



KATJA AHINKO

Successful Intrauterine
Insemination Treatment



ACADEMIC DISSERTATION

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University of Tampere, Medical School

Tampere University Hospital, Department of Obstetrics and Gynecology

Finland

Supervised by

Helena Tinkanen, MD, PhD

University of Tampere

Finland

Professor Pertti Kirkinen

University of Tampere

Finland

Reviewed by

Docent Maritta Hippeläinen

University of Kuopio

Finland

Professor Outi Hovatta

Karolinska Institutet, Stockholm

Sweden

Distribution

Bookshop TAJU

P.O. Box 617

33014 University of Tampere

Finland

Tel. +358 3 3551 6055

Fax +358 3 3551 7685

taju@uta.fi

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To my daughters

Emma and Iida

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1 LIST OF ORIGINAL COMMUNICATIONS

This thesis is based on the following original publications, referred to in the text by their Roman numerals.

I Ahinko-Hakamaa K, Huhtala H and Tinkanen H (2007): The validity of air and saline hysterosalpingo-contrast sonography in tubal patency investigation before insemination treatment. *Eur J Obst Gyn Repro Biol* 132: 83-87.

II Ahinko-Hakamaa K, Huhtala H and Tinkanen H (2009): Confirmation of tubal patency in hysterosalpingo-contrast sonography by transvaginal hydrolaparoscopy. *Acta Obstet Gynecol Scand* 88: 286-90.

III Ahinko-Hakamaa K, Huhtala H and Tinkanen H (2007): Success in intrauterine insemination: the role of etiology. *Acta Obstet Gynecol Scand* 86: 855-860.

IV Ahinko K, Ahola A, Huhtala H and Tinkanen H: Impact of post-wash sperm morphology on the success of intrauterine insemination. Submitted.

2 ABBREVIATIONS

AI	Aromatase inhibitor
ART	Assisted reproductive technique
CC	Clomifene citrate
CI	Confidence interval
CPR	Cumulative pregnancy rate
FSH	Follicle-stimulating hormone
GnRH	Gonadotrophin-releasing hormone
hCG	Human chorionic gonadotrophin
HMG	Human menopausal gonadotrophin
HSG	Hysterosalpingography
HyCoSy	Hysterosalpingo-contrast sonography
ICSI	Intracytoplasmic sperm injection
IMC	Inseminating motile sperm count
IUI	Intrauterine insemination
IVF	In vitro fertilization
L	Letrozole
LH	Luteinizing hormone
MPR	Multiple pregnancy rate
NPV	Negative predictive value
PPV	Positive predictive value
PR	Pregnancy rate
rFSH	Recombinant follicle-stimulating hormone
THL	Transvaginal hydrolaparoscopy
TMC	Total motile sperm count
TSH	Thyroid-stimulating hormone/thyrotrophin
TZI	Teratozoospermia index
uFSH	Urinary follicle-stimulating hormone
WHO	World Health Organization

3 ABSTRACT

Intrauterine insemination (IUI) is defined as direct transfer of motile spermatozoa into the uterine cavity, after semen preparation and concentration in a small volume of medium. It has been considered as a treatment option for mild to moderate male infertility, and also for couples with unexplained infertility or ovulatory dysfunction and mild endometriosis.

The aim of the present study was to make an attempt to find out factors affecting the success of IUI treatment. Special interest was focused on the evaluation of tubal patency and the aetiology of infertility. In addition, the impact of post-wash sperm morphology as regards successful IUI was investigated.

In a retrospective study the validity of air and saline hysterosalpingo-contrast sonography (HyCoSy) in tubal patency investigation in relation to IUI success was evaluated in a cohort of 559 women. Altogether, 1240 cycles were investigated by hysterosalpingography (HSG) (n=175), laparoscopy (n=571) and HyCoSy (n= 494). There were no statistically significant differences in pregnancy rates (PRs) per cycle between these three subgroups. After two IUI cycles the cumulative pregnancy rate (CPR) was significantly better in the HyCoSy group (41%) compared with the laparoscopy group (30%) and it was similar to that in the HSG group (38%). However, there were no significant differences in delivery rates (31%; 22%; 26%) between the subgroups. Neither were the numbers of tubal pregnancies significantly different in the subgroups. Even in cases of unilateral tubal patency the CPR was 29% in the HyCoSy and HSG groups and 18% in the laparoscopy group.

The accuracy of HyCoSy was confirmed by transvaginal hydrolaparoscopy (THL) in a prospective series of 56 infertile women with a history of tubal risk factors. The concordance between the two procedures was 77%. With HyCoSy the sensitivity was 95%, the specificity 73%, the negative predictive value 98% and the positive predictive value 45%. Suspected tubal occlusion in HyCoSy was confirmed by THL to be due to benign tubal spasm in every second case. Transvaginal hydrolaparoscopy appeared to be a safe and inexpensive procedure to confirm the findings in HyCoSy.

To assess the role of aetiology in IUI success, 532 subjects with a total of 1171 IUI cycles were retrospectively analysed. Five subgroups were formed according to the cause of infertility: unexplained (54.4%), ovulatory disorders (16.8%), male factor (13.2%) endometriosis (10.8%) and multiple reasons (4.8%).

In cases of unexplained infertility the stimulation protocol was not crucial but the PR turned out to be highest with three follicles (24.2%) compared with one follicle (9.2%), the multiple pregnancy

rate (MPR) being only 8.9%. A sperm count of $> 30 \times 10^6/\text{mL}$ was also a statistically significant positive prognostic factor.

Women with anovulatory infertility had the best PRs (19.2%), but also the highest risk of multiple pregnancy (13.2%). Stimulation with sequential clomifene citrate (CC) and human menopausal gonadotrophin (HMG) resulted in the best PR. Therefore, these women have to be stimulated with caution, aiming at a monofollicular response.

In male infertility there were no significant predictors of best PR to be found. Female age up to 39 years was not a prognostic factor, nor was the duration of infertility. The PR was 18.2%, with a low MPR of 3.6%. Even a sperm count of less than $5 \times 10^6/\text{mL}$ resulted in a PR of 16.5%. In women with endometriosis, young age and a partner's good total motile sperm count (TMC) of $> 30 \times 10^6/\text{mL}$ seemed to increase the PR. Stimulation with CC with more than one follicle yielded the highest PR. In this subgroup the PR was 11.9%, being the lowest of all. The remaining subgroup of 'multiple reasons' was too small to draw any conclusions.

The impact of post-wash sperm morphology on PR was assessed in 170 cases with male infertility. Teratozoospermia was found in 253 IUI cycles, of which 125 (49%) were classified as isolated teratozoospermia. Even a small increase in the percentage of normal forms in post-wash sperm sample, when accompanied by a decrease in head anomalies, was a positive prognostic factor as regards pregnancy after IUI. However, isolated teratozoospermia did not affect the success rate of IUI.

In the light of these results, it appears that HyCoSy is a reliable and cost-effective tubal investigation method in selection of subjects for insemination. The change of clinical practice from laparoscopy to HyCoSy as a first-line tubal investigation method has not impaired the results of IUI as regards the cumulative live birth rate. Insemination is also a valid treatment in cases of unilateral tubal patency. However, in cases of suspected tubal occlusion in HyCoSy, an invasive method should be applied to exclude benign tubal spasm. Nowadays THL is rarely used as an investigation method in Finland but its introduction could decrease the need of laparoscopic procedures. The combination of HyCoSy and THL is a novel and time-saving method to verify tubal patency. The present results suggest a new way to plan an ideal and individual infertility treatment by considering the cause of infertility. This is a result of the finding in the present study that the factors affecting PR, such as the number of follicles, the stimulation protocol and sperm count are not identical as regards the different aetiologies of infertility. This makes it possible to achieve the final goal of IUI: one child at a time. Sperm morphology seems to be more crucial in IUI than in IVF/ICSI treatment, but teratozoospermia as the only sperm defect can be ignored when planning appropriate infertility treatment.

Despite the rapid development of assisted reproductive techniques, as a low-cost and low-tech method, IUI treatment still has a remarkable place in the field of infertility treatment. The future of IUI treatment will depend on our ability to maintain a low rate of multiple pregnancies without affecting the success rate of IUI.

4 TIIVISTELMÄ

Inseminatiohoito valitaan usein ensimmäiseksi lapsettomuuden hoitomuodoksi, kun siemennesteessä on todettu lievästi tai kohtalaisesti alentunut hedelmöittämiskyky. Sitä voidaan käyttää myös silloin, kun kyseessä on selittämätön lapsettomuus, munasolun irtoamishäiriö tai lievä endometrioosi. Inseminatiossa pesty siemenneste ruiskutetaan kohtuun ovulaatioajankohtana. Hoidon edellytyksenä on munasolun kypsyminen ja irtoaminen, avoimet munajohtimet ja riittävä liikkuvien siittiöiden määrä.

Tämän väitöstutkimuksen tavoite oli selvittää inseminatiohoidon onnistumiseen johtavia tekijöitä, erityisesti raskauden ennustetekijöitä eri syistä johtuvassa lapsettomuudessa kuin myös munajohdinten tutkimusmenetelmän ja siemennesteen morfologian ennusteellista arvoa.

Ensimmäinen retrospektiivinen tutkimus selvitti onko munajohdinten tutkimusmenetelmällä merkitystä inseminatiohoidon onnistumiseen erityisesti silloin, kun ensisijaisena tutkimusmenetelmänä on ei-invasiivinen (HyCoSy). Tutkimuksen kohteena oli 559 lapsetonta paria, joille oli tehty 1240 inseminatiohoitoa. Tutkimuksessa verrattiin kolmen eri munajohdintutkimusmenetelmän, HyCoSyn (n=494), vatsaontelon tähystyksen (n=571) ja röntgentutkimukseen perustuvan varjoainetutkimuksen (HSG) (n=175) luotettavuutta suhteessa inseminatiohoitojen raskaustuloksiin. Osoittautui, että kaikki kolme menetelmää johtivat yhtä hyvään tulokseen, jos mittarina käytettiin elävänä syntyneiden lasten määrää pariskuntaa kohti. Munajohdinraskauksia esiintyi kaikissa ryhmissä yhtä paljon. Kumulatiiviset raskaustulokset kahden hoidon jälkeen olivat hyviä myös tapauksissa, joissa vain toinen munajohdin oli auki (HyCoSy ja HSG 29% ; vatsaontelon tähystys 18%).

Toinen munajohtimien aukioloa selvittävä prospektiivinen tutkimus käsitteli HyCoSy:n luotettavuutta varmistettuna emättimen kautta tapahtuvalla vatsaontelontähystyksellä (THL). Osatyön aineistona oli 56 lapsettomuudesta kärsivää naista, joilla oli taustalla jokin munajohdinvaurioon liittyvä riskitekijä (leikkaus, tulehdus tai kohdunulkopuolinen raskaus). Tutkimusten yhtäpitävyys kahden eri menetelmän välillä oli 77%. HyCoSy:n herkkyys oli 95%, tarkkuus 73%, negatiivinen ennustearvo 98% ja positiivinen ennustearvo 45%. Tulosten mukaan HyCoSy osoittaa luotettavasti munatorvien aukiolon. Sen sijaan epäilty munajohtimien tukos osoittautui varmistavalla THL-tutkimuksella lähes puolessa tapauksissa hyvänlaatuisiksi munajohtimien supistustilaksi.

