on endometrial hyperplasia and luteal phase deficiencies, the reproducibility of diagnosing chronic endometritis is similar or slightly more promising.

The impact of chronic endometritis on fertility is controversial. The described prevalence varies widely and trials investigating the reproductive outcome of patients with chronic endometritis reported contrary results (Czernobilsky, 1978). The prevalence of chronic endometritis has been described to be between 0.2% and 46% amongst infertile women (Wild et al., 1986; Sahmay et al., 1995; Féghali et al., 2003; Polisseni et al. 2003; Cicinelli et al., 2005; Johnston-Macananny et al., 2010). Most recent studies investigated a hysteroscopy guided biopsy and found chronic endometritis in 12% - 46%. Those studies concern small patient populations, generally in which women had clinical symptoms justifying diagnostic hysteroscopy. Since the current study investigated a large group of consecutive women, without specific symptoms suggestive of uterine pathology, prior to starting a first IVF/ICSI cycle, the relatively low prevalence was to be expected. Regarding the impact of chronic endometritis on fertility, limited research is published. Recently published literature has shown that in 33 patients with two or more failed IVF attempts, the implantation rate of patients with chronic endometritis was significantly lower than controls without chronic endometritis, 12% versus 33% (Johnston-Macananny et al., 2010). However, the ongoing pregnancy rate did not significantly differ between the patients of both groups. Moreover, in patients clinically suspected for pelvic inflammatory disease, the prevalence of infertility did not significantly differ between patients with or without chronic endometritis (Haggerty et al., 2003).

The uncertainty on the impact of chronic endometritis on reproductive outcome also determines the importance of its detection. Future research should

be ideally a sufficiently powered prospective study in infertile women indicated for assisted reproductive technology (ART) in whom a standard biopsy is taken and the histopathology is related to subsequent outcome of treatment. Possibly, the inSIGHT trial, in which hysteroscopy with direct treatment of visible pathology is performed with the addition of a standard biopsy, will contribute to such data (trail register number NCT01242852). This multicenter trial has recently been started in The Netherlands. Moreover, a systematic review on individual patient data may allow for larger data acquisition that will help assessing the exact significance of endometritis.
Once the true impact is clarified by this future research, the usefulness of an endometrial biopsy in patients suffering from infertility can be determined. Nevertheless, the present interobserver agreement study has demonstrated that histological examination of an endometrial biopsy is a reproducible method for the diagnosis of chronic endometritis.



Chapter 6

Cost-effectiveness of hysteroscopy screening for infertile women

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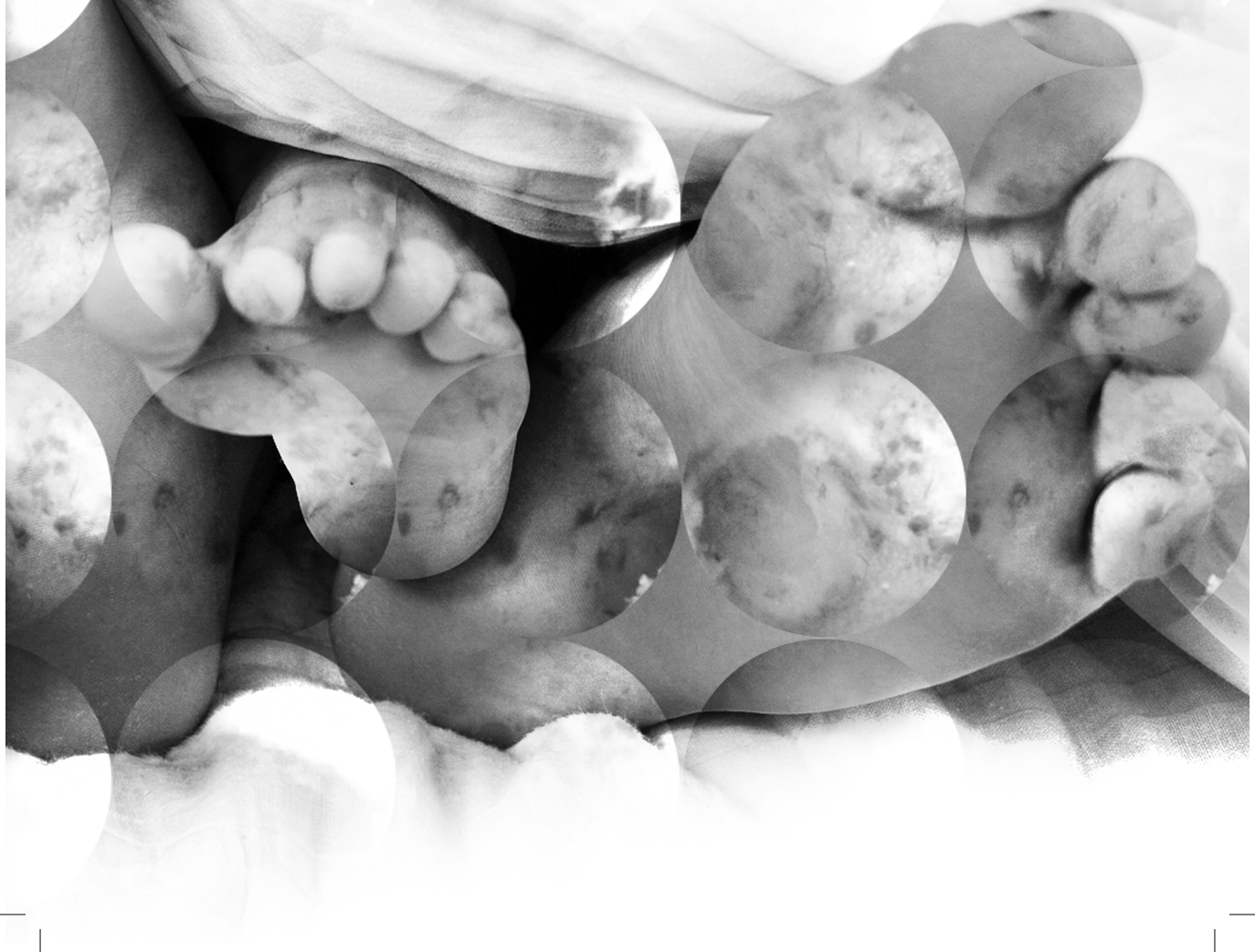
Submitted



Abstract

Background Minor intrauterine pathology is considered to have a negative impact on in vitro fertilization (IVF) outcome. It is advocated to diagnose and treat this pathology by hysteroscopy in order to optimize IVF treatment. The aim of this study was to assess the cost-effectiveness of office hysteroscopy screening prior to IVF.

Methods The cost-effectiveness of two distinct strategies (hysteroscopy after 2 failed IVF cycles, [Failedhyst] and routine hysteroscopy prior to IVF, [Routinehyst]) was compared to the reference strategy (no hysteroscopy, [Nohyst]). When present, intrauterine pathology (polyps, myoma, adhesions, septa) was treated during the hysteroscopy procedure. Two models were constructed and evaluated in a decision analysis. In Model I, which was based on the current literature, it was assumed that all patients who underwent screening hysteroscopy prior to IVF, would benefit of an increase in pregnancy rate. Model II -more hypothetical- assumed that the pregnancy rate solely increased in patients with intrauterine abnormalities, which were subsequently corrected by hysteroscopic treatment. For the three strategies, the total costs and live birth rates after a total of 3 IVF cycles were assessed. Also, sensitivity analysis was performed. Results were visualized in an incremental cost-effectiveness plane and a cost-effectiveness acceptability curve.

Results For Model I (all patients benefit from hysteroscopy), strategy [Routinehyst] was always cost-effective compared to strategy [Nohyst] or [Failedhyst]. For this strategy, a monetary profit would be obtained in case hysteroscopy would increase the live birth rate after IVF by ≥2.8%. In Model II (only patients with abnormalities will benefit from hysteroscopic correction), the three strategies showed less divergence. [Routinehyst] dominated [Failedhyst], however, hysteroscopy performance was accompanied with considerable costs. Sensitivity analysis demonstrated that variation in the increase in live birth rate by performing hysteroscopy was the only model variable that influenced the cost-effectiveness considerably.

Conclusions According to the published literature, the application of a routine hysteroscopy prior to IVF seems to be cost-effective. Randomized clinical trials confirming the effectiveness of hysteroscopy are needed.

Introduction

Despite progressing improvement of in vitro fertilization (IVF), the maximum implantation rate per embryo transferred usually does not exceed 30% (Andersen et al., 2008). Even if both ovum pick-up and fertilization occur successfully in the process of IVF, there is a large unexplained drop between embryo transfer and occurrence of pregnancy. Implantation failure presents a major clinical challenge and is a cause of considerable stress to patients and their carers in assisted reproductive technology. Next to the physiological and physic burden that comes with every IVF cycle, implantation failure also adds up to the considerable costs associated with fertility treatment (Bouwmans et al., 2008). If progress is to be made in improving implantation rates, a greater understanding of the factors that determine successful im plantation is required. Implantation failure could be due to the embryo, uterine environment, or a combination of both. Even minor uterine cavity abnormalities, such as endometrial polyps, small submucous myomas, adhesions, and septa are considered to have a negative impact on the chances to conceive through IVF (Rogers et al., 1986). The prevalence of unsuspected intrauterine abnormalities, diagnosed by hysteroscopy prior to IVF, has been described to be 11% - 45% (Shamma et al., 1992; Balmaceda and Ciuffardi, 1995; La Sala et al., 1998; Oliveira et al., 2003; Hinckley and Milki, 2004; Demirol and Gurgan, 2004; Doldi et al., 2005; Rama Raju et al., 2006; Fatemi et al.,2010). Therefore, it is advocated to diagnose and treat these abnormalities in order to optimize the condition of the uterine environment and thereby the outcome of IVF treatment. However, high quality evidence that defines the influence of screening for intrauterine pathology on reproductive outcome is absent (Shamma *et al.*, 1992; Oliveira *et al.*, 2003; Demirol and Gurgan, 2004; Doldi *et al.*, 2005; Rama Raju *et al.*, 2006).

At present, the basic work-up for evaluation of the uterine cavity prior to IVF consists of transvaginal ultrasound (TVS), possibly followed by gel or saline infusion sonography (SIS), hysterosalpingography (HSG) or hysteroscopy. The accuracy of HSG in assessment of the uterine cavity integrity in infertile patients has been reported to be rather disappointing (Gaglione *et al.*, 1996; Golan *et al.*, 1996). Whereas GIS/SIS are increasingly considered to be useful in diagnosing intrauterine abnormalities, hysteroscopy is still known as the gold standard (Bozdag *et al.*, 2008). It is easy to perform in an outpatient clinic without anesthesia. Moreover, hysteroscopy enables diagnosis and treatment of intrauterine pathology in the same setting.

Due to paucity of high quality evidence on the impact of unsuspected intrauterine abnormalities on IVF outcome in asymptomatic infertile patients, there is the possible widespread introduction of hysteroscopy and other imaging tech-

niques prior to IVF, without the certainty that this policy is truly (cost-)effective. Therefore, the aim of the current study was to provide the cost-effectiveness analysis of hysteroscopy as a routine procedure for assessment of the uterine cavity prior to IVF treatment.

Materials and Methods

Decision analytic model

To determine whether implementation of routine hysteroscopy prior to IVF treatment would be cost-effective, a decision making model was made. The hypothetical patient population consisted of infertile women, indicated for IVF/ICSI treatment, with no symptoms of intrauterine pathology and a normal transvaginal sonography. The decision model contained three strategies, according to the most commonly used scenarios in daily clinical practise.