Yksi tavoitteistamme oli löytää mitkä tekijät inseminatiohoidossa ennustavat raskauden alkamista eri syistä johtuvassa lapsettomuudessa. Tutkimukseen osallistui osa (532) ensimmäisen tutkimuksen potilaista, joille oli suoritettu 1170 keinohedelmöityshoitoa. Parit jaettiin viiteen eri alaryhmään lapsettomuuden syyn perusteella: selittämätön (54.4%), munasolun irtoamishäiriöstä

johtuva (16.2%), miehestä johtuva (13.2%), endometriosisista johtuva (10.8%) ja useasta syystä johtuva (4.8%).

Selittämättömässä lapsettomuudessa raskausprosentti oli 14.1% ja kypsien munarakkuloiden määrä ennusti raskaustulosta tilastollisesti merkittävästi, ollen 24.2% kolmella munarakkulalla ja vain 9.2% yhdellä munarakkulalla. Monisikiöraskauksia tässä ryhmässä oli 8.9%. Pesunjälkeinen liikkuvien siittiöiden määrä ($>30 \times 10^6$) oli myös tilastollisesti merkittävä positiivinen ennustetekijä raskauden suhteen. Ovulaatiohäiriöryhmässä sekä raskausprosentti (19.2%) että riski monisikiöraskauteen oli korkein (13.2%). Tilastollisesti merkittävä ennustetekijä oli klomifeenin ja virtsaperäisen gonadotropiinin yhdistelmä munasarjojen stimulaatiossa. Näillä potilailla on stimulaatiossa pyrittävä yhden munarakkulan vasteeseen. Miehestä johtuvassa lapsettomuudessa tilastollisesti merkittäviä raskautta ennustavia tekijöitä ei tullut esiin. Huomattavaa oli, että tässä ryhmässä naisen iällä (<39 -v) ei ollut merkitystä hoidon tulokseen. Raskaustulokset olivat kuitenkin hyvät (18.2%) ja monisikiöraskauksia oli vähiten (3.6%). Raskausprosentti oli hyvä (16.5%) myös silloin, kun pesunjälkeinen liikkuvien siittiöiden määrä oli alle 5×10^6 . Endometriosisiryhmässä raskauserot olivat matalimmat (11.9%) ja positiivisia ennustetekijöitä olivat nuori ikä sekä korkea pesunjälkeinen liikkuvien siittiöiden määrä ($>30 \times 10^6$). Pareja, joilla oli useasta syystä johtuva lapsettomuus, oli tutkimuksessamme niin vähän, että luotettavia johtopäätöksiä ei voitu vetää.

Neljäs prospektiivinen osatyö paneutui siittiöiden rakenteellisten poikkeavuuksien vaikutukseen inseminaatiohoidon onnistumisessa. Tutkimukseen osallistui 170 paria, jotka kärsivät miehestä aiheutuvasta lapsettomuudesta. Heiltä analysoitiin yhteensä 268 hoitosykliä. Rakenteeltaan poikkeavien siittiöiden osuus oli epänormaali 253 hoitosyklissä ja näistä 125 (49.4%) tapauksessa se oli ainoa löydös. Havaitsimme, että jo muutaman prosenttiyksikön nousu rakenteeltaan normaalien siittiöiden määrässä siemennesteen pesun jälkeen oli tilastollisesti merkittävä tekijä onnistuneen inseminaatiohoidon suhteen. Tämä näytti johtuvan siittiöiden pääepämuodostumien vähenemisestä. Jos siemennesteen muut tekijät olivat normaalit, ei siittiöiden rakennepoikkeavuuksilla näyttänyt olevan merkitystä inseminaatiohoitojen onnistumisessa.

HyCoSy on luotettava ja erittäin kustannustehokas munajohtimien tutkimusmenetelmä valittaessa potilaille sopivaa hedelmöityshoitomenetelmää. Vatsaontelon tähytyksen korvaaminen HyCoSy:llä ensisijaisena munajohdinten tutkimusmenetelmänä ei vaikuttanut IUI-hoidon onnistumiseen, kun tuloksia verrattiin elävänä syntynyttä lasta kohti. Inseminaatiohoito kannattaa toteuttaa myös silloin, kun vain toinen munajohdin on auki. HyCoSy osoittaa luotettavasti munajohtimien aukiolon, mutta jos epäillään munajohtimien tukosta, täytyy löydös varmistaa invasiivisella menetelmällä munajohdinten supistustilan poissulkemiseksi. Vaikka THL:n käyttö Suomessa on vielä vähäistä, sen käytön lisääntyminen tulisi vähentämään vatsaontelon tähytyksen

tarvetta. HyCoSyn ja THL:n yhdistelmä osoittautui luotettavaksi sekä aikaa ja kustannuksia säästäväksi menetelmäksi munajohdinten tilan varmistamisessa. Tämän tutkimusmenetelmän luotettavuutta ei ole aikaisemmin selvitetty.

Suunniteltaessa ihanteellista ja yksilöllistä hoitoa on tärkeää huomioida lapsettomuuden syy, koska onnistuneeseen hoitoon vaikuttavat tekijät, kuten munarakkuloiden määrä, siittiöiden määrä tai munasarjojen stimulaatiomenetelmä, vaihtelevat lapsettomuuden syyn mukaan ja myös riski monisikiöraskauteen oli eri etiologiaryhmissä erilainen. Tämän lähestymistavan avulla voidaan saavuttaa inseminaatiohoidon tärkein tavoite: yksi lapsi kerrallaan. Siittiöiden rakennepoikkeavuudella näyttää olevan enemmän merkitystä IUI-hoidoissa kuin koeputkihedelmöityshoidoissa, mutta alentunut normaalimuotoisten siittiöiden määrä ainoana löydöksenä voidaan jättää huomioimatta hoitoa suunniteltaessa.

Huolimatta koeputkihedelmöityshoitojen nopeasta kehitymisestä, on inseminaatiohoidolla halpana ja yksinkertaisena menetelmänä merkittävä paikka lapsettomuushoidoissa. Tulevaisuudessa inseminaatiohoitojen suurin haaste on monisikiöraskauksien välttäminen vaikuttamatta hoitotuloksiin.

5 INTRODUCTION

Infertility or subfertility is defined as a condition in which a couple will not achieve a pregnancy within one year. Subfertility generally describes any form of reduced fertility with a prolonged time of non-conception. Infertility may be used synonymously with sterility, with only sporadically occurring spontaneous pregnancies. The prevalence of infertility in Finland is thought to be approximately 15%, which means that every sixth couple will suffer from infertility in some part of their potentially fertile years (Tiitinen 2008). Even though infertility has no effect on physical health, it influences the mental health and social life of infertile couples.

Approximately 30% of cases of infertility are the result of female factors, 30% are connected with male factors, 30% are connected with both male and female factors and in 10–20% of cases the reason for infertility remains unclear. Of infertile couples, 22% are regarded as sterile. Female sterility caused by bilateral tubal occlusion or amenorrhoea is observed in 16%, and male sterility caused by aspermia or azoospermia is responsible for 6% of cases (Collins 1995). The tests needed for the diagnosis of infertility vary between clinics and diagnosis also depends on the experience of the clinician. The most important assessments of infertility include assessment of ovulation, semen analysis and an evaluation of tubal patency.

Examination of tubal patency is essential because tubal disorders cause infertility in 15–30% of infertile couples and – additionally – they also have an impact on the choice of treatment. The risk factors of tubal infertility are sexually transmitted diseases such as chlamydial and gonococcal infections, endometriosis, pelvic surgery and ectopic pregnancies. First hysterosalpingography (HSG) and then later laparoscopy with chromopertubation were gold standards as regards the investigation of tubal patency. Ultrasound-based hysterosalpingo-contrast sonography (HyCoSy; also called TSSG), was presented over 20 years ago and since then it has taken its place as a first-line method in the assessment of tubal patency in Finland (Tiitinen 2008). Although HyCoSy is an outpatient procedure, a safe and cost-effective alternative and has shown good correlation with conventional laparoscopy, as a non-invasive method it might give false information that affects the choice of treatment and could have a negative effect on pregnancy rates.

Transvaginal hydrolaparoscopy (THL) is an old tubal investigation method that was newly presented at the end of the 1990s by Gordts et al. (1998) and by Watrelot et al. (1999). It is an invasive method, but it does not require operating theatre facilities and can be performed under local anaesthesia. It allows the direct visualization of the female genitals and without time delay and

anaesthetic risks it was thought to be a good alternative in the investigation of tubal patency. In Finland, this technique is at present not widely applied.

The choice of the most appropriate assisted reproductive treatment for an individual couple is often a difficult one. The aim of the clinician is to achieve a live birth with the least invasive technology available. Intrauterine insemination is widely used for treating infertility in couples because it is a simple, inexpensive, and acceptable assisted reproductive technique (Zhao et al. 2004). Intrauterine insemination (IUI) is generally attempted before proceeding to more expensive and invasive assisted reproductive techniques such as *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI). Pregnancy rates after IUI differ from one study to another according to patient selection criteria, the presence of various infertility factors, methods of ovarian stimulation, number of cycles performed, different sperm parameters, and technique of preparation. However, the effect of the aetiology of infertility on the prognostic factors associated with IUI success has not been evaluated in the literature.

Several semen parameters have been shown to correlate with IUI outcome, such as number of motile sperm and the degree of normal morphology. Analysis of post-wash semen parameters can provide useful prognostic information for women undergoing IUI. Parameters of raw samples, however, do not correlate with cycle fecundity. In the literature, post-wash total motility sperm count is usually used as prognostic information for IUI, but only in a few studies has there been analysis of the impact of post-wash morphology on IUI outcome (Hauser et al. 2001, Badawy et al. 2008).

6 REVIEW OF THE LITERATURE

6.1 Intrauterine insemination

In the literature the first report on the use of an artificial insemination was published in 1790. However, it took almost 80 years before the first pregnancy achieved by way of intrauterine insemination was reported by Sims in 1867 (Cohlen 1997). Despite the rapid development of assisted reproductive techniques (ARTs), IUI has still remained a popular treatment of subfertility. In the past the whole semen ejaculate with harmful prostaglandins, immunocompetent cells and bacteria was used in insemination, but with time, sperm preparation techniques have developed and they have been used in IUI as well as in other forms of ART.

Intrauterine insemination is defined as direct transfer of motile spermatozoa into the uterine cavity, after semen preparation and concentration in a small volume of medium. Sperm preparation increases the number of highly motile spermatozoa with a high proportion of normal forms at the site of fertilization and removes dead spermatozoa and leukocytes that generate oxygen radicals, which reduce the capacity of spermatozoa to fertilize (Aitken and Clarkson 1988).

Insemination has been regarded as a first-line treatment in cases of male subfertility, but it has also been applied among women with unexplained infertility, ovulatory dysfunction, mild endometriosis and cervical factors. The benefit of this therapy is based on the assumption that the conception rate will increase if multiple oocytes can be induced, and if with accurate timing an adequate concentration of mobile spermatozoa can be introduced directly into the uterine cavity.

In the literature, three to six IUI treatments are recommended for infertile couples when tubal patency has been addressed and there are no severe male factor parameters. Fulfilling these criteria, the pregnancy rate (PR) after IUI treatment has varied between 9 and 21% per cycle (Hamilton et al. 2003, Claman et al. 2004, Iberico et al. 2004, Osuna et al. 2004). There is no reliable data on IUI results in Finland until 2006 as a result of the lack of a national register. A total of 4 400 IUI cycles were performed in Finland in 2006, with a PR of 12.2 % and a multiple pregnancy rate (MPR) of 11.0 % (Official statistics of Finland 2006). A recent meta-analysis evaluated the effectiveness of IUI and showed the PR of 7% with clomiphene citrate and IUI and the PR of 12% with FSH and IUI the MPR being 13%. They concluded that ovarian stimulation is needed for IUI treatment to achieve modest results, but the high MPR makes IUI a poor substitute for IVF treatment (ESHRE Capri Workshop 2009).

In planning infertility treatment, the cost-effectiveness of the treatment and MPRs should be considered. Nowadays, when IVF and ICSI are widely available, over-treatment should also be avoided and IUI appears to be a more cost-effective option than immediate IVF (Garceau et al. 2002). It has been demonstrated that three cycles of IUI result in the same cumulative pregnancy rate as IVF, and IUI is more cost-effective as regards unexplained infertility and moderate male factor infertility (Goverde et al. 2000, Philips et al. 2000).

The multiple pregnancy rate is the most important concern as regards IUI nowadays. Therefore, ovarian hyperstimulation should be avoided. On this basis the future of IUI will depend on our ability to maintain an acceptable MPR without affecting the overall PR.

6.2. Investigation of tubal patency

Tubal disorder is one of the most frequent female factors associated with infertility, occurring in up to 20% of infertile couples (Tiitinen 2008). Therefore, the assessment of tubal patency plays an important role in the diagnosis and treatment of female infertility. However, it seems that the proportion of cases of tubal infertility among infertile couples has decreased recently and among couples treated by means of IVF it has become reduced from 33.3% to 13.7 % between 1992 and 2005 in our country (Official statistics of Finland 2006).

Traditionally, X-ray hysterosalpingography and laparoscopy with dye have been regarded as the gold standards for testing tubal patency. However, recent advances in high-resolution ultrasonography with contrast medium (HyCoSy) have made it possible to assess the Fallopian tube without surgical risk and exposure to ionizing radiation. At the end of the 1990s an old 'culdoscopy technique' was introduced again as the transvaginal hydrolaparoscopy (THL) technique. This can replace diagnostic laparoscopy in carefully selected women. An ideal investigation method of tubal patency would avoid waiting list time delay, expense, anaesthetic and operative risks and gonadal exposure to X-irradiation.

6.2.1 Laparoscopy

The first visualization of the human peritoneal cavity by endoscopy was recorded in 1910 by Jacobaeus (Murphy 1987), but the first written laparoscopic monographs were published by Steptoe in 1967 and by Cohen in 1968. Operative laparoscopy was introduced in 1974 by Semm (Murphy 1987). During the 1980s and 1990s the laparoscopic technique was arising over the world and it is now considered as a gold standard in the assessment of tubal patency.

Laparoscopy allows direct visualization of the female pelvis and with methylene blue or indigo carmine installation, tubal patency can also be assessed at the same time. Laparoscopy is very accurate in detecting peritubal and periovarian adhesions, and hydrosalpinx, which can be missed when using indirect techniques such as HSG and HyCoSy (Cundiff et al. 1995, Swart et al. 1995, Mol et al. 1996). The agreement between non-invasive methods has varied from 46 to 90% (Spalding 1998).

Laparoscopy is obligatory in the diagnosis of peritoneal endometriosis, but there is no firm evidence that medication or surgery enhances fertility in women with diagnosed endometriosis (Hughes et al. 1999, Olive et al. 2002). The role of surgery in the treatment of endometriosis remains unsettled. However, the effect of surgery seems to be small and in infertile women IUI and IVF appear to be useful at all stages of endometriosis (Ekerhovd et al. 2004). In the evaluation of tubal patency even small irritative lesions of endometriosis can lead to adhesions and on the other hand very extensive disease may be present without a significant effect on tubal structures. However, if peritubal adhesions are detected by laparoscopy, in most cases IVF will be more successful than treating adhesions through the laparoscope. Recently, the main indication for operative laparoscopy in infertile woman has seemed to be pelvic pain rather than infertility (Kennedy et al. 2005).

Although laparoscopy with dye is considered to be the gold standard for evaluating tubal pathology, it requires general anaesthesia, operating theatre facilities and absence from professional activities. Among infertile women, laparoscopy very seldom reveals any pathological conditions, but it can reveal some potentially serious risks. In the literature the incidence of complications has varied between 0.3–1.5% (Spalding 1998). In more recent study, of 3572 woman, overall 67 (1.88%) complications occurred, including one death. Complications during insertion of the Verres needle and the principal and accessory trocar were noted in 25 cases (0.7%). Intra-operative complications occurred in 32 patients (0.9%) and acute postoperative complications in 10 (0.3%) patients (Tarik and Fehmi 2004). The complication rate depends to a degree on the skills and experience of the operating gynaecologist, as well as on the extent of the procedure. However,

complications associated with all types of laparoscopic procedures should not be underestimated. Given the risks associated with laparoscopy, this should be reserved for cases where pathology is suspected and it should be scheduled to be combined with laparoscopy surgery (Vyjayanthi et al. 2004).

6.2.2 X-ray hysterosalpingography (HSG)

X-ray hysterosalpingography has been used in the evaluation of tubal patency since 1914, when a radiopaque dye was first introduced as a contrast medium for radiological diagnosis of tubal patency. It remained a gold standard for evaluating tubal patency over many decades (Maathuis et al. 1972). In recent decades laparoscopy has taken the place of a gold standard in evaluation of tubal patency and it has mainly replaced the use of HSG. Nowadays, in Finland the use of HSG has disappeared, mainly because HyCoSy is easier to perform at outpatient clinics and it does not expose a woman to unnecessary radiation. However, HSG is still widely used in some parts of the world, e.g. the United Kingdom and Middle-East countries (Vyjayanthi et al. 2004, Sakar et al. 2008).

X-ray hysterosalpingography is performed in the early follicular phase of the menstrual cycle by transcervical injection of radiopaque medium into the uterine cavity under fluoroscopic visualization. In addition to investigation of tubal patency, HSG is also used to exclude Müllerian tube malformations and other uterine cavity defects (Simon and Laufer, 1993).

In the literature, the concordance between HSG and laparoscopy has varied between 46–90% (Spalding 1998). In a meta-analysis of 20 studies in which HSG and laparoscopy were compared as regards tubal patency, the sensitivity of HSG was 65% and the specificity 83% (Swart et al. 1995). It can be concluded that although HSG in detecting tubal patency is of limited use because of its low sensitivity, its high specificity makes it a useful test for ruling out tubal obstruction (Swart et al. 1995). Low sensitivity could be a result of tubal spasm caused by the pain and discomfort of HSG. The sensitivity of HSG in detecting peritubal adhesions has been reported to be 34–75% (Rice et al. 1986) and in meta-analysis HSG was not reliable for the evaluation of peritubal adhesions (range of sensitivity 0.13–0.79) (Swart et al. 1995). Therefore, even when tubal patency has been demonstrated by HSG, laparoscopy has been suggested as a mandatory step to rule out the existence of peritubal adhesions as well as endometriosis as causes of infertility (Simon and Laufer, 1993). Endometriosis rarely causes radiographic abnormalities in HSG and therefore it can only be diagnosed by laparoscopy (Johnson et al. 1994). On the other hand, considering cost-effective

infertility care, it has been suggested that in the case of normal results in a gynaeco-radiological procedure, the probability of clinically relevant tubal disease or endometriosis is so low that laparoscopy does not seem warranted (Gleicher 2000). In a review article (Fatum et al. 2002), the authors concluded that in a woman with no tubal risk factors in her history and with normal results in HSG, the probability of clinically relevant tubal disease or endometriosis is so low that laparoscopy does not seem to be justified or cost-effective. However, diagnostic laparoscopy is needed after abnormal results in HSG to prevent over-treatment by means of IVF (Lavy et al. 2004, Tanahatoc et al. 2008). In the case of unilateral distal tubal occlusion in HSG, laparoscopy may be omitted because it does not lead to changes in the original treatment plan (Lavy et al. 2004).

Comparing HyCoSy with air and saline as contrast media with HSG, a concordance of 85% was found, with ultrasonography being more sensitive in detecting uterine pathology (Chenia et al. 1997). Similar rates have also been reported in other studies (Reis et al. 1998, Dijkman et al. 2000, Exacoustos et al. 2003), although some authors have reported limitations of HSG, especially when diagnosing distal tubal obstruction and hydrosalpinx (Cundiff et al. 1995, Mol et al. 1996).

6.2.3 Hysterosalpingo-contrast sonography (HyCoSy)

In the early 1980s hysterosalpingo-contrast sonography (HyCoSy) was presented by Nannini et al. (1981), Richman et al. (1984) and Randolph et al. (1986). Those studies were first performed transabdominally, but the introduction of transvaginal probes increased the accuracy of HyCoSy. Healthy Fallopian tubes are not visible in ultrasonography and therefore a contrast medium is required. The preliminary studies were performed using saline as a contrast medium and fluid in the abdominal cavity was considered as a sign of at least unilateral patency (Nannini et al. 1981, Richman et al. 1984, Randolph et al. 1986, Rasmussen et al. 1986, Deichert et al. 1989, Volpi et al. 1991, Deichert et al. 1992). Today, the use of HyCoSy is widespread, especially in Northern Europe and in Finland it is considered as a first-line procedure in the evaluation of tubal patency.

The procedure is performed in the follicular phase of the menstrual cycle and a catheter with a balloon is inserted into the uterine cavity. A contrast medium is injected through the catheter during continuous transvaginal ultrasonographic scanning. In HyCoSy the tubes are considered patent if one or all of the following criteria (Volpi et al. 1996) are fulfilled:

1. Air can be detected passing through the saline solution for at least 8–10 seconds in the isthmic part of the tube. This is the minimum criterion to diagnose tubal patency.
2. Visualization of the tube and confirmation of displacement of air within it by saline solution.