In strategy [Nohyst], all patients underwent IVF treatment cycles without hysteroscopy screening. In case of a normal TVS, a maximum of three IVF treatment cycles were performed. This strategy was considered as the reference strategy.

In strategy [Failedhyst], patients with two failed IVF treatment cycles, underwent screening hysteroscopy. In case of a normal TVS and if a pregnancy had not been achieved after two subsequent IVF treatment cycles, hysteroscopy screening was performed. Also, intrauterine abnormalities (endometrial polyps, submucous myoma, adhesions, septa) were treated during the same hysteroscopy procedure. In addition, a third IVF treatment cycle was performed.

In strategy [Routinehyst], all patients underwent a hysteroscopy prior to the first IVF treatment cycle. All women with a normal TVS underwent a screening hysteroscopy. Intrauterine abnormalities, predefined as endometrial polyps, submucous myoma, adhesions or septa, were treated during the same hysteroscopy procedure. Afterwards, a maximum of three IVF treatment cycles was performed.

Model input; probabilities

The probability data of the decision model were obtained from the best available evidence concerning hysteroscopy in fertility treatment. The prevalence of minor intrauterine abnormalities has been widely investigated. However, the results of prevalence studies are rather diverse. The prevalence in studies among asymptomatic, infertile patients, with a normal TVS or HSG, is reported to be between 11% - 40% (Balmaceda and Ciuffardi, 1995; La Sala *et al.*, 1992; Hinckley and Milki, 2004: Fatemi *et al.*, 2010).

The exact effect of detection and treatment of these abnormalities by hysteroscopy prior to IVF has not been clarified yet. The best available evidence

consists of only two randomized trials (Bosteels *et al.*, 2009; El-Toukhy *et al.*, 2008; Bozdag *et al.*, 2008). In a population of women with ≥2 failed IVF cycles, both Demirol and Gurgan and Rama Raju et al. assessed the difference in pregnancy rate between a group without hysteroscopy (I) and a group with hysteroscopy and immediate treatment of detected pathology (II) (Demirol and Gurgan, 2004; Rama Raju *et al.*, 2006). In both studies a firm increase was observed in pregnancy rates between group I and the part of group II with pathology (22% versus 30%, and 26% versus 40%). Moreover, Rama Raju et al. reported an increase in live birth rate of 8.4% (16.6% versus 25%).
For the population based pregnancy rates, the Dutch IVF results of the yearly report by the Dutch Foundation of Infertility Registration of were used. The live birth rate after IVF in 2007/2008 was 23.3% (Kremer, 2009).

Model input; costs
The cost-effectiveness analysis was conducted from a health care provider perspective. The costs per IVF treatment cycle in the Netherlands in 2004 were calculated to be €2381 (Bouwmans *et al.*, 2008). These costs were transferred to the costs per IVF cycle in 2008 by taking into account the health specific increase in expenses of 1.2% - 2.5% per year (Hakkaart-Roijen van *et al.*, 2010). The costs of one outpatient screening hysteroscopy has not previously been assessed. Therefore, the directs costs in health care sector were estimated, making use of standard prices (Table 1) (Hakkaart-Roijen van *et al.*, 2010). Also, questionnaires on the costs of a hysteroscopy were obtained from three different non-academic hospitals. The average costs of one hysteroscopy based on the questionnaires was compared to the costs of one hysteroscopy based on the standard prices. The indirect costs were not assessed, as it would surpass the objective of the current study.

Model analysis and outcomes
For analysis of the cost-effectiveness of hysteroscopy prior to IVF, two models were used. Model I was exclusively based on the available evidence, which postulates that all infertile patients undergoing hysteroscopy prior to IVF encounter an increase in pregnancy rate, disregarding the presence or absence of intrauterine abnormalities. This increase in pregnancy rate for patients who underwent hysteroscopy was recharged for every subsequent IVF treatment cycle.
Model II is a more hypothetical, however potentially more realistic model. In this model, the assumption was made that the patients with intrauterine abnormalities had a reduced chance to conceive. The pregnancy rate through IVF would convert to the normal pregnancy rate in case the patient underwent hysteroscopy. Thus, in Model II, the increase in pregnancy rate trough hysteroscopy

was solely calculated for patients with intrauterine abnormalities. The increase in pregnancy rate for patients who underwent hysteroscopic treatment was recharged for every subsequent IVF treatment cycle.
Models I and II were analysed using Microsoft Excel. The primary study outcome parameters for both models were the total costs per live birth and effects, expressed as cumulative live birth rate after 3 IVF cycles, for each of the three strategies. The base-case analysis was performed making use of the average value of all model variables (Table 2). Sensitivity analysis was done, to analyse the effect of variation of the baseline assumptions of each of the variables in the model separately.
Forthcoming out of the primary study outcomes, the extra costs for achieving an additional live birth in relation to the reference strategy [Nohyst], the incremental cost effectiveness ratio's (ICERs), were calculated. To test the uncertainty of the estimated costs and effects of strategy [Failedhyst] and [Routinehyst] in relation to the reference strategy ([Nohyst]), Monte Carlo simulation using 1.000 combinations of the values randomly drawn from uniform distributions within the preset range of the variables (Table 2) was performed and illustrated in a scatter plot in the 'incremental cost-effectiveness plane'. From these results the probability of a strategy being more cost-effective than the reference strategy at a given threshold for society's willingness to pay was visualized in a cost-effectiveness acceptability curve. This curve illustrates the proportion of the 'costs and effects pairs' -shown in the incremental cost-effectiveness plane- (y-axis) which are cost-effective for a range of monetary values (x-axis).

Results

Model input, probabilities and costs

The estimated costs of a screening hysteroscopy, based on standard prices was €126 (Table 1). The overall costs were comparable to the average costs of a hysteroscopy in the three reference hospitals, which was on average €124. Calculating the health specific increase in expenses per year, the costs of IVF in 2008 were estimated to be on average €2550. An overview of the assumed distribution of all variables of the analyzed models is shown in Table 2.

Model I

In Model I, the live birth rate of all patients increased after hysteroscopy. Base-case analysis, making use of the average values of the variables, showed that the cumulative birth rate in three cycles was 46.7%, 48.4% and 50.4% for strategy [Nohyst], [Failedhyst] and [Routinehyst], respectively. The accom-

Table 1 Costs for hysteroscopy, based on estimated standard costs

Variables	€
Personel	
- Gynecologist	34
- Assisting personel	14
Hysteroscopic procedure	
- Hysteroscope (and maintainance)	7
- Other material used during procedure (infusion fluid, gloves, paper etc.)	2
- Other hospital services used (histopathologic examination)	50
Supporting facilities (reception, hospital cleasising, etc.)	19
Total	126

Note The directs costs in health care sector were estimated, making use of standard prices (Hakkaart-Roijen van et al., 2010)

Table 2 Distribution of each of the variables use in the model analysis

	Lower range -	Upper range -	Average
Increase in live birth rate trough hysteroscopy[a]	1%	9%	4.5%
Prevalence of intrauterine abnormalities	11%	40%	25.5%
Population based live birth rate after IVF	23%	24%	23.5%
Costs of one screening hysteroscopy[a]	€100,-	€150,-	€125,-
Costs of one IVF treatment cycle	€2500,-	€2600,-	€2550,-
Drop-out rate	2%	2%	2%

[a]: Costs for screening hysteroscopy and see and treat in case of abnormalities (Table 1)

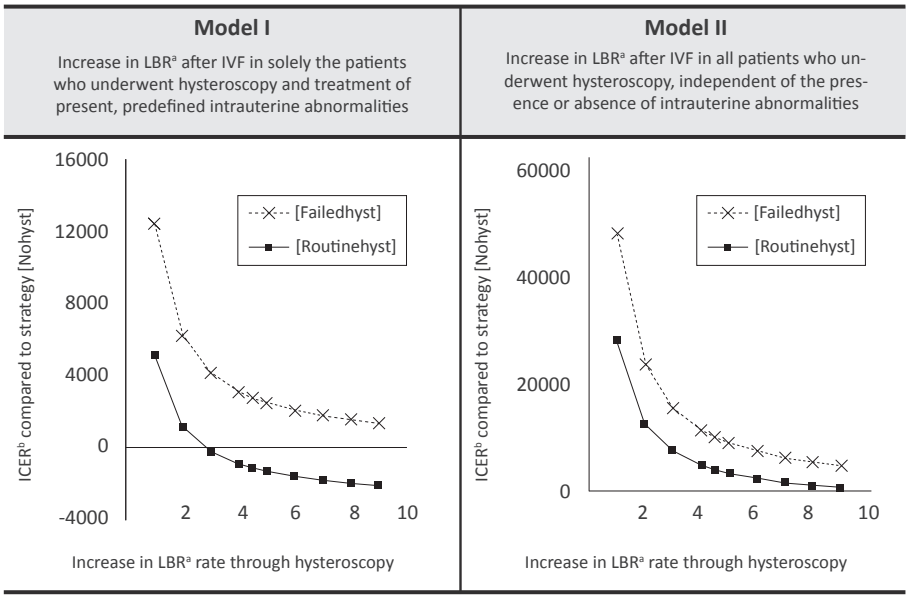
Table 3 Sensitivity analysis, showing the base-case analysis, the analysis of a scenario in which all model variables are in favor of hysteroscopy and the analysis of a scenario in which all model variables are out of favor of hysteroscopy

	Model I			Model II		
	Out of favor of HY[e]	Baseline[f]	In favor of HY[g]	Out of favor of HY[b,e]	Baseline[f]	In favor of HY[g]
Population based LBR[a]	23%	23.5%	24%	23%	23.5%	24%
Prevalence intrauterine pathology	-	-	-	11%	23.5%	40%
Increase in LBR[a] trough HY[b]	1%	4.5%	9%	1%	4.5%	9%
Costs of one HY[b]	€150	€125	€100	€150	€125	€100
Costs of one IVF cycle	€2500	€2550	€2600	€2500	€2550	€2600
Cumulative LBR[a]						
[Nohyst]	45.9%	46.7%	47.4%	45.9%	46.6%	47.1%
[Failedhyst]	46.3%	48.4%	50.7%	46.0%	47.1%	48.7%
[Routinehyst]	47.5%	50.4%	60.2%	46.1%	48.5%	52.8%
Total costs for one LB[c]						
[Nohyst]	€10870	€10851	€10833	€10870	€10867	€10913
[Failedhyst]	€10903	€10570	€10197	€10984	€10859	€10647
[Routinehyst]	€10733	€9341	€8045	€11143	€10604	€9610
ICER[d]						
[Failedhyst]	15000	2778	1111	133267	10004	2430
[Routinehyst]	6728	-1127	-2372	82554	3938	-1176

[a]: LBR: Live birth rate, [b]: HY: Hysteroscopy, [c]: LB: Live birth, [d]: ICER: Incremental cost-effectiveness ratio compared to the reference strategy [Nohyst], [e]: All variables in the model are out of favor of hysteroscopy, [f]: Base-case analysis, making use of all the average values of the variables in the model (Table 1) , [g]: All variables in the model are in of favor of hysteroscopy

panying costs were €10,851, €10,570, and €9341 per live birth, making strategy [Routinehyst] the least expensive and the most effective one of the three strategies (Table 3). Sensitivity analysis was performed, comparing the analysis of a scenario in which all model variables were in favor of routine hysteroscopy to a scenario in which all model variables were out of favor of routine hysteroscopy (Table 3). This illustrated that strategy [Routinehyst] was always dominant over strategies [Nohyst] and [Failedhyst]. In case only the increase in live birth rate through hysteroscopy was varied in the base-case analysis, strategy [Routinehyst] was found to even give a monetary profit over strategy [Nohyst] from an increase in live birth rate of 2.8% onwards (Figure 1).
The Monte Carlo uncertainty analysis, illustrated in an incremental cost-effectiveness plane is visualized in Figure 2. The interventions falling in a south-east