3. Detection of air bubbles moving around the ovary. This may be possible even without visualization of the entire course of the tube.

4. Detection of fluid and air bubbles in the pouch of Douglas.

Of the criteria listed above, numbers 2 and 3 are the most important ones for the demonstration of tubal patency (Volpi et al. 1996).

The role of HyCoSy as a first-line procedure for the assessment of tubal patency has been examined in several studies and in the earliest studies X-ray HSG was used as a reference method, with agreement of 83–84% (Rasmussen et al. 1986, Volpi et al. 1991). Most of the studies have been performed by comparing HyCoSy with laparoscopy and HSG, or with laparoscopy alone in the diagnosis of tubal pathology, with a high concordance rate (80–91%) (Volpi et al. 1991, Heikkinen et al. 1995, Ayida et al. 1996, Volpi et al. 1996, Spalding et al. 1997, Hamilton et al. 1998, Reis et al. 1998, Strandell et al. 1999, Hauge et al. 2000, Tanawattanachaoren et al. 2000, Exacoustos et al. 2003, Shahid et al. 2005). In a meta-analysis of three comparative studies of 1007 women, a concordance rate of 83% was found when HyCoSy was compared with either HSG or laparoscopy with dye (Holz et al. 1997). Compared with laparoscopy, HyCoSy has been shown to have a sensitivity of 92.8%, a specificity of 96.2%, a positive predictive value (PPV) of 92.8% and a negative predictive value (NPV) of 98.1 % (Hauge et al. 2000). Using three-dimensional HyCoSy, a sensitivity of 100%, a specificity of 67%, a PPV of 89%, an NPV of 100% and a concordance rate of 91% were detected when compared with laparoscopy (Chan et al. 2005).

As mentioned above, the first studies by HyCoSy were performed with saline as a contrast medium. Air combined with saline solution as a contrast medium was first introduced by Volpi and associates (Volpi et al. 1991). Echovist[®] (Schering AG, Berlin), which contains galactose microbubbles in galactose solution, was introduced by Deichert et al. (1989). In the majority of studies Echovist[®] has been used as the ultrasonographic contrast medium (Campell et al. 1994, Holz et al. 1997, Hamilton et al. 1998, Reis et al. 1998, Strandell et al. 1999, Dijkman et al. 2000, Hauge et al. 2000, Skinner et al. 2000, Hamilton et al. 2003, Chan et al. 2005, Shahid et al. 2005, Tamasi et al. 2005), but investigations involving the use of air and saline as a contrast medium have concordance rates (vs. laparoscopy or HSG) as high as in studies with positive contrast medium (Heikkinen et al. 1995, Chenia et al. 1997, Spalding et al. 1997a, Exacoustos et al. 2003). In studies in which the contrast media used in HyCoSy have been compared, some investigators found a mixture of air and saline to be comparable with Echovist[®] (Spalding et al. 1997a, Deichert et al. 1989), whereas in one report saline was found to be worse than a positive contrast agent (Boudghene et al. 2001).

Doppler techniques have been introduced to increase the diagnostic efficacy of ultrasonography (Schlief and Deichert 1991, Deichert et al.1992) and saline solution has been used as a contrast medium in many studies. When a continuous flow of saline in the Fallopian tube was detected by using a high-resolution Doppler technique, the tube was regarded as patent. Doppler assessment seems useful, especially in cases with very slow flow or with poor visualization of the flow in the tube (Schlief and Deichert 1991). The concordance between colour-Doppler assessment and laparoscopy with dye has been 81–86% (Spalding 1998).

Hysterosalpingo-contrast sonography seems to be a well-tolerated investigative method in the assessment of tubal patency. The most important side-effects are abdominal pain and vasovagal reactions. In a meta-analysis, mild pain was detected in 42.3 % of subjects, severe pain in 10.1% of subjects and other adverse events such as nausea and vasovagal reactions in 7.1% of subjects and only 1.9% of women needed treatment (Holz et al. 1997). In a recent report on 660 infertile women, 4.1% (n=20) had mild vasovagal reactions and 0.8% (n=4) had severe vasovagal reactions without late complications requiring atropine or anti-inflammatory drugs (Savelli et al. 2008). In a study by Chan et al. (2005), 71% of women regarded HyCoSy at least as acceptable. Warming a contrast medium to a body temperature may also reduce the pelvic pain (Nirmal et al. 2006).

In the majority of women, pelvic status is normal and therefore, considering HyCoSy as a first-line procedure, in 86% of cases an invasive procedure can be avoided (Skinner et al. 2000). It seems that laparoscopy should be reserved as a second-line investigative method if tubal occlusion is demonstrated in HyCoSy, or a first-line method if endometriosis is suspected (Skinner et al. 2000).

6.2.4 Transvaginal hydrolaparoscopy (THL)

Endoscopic examination of the female genital tract may be performed through either the abdominal or vaginal route. The vaginal technique was initially introduced in the USA in 1944 and was subsequently described using the term “culdoscopy”, a technique in which the endoscope is introduced through the posterior vaginal fornix (Watrelet et al. 2003). Later, transabdominal laparoscopy displaced culdoscopy, because it provided a view of the abdominal cavity, better access for surgical treatment and it also reduced the risk of infection. Hydroculdoscopy was introduced in the 1970s (Odent 1973) and the procedure of transvaginal hydrolaparoscopy was described by Gordts et al. (1998), with abdominal distension with saline and exploitation of smaller endoscopes.

Fertiloscopy, described by Watrelot et al. (1998), includes THL as well as salpingoscopy, microsalingoscopy and hysteroscopy (Watrelot et al. 2003).

Transvaginal hydrolaparoscopy and dye test with optional salpingoscopy and hysteroscopy allows complete evaluation of the reproductive tract. It has been proposed as an alternative to diagnostic laparoscopy in the routine assessment of infertile woman, because most subfertile women who undergo diagnostic laparoscopy have normal findings (Watrelot et al. 1998). It has also been reported that THL provides a better opportunity to detect fine peritubal or peri-ovarian adhesions than laparoscopy (Brosens et al. 2001, Campo et al. 2002). However, diagnostic laparoscopy will obviously remain the procedure of choice in women with obvious pelvic pathology.

The results of THL have shown good correlation with those of conventional laparoscopy (Darai et al. 2000, Casa et al. 2002, Watrelot et al. 2003). In a report on 60 women, diagnosis by means of THL correlated with that of laparoscopy in 92.3% of cases and in cases of abnormal findings in THL, there were no normal laparoscopic results (Darai et al. 2000). In another study, on tubal pathology, 77.8% agreement was found between the two techniques. Overall, THL findings correlated closely (92.8%) with laparoscopic findings, but the diagnostic accuracy of THL was 100% in cases of complete pelvic evaluation. The authors concluded that conventional laparoscopy is indicated in cases of incomplete pelvic evaluation or abnormal findings (Casa et al. 2002).

Transvaginal hydrolaparoscopy has several advantages over conventional laparoscopy. It is an outpatient procedure and can be performed under local anaesthesia. This technique avoids the pneumoperitoneum and the need of abdominal incision and subsequent scars (Watrelot et al. 2003). Furthermore, there is hardly any risk of vessel injury and the risks of general anaesthesia are avoided (Gordts et al. 1998). The greater accuracy in detecting the fimbria region of the tubes is possible because of the high magnification used and manipulation of adnexal structures can be avoided.

The most feared complication of THL is bowel injury. In the literature a total of 4232 procedures (ten studies) resulted in 26 bowel injuries (0.61%) (Gordts et al. 2004), but in a recent study by Shibahara et al. (n=177) the incidence of rectal perforation was 1.1% (n=2), both cases diagnosed during diagnostic THL (Shibahara et al. 2007). It has also been reported that after initial experience, the prevalence of bowel injury was 0.25% (Gordts et al. 2001). Most investigators have reported a success rate of over 90% in accessing the pouch of Douglas (Campo et al. 2002, Casa et al. 2002, Watrelot et al. 2003). The use of ultrasonography seems to increase the rate of successful access to the pouch of Douglas and minimizes complications (Sobek et al. 2008). The contraindications of THL are pelvic infection, pelvic tumour, bleeding or fixed retroverted uterus

and grave obesity. When pelvic pathology is obvious, laparoscopy is recommended (Gordts et al. 1998).

The transvaginal approach allows easy and direct access to the tubo-ovarian structures and the fossa ovarica without manipulation and thus also makes tubo-ovarian surgery possible. The proximity of tubo-ovarian structures allows operative procedures for treatment of superficial and cystic ovarian endometriosis, drilling of the ovarian capsule, adhesiolysis and salpingostomy (Gordts et al. 2002).

Transvaginal hydrolaparoscopy is not as widely used in Finland as it is in many other European countries such as Belgium and France.

6.3 Controlled ovarian hyperstimulation

6.3.1 Clomifene citrate

Clomifene citrate (CC) has been used for over 40 years to correct ovulatory dysfunction in infertile women. It is a non-steroidal selective oestrogen receptor modulator, which acts primarily by binding to oestrogen receptors at the hypothalamus (Kurl and Morris, 1978). It is both an agonist and antagonist of oestrogen, generally acting as a competitive antagonist at physiological female oestrogen levels. By diminishing oestrogen receptors in the hypothalamus, the negative feedback signal induced by oestrogen is blocked and then the altered GnRH secretion in turn increases gonadotrophin secretion from the pituitary, leading to folliculogenesis in the ovary (Clark and Markaverich 1982).

Although clomifene citrate results in ovulation in most cases, pregnancy rates are disappointing. This has been attributed to its peripheral anti-oestrogenic effects, mainly on the quality and quantity of cervical mucus, and endometrial growth and maturation (Fritz et al. 1991) that could prevent pregnancy in the face of successfully induced ovulation. Long-lasting oestrogen receptor depletion has been involved in the anti-oestrogenic mechanism of action of CC. It also appears that CC accumulates in the body because of its long half-life (Mitwally and Casper, 2001).

Controlled ovarian hyperstimulation combined with intrauterine insemination has been demonstrated to be an effective form of treatment in subfertile couples (Hughes 1997), but no consensus of opinion exists concerning the drug of first choice to be used for hyperstimulation. However, clomifene citrate is considered as a first-line medical treatment for oligo-ovulatory and

anovulatory patients (Beck et al. 2005, Palomba et al. 2006). A mini-review of seven studies indicated an ovulation rate of 73%, with a pregnancy rate of 36% (Homburg 2005). In a meta-analysis of 12 randomized controlled trials it was concluded that CC is effective in increasing the PR when compared with placebo (Beck et al. 2005).

Based on good experiences in improving fertility success of anovulatory women, CC has been more recently used as an approach to initial treatment in subjects with unexplained infertility. This is based on the idea that multiple oocytes will be induced monthly and thus the conception rate will increase. Furthermore, it has been claimed that CC may overcome a subtle defect in ovulatory function not uncovered by conventional testing (Homburg 2005). On the other hand, CC has an adverse influence on cervical mucous and the endometrium because of its anti-oestrogenic effect. Hence pregnancy rates in connection with the use of CC are lower than expected on the basis of achieved ovulation rates.