Figure 1 Sensitivity analysis illustrating the effect of the increase in live birth rate after hysteroscopy (x-axis) on the incremental cost effectiveness ratio (additional costs/additional live birth) (y-axis) for strategy [Failedhyst] and [Routinehyst] in relation to the reference strategy [Nohyst]

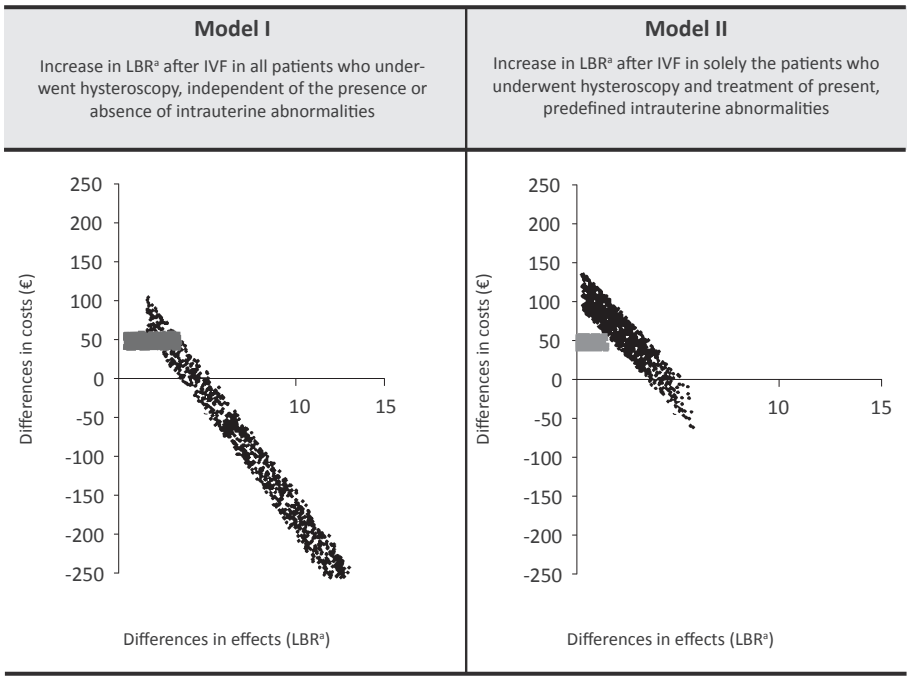


Note For this chart, only the variable 'increase in live birth after hysteroscopy' was varied in the base-case analysis, thereby showing the effect of this variable on the ICER. Remarkable are the exponential character of the chart and the fact that strategy [Routinehyst] is always positioned south from strategy [Failedhyst]. Strategy [Routinehyst] is thus accompanied with lower costs per additional live birth and, in Model I, even a monetary profit from an increase in live birth rate from ≥2.8%.

[a]: LBR: Live birth rate, [b]: ICER: Incremental cost effectiveness ratio (additional costs/additional live births)

quadrant are by definition cost-effective, as they combined positive effects with a decrease in costs. Interventions falling in a north-east quadrant are relatively cost-effective: increase in effects and increase in costs. Figure 2 illustrated that strategy [Routinehyst] is mainly positioned in the south-east quadrant, resulting in a monetary profit. Also, strategy [Routinehyst] is generally positioned south east in relation to strategy [Failedhyst], thereby visualizing that [Routinehyst] is the most cost-effective strategy. The probability of the strategies being cost-effective compared to the reference strategy [Nohyst] in relation to the willingness to pay for one additional live birth was shown in the cost-effectiveness acceptability curve (Figure 3). Strategy [Routinehyst] is shown to have the highest probability of being the strategy with the highest health benefit compared to the other two strategies From €2000 per added live birth onwards, there is a 90% probability that strategy [Routinehyst] is cost-effective compared to strategy [Nohyst].

Figure 2 The incremental cost-effectiveness plane: showing the difference in live birth rate in relation to the difference in costs for strategy [Failedhyst] (in grey) and [Routinehyst] (in black) versus strategy [Nohyst] (reference strategy). Data built up from 1000 random combinations of the values within the range of the variables that the model contains (Table 2).



Note Interventions falling in a south-east quadrant of the chart are by definition cost-effective, as they combined positive effects with a decrease in costs. Interventions falling in a north-east quadrant are relatively cost-effective: increase in effects accompagnied with an increase in costs. In both models, strategy [Routinehyst] is generally positioned (south-)east in relation to strategy [Failedhyst]. Strategy [Routinehyst] is thereby relatively cost-effective compared to strategy [Failedhyst].

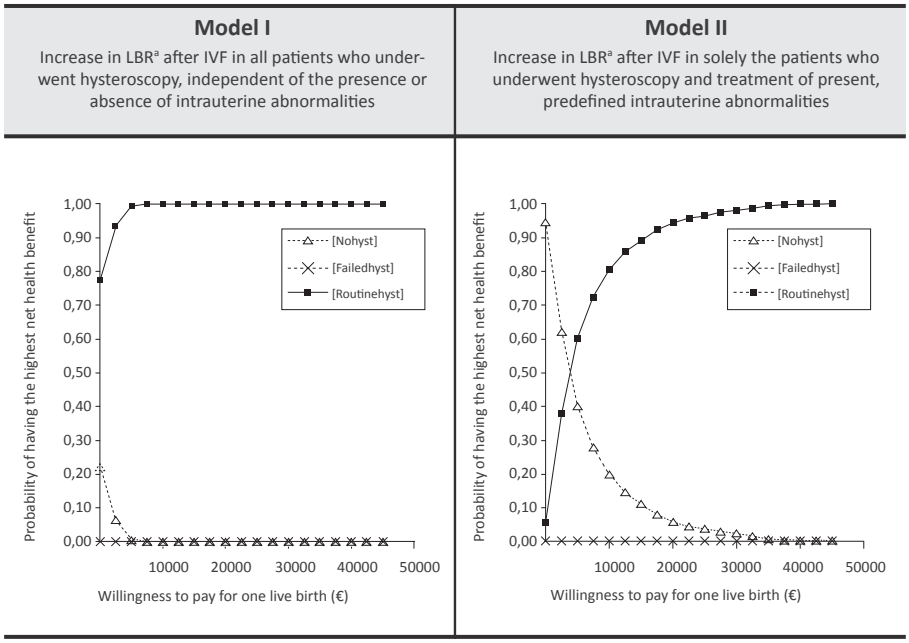
[a]: LBR: Live birth rate

Model II

In Model II, the live birth rate after IVF of solely patients with an intrauterine abnormality was assumed to increase in case they underwent screening hysteroscopy.

Making use of the average values of the model variables, base-case analysis showed that the cumulative birth rate were 46.6%, 47.1% and 48.5% for strategy [Nohyst], [Failedhyst] and [Routinehyst] respectively. The accompanying costs per live birth rate were €10,867, €10,859, and €10,604. Strategy [Routinehyst] was found to dominate strategy [Failedhyst], however hysteroscopy performance was accompanied with extensive costs (Table 3).

Figure 3 Acceptability curve showing the probability of having the highest net health benefit in relation to the willingness to pay for one additional live birth for the three strategies [Nohyst], [Failedhyst], [Routinehyst]



Note This chart illustrates that in Model I, strategy [Routinehyst] is most probably the strategy with the highest health benefit compared to the other two strategies. In Model II, the probability of strategy [Routinehyst] having the highest health benefit is always dominating the probability of strategy [Failedhyst] having the highest health benefit. From a certain willingness to pay for one additional live birth onwards, this also accounts for strategy [Routinehyst] compared to strategy [Nohyst].

[a]: LBR: Live birth rate

The cost-effectiveness plane shows that the greater part of the strategy [Routinehyst] did not fell in the south-east quadrant, implying that this strategy will probably not provide monetary profit in relation to strategy [Nohyst] (Figure 2). The cost-effectiveness acceptability curve showed that the probability of strategy [Routinehyst] having the highest health benefit was always dominating the probability of strategy [Failedhyst] having the highest health benefit. From €15,800 per added live birth onwards, there is a 90% probability that strategy [Routinehyst] is cost-effective compared to strategy [Nohyst] (Figure 3).

Discussion

Increasingly, it is recommended to perform a routine office hysteroscopy prior to an IVF treatment cycle (Bozdag *et al.*, 2008; El-Toukhy *et al.*, 2008). The rationale behind this suggestion is however based on limited research, which is of suboptimal quality and concerns a very specific patient population. Clinical investigation into the significance of hysteroscopy in the fertility work-up is time consuming. Model analysis can be useful by giving insight in the relationship between different parameters that influence the outcome after fertility treatment. The current study showed that the cost-effectiveness of a hysteroscopy in the fertility work-up mainly depends on its specific impact on the live birth rate and whether the model variable 'abnormality prevalence' was taken into account.

In Model I, the abnormality prevalence was not involved in the decision model. All patients who underwent hysteroscopy prior to IVF were considered to benefit from an increase in live birth rate. In such a model, strategy [Routine-hyst] seemed to generally dominate the other strategies. Strategy [Routine-hyst] was with a probability of 90% cost-effective over the reference strategy in case the willingness to pay for one added live birth is ≥€2000. This means that, according to the acceptability curve originating from the Monte Carlo simulation, performance of screening hysteroscopy is most probable cost-effective in case society is willing to pay at least €2000 per live birth on top of the costs of a live birth after IVF without screening hysteroscopy.

If the abnormality prevalence is taken into account in the decision analysis and solely patients with intrauterine abnormalities gained increase in pregnancy rate after hysteroscopic treatment, as was applied in Model II, the cost per live birth were considerably higher for all three strategies. This resulted in a considerably increase in willingness to pay for one additional live birth to the amount of >€15,800 to make routine hysteroscopy cost-effective compared to no hysteroscopy prior to IVF with a probability of 90%.