Compared with natural cycle IUI, ovarian hyperstimulation improves treatment outcome in couples with unexplained and mild male subfertility (Cohlen et al. 1998, Guzick et al. 1999). Pregnancy rates obtained with CC are lower in comparison with FSH, mainly when ovarian stimulation is associated with IUI (Balasch et al. 1994, Hughes et al. 1997, Cohlen et al. 1998, Guzich et al. 1999, Costello et al. 2004). In a review of 43 trials and 3957 women (Cantineau et al. 2007), seven studies (n=556) showed that gonadotrophins are more effective drugs (OR 1.8, 95% CI 1.2 –2.7) than anti-oestrogens when IUI is combined with ovarian hyperstimulation. However, in a recent randomized trial carried out by Dankert et al. (2007) no significant difference in live birth rates as regards CC and recombinant human FSH (rFSH) treatment was observed. The authors concluded that CC is a more cost-effective drug and therefore can be used as the drug of first choice.

6.3.2 Gonadotrophins

Since 1962, when the first pregnancy after ovulation induction with HMG and hCG was reported, the use of gonadotrophins has increased tremendously. Until rFSH was marketed, all available human FSH pharmaceutical preparations were made from post-menopausal urine extracts. Recombinant FSH preparations differ from urinary FSH preparations in that they completely lack any LH activity and extraneous human protein. Both preparations are widely used in IUI treatments.

Over the past 20 years, there has been a marked increase in the use of ovulation induction and IUI for the treatment of unexplained infertility. The results of several studies indicate that ovulation induction with gonadotrophin significantly improves the probability of conception in couples with unexplained infertility, particularly when associated with IUI (Hughes, 1997, Cohlen et al. 1998, Guzick et al. 1999). It seems that both treatments (FSH and IUI) increase the likelihood of conception approximately two-fold (ESHRE Capri Workshop 1996). Indeed, it has been shown that addition of gonadotrophins to IUI was more effective than clomifene citrate in cases of unexplained and male-factor subfertility (Practice Committee of the American Society for Reproductive Medicine. 2006). On the other hand, it has been suggested that in the absence of any results of a randomized trial showing a clinical benefit over natural cycle IUI for subjects with unexplained infertility, gonadotrophin treatment might not be recommended (Cohlen 2005).

Comparison of different gonadotrophin preparations has been performed in several studies of IVF cycles (Out et al. 1995, Brinsden et al. 2000, Harlin et al. 2000), but there is little data in IUI cycles. In a prospective randomized study, when urinary FSH (uFSH) and rFSH were compared in IUI cycles, both seemed equally effective, but uFSH appeared to be a more cost-effective choice as a result of its lower cost per cycle (Gerli et al. 2004). On the other hand, better efficacy of rFSH (PR= 25.9%) over uFSH (PR=13.8%) and HMG (PR=12.5%) in terms of pregnancy rate has been suggested, with a lower total dose in the rFSH group (Demiroglu et al. 2006). In a review article of nine studies and 576 women in which different types of gonadotrophins were compared, no significant difference was reported (OR 1.2; 95% CI 0.64–2.1) (Cantineau et al. 2007). It also seems that combination protocols (CC + FSH) are less costly and equally effective as gonadotrophins alone, with fewer multiple births (Ryan et al. 2005).

The high iatrogenic multiple pregnancy rate associated with IUI is becoming less acceptable. It has been postulated that higher MPRs result if gonadotrophins are used in the induction of ovulation (Goverde et al. 2005). Doubling the dose of gonadotrophins does not enhance the pregnancy rate, but adverse outcomes such as ovarian hyperstimulation syndrome (OHSS) as well as multiple pregnancy rate are increased (Cantineau et al. 2007). In addition, the best gonadotrophin regimen that achieves the highest pregnancy rates together with the lowest MPR in IUI cycles has remained unsettled. In a recent study, it was found that the application of a mild hyperstimulation protocol as an alternative to a standard hyperstimulation protocol in IUI cycles did not result in higher pregnancy rates in comparison with natural cycles (40% vs. 35%), while at the same time multiple pregnancies could not be avoided (27% vs. 4%) in cases of male and unexplained infertility. Therefore, the authors suggested that there was no place for the use of gonadotrophins in IUI treatment (Goverde et al. 2005).

6.3.3 Aromatase inhibitors

The concept of using aromatase inhibitors (AIs) for ovulation induction, as a new method that could avoid many of the adverse effects of CC, has recently been explored. The third-generation AIs include two non-steroidal inhibitors, anastrozole and letrozole (L), which are both selective. Aromatase inhibitors induce ovulation by inhibiting oestrogen production; the consequent hypoestrogenic state increases GnRH release and pituitary FSH synthesis. Furthermore, an increase in intra-ovarian androgens secondary to aromatase inhibition has also been shown with AIs, leading to improved early follicular growth (Weil et al. 1998). Because the central feedback mechanism remains intact, mono-follicular responses are normal and a lower rate of multiple pregnancies may be expected (Casper and Mitwally, 2006).

Letrozole is the most widely used AI in assisted reproduction. In most studies, it has been administered once daily at doses of 2.5–5 mg for 5 days. In a recent meta-analysis, nine studies were included and three comparisons were performed. First the effects of letrozole and CC in patients with polycystic ovary syndrome (PCOS) were compared in four trials and the results were not significantly different as regards ovulatory cycles (OR = 1.17; 95% CI 0.66–2.09), or pregnancy rate per cycle (OR = 1.47; 95% CI 0.73–2.96). Secondly, three retrospective studies of L + FSH versus FSH alone in ovarian stimulation for IUI were compared in three studies and OR was 1.15 (95% CI 0.78–1.71). Thirdly, a final meta-analysis included one randomized controlled trial and one cohort study in which letrozole + gonadotrophin versus gonadotrophin alone were compared, showing no difference in pregnancy rates per subject (OR = 1.40; 95% CI 0.67–2.91). The authors concluded that in ovulation induction letrozole is as effective as other methods (Requena et al. 2008). It is also notable that in women with PCOS, the percentage of mono-follicular cycles achieved in women treated with AIs is higher than in those treated with CC, as a result of which a lower rate of multiple pregnancies may be expected (Requena et al. 2008).

Cohort studies have not revealed an increase in the rate of congenital malformations among the offspring of mothers who conceived with letrozole treatment for infertility. Because of the short half-life of AIs, the biological plausibility of teratogenic effects of these drugs, which are used in the early follicular phase, can be discarded (Requena et al 2008). In Finland, however, AIs have not gained official approval for ovulation induction.

6.3.4 Natural cycles

There is evidence that ovarian hyperstimulation (OH) improves IUI outcome in couples with unexplained and mild male subfertility, compared with natural cycle IUI (Cohlen et al. 1998, Guzick et al. 1999). However, the main concern about IUI treatment with OH is the increase in the multiple pregnancy rate. The results of a meta-analysis carried out by Cohlen et al. (2004), with 18 randomized studies, showed that IUI in natural cycles does not improve the probability of conception in couples with unexplained infertility and OH seems to be important. Thus, it has been postulated that the worse the semen factor, the more important IUI becomes, and the better the semen factor, the more important OH becomes (Balasch et al. 2004). In a recent Cochrane review of three randomized studies, a significant increase in pregnancy rate was found among women with unexplained infertility when IUI with OH was compared with IUI in natural cycles (OR 2.33; 95% CI 1.46–3.71), but there was insufficient data on multiple pregnancies and other adverse events as regards treatment with OH (Verhulst et al. 2006). However, in a Cochrane review of male subfertility there was no statistically significant difference between pregnancy rates per couple when IUI + OH was compared with IUI in natural cycles (OR 1.47; 95% CI 0.92–2.37) (Bensdorp et al. 2007). It seems logical to suggest that the better the semen quality, the more likely female factors have an influence on subfertility in a couple (Cohlen et al. 1998).

Nowadays ovarian induction seems acceptable only when it leads to a singleton pregnancy. The ESHRE Capri Workshop Group (2000) reported that MPRs in ovulation induction cycles could be reduced by using lower dosage gonadotrophin regimens. In a study of 3219 IUI cycles and 1256 women receiving minimal ovarian stimulation with rFSH (50–75 IU/day) and accepting only two dominant follicles (> 15 mm) on the day of hCG injection, a reduced rate of twins (< 10%) and high order multiples (< 0.5%) was achieved while maintaining acceptable singleton pregnancy rates (Papageorgiou et al. 2004).

6.4. Sperm parameters

The definition of fertile, subfertile and infertile men has been difficult to establish. Although pregnancy is the only proof of fertility, absence of pregnancy is not helpful for defining male subfertility because female factors play an important role as well. Semen analysis is the most important tool in the assessment of male fertility. Male subfertility is defined by the World Health Organization (WHO) as a lack of conception after at least 12 months of unprotected intercourse in

combination with at least two separate semen samples not reaching the criteria of normality as defined by the WHO (Table 1). Although semen analysis is only a guiding instrument in assessing male subfertility, it should not be used as an absolute indication of subfertility (Aitken et al. 1995). Biological variability in semen quality and variation between and within laboratories in examining semen samples also confuse the interpretation of semen analysis.

Table 1. Classical criteria of normal semen (WHO 1999)

Volume	≥ 2.0 mL
Concentration	$\geq 20 \times 10^6$ /mL
Total count	40×10^6
Total progressive motility (A+B)	$> 50\%$
Normal morphology	$\geq 15\%$
Anti-sperm antibodies	$\leq 10\%$

6.4.1. Sperm morphology

The morphological variability of the human spermatozoon makes sperm morphology assessment difficult. A normal spermatozoon must have the structure of sperm head, neck, midpiece and tail as described in laboratory manuals.

Evaluating the impact of sperm morphology on pregnancy rates is very difficult as a result of different standardization of sperm morphology used all over the world. The most widely used criteria are published by the World Health Organization (WHO 1992, WHO 1999). In the 1992 edition sperm samples were considered to be normal if the percentage of normal forms was $\geq 30\%$ and in 1999 this was changed to $\geq 15\%$. The latter edition reflects 'strict criteria'. The main difference between the criteria in these two editions is that classification by strict criteria requires that all borderline forms be considered abnormal. This results in the situation where the amount of normal forms is considerably low in most semen samples. Furthermore, variations between laboratories are also crucial. In the current WHO classification (WHO 1999) the following categories of defects should be noted:

1. Head defects:

- large, small, tapered, pyriform, round, amorphous or vacuolated heads, heads with small acrosomal areas, double heads, or any combination of these

2. Neck and midpiece defects:

- 'bent' neck, asymmetrical insertion of the midpiece into the head, thick or irregular midpiece, abnormally thin midpiece, or any combination of these

3. Tail defects:

- short, multiple, hairpin, broken tails, bent tails, tails of irregular width, coiled tails, or any combination of these

4. Cytoplasmic droplets:

- greater than 1/3 of the area of a normal sperm head

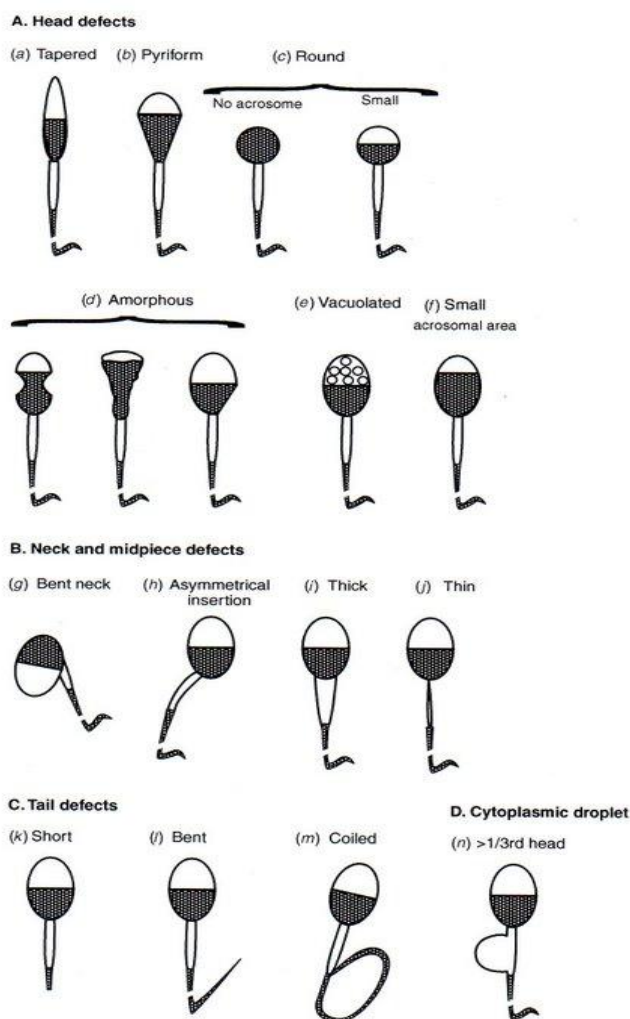


Figure 1. Schematic drawing of some abnormal forms of human spermatozoa (WHO 1999)

Normal sperm morphology, assessed by strict criteria, seems to correlate positively with pregnancy rates in IUI-treated couples in cases of male infertility (Hauser et al. 2001). The threshold of the proportion of spermatozoa with normal morphology below which IVF is recommended varies widely in the literature from 4% to 50% (Francavilla et al. 1990, Burr et al. 1996, Hauser et al. 2001). In a meta-analysis of six studies, a significant improvement in PRs above

the 4% threshold (strict criteria) was reported (van Waart et al. 2001). A clear drop in pregnancy rate after IUI has also been reported in other studies in the presence of severe teratozoospermia (Lindheim et al. 1996, Ombelet et al. 1997, Lee et al. 2002), but other investigators have reported no such association (Matorras et al. 1995, Karabinus et al. 1997). Ombelet et al. also reported that sperm morphology became a useful predictive tool in men with a TMC $< 1 \times 10^6$ /mL (Ombelet et al. 1997). In a recent study it was concluded that intrauterine insemination used for treating male factor infertility has little chance of success when the woman is older than 35 years, the number of motile spermatozoa inseminated is $< 5 \times 10^6$, or the proportion of sperm with normal morphology is $< 30\%$ assessed by WHO 1992 criteria (Badawy et al. 2008).

The impact of isolated teratozoospermia (other sperm parameters normal) on IUI has been reported in only a few studies. The earliest report showed that the PR after IUI was significantly lower (1%) when the morphology score was $< 4\%$ (strict criteria) than when the morphology score was $\geq 4\%$ (19%) (Lindheim et al. 1996). In a retrospective study of 440 couples and 872 cycles the cumulative live birth rate after four IUI cycles was significantly and similarly reduced in cases of isolated teratozoospermia (33.4%) and in cases with other sperm defects (31.4%), when compared with normozoospermia (52.8%) (Spiessens et al. 2003). Similar results were also shown in a more recent study and an adverse impact became clearly evident after the first IUI failure. Thus, the authors suggested that couples in which the male partner has isolated teratozoospermia should be offered only a limited number of IUI attempts (Grigoriou et al. 2005).

Morphologically abnormal spermatozoa often have multiple defects. In the teratozoospermia index (TZI) the number of defects is divided by the number of defective spermatozoa. Teratozoospermia index values should be between 1.0 and 3.0. It has been suggested that a TZI index of more than 1.6 is associated with lower pregnancy rates in untreated infertile couples (WHO 1999). On the other hand, it has been reported that the TZI index had no predictive value as regards the outcome of IVF or ICSI (Host et al. 2001).

6.4.2. Post-wash total motile sperm count

There is good evidence in the literature that intrauterine insemination is the best first-line treatment and most cost-effective procedure in cases of moderate male factor subfertility. However, it seems very difficult to identify individual semen parameters predicting the likelihood of pregnancy after IUI, because of a lack of standardization of semen analyses and other variables affecting IUI success. A review of the literature confirmed that sperm morphology using strict criteria and post-

wash total motile sperm count (TMC) after sperm preparation are the two most important sperm parameters impacting on IUI outcome (Ombelet et al. 2003).

The post-wash TMC has been proposed as a test to help distinguish the couples who would benefit from IUI from the couples who would benefit more from IVF or ICSI. The post-wash TMC represents the total number of motile sperm that are present after preparation and are subsequently available for insemination in IUI. It reflects sperm concentration and motility, as well as the effects of sperm processing.

Increasing inseminating motile sperm count seems to enhance conception, but the cut-off value above which IUI seems to be more successful is still unclear, mostly as a result of an assortment of variables such as female fertility factors, methods of ovulation induction and sperm preparation techniques (Matorras et al. 1995, Ombelet et al. 1995). The proposed cut-off values below which IUI is not advised range from 0.3 to 20 million post-wash progressively motile spermatozoa (Van Weert et al. 2004). In that meta-analysis, an optimal cut-off value for the post-wash TMC at insemination to use for patient counselling could not be identified, but the cut-off values varied between 0.8 and 5 million processed motile sperm. The best predictive cut-off value of the post-wash TMC can probably be achieved when this cut-off value is based on the clinical population and the sperm preparation technique used by the individual clinic. Authors have postulated that the value of the post-wash TMC at insemination lies in the enhancement of subject selection by identifying couples unlikely to conceive by means of IUI and not in the selection of those most likely to conceive after IUI (Van Weert et al. 2004).

6.4.3 Sperm preparation techniques

In order to be able to fertilize, spermatozoa must be separated from the seminal plasma. In the natural environment spermatozoa achieve this by means of their own motility and they penetrate the cervical mucus.

Semen preparation techniques for assisted reproduction, including intrauterine insemination, were developed to separate motile morphological normal spermatozoa. These procedures remove leucocytes, bacteria and dead spermatozoa, which produce oxygen radicals that negatively influence the ability to fertilize the egg. The yield of as many motile, morphologically normal spermatozoa as possible might influence treatment choices and therefore outcomes. Several methods of sperm preparation have been introduced, swim-up and gradient techniques being the most popular.

In a recent Cochrane review, there was no evidence of a difference between pregnancy rates after a swim-up versus a gradient, or wash and centrifugation technique (OR 1.57; 95% CI 0.74–3.32; OR 0.41; 95% CI 0.15–1.10, respectively), or in two studies in which a gradient technique versus wash and centrifugation were compared (OR 1.76; 95% CI 0.57–5.44). The authors concluded that there is insufficient evidence to recommend any specific preparation technique (Boomsma et al. 2007).

6.5. Other prognostic factors for IUI success

The important differences observed in predictors of pregnancy rates, which usually range between 8% and 26% (Tomlinson et al. 1996), may be mainly due to the influence of various factors on cycle outcome (Stone et al. 1999, Khalil et al. 2001). In this sense, some factors such as sperm count (Van Voorhis et al. 2001) and follicle development (Nuojuua-Huttunen et al. 1999) have been positively related to PRs, whereas others, such as a high cycle number and higher female age have been negatively associated (Tomlinson et al. 1996).

An advanced maternal age is a known risk factor as regards infertility. This decline in fecundity has been suggested to be a result of decreased oocyte quality. In an analysis of 9963 cycles, maternal age was the single most important determinant of the likelihood of pregnancy, with PRs decreasing when a woman's age at the time of IUI exceeded 32 years (Stone et al. 1999). This has been shown in earlier reports (Allen et al. 1985, Horbay et al. 1991) and also in a more recent report (Ghosh et al. 2003). In a report by Nuojuua-Huttunen et al. (1999) age was not a prognostic factor of IUI outcome when a woman was under 40 years old, but positive results after IUI declined rapidly after that age. It has also been reported that a link between increasing age and decreasing PR can be identified, but it is not statistically significant (Khalil et al. 2001, Dickey 2002). It can be concluded that there is sufficient evidence to assume a negative linear association between a woman's age and pregnancy rate after IUI, although PRs seem to be acceptable up to the age of 40 (Nuojuua-Huttunen et al. 1999, Goverde et al. 2000).

The precise limits of infertility duration as regards recommending IUI have not been clearly established. In many studies pregnancy rates have decreased with increasing duration of infertility (Tomlinson et al. 1996, Nuojuua-Huttunen et al. 1999, Iberico et al. 2004, Erdem et al. 2008). Intrauterine insemination cannot be recommended after a long-standing duration of infertility and PR may be clearly decreased when it is over three years (Iberico et al. 2004).

The effect of infertility aetiology on IUI outcome has seldom been assessed as a prognostic factor. Many investigators have reported a significantly lower pregnancy rate in women with

endometriosis compared with other reasons for infertility (Nuojuua-Huttunen et al. 1999, Dickey et al. 2002) and in a meta-analysis (Hughes, 1997) endometriosis decreased the effectiveness of controlled ovarian hyperstimulation (COH) and IUI by half in the treatment of persistent infertility.

Intrauterine insemination seems to be an efficient treatment in cases of unexplained and male factor infertility, and also in cases of anovulatory disorders after unsuccessful ovulation induction treatment (Nuojuua-Huttunen et al. 1999, Khalil et al. 2001, Dickey et al. 2002). In a recent Dutch study it was reported that an additional beneficial effect of IUI together with COH in couples with unexplained subfertility can be excluded. Expectant management for a period of six months therefore appears justified in these couples (Steures et al. 2008).

The number of dominant follicles before hCG administration has been a positive predictive factor for IUI success in many studies (Tomlinson et al. 1996, Nuojuua-Huttunen et al. 1999, Stone et al. 1999, Khalil et al. 2001, Dickey et al. 2002, Iberico et al. 2004, Erdem et al. 2008). The presence of three or more dominant follicles seems to increase PRs two- or three-fold when compared with mono-follicular responses in stimulated IUI cycles (Nuojuua-Huttunen et al. 1999, Dickey et al. 2002, Iberico et al. 2004). However, the major drawback of multi-follicular responses in IUI cycles is the increased risk of multiple pregnancies, which is nowadays becoming less acceptable.