In Model I, the degree of increase in live birth rate after hysteroscopy was the only model variable which had an exponential effect on the incremental cost-effectiveness ratio of the strategies. This occurred due to the fact that the impact of this variable was accounted for in all subsequent IVF cycles. Unfortunately, the data on the increase in live birth rate after IVF treatment by hysteroscopy is based on sparse research, performed amongst women with ≤2 failed IVF cycles. Moreover, after hysteroscopy, the pregnancy rate between women with or without intrauterine pathology was similar. The explanation of the improvement of fertility after hysteroscopy is a matter of debate. It has been suggested that the hysteroscopy procedure itself may have a positive effect on the chance to conceive (Mooney and Milki, 2003). As the cost-ef-

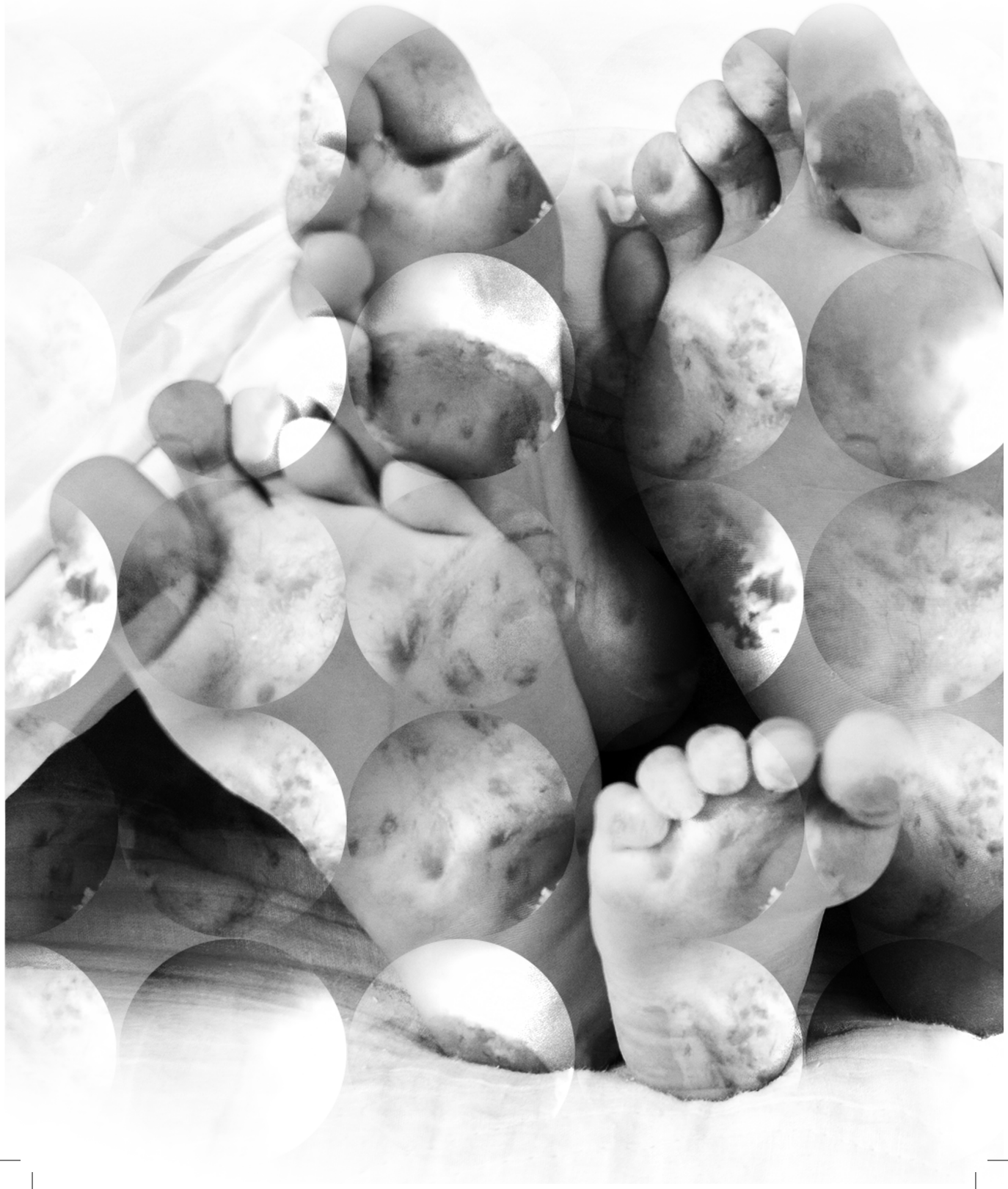
fectiveness of a strategy was most sensitive to the variation in the model assumptions concerning the increase in live birth rate, which was based on questionable data, Model II was designed. By analyzing Model II, it was tried to put the possible excessive effect of a routine hysteroscopy in perspective by taking into account the abnormality prevalence. Thereby it was found that variation in the abnormality prevalence also had exponential effect on the incremental cost-effectiveness ratio of a strategy, however, still not as excessive as the degree of improvement in live birth rate after hysteroscopy performance. Besides the debatable data on which the impact of routine hysteroscopy prior to IVF was based, another study weakness is the lack of research into the costs of outpatient hysteroscopy. The costs of an outpatient hysteroscopy versus a day case hysteroscopy have been assessed. However, the standard costs (travel expenses, overhead etc.) have not been analyzed (Marsh *et al.*, 2004). Therefore, questionnaires on the costs accompanying hysteroscopy in combination with standard costs had to be used. It appeared from the sensitivity analysis that the influence of the costs of one hysteroscopy on the study outcome was rather small.

A final study limitation could be the restricted amount of three IVF cycles analyzed. Thereby, the performance of hysteroscopy after 2 failed IVF cycles in strategy [Failedhyst] led to a significant increase in total costs per live birth, whereas the effect was only accounted for in one IVF cycle. For strategy [Routinehyst] relatively more hysteroscopies were carried out, resulting in slightly higher costs, but also in an increase in live birth rate for a total of three following IVF cycles. Nevertheless, analysis of three IVF cycles was thought to be closest to reality. Moreover, the considerable difference in cost-effectiveness between [Routinehyst] and [Failedhyst] would probably also remain by analyzing an extra IVF cycle and not have changed the study recommendations.

The Dutch society of Gynaecology as well as the ESHRE (European Society for Human Reproduction and Embryology) and RCOG (Royal College of Obstetricians and Gynaecologists) do not recommend hysteroscopy as initial investigation prior to starting IVF (Crosignani and Rubin, 2000; NVOG, 2004; RCOG, 2004). It has been argued that the significance of treating unsuspected intrauterine abnormalities has not yet been fully proven. The cost-effectiveness of the three strategies in both models was indeed mainly influenced by the impact of hysteroscopy on the chance to conceive. However, assuming that the impact of hysteroscopy passes through three IVF cycles, performance of hysteroscopy seems to be promising for improving the cost-effectiveness of IVF treatment.

It was shown, that the costs per live birth mainly increased, if the effect of hysteroscopy on the chance to conceive declined. Therefore, the question rises what society is willing to pay for one additional live birth. In 2004, the costs

per ongoing pregnancy resulting from IVF treatment (including the pre-IVF diagnostic work-up) was calculated to be €10,768 (Bouwmans *et al.*, 2008). Taking into account the health specific increase in expenses per year, the costs for an ongoing pregnancy in 2008 would be €11,532. According to Model I, these costs most probably increase by a maximum of €2000 in case hysteroscopy screening is performed. As stated in Model II, the costs per ongoing pregnancy resulting from IVF could raise by €15,800 -and therefore double-, making strategy [Routinehyst] not preferable. The general accepted amount to be paid for one additional QALY (quality-adjusted life year) was found to be >€20,000 (Hirth *et al.*, 2000). Therefore, it is to the healthcare physicians to decide whether the extra amount of €15,800 per live birth would justify the implementation of routine hysteroscopy prior to IVF treatment. As our study results mainly rely on the true effect of a screening hysteroscopy on the outcome of fertility treatment, further study on this subject is warranted. Currently, the inSIGHT trial is conducted, which will investigate the significance of routine hysteroscopy prior to a first IVF/ICSI treatment cycle in asymptomatic patients with a normal transvaginal ultrasound. With the outcomes of this trial it will be possible to further define the cost-effectiveness of that strategy. Thus, according to the published literature, the application of a routine hysteroscopy prior to IVF seems to be cost-effective. However, sensitivity analysis has shown that the cost-effectiveness of a scenario is most influenced by the variance in increase in ongoing pregnancy rate by performing a hysteroscopy. Moreover, in case solely patients with intrauterine abnormalities experience the positive effect of hysteroscopy on fertility, hysteroscopy is accompanied with significant extra costs for an additional live birth. Therefore, high quality data on this subject is crucial to recommend the most cost-effective strategy for daily practice.



Chapter 7

General Discussion

The uterus is the reproductive organ that plays a role in semen migration, embryo implantation and foetal nutrition. Embryo implantation is a multifactorial, highly complex procedure and concerns a continuous dialogue between a competent embryo and receptive endometrium. Increasingly, the factors involved in implantation are studied (Boomsma et al., 2009; Teklenburg et al., 2010, Horcajadas et al., 2007). However, a thorough understanding of the implantation process may not be within reach for the next decade. This also accounts for most of the presumed negative effect of congenital or acquired uterine cavity pathology on the chance to achieve a successful pregnancy.
The most frequent acquired abnormalities, the endometrial polyp and the submucous leiomyoma, may prevent embryo implantation by their mechanical effects, but also by causing an inflammatory response, release of biochemical factors or abnormal vascularisation. Congenital uterine malformations, for example the septate uterus, are thought to impair the implantation rate also by a decrease of vascularisation at site, as well as by the anatomical change of the uterine cavity. Finally, subacute or chronic endometritis forces an inflammatory reaction, inducing a uterine environment that becomes hostile for the implanting embryo (Bettocchi et al., 2011).

The implantation rate after embryo transfer in in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatment does not exceed 30%, warranting methods to optimize fertility treatment (Andersen et al., 2008). Increasing receptivity of the uterine cavity by treatment of uterine abnormalities has been posed. Up to now, hysteroscopy is solely advised to use as a treatment tool in case uterine pathology is suspected or after repeated IVF/ICSI treatment failure (Crosignani and Rubin, 2000; NVOG, 2004; RCOG, 2004). Hysteroscopy as a screening test prior to the start of fertility treatment or even as a standard part of the infertility work-up is under debate. The aim of this thesis was to explore the value of hysteroscopy as a screening tool in infertile patients, indicated for IVF treatment and not suspected of any intrauterine pathology based on history taking and a normal transvaginal sonography (TVS) examination. Focus was on the following topics:

- The prevalence of unsuspected intrauterine pathology
- The reliability of screening hysteroscopy as a diagnostic tool for unsuspected pathology
- The clinical significance and reliability of diagnosing endometritis by hysteroscopy guided endometrial biopsy
- The cost-effectiveness of screening hysteroscopy in assisted reproductive technology

Prevalence

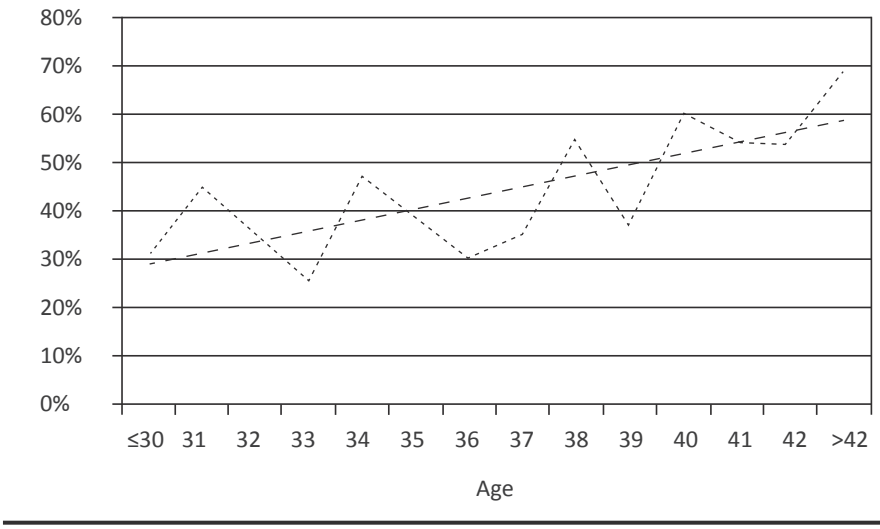
In infertility patients scheduled to undergo IVF, the prevalence of unsuspected intrauterine pathology according to cavity assessment by TVS or hysterosalpingography (HSG), was found to be between 20% and 45% (Balmaceda and Ciuffardi, 1995; La Sala et al., 1998; Hinckley and Milki, 2004). In *chapter 2*, the abnormality rate in our patient population was also evaluated. In 11% of the 678 examined asymptomatic women, with normal findings at TVS, some form of intrauterine pathology was detected. This finding even enlarged the range of the observed prevalence of intrauterine abnormalities at screening hysteroscopy in the current literature.