Cycle fecundity has been shown to be relatively constant for the first three to six cycles (Sahakyan et al. 1999, Dickey et al. 2002), although decreasing PRs with an increased number of cycles have also been reported (Nuojuua-Huttunen et al. 1999, Khalil et al. 2001, Erdem et al. 2008). A maximum of four IUI cycles has been shown to be the most optimal (Nuojuua-Huttunen et al. 1999, Khalil et al. 2001). However, a recent study showed that ongoing PRs in high-order IUI cycles are acceptable and it may be reasonable to conduct up to nine cycles (Custers et al. 2008).

Endometrial thickness and pattern have been implicated in the successful outcome of infertility treatment. Although assessment of the endometrium by means of ultrasonography has become a standard procedure during the diagnostic work-up and treatment of infertile women, the clinical significance of differences in endometrial thickness has remained controversial. Some investigators have reported an association between endometrial thickness and IUI outcome (Tomlinson et al. 1996, Esmailzadeh et al. 2007), but not all (Hock et al. 1997, Nuojuua-Huttunen et al. 1999, Tsai et al. 2000, Kolibianakis et al. 2004). A trilaminar endometrial pattern on the day of IUI provides a favourable prediction of pregnancy (Hock et al. 1997, Tsai et al. 2000). In addition, an endometrial volume of < 2 mL measured by three-dimensional ultrasonography on the day of IUI correlated negatively with IUI success (Zollner et al. 2003).

6.6. Timing of IUI

Accurate timing is probably one of the most important variables that affect the results of IUI. Insemination that bypasses the cervix has to be performed as close to the moment of ovulation as possible, because the spermatozoa after IUI probably survive for a much shorter period compared with normal sperm after intercourse because the latter can survive in cervical crypts (Cohlen et al. 1997). Furthermore, oocytes are fertilizable for only 12–16 hours (Edwards and Brody, 1995). In analysis of natural cycles, ovulation occurs 24–56 hours after the onset of the LH surge, with a mean time of 32 hours (WHO 1980). In stimulated cycles, ovulation usually begins 36–38 hours after hCG administration (Edwards and Steptoe, 1974). However, a premature LH surge, which can interfere the adequate timing, has been shown to occur in 24% of stimulated cycles with gonadotrophins (Cohlen et al. 1998).

Many different time intervals have been suggested for IUI after hCG injection. It is currently believed that IUI at 32–38 hours after hCG administration is the most optimal (Ragni et al. 2004). In a randomized prospective trial, PRs did not differ between short (33 h) and long (39 h) hCG–IUI interval cycles (Claman et al. 2004), although one earlier study has shown higher PRs in connection with an hCG–IUI interval of 38–40 h compared with 32–34 h (Pryor et al. 2001).

An alternative strategy to solve the timing problem in IUI is to perform two IUI procedures at different intervals after hCG injection. The results of five prospective randomized studies are controversial. Two of them indicated higher PRs with a double insemination strategy (Silverberg et al. 1992, Ragni et al. 1999), whereas in the other three studies there was no difference (Ransom et al. 1994, Alborzi et al. 2003, Casadei et al. 2006). Consequently, in two reviews there were no significant differences between these two methods (Cantineau et al. 2003, Osuna et al. 2004). With a lack of sufficient data to support a double insemination strategy, one insemination in each treatment cycle saves both time and expense. It has also been reported that a bed rest of 10 min after IUI has a positive effect on the pregnancy rate (Saleh et al. 2000).

The clinician can follow two tactics for intrauterine insemination timing: hCG administration or LH recording. As discussed above, there are difficulties in finding ideal timing for the hCG–IUI interval. A certain follicle diameter, usually > 18 mm, is required for hCG administration and follicle diameter as a single measurement may be misleading. However, with hCG administration there is the theoretical risk of release of immature follicles or unruptured luteinized follicles. An attempt at better timing of IUI has led to the method of LH surge detection in IUI treatment. At a clinical level, once-daily morning LH measurements are satisfactory, after the follicle has reached the size of 14–15 mm in ultrasonography. When an LH surge is detected, insemination is

usually performed after 24 hours. In a meta-analysis of seven studies and 2623 patients it was reported that hCG-induced ovulation did not show any clinical benefit over spontaneous ovulation as regards IUI timing (Kosmas et al. 2007). However, it seems that women with ovulatory dysfunction may benefit from hCG administration (Vlahos et al. 2005, Kosmas et al. 2007), whereas women with unexplained or male-factor infertility showed higher PRs with LH recording (Kosmas et al. 2007). It should also be remembered that the cancellation rate with urinary LH surge detection is higher than in IUI cycles with hCG injection (Lewis et al. 2006).

To avoid weekend insemination, GnRH-antagonist has been administered in IUI treatment to inhibit LH rise. In a meta-analysis of six studies and 1069 women higher PRs were found (OR 1.56, 95% CI 1.15-4.63) when a GnRH-antagonist was added to a gonadotrophin superovulated IUI protocol (Kosmas et al. 2008).

6.7. Multiple pregnancies in IUI treatment

The balance between the number of follicles in women undergoing IUI with COH and a still acceptable pregnancy rate is extremely important. The drawback of multi-follicular growth in IUI and COH is the risk of multiple pregnancies. Although there is debate over whether a multiple pregnancy should be seen as a complication of assisted reproductive techniques, there is consensus that a multiple pregnancy carries increased risks of pregnancy complications, such as preterm delivery, growth retardation and pre-eclampsia (Land and Evers, 2003, van Wely et al. 2006).

The majority of the studies indicate a positive association between the number of follicles and pregnancy rate (Tomlinson et al. 1996, Nuojua-Huttunen et al. 1999, Stone et al. 1999, Dickey et al. 2002, Ibérico et al. 2004). However, in a recent retrospective cohort study, it was stated that in ovulation induction for unexplained non-conception, induction of more than one follicle did not improve the ongoing pregnancy rate but increased the risk of multiple pregnancies (van Rumste et al. 2006). Therefore, to reduce the number of multiple pregnancies, in all IUI cycles for unexplained non-conception, mono-follicular growth was suggested (van Rumste et al. 2006).

In a meta-analysis of 14 studies and 11 599 cycles, the pooled OR for pregnancy after the growth of two follicles as compared with mono-follicular growth was 1.6 (99% CI 1.3–2.0), whereas for three and four follicles, the ORs were both 2.0. The pooled OR for multiple pregnancies after the growth of two follicles was 1.7 (99% CI 0.8–3.6), whereas for three and four follicles the figures were 2.8 and 2.3, respectively. Since in cycles with three or four follicles, the multiple pregnancy rate increased without substantial gain in the overall pregnancy rate, ovarian stimulation

in IUI should not be aimed at producing more than two follicles. One stimulated follicle should be the goal if safety is the primary concern, whereas two follicles may be accepted after careful counselling of the couple (van Rumste et al, 2006).

A recent report showed that the strategies successful in reducing the high-order multiple pregnancy rate (to < 2%) in IUI include: use of clomifene (CC) before gonadotrophins, minimal gonadotrophin doses, cancellation if there are more than three follicles >10–15 mm, and aspiration of excess follicles. Pregnancy rates per subject will not be reduced if low doses are continued for 4–6 cycles (Dickey et al. 2009).

7 AIMS OF THE STUDY

The purpose of this study was to determine the factors influencing subject selection for successful intrauterine insemination treatment.

The specific aims were:

1. to assess the influence of HyCoSy in selecting subjects for IUI treatment,
2. to estimate the clinical usefulness of transvaginal hydrolaparoscopy instead of laparoscopy in verifying the HyCoSy findings,
3. to study the impact of the aetiology of infertility on the success of IUI treatment,
4. to evaluate the impact of post-wash sperm morphology on the outcome of IUI treatment.

8 SUBJECTS AND METHODS

8.1 SUBJECTS AND STUDY DESIGN

All subjects were recruited to the study protocols from the Department of Obstetrics and Gynaecology, Tampere University Hospital, Finland. Table 2 describes the study population, objectives and study design.

Table 2. Study material and design of Studies I–IV

Study	Study I	Study II	Study III	Study IV
Number of subjects	559	56	532	170
Number of cycles	1240		1171	268
Objectives	To compare the prognostic significance of tubal patency investigation by laparoscopy, air and saline HyCoSy and HSG before IUI	To investigate the accuracy of HyCoSy confirmed by THL	To study the prognostic factors regarding IUI success in different infertility aetiology groups	To evaluate the impact of sperm morphology on IUI PR before and after sperm preparation
Design	retrospective	prospective	retrospective	prospective
Main outcome measures	Pregnancy rate Cumulative PR	Tubal patency Accuracy of HyCoSy Usefulness of THL	Pregnancy rate Multiple PR	Sperm morphology Sperm anomalies PR

8.1.1 Studies I and III

The subjects in Study I comprised all 559 consecutive infertile women who attended Tampere University Hospital Infertility Clinic and who underwent intrauterine insemination treatment, the time period being from January 1996 to August 2003. Tubal patency was evaluated by means of laparoscopy and dye in 261 cases, by HyCoSy in 217 and by HSG in 81. Altogether, 1240 insemination cycles were evaluated: 571 cycles among women investigated by means of laparoscopy (46%), 494 cycles among women investigated by HyCoSy (40%) and 175 cycles among women investigated by means of HSG (14%) and the clinical pregnancy rates were compared in these three study groups. In cases of azoospermia, 27 women underwent AID (donor sperm insemination) in 69 cycles.

All the study couples had experienced at least 1 year of infertility (median 2.0 years (1–12)). The median age was 30.0 years (20–40) and 65.2% of the couples suffered from primary infertility. All couples underwent standard infertility investigation consisting of anamnesis, assay of FSH, prolactin, thyroid hormone and mid-luteal progesterone concentrations, and sperm analysis. If at least one Fallopian tube was patent and the sperm was normal or slightly subnormal, one to five (median 2.0) insemination treatments were carried out for each couple.

Infertility was considered male if the inseminated motile sperm count was $\leq 5 \times 10^6/\text{mL}$ in at least 50% of the IUI cycles. Ovulatory disorder was diagnosed when the menstrual cycle was not regular (21–35 days) and/or mid-luteal serum progesterone concentrations were $<10 \text{ nmol/L}$. For these subjects IUI was offered if ovulation induction did not result in pregnancy. The diagnosis of mild or moderate endometriosis was based on laparoscopic findings, observation of rectovaginal endometriosis in pelvic examination or endometrioma(s) in ultrasonographic scanning. The demographic data on the subjects showed no statistically significant differences between the subgroups (Studies I and III).