Consequently, investigation was initiated to the cause for this wide variance in abnormality prevalence between studies with a comparable study design. An explanation could be found in the diversity amongst the characteristics of the studied patient population. In the current study, the only correlation between the presence of abnormalities and a patient characteristic was found for female age. Most other researchers analysing this issue also found an increase of intrauterine pathology with age (Dicker et al., 1990; El-Mazny et al., 2009; Koskas et al., 2010). Koskas et al. observed that the risk of intrauterine pathology multiplied by a factor 1.5 every 5 years (Figure 1). Moreover, Body Mass Index (BMI) was found to have a positive correlation with the occurrence of endometrial polyps. In patients with a BMI of ≥30, 52% was diagnosed with endometrial polyps, compared to 15% of the patients with a lower BMI. It was hypothesized that there could be a connection between the increased levels of cytokines in the circulation of women with a higher BMI and the elevated levels of the same cytokines in the uterine washings when endometrial polyps were present (Onalan et al., 2009). Finally, in a population of patients, who suffered from secondary infertility, a higher incidence of intrauterine pathology was observed. Submucous leiomyoma, as well as adhesions and abnormalities at sight of the tubal ostia, were found to be more common in this specific group (Pansky et al., 2006; Shokeir et al., 2004).

Studies reporting the prevalence of intrauterine abnormalities in patients unsuspected of intrauterine pathology, all describe normal HSG findings (Shamma et al., 1992; Balmaceda and Ciuffardi, 1995; La Sala et al., 1998; Oliveira et al., 2003; Hinckley and Milki, 2004; Demirol and Gurgan, 2004; Doldi et al., 2005; Rama Raju et al., 2006; Lorusso et al., 2008). None of the studies, except Fatemi et al., mention the findings at TVS (Fatemi et al., 2010). As described by many authors, the accuracy of the diagnostic tests used to evaluate the uterine cavity may be rather diverse (*chapter 1*). Though certain studies have found HSG to be accurate, most of them described it to be insensitive for detection of intrauterine pathology (Gaglione et al., 1996; Golan et al., 1996). In case a HSG

instead of a TVS was performed as previous uterine cavity evaluation, one would expect to detect a higher prevalence of abnormalities at hysteroscopy. In general, not much effort has been made to describe patient history in detail. Overall, it remained uncertain whether the studied population concerned an unselected patient population. Each step of the fertility work-up, including all patient information, the semen analysis, diagnostic test etc. determines the a-priori chance for intrauterine abnormalities. The knowledge of certain -maybe non recorded- patient information could therefore influence the recognition of an abnormality by the hysteroscopy performer and with that, the study results. Therefore, possible selection or observation bias should be taken into account when comparing results of prevalence studies.

Figure 1 Rates of abnormal findings in 557 infertile women during office hysteroscopy according to age (Koskas et al., 2010)



Reliability

Next to a difference in patient variables, lack of reliability of the investigated diagnostic test, the hysteroscopy examination, could also cause variation in study results. During hysteroscopy it is possible to visualize the endometrial

lining directly and therefore detect the smallest abnormalities. The findings at hysteroscopy were found to be highly comparable to endometrial biopsy or histopathology examination after hysterectomy in case of gross pathology (Widrich et al., 1996; Fabres et al., 1998; Ceci et al., 2002; Dueholm et al., 2002[b]; Karageyim Karsidag et al., 2009). *Chapter 3* describes the accuracy of hysteroscopy in patients, who were unsuspected of any intrauterine pathology as they had no clinical signs or complaints and a normal TVS. The reproducibility between experienced physicians in diagnosing unsuspected intrauterine pathology from hysteroscopy recordings in infertile patients was found to be only moderate. The one other study, focussing on the interobserver agreement of hysteroscopy findings was performed in patients who underwent hysterectomy for symptomatic benign uterine diseases (Dueholm et al., 2002[a]). Though more profound cavity pathology was to be expected in such a patient population, the observed agreement was only slightly better. Moreover, the preliminary results of a recent international observer study seem to be in line. Eight hysteroscopy recordings of uterine cavities that were assumed to be normal, arcuate or septate, were shown on the European Society for Gynaecological Endoscopy (ESGE) website and evaluated by 65 ESGE members. Even interobserver agreement on an obvious feature like a septate uterus was found to be rather disappointing, as frequently an arcuate uterus was scored as a uterine septum (Table 1).
The validity and therefore the significance of routine hysteroscopy in asymptomatic infertile patients prior to IVF/ICSI may therefore still be questioned and may certainly affect the prevalence of findings at screening hysteroscopy in asymptomatic infertility patients.

Endometrial biopsy

Hysteroscopy offers the ability of performing a direct biopsy of suspected endometrial areas. With that, the diagnosis based on the visual observations can be confirmed and pathology of microscopic level can be detected. Polypoid endometrium is a relatively frequent detected feature of the endometrium. To reveal its origin, for example endometrial polyps, artefact, endometritis or hyperplasia, the endometrial biopsy could be helpful.
One of these minor abnormalities, chronic endometritis, is hardly ever clinically suspected. Though the presence of atypical polypoid structures, oedema or a special staining pattern after introducing methylene blue dye, may direct towards the presence of chronic endometritis, the diagnosis is ultimately based on histology examination (Cicinelli et al., 2005; Küçük and Safali, 2008). The prevalence of chronic endometritis in infertile patients is reported to be

Table 1 Preliminary results of the international observer study, showing the interobserver agreement on 8 hysteroscopy recordings of uterine cavities by 65 members of the European Society for Gynaecological Endoscopy (ESGE)

Variable (Sub)group	N	Interobserver agreement[b]	κ-value
Uterine shape (normal, arcuate, septate)			
- Overall	65	Slight	0.17
- Members who performed >4000 HYs[a]	16	Fair	0.25
- Members who had >10 years HY[a] experience	32	Slight	0.16
Metroplasty recommended			
- Overall	58	Slight	0.16
- Members who performed >4000 HYs[a]	17	Slight	0.15
- Members who had >10 years HY[a] experience	31	Slight	0.08

[a]: Hysteroscopy, [b]: According to Landis and Koch, 1977

between 0.2% and 46% (Polisseni et al., 2003; Wild et al., 1986; Feghali et al, 2003; Cicinelli et al., 2005; Johnston-Macananny et al., 2009). In *chapter 4* the prevalence was verified in our patient population and its impact assessed. Although the observed prevalence was as low as 2.8% and therefore the analysed population on IVF/ICSI outcome small, no trend towards a negative impact of chronic endometritis on reproductive oucome could be found. This was similar to the results of two studies that investigated patients with symptoms of pelvic inflammatory disease or repeated IVF failure (Haggarty et al., 2003; Johnson-Macananny et al., 2009).

The diagnosis chronic endometritis is difficult for a pathologist as the one specific criterion is the presence of plasma cells in the endometrial stroma. The interobserver agreement, discussed in *chapter 5* however does prove that the existence of this well circumscribed definition results in substantial reproducibility. Although the diagnosis of chronic endometritis was found to be reliable, endometrial biopsy is not proven to be indicated for diagnosis of the otherwise undetectable chronic endometritis in patients not suspect of intrauterine pathology. Thus, concerning the hysteroscopy guided endometrial biopsy, it seems to be only of value for histopathology examination or resected pathology.

Cost-effectiveness

Chapter 2 describes the evolvement of the TEA trial, which stands for "Treatment Efficacy of uterine Abnormalities". The purpose of this multicenter randomized controlled trial was to provide high quality evidence on the beneficial effects of diagnosis and treatment of intrauterine abnormalities by hysteroscopy on IVF/ICSI outcome. During the hysteroscopy, randomization was applied for immediate treatment versus no treatment in case intrauterine pathology was detected. Seen the low prevalence and the shortage of patients, willing to undergo randomization instead of direct treatment, the TEA trial was prematurely interrupted.

Two prospective randomized trials did succeed, due to the difference in timing of the randomization. Demirol and Gurgan as well as Rama Raju et al. investigated patients with ≥2 failed IVF treatment cycles and a normal HSG (Demirol and Gurgan, 2004; Rama Raju et al., 2006). Randomization took place for no hysteroscopy (group I) versus hysteroscopy (group II) and treatment in case intrauterine pathology was detected (group IIb). Abnormalities were found in 26% - 38% of all patients. The authors reported a 9% - 13% increase in clinical pregnancy rate between group I and group IIb (from 21.6% to 30.4% and from 26.2% to 39.6%, respectively). No significant difference was found in clinical pregnancy rate between patients who did have or did not have abnormalities at hysteroscopy. Three other observational studies reported similar results (Shamma et al., 1992; Oliveira et al., 2003, Lorusso et al., 2008).

Unfortunately, despite the results of the two randomized trials, high quality evidence on the effect of hysteroscopy on fertility is still absent due to some important study limitations (Demirol and Gurgan, 2004; Rama Raju et al., 2006). First, the authors of both trials did not describe all relevant patient characteristics, for example primary or secondary infertility, smoking and symptoms suggestive of intrauterine pathology. Second, it was not reported whether abnormal findings at TVS were present prior to deciding to perform the hysteroscopy. Due to the relative inaccuracy of HSG (which had been performed in these two studies), this could have led to an increased number of abnormalities, observed at hysteroscopy. Moreover, (if performed) it could not be assessed whether the hysteroscopy performer was blinded for the findings at TVS or not. Third, in a group of 210 patients, Demirol and Gurgan solely observed polyps and adhesions at hysteroscopy, the presence of leiomyoma or septa has not been commented upon. Thus, pathology that is most likely to affect pregnancy chances in IVF was not present in this study group.

The reported, major increase in pregnancy rates after hysteroscopy, regardless of hysteroscopy treatment, could be explained by two hypotheses. The hysteroscopy treatment of pathology leads to an increase in pregnancy rate, the

hysteroscopy procedure itself has an impact on fertility, or both hypotheses might play a role. The pregnancy rate in the group of patients without hysteroscopy (group I) is composed of the pregnancy rate of patients with and without abnormalities (group II and IIb). The group of patients with pathology (group IIb) is a lot smaller than the group of patients without pathology (group II). Therefore, in case the increase in pregnancy rate after hysteroscopy would solely be related to the treatment of pathology, the pregnancy rate prior to treatment in the group of patients with pathology (group IIb) would be (close to) zero. On the other hand, the hysteroscopy procedure itself could lead to a higher chance to conceive. To the best of our knowledge, only one published study has been performed on the impact of hysteroscopy itself on fertility. Mooney and Milki compared the pregnancy rate of patients who underwent hysteroscopy long before starting IVF and in the menstrual cycle prior to start ovarian hyperstimmulation. The pregnancy rates were found to be better in the latter group (Mooney and Milki, 2003). It was postulated that dilatation of the uterine cavity and tubes may be effective in improving fertility.
Thus, even through the literature reports a major effect of screening hysteroscopy prior to fertility treatment, good quality evidence to prove the true effect is still absent. Further study, preferably by performing multicenter randomized controlled trials, is still necessary to reveal the precise effect of hysteroscopy on fertility.

Making use of the effect size forthcoming out of the two randomized trials, the cost-effectiveness analysis of routine hysteroscopy prior to IVF was performed and described in *chapter 6*. It was found that screening hysteroscopy was always preferable over hysteroscopy after two IVF treatment cycles. Moreover, the profit of screening hysteroscopy was shown to be mainly influenced by the increase in pregnancy rate it caused, and the assumed aetiology behind the effect. In case hysteroscopy would result in an increase in pregnancy rate in both patients with and without treatment, hysteroscopy would be monetary profit if it increases the pregnancy rate by 2.8%. In case hysteroscopy would only result in a higher pregnancy rate in patients who undergo hysteroscopic treatment of detected pathology, hysteroscopy never gives a monetary benefit. In the latter situation, the question rises how much an additional pregnancy is worth paying for. The general accepted answer would be ≥€20.000 (Hirth et al., 2000). For that extra amount of money, the increase in pregnancy after hysteroscopy does not have to be of a major extent to be of value for the Dutch society (*chapter 6*). Thus, despite the true impact of hysteroscopy remains uncertain, the positive results of the available randomized trials make screening hysteroscopy prior to IVF most likely cost-effective. However, the exact abnormality prevalence and effect of hysteroscopy in patients initiating IVF/ICSI as well as the aetiology be-

hind the positive effect of hysteroscopy still is unknown. Therefore, thorough research to the true abnormality prevalence and impact of hysteroscopy on fertility is necessary before implementing hysteroscopy in daily fertility practice.

Future perspectives

International definitions

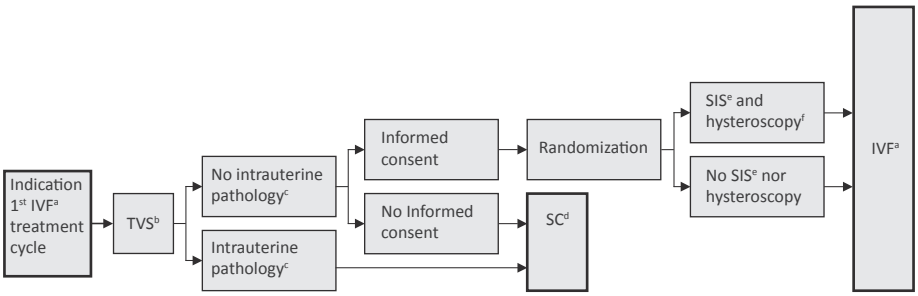
The prevalence of intrauterine abnormalities at hysteroscopy amongst infertile patients prior to fertility has a wide range (Balmaceda and Ciuffardi, 1995; La Sala et al., 1998; Hinckley and Milki, 2004; Fatemi et al., 2010). To provide the possibility to legitimately compare different study results, first, detailed description of patient characteristics and preliminary diagnostic work-up are necessary. Second, the reproducibility of screening hysteroscopy should be optimized. So far, it is assumed that direct visualization results in an obvious diagnosis and thereby interobserver agreement. In *chapter 2* it is reported not to be the case. International definitions of a normal and abnormal uterine cavity are absent. Recently, a proposal for a new subclassification of congenital Müllerian duct abnormalities at hysteroscopy has been posed (Gubbini et al., 2009). The author emphasized the lack of definitions to differentiate the septate uterus from an arcuate uterus. The difference is important as the septate uterus is thought to influence fertility and pregnancy outcome, whereas the arcuate uterus is said to be a variance of a normal uterine cavity. The definition was based on the outer contour of the uterus, the fundal thickness and endocavitary development of the 'septum', measured by 3D sonography and substantiated by hysteroscopy. It was shown that the new classification system led to more homogeneous data and the right treatment option. Similar studies to classify hysteroscopy findings such as endometrial polyps, leiomyoma, and adhesions are required too. Large series of comparisons between hysteroscopy findings and their correlation with histopathology examination of the biopsies of those findings should help make proper definitions.

InSIGHT-trial

Due to paucity of high quality evidence on the impact of unsuspected intrauterine abnormalities on IVF outcome in asymptomatic, infertile patients, there is the threat of widespread introduction of hysteroscopy and other imaging techniques prior to IVF/ICSI, without the certainty that this policy is (cost-)effective. Therefore, the inSIGHT trial proposal was made. "InSIGHT" stands for "SIGnificance of routine Hysteroscopy prior to a first IVF Treatment cycle" and obviously aims at assessment of the costs and effects of saline infusion sonography (SIS) and/or hysteroscopy prior to a first IVF/ICSI treatment

cycle in patients unsuspected of intrauterine pathology. In this multicenter trial, patients indicated to start fertility treatment would be randomized for the additional diagnostic tests, SIS and hysteroscopy, or not (Figure 2). Hysteroscopy will be performed in an outpatient setting and combined with endometrial biopsy to ascertain the hysteroscopy diagnosis. Primary outcome measures will be the live birth rate within 18 months after randomization. Secondary outcome measures will be the implantation rate and miscarriage rate in the same time span. Moreover, the total costs of the fertility treatment with and without the additional costs will be evaluated. As certain patient characteristics seem to be associated with a higher intrauterine abnormality rate, subgroup analysis will be performed to find out whether certain patients require specific diagnostic tests (Dicker et al., 1990; Shokeir et al., 2004; Pansky et al., 2006; El-Mazny et al., 2009; Onalan et al., 2009; Koskas et al., 2010). El-Toukhy has published the proposal for a similar trial amongst patients with repeated IVF/ICSI failure (El-Toukhy et al., 2009). The results of these trials are urgently needed, so that a proper proposal can made for the ideal pre-IVF work-up.

Flowchart



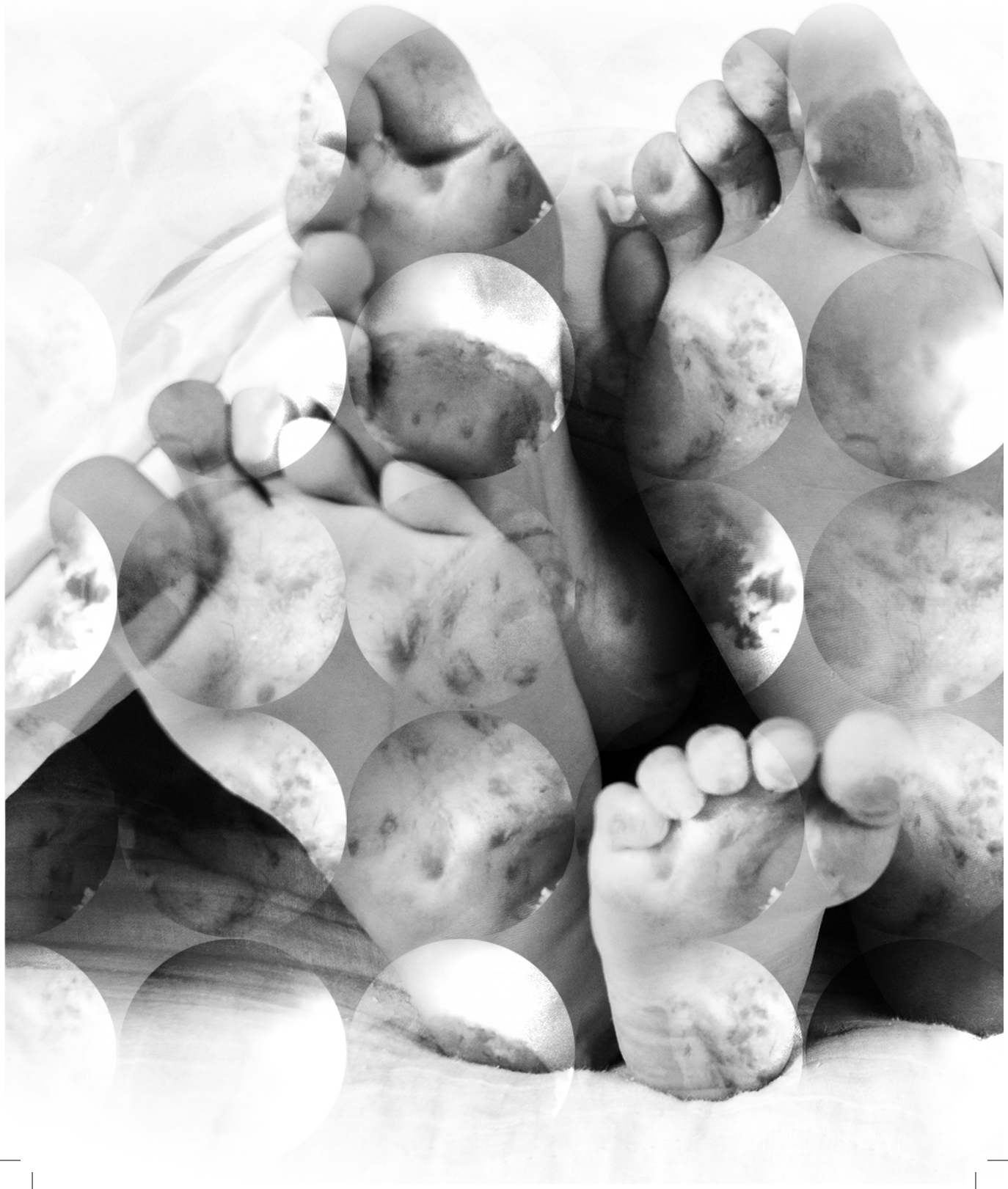
[a]: In Vitro Fertilization, [b]: Transvaginal Sonography, [c]: Predefined as endometrial polyps, submucous myoma, intrauterine adhesions or septa, [d]: Standard Care, [e]: Saline Infusion Sonography, [f]: Including instant treatment of possible detected abnormalities

Conclusion

Based on the available literature, the prevalence of intrauterine abnormalities detected by office hysteroscopy is relatively high, between 11% and 45% (Balmaceda and Ciuffardi, 1995; La Sala et al., 1998; Hinckley and Milki, 2004; Demirol and Gurgan, 2004; Rama Raju et al., 2006; Lorusso et al., 2008; Fatemi et

al., 2010). However, the true effect of routine hysteroscopy prior to infertility treatment has not been reliably assessed. Moreover, the validity of hysteroscopy in patients not suspected of intrauterine pathology is doubtful and the value of a routine endometrial biopsy for diagnosing endometritis is questionable. Making use of the available study results on the cost-effectiveness impact of screening hysteroscopy, hysteroscopy performance prior to IVF was found to be remarkably beneficial for the Dutch health care provider. Moreover, even if hysteroscopy would be less efficacious in improving fertility, it is plausible that screening hysteroscopy would still be cost-effective.

Seen the rapid evolvement of hysteroscopy towards a minimal invasive diagnostic and therapeutic tool, the execution of screening hysteroscopy in future might be expected (Bettocchi et al., 2011). Nevertheless, the need for high quality evidence to ascertain the exact costs and effects must be underlined.



Chapter 8

Summary in English
Summary in Dutch (Nederlandse samenvatting)

Summary

Chapter 1, the introduction, underlines the burden of IVF, describes the routine uterine cavity evaluation prior to in vitro fertilization (IVF) in the current fertility practice, and the continuous debate on the correct pre-IVF work-up. In the Netherlands, yearly 7000 couples rely on IVF or intracytoplasmic sperm injection (ICSI) treatment as a tool to enhance their chances for a live born child. Despite the numerous advances in the field of IVF/ICSI, there still exists a maximum implantation rate per embryo transferred of about 30%. Implantation failure could be due to the embryo, the uterine environment or a combination of both. Uterine cavity evaluation is generally performed by a transvaginal sonography (TVS) in combination with a hysterosalpingography (HSG) in case tubal pathology is suspected. Upcoming procedures are saline infusion/gel instillation sonography and hysteroscopy. Since hysteroscopy has become easy to perform in an outpatient setting, it is increasingly recommended to implement routine hysteroscopy screening prior to a first IVF/ICSI. However, high quality evidence for this recommendation is absent.
The aim of this thesis was to assess the true value of routine hysteroscopy in infertile, IVF/ICSI indicated patients, who are not suspected of intrauterine pathology. Focus was on the prevalence of intrauterine pathology, the observer agreement in evaluation of the uterine cavity, the endometrial biopsy and the cost-effectiveness of screening hysteroscopy.

Chapter 2. The prevalence of unsuspected intrauterine abnormalities at office hysteroscopy among infertile patients is rather diverse, between 20% and 45%. At the University Medical Center Utrecht (UMCU) and the Academic Hospital at the Dutch-speaking Brussels Free University (AZVUB), a group of 678 consecutive, unselected patients, indicated for IVF/ICSI and allocated for a randomized trial, underwent office hysteroscopy. Only asymptomatic patients, aged ≤42 years, with normal TVS findings and no previous hysteroscopy were included. At hysteroscopy, endometrial polyps were identified in 41 patients (6%) and submucous myomas in 6 patients (1%). Also intrauterine adhesions (2%) and septa (2%) were detected. Thus, the overall prevalence of any predefined intrauterine abnormality in this IVF/ICSI population was 11%, which was clearly lower than the previously reported prevalence. Such a low prevalence may have implications for the significance of intrauterine abnormalities regarding prospects in IVF/ICSI treatment cycles.

Chapter 3. Literature on the reproducibility of screening hysteroscopy is lacking. In the current study, screening hysteroscopies of 123 unselected, asymptomatic, infertile women with an indication for IVF/ICSI treatment were recorded

on DVD. After editing, the hysteroscopy performer and three other experienced gynaecologists independently assessed all recordings, focusing on the appearance of predefined intrauterine abnormalities (i.e. endometrial polyps, myomas, adhesions or septa). A total of 107 recordings remained for assessment and analysis. The intraobserver agreement on the appearance of any of the predefined intrauterine abnormalities was substantial (kappa = 0.707), whereas the interobserver agreement was moderate (kappa = 0.491). Perfect agreement occurred only in 77.6% of the cases. In conclusion, the interobserver agreement among experienced gynaecologists appeared to be rather disappointing. This may affect the diagnostic accuracy of screening hysteroscopy prior to IVF, as well as for the clinical significance in IVF programs.

Chapter 4. The prevalence of chronic endometritis has a big range and its impact on fertility is unclear. At the UMCU and the AZVUB a group of 678 patients, unsuspected of intrauterine pathology, underwent hysteroscopy guided endometrial biopsy prior to IVF/ICSI. The endometrial samples obtained were histological examined by two pathologists for the diagnosis chronic endometritis. Moreover, the live birth rate after initiation of IVF/ICSI treatment of the patients diagnosed with chronic endometritis was compared to the live birth rate of a randomly selected, matched control group of patients without endometritis. Chronic endometritis was diagnosed in 2.8% of all patients (17/606). The live birth rate did not significantly differ between patients with or without chronic endometritis, 76% versus 54% (P-value: 0.11). Also, the cumulative live birth rate per embryo transfer was not significantly different (Hazard Ratio 1.456, 95% CI: 0.770 - 2.750, P-value: 0.2). Therefore, the clinical significance of chronic endometritis on reproductive outcome seemed to be minimal.

Chapter 5. Chronic endometritis is a subtle condition, which is difficult to diagnose. The diagnosis is ultimately based on the presence of plasma cells in the endometrial stroma on histopathological examination. In a group of 678 infertile patients, unsuspected of intrauterine pathology, a hysteroscopy guided endometrial biopsy was obtained prior to IVF/ICSI treatment. All endometrial samples were independently examined by two pathologists. The slides diagnosed with chronic endometritis, replenished with a random sample of the remaining slides up to a total of 100, were exchanged between the two pathologists and reassessed. The prevalence of chronic endometritis was 2.8%. Perfect agreement between the pathologists was found in 86%. The interobserver agreement reached to substantial, with a kappa-value 0.66. It was concluded that the interobserver agreement was substantial and thereby the diagnosis chronic endometritis reproducible.

Chapter 6. The aim of the present study was to analyze the cost-effectiveness of screening hysteroscopy prior to IVF. The cost-effectiveness of two distinct strategies (hysteroscopy after 2 failed IVF cycles, [Failedhyst] and routine hysteroscopy prior to IVF, [Routinehyst]) was compared to the reference strategy (no hysteroscopy, [Nohyst]). When present, intrauterine pathology (polyps, leiomyoma, adhesions, septa) was treated during the hysteroscopy procedure. Two models were constructed and evaluated in a decision analysis. In Model I, which was based on the current literature, it was assumed that all patients who underwent screening hysteroscopy prior to IVF, would benefit of an increase in pregnancy rate. Model II -more hypothetical- assumed that the pregnancy rate solely increased in patients with intrauterine abnormalities, which were subsequently corrected by hysteroscopic treatment. For Model I (all patients benefit from hysteroscopy), strategy [Routinehyst] was generally cost-effective compared to strategy [Nohyst] or [Failedhyst]. In Model II (only patients with abnormalities benefit from hysteroscopic correction) [Routinehyst] also dominated [Failedhyst], however, hysteroscopy performance was accompanied with considerable costs. Sensitivity analysis demonstrated that the only model variable that influenced the cost-effectiveness considerably, was the variation in increase in live birth by performing hysteroscopy. In conclusion, screening hysteroscopy seems to be a cost effective procedure, however, high quality data on the true effect of hysteroscopy on fertility is necessary.

Chapter 7, the general discussion emphasizes the conclusions and clinical implications of this thesis. It was concluded that the prevalence of intrauterine abnormalities varies widely among studies with a comparable set-up and that the observed prevalence in our patient population of 11% was beneath average. Next to differences in the investigated patient populations, one of the explanations for this discrepancy could be found in the unsatisfying interobserver agreement on evaluation of the uterine cavity. Histopathology examination of the endometrial biopsy for diagnosing chronic endometritis was found to be accurate. However it seemed not useful as a routine procedure, due to the low prevalence of chronic endometritis and lack of trend towards a negative effect of the chance to conceive.
The literature on the impact of screening hysteroscopy in patients with a normal HSG shows promising results. In a group of patients with repeated IVF failure, hysteroscopy increases the pregnancy rate by 9% - 13%. Unfortunately, the studies are concerned with considerable weaknesses. However, the cost-effectiveness analysis showed that the effect of hysteroscopy does not have to be of that extent to still be cost-effective over a strategy without performance of a hysteroscopy.

Therefore, the trend towards a positive effect of hysteroscopy screening on fertility outcome cannot be denied. However, thorough research to the true prevalence of intrauterine abnormalities, true increase in pregnancy rate by hysteroscopy and etiology behind the positive effect are warranted, prior to implementation of routine hysteroscopy in the daily fertility practice.

Samenvatting

Hoofdstuk 1, de introductie van dit proefschrift, benadrukt de mentale en lichamelijke belasting die een in vitro fertilisatie (IVF) behandeling met zich meebrengt. Ook wordt het debat uiteengezet over het juiste aanvullend onderzoek ter evaluatie van de baarmoederholte, dat standaard zou moeten worden uitgevoerd voorafgaand aan IVF. Jaarlijks ondergaan in Nederland ongeveer 7000 paren IVF/ICSI (intra-cytoplasmatische sperma injectie) behandeling om hun zwangerschapskans te optimaliseren. Ondanks de vele ontwikkelingen op het gebied van IVF/ICSI is de kans op innesteling na embryo na terugplaatsing slechts 30%. Zowel eigenschappen van het embryo als de baarmoeder kunnen innesteling voorkomen. In de dagelijkse praktijk wordt de baarmoederholte gescreend op afwijkingen door middel van transvaginale echografie (TVE). Eventueel vindt er vervolgens een hysterosalpingografie (HSG) plaats, als er een verdenking bestaat op occlusie van de eileiders. Relatief nieuwe procedures waarmee de baarmoederholte onderzocht kan worden zijn de water- of gel-echo en de hysteroscopie (een kijkonderzoek naar de binnenzijde van de baarmoeder). Aangezien de hysteroscopie steeds eenvoudiger uitvoerbaar is, wordt er steeds vaker geadviseerd om dit onderzoek standaard uit te voeren voorafgaande aan een eerste IVF/ICSI cyclus. Echter, wetenschappelijk onderzoek van hoge kwaliteit, waarmee een dergelijk advies kan worden onderbouwd, ontbreekt. Het doel van dit proefschrift was om te achterhalen wat de waarde is van een routinematige hysteroscopie bij infertiele patiënten met een indicatie voor IVF/ICSI, die niet worden verdacht van afwijkingen in de baarmoeder. De focus was op de prevalentie van afwijkingen in de baarmoederholte, de overeenkomst tussen beoordelaars in het evalueren van de baarmoederholte, het baarmoederslijmvlies-biopt en de kosteneffectiviteit van een routine hysteroscopie.

Hoofdstuk 2. De prevalentie van afwijkingen in de baarmoederholte bij vrouwen zonder verdenking daarop, varieert aanzienlijk en is beschreven tussen de 20% en 45%. Een groep van 678 opeenvolgende, niet geselecteerde patiënten met een indicatie voor IVF/ICSI behandeling aan het Universitair Medisch Centrum Utrecht (UMCU) en het Academisch Ziekenhuis aan de Vrije Universiteit Brussel (AZVUB) werden geworven voor een gerandomiseerde trial en ondergingen poliklinisch een hysteroscopie. Alleen patiënten zonder klachten, een leeftijd ≤42 jaar, normale bevindingen op de TVE en nooit eerder een hysteroscopisch onderzoek, werden geïncludeerd. Tijdens de hysteroscopie werden bij 41 patiënten baarmoederslijmvlies poliepen gediagnosticeerd (6%) en submuceuze vleesbomen bij 6 patiënten (1%). Daarnaast werden verklevingen in de baarmoederholte (2%) en een tussenschot (2%) gedetecteerd. De prevalentie van alle vooraf gedefinieerde afwijkingen in de baarmoederholte was samen-

genomen 11%. De geobserveerde prevalentie was daarmee duidelijk lager dan de prevalentie zoals beschreven in de beschikbare literatuur. Een dergelijk lage prevalentie heeft mogelijk consequenties voor de betekenis van afwijkingen in de baarmoederholte aangaande de uitkomstverwachting van een IVF/ICSI behandeling.

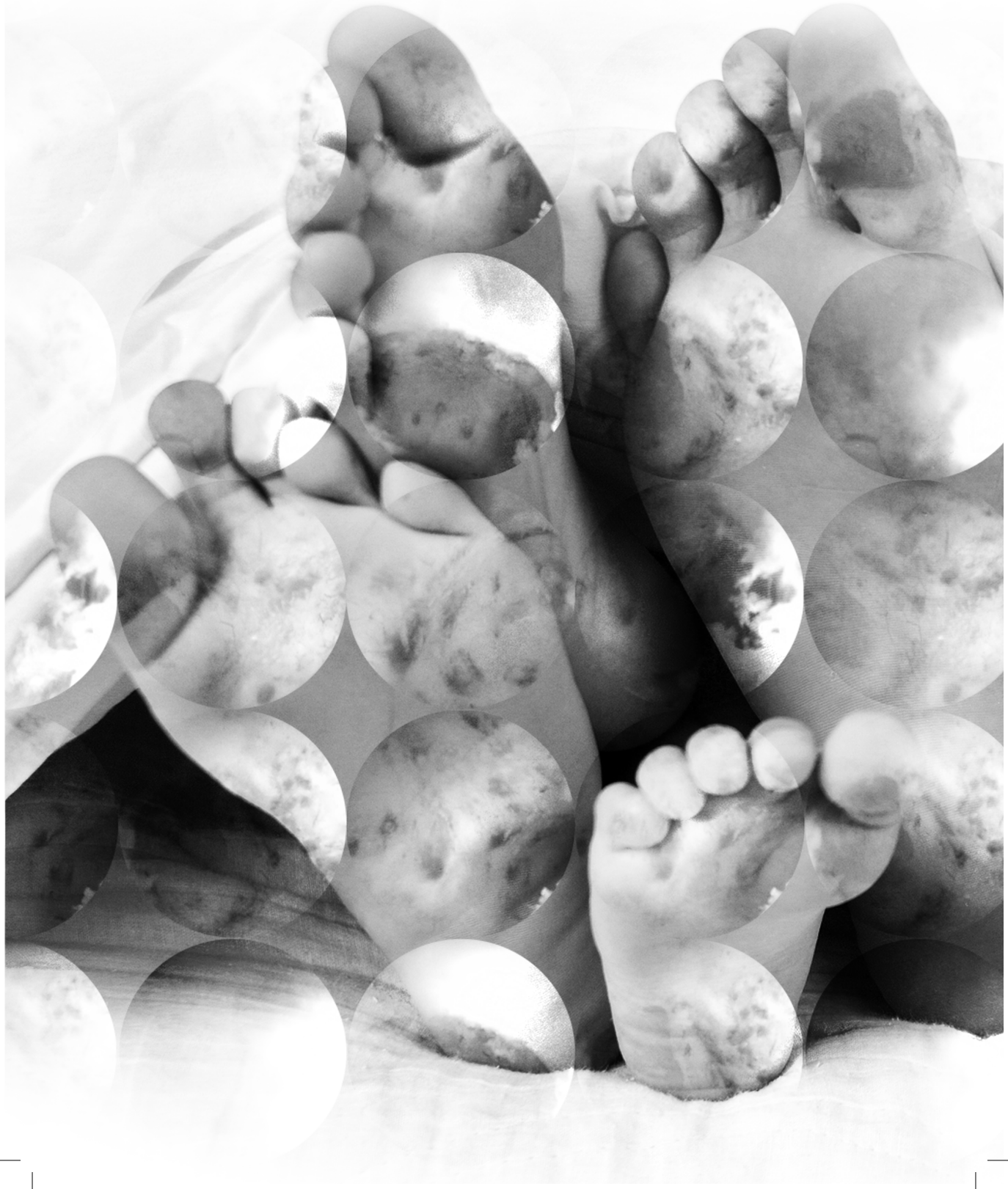
Hoofdstuk 3. Wetenschappelijk onderzoek naar de reproduceerbaarheid van een screenende hysteroscopie ontbreekt. In de huidige studie werden opnames gemaakt van 123 screenende hysteroscopiën bij infertiele vrouwen met een IVF/ICSI indicatie. Na bewerken van de DVD opnames werden ze onafhankelijk van elkaar geëvalueerd door de gynaecoloog die de hysteroscopie uitvoerde en drie andere, ervaren gynaecologen. Daarbij werd gelet op de aard van eventueel aanwezige, vooraf gedefinieerde afwijkingen in de baarmoederholte (baarmoederslijmvlies poliepen, vleesbomen, verklevingen en het tussenschot). Er bleven 107 DVD opnames over voor beoordeling en analyse. De overeenkomst tussen de originele bevindingen van de uitvoerende gynaecoloog en zijn herevaluatie over het aanwezig zijn van een afwijking of niet was aanzienlijk (kappa = 0.707). Daarentegen was de overeenkomst tussen de drie andere gynaecologen op dit punt slechts gemiddeld (kappa = 0.491). Precies dezelfde mening hadden zij in 77.6% van de gevallen. Concluderend was de overeenkomst in beoordeling van de baarmoederholte tussen drie gynaecologen teleurstellend. Dit heeft mogelijk effect op de diagnostische accuratesse en daarmee de klinische betekenis van een screenende hysteroscopie voorafgaand aan IVF.

Hoofdstuk 4. De prevalentie van een chronische ontsteking van het baarmoederslijmvlies, chronische endometritis, heeft een grote variatie en het effect ervan op de fertiliteit is onduidelijk. Een groep van 678 vrouwen, zonder verdenking op afwijkingen in de baarmoederholte, ondergingen een baarmoederslijmvlies-biopt tijdens een hysteroscopie aan het UMCU of AZVUB, voorafgaand aan IVF/ICSI. De verkregen biopten werden histopathologisch onderzocht door twee pathologen op de aanwezigheid van chronische endometritis. Vervolgens werd de kans op een levend geboren kind na IVF/ICSI van patiënten met chronische endometritis vergeleken met de kans op een levend geborene van een willekeurig geselecteerde, gematchte controle groep van patiënten zonder chronische endometritis. Chronische endometritis werd gediagnosticeerd in 2.8% van alle patiënten (17/606). De cumulatieve zwangerschapskans tussen de patiënten met of zonder endometritis verschilde niet significant, 76% ten opzichte van 54%. Ook was de zwangerschapskans per teruggeplaatst embryo niet significant verschillend (Hazard Ratio 1.456, 95% CI: 0.770 - 2.750, P-value: 0.2). Daarmee is aangetoond, dat een duidelijk effect van chronische endometritis op de vruchtbaarheid van een vrouw onwaarschijnlijk is.

Hoofdstuk 5. Chronische endometritis is een subtiele aandoening, die moeilijk te diagnosticeren is. De diagnose is uiteindelijk slechts gebaseerd op de aanwezigheid van plasma cellen bij het histopathologisch onderzoek van een baarmoederslijmvlies-biopt. Bij een groep van 678 infertiele patiënten, die niet werden verdacht van afwijkingen in de baarmoederholte, werd een baarmoederslijmvlies-biopt afgenomen tijdens een hysteroscopie voorafgaande aan IVF/ICSI behandeling. De biopten werden onderzocht door twee pathologen. De biopten van de patiënten, bij wie chronische endometritis was gedetecteerd, werden aangevuld met een willekeurige selectie van de biopten van de andere patiënten, tot een totaal aantal van 100. Het bioptmateriaal van deze 100 patienten werd uitgewisseld tussen de twee pathologen en herbeoordeeld. De pathologen hadden exact dezelfde beoordeling in 86%. De overeenkomst tussen de pathologen kwam tot substantieel (kappa-waarde: 0.66). Concluderend was de overeenkomst tussen twee pathologen substantieel en daarmee de diagnose chronische endometritis reproduceerbaar.

Hoofdstuk 6. Het doel van deze studie was om de kosteneffectiviteit van een hysteroscopie voorafgaande aan IVF te analyseren. De kosteneffectiviteit van twee strategieën (een hysteroscopie na 2 mislukte IVF behandelingen, [Gefaaldehyst] en een routinematige hysteroscopie voorafgaande aan de eerste IVF behandeling, [Routinehyst]) werd vergeleken met die van de referentie strategie (geen hysteroscopie, [Geenhyst]). Indien aanwezig, werden afwijkingen in de baarmoederholte (baarmoederslijmvlies poliepen, vleesbomen, verklevingen en een tussenschot) verwijderd tijdens de hysteroscopie. Er werden twee modellen geanalyseerd. In Model I, die berustte op gegevens uit de literatuur, werd verondersteld dat alle patiënten stegen in hun kans op zwangerschap na hysteroscopisch onderzoek. In Model II, een meer hypothetisch model, werd ervan uit gegaan dat alleen de patiënten met afwijkingen in de baarmoederholte na hysteroscopische verwijdering een toename in zwangerschapkans ondervonden. Uit analyse van Model I (alle vrouwen hebben baat bij hysteroscopie) bleek dat strategie [Routinehyst] doorgaans kosteneffectief was ten opzichte van strategie [Gefaaldehyst] en [Geenhyst]. In Model II (alleen patiënten met afwijkingen hebben baat bij hysteroscopie) domineerde [Routinehyst] ook [Gefaaldehyst], echter, ging dat gepaard met aanzienlijk hogere kosten. Sensitiviteit analyse liet zien dat de variatie in toename in zwangerschapskans door hysteroscopie de meeste invloed had op het verschil in kosteneffectiviteit. Concluderend lijkt een screenende hysteroscopie voorafgaand aan IVF kosteneffectief, echter, wetenschappelijk onderzoek van hoge kwaliteit naar het werkelijke effect van hysteroscopie op de vruchtbaarheid is noodzakelijk.

Hoofdstuk 7, de algemene discussie, zet de conclusies en klinische consequenties uiteen van het onderzoek dat is beschreven in dit proefschrift. Er werd geconcludeerd dat de geobserveerde prevalentie van afwijkingen in de baarmoederholte tamelijk verschilt tussen vergelijkbare studies. De door het huidige onderzoek bevonden prevalentie van 11% is lager dan de gemiddelde prevalentie. Naast het verschil in de onderzochte patiënt populaties zou een verklaring voor de discrepantie een lichte tekortkoming aan overeenkomst tussen gynaecologen kunnen zijn in hun beoordeling van de baarmoederholte. Histopathologisch onderzoek van het baarmoederslijmvlies biopt bleek accuraat voor de diagnose chronische endometritis. Het lijkt echter niet nuttig als routine procedure, vanwege de lage prevalentie van chronische endometritis en gebrek aan aanwijzingen voor een negatief effect ervan op de zwangerschapskans.
Het wetenschappelijk onderzoek naar de waarde van een screenende hysteroscopie bij patiënten met een normale HSG toont veelbelovende resultaten. In een groep patiënten met herhaaldelijk IVF falen, stijgt de zwangerschapskans na hysteroscopie met 9% - 13%. Helaas gaan deze studies gepaard met zekere tekortkomingen. Desondanks laat de kosteneffectiviteit analyse zien, dat het effect van een hysteroscopie niet tot aanzienlijk veel meer zwangerschappen hoeft te leiden om kosteneffectief te zijn in vergelijking met een strategie zonder hysteroscopie.
Dus, de trend richting een positief effect van screenende hysteroscopie bij infertiele patiënten kan niet worden genegeerd. Desalniettemin is grondig wetenschappelijk onderzoek naar de werkelijke prevalentie van afwijkingen in de baarmoederholte, werkelijke stijging in zwangerschapskans na hysteroscopie en de etiologie van het positieve effect van een hysteroscopie noodzakelijk, voordat een routine hysteroscopie in de dagelijkse voortplanting kliniek wordt ingevoerd.



Chapter 9

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Words of Appriciation (Dankwoord)
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Curriculum Vitae

Jenneke Cornelia Kasius was born on the 12th of September 1985 in Driebruggen, The Netherlands. There, she grew up with her four sisters; Karin, Marjan, Thera en Irene. At the age of eleven, her parents got divorced. They both remarried and on her father's side the family was expended with a half-sister and brother, twins; Jorie and Jurriën.

Jenneke attended the gymnasium at the secondary school, 'De Goudse Waarden' in Gouda, from which she graduated in 2003. Subsequently, she started medical school at the University Utrecht. Early in her studies gynaecology and obstetrics caught her interest. Consequently, she started her scientific carrier at the department 'Woman and Baby' at the University Medical Center Utrecht under supervision of Prof. dr. F.J.M. Broekmans. Her focus was on the "Significance of hysteroscopy screening prior to assisted reproduction". Jenneke also has an interest for international differences in health care and therefore she travelled to Nepal, South Africa and the United Kingdom for internships at different departments. After obtaining her medical degree in September 2009, she continued her research project as a PhD thesis (supervisor Prof. dr. F.J.M. Broekmans, Prof. dr. H.M. Fatemi and Prof. dr. B.C.J.M. Fauser). In March 2010 she started to combine her research with clinical work at the department of Gynaecology and Obstetrics at the "St. Antonius Hospital", Nieuwegein (supervisor Dr. J. H. Schagen van Leeuwen). In august of the same year she started her official residency Gynaecology and Obstetrics at the "Twee Steden Ziekenhuis" Tilburg (supervisor Dr. A.E.M. Roosen and Dr. H.J.H.M. van Dessel). From august 2011 she will continue her residency at the University Medical Center Utrecht (supervisor Prof. dr. G.H.A. Visser).