Studies I and III mostly involved the same subjects. In study III, donor inseminations were excluded, meaning that altogether 532 subjects and 1171 IUI cycles were included and five subgroups were formed according to aetiology: unexplained (54.4%), male factor (13.2%), ovulatory disorders (16.8%), endometriosis (10.8%) and multiple reasons (4.8%). Standard infertility and tubal investigations were performed and the different aetiology groups were formed as described above. There were no statistically significant differences in the baseline characteristics of the women in the various subgroups.

8.1.2 Study II

The study population consisted of 56 consecutive infertile women referred to the infertility clinic of Tampere University Hospital between October 2003 and September 2006. We included subjects who had previous known tubal risk factors such as pelvic inflammatory disease (PID), tubal pregnancy or pelvic operation (52%) or who had a history of minor genital infections or abdominal pain (48%). Women with no tubal risk factors in their past and who had bilateral patent tubes in HyCoSy were excluded due to potential complication risks associated with invasive investigation methods. None of these women had clinical evidence of tubal pathology. Subjects with endometriosis or sactosalpinx suspected in pelvic examination or in transvaginal ultrasonographic scanning were also excluded from the study and laparoscopy was carried out to make operative treatment possible. The other contraindications of THL were acute situations (bleeding or infection), obliterated cul-de-sac, massive obesity and fixed retroverted uterus.

Tubal investigation was started with HyCoSy carried out by the same experienced gynaecologist, using air and saline as a contrast medium and within one month THL with a dye test was carried out by another gynaecologist to confirm the findings. All THLs were performed without general anaesthesia in the operating theatre of the outpatient clinic.

The mean age was 31 (20–39) years and 73.2% of the subjects were nulliparous. All the couples had suffered from infertility for at least one year. Sperm quality was evaluated and women whose partners had normal or mildly subnormal sperm were included in the study.

8.1.3 Study IV

From April 2006 to June 2007, 268 homologous artificial inseminations were performed among 170 subfertile couples at the Infertility Clinic of Tampere University Hospital, with indications of mild or moderate male factor infertility. All couples underwent standard infertility investigation, consisting of anamnesis, assay of FSH, prolactin, thyroid hormone and mid-luteal progesterone concentrations of the women, and sperm analysis. Tubal patency was evaluated by hysterosalpingo-contrast sonography. One to four (mean 1.6) IUI cycles were performed per couple. The mean age of the women was 30.0 (19-40) years and the mean duration of infertility 2.0 (1-10) years. Of the couples, 70.1% suffered from primary infertility.

The sperm were evaluated according to WHO guidelines (1992). Accordingly, sperm was characterized to be normal when the concentration was ≥ 20 million/mL, motility $\geq 50\%$ and the

percentage of normal forms $\geq 30\%$. The most common finding was isolated teratozoospermia (46.6%), meaning that all the other sperm parameters were normal. In cases of severe male factor infertility the couples were advised to undergo either IVF or ICSI treatment.

8.2 METHODS

8.2.1 Techniques of HyCoSy (Studies I and II)

The HyCoSy procedures were performed between days 5 and 10 of the menstrual cycle. The subjects were advised to take analgesic drugs one hour before the procedure. The cervix was cleansed with sterile saline and a catheter was inserted into the uterine cavity.

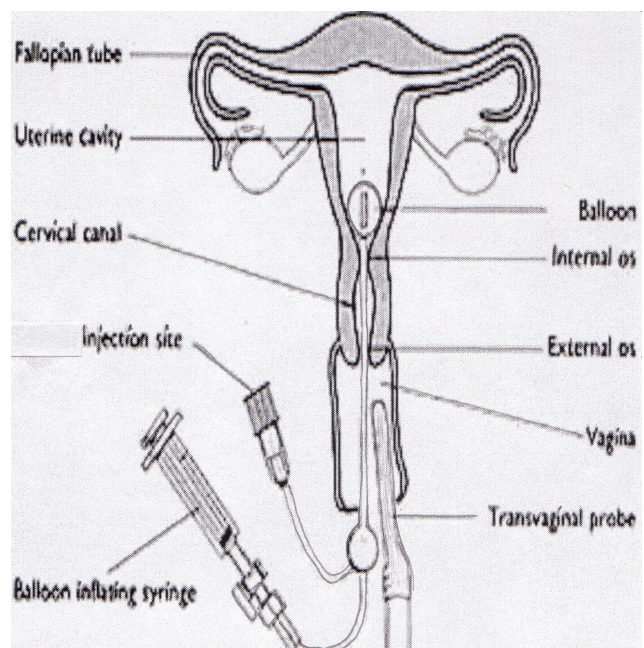


Figure 2. The technique of HyCoSy

The balloon was inflated with 1–2 mL of saline and positioned at the internal os of the cervical canal. A mixture of air and physiological saline, 2–5 mL at a time, was injected through the catheter during continuous transvaginal ultrasonographic scanning. The total amount of saline used during the procedure was between 10–15 mL. The uterine cavity was evaluated first and after that the field of view was adjusted so that the area between the uterine horn and the ovary was kept under observation. When flow through the Fallopian tubes emerging into the abdominal cavity was demonstrated, the tubes were considered patent.

8.2.2 Techniques of THL (Study II)

Transvaginal hydrolaparoscopy was also performed in the proliferative phase of the menstrual cycle, after bleeding had ended. All women received 1.5 g cefuroxime (Kefurion®) intravenously before the procedure. All the THL procedures were carried out by one gynaecologist (K. A-H) with one nurse as an assistant. All the procedures were carried out without general anaesthesia in the operating theatre of the outpatient clinic.

All procedures were performed using the technique described previously by Watrelot et al. (1998). Briefly, with the subject in a dorsal decubitus position, a Colin's speculum was inserted and the vagina was cleansed with sterile saline. Local anaesthesia was carried out by means of 5–10 mL of lidocaine applied about 1–1.5 cm below the cervix. The posterior lip of the cervix was then grasped with a tenaculum. For chromopertubation, the first balloon introducer (FT 1-29 Soprane SA, France) was inserted into the uterine cavity and the balloon inflated with 3 mL of air. Before entering the peritoneal cavity, 0.5–1.0 mL alfentanil (Rapifen®) was injected intravenously and analgesia was repeated at a later stage of the procedure if needed. A Veress needle was inserted into the pouch of Douglas and 200–400 mL of pre-warmed saline solution (37 °C) with lidocaine was freely injected into the abdominal cavity. The Veress needle was then removed and the second balloon introducer with a dilatator trocar (4 mm) was inserted into the pouch of Douglas. The balloon maintaining the introducer in the abdominal cavity was inflated with 5 mL of air. A 2.9 mm scope with a 30° lens and a flow channel (Karl Storz SA, Germany) was put through the trocar and a camera was attached to the scope. To allow free movement of the scope the tenaculum and the speculum were removed and saline solution was allowed to flow freely through the channel. The total amount of saline solution needed in the procedure varied from 500 to 700 mL. Evaluation started from the posterior wall of the uterus, moving toward the adnexae. The tubes were followed and every tubal part and the ovaries were examined. Methylene blue was then injected through the intrauterine introducer and chromopertubation was performed. A flow of blue dye at the fimbriae was considered as evidence of tubal patency. After examination of the pelvic cavity, as much of the fluid as possible was removed through the trocar. The vaginal posterior fornix was left to heal and the women were advised to avoid intercourse and tampons for 5 days. The women were discharged 2–3 hours after the procedure. All the findings were recorded.

8.2.3 Controlled ovarian stimulation (Studies III and IV)

Ovarian stimulation was accomplished with clomifene citrate, HMG or a combination regimen. Clomifene citrate (50–150 mg/d; Clomifen; Leiras, Turku, Finland) was given between days 3 and 7 of the menstrual cycle. Human menopausal gonadotrophin (Menogon; Ferring, Copenhagen, Denmark; or Humegon; Organon, Oss, the Netherlands) was started at 75–150 IU on cycle day 5 and the dose was adjusted according to the results of ultrasonographic monitoring. Clomifene citrate was the first alternative for ovarian stimulation and ovarian and endometrial responses were monitored by vaginal ultrasonography on cycle days 10 and 12. If the responses were unsatisfactory with CC, stimulation was continued with HMG. When the leading follicle reached 18 mm, 5000–10 000 IU of hCG (Profasi; Serono Laboratories, Rome, Italy) was administered and after 39 hours intrauterine insemination was performed with 1.0 mL of the partner's washed sperm. Luteal support (progesterone vaginal suppositories, 200 mg × 3) was used in cases of irregular bleeding in the luteal phase in previous cycles.

8.2.4 Sperm preparation and assessment of morphology (Studies III and IV)

Semen samples were collected by masturbation after 3–5 days of sexual abstinence. Sperm were prepared for swim-up after 30–60 minutes of room temperature liquefaction. After liquefaction each sample was analysed using WHO guidelines. The sample was then centrifuged at $500 \times g$ for 10 min, the seminal plasma was carefully removed and the pellet diluted in 2–3 mL of IVF medium (MediCult/Universal IVF-Medium with Phenol Red; Copenhagen, Denmark). After re-centrifugation at $500 \times g$ for 5 minutes the supernatant was discarded, 1 mL of IVF medium was added and the sperm suspended. The same protocol was repeated once more and the final pellet was gently covered with 1 mL of medium and incubated for 30–60 min at 37 °C in a leaning position. After incubation, swim-up was very carefully separated and analysed. Morphology was evaluated using WHO 1992 guidelines instead of other (strict) criteria in order to find more differences in morphology before and after preparation. This was done because at present almost every sperm sample has a very low amount of normal forms (< 5%) when analysed by using strict criteria and it is impossible to achieve statistical differences in sperm morphology. In assessing sperm morphology, 200 spermatozoa were looked at before and after the washing procedure. The smears

were stained using Mayer's haematoxylin-eosin. All sperm preparations and evaluation of morphology were carried out in the sperm laboratory of Tampere University by a single laboratory technician with substantial experience. Our laboratory has annually taken part in Labquality Andrological External Quality Assessments since 1995. Labquality data shows that the differences between Labquality reference values and the values of our laboratory have never exceeded 10%.

8.2.5 Insemination (Studies I, III and IV)

Before insemination, endometrial thickness and the presence of ovulation were assessed by transvaginal ultrasonography. The procedure was carried out by using an intrauterine catheter (Gynetic, Gynetics Medical products; Hamont-Achel, Belgium) with a 2 mL syringe. With the woman in the lithotomy position, the IUI catheter was gently directed into the uterine lumen and the sperm suspension slowly infused. The women were allowed to stand up and leave immediately. They were advised to perform a urinary pregnancy test 14 days after insemination. Ultrasonographic scanning was carried out in all instances of pregnancy.

8.3 STATISTICAL ANALYSIS

In Studies I and III categorical variables between the groups were compared using the Chi square test and results were presented as median values. In Studies III and IV associations between PR and independent variables were assessed by means of binary logistic regression analysis and odds ratios (ORs) with their 95% confidence intervals were presented. In Study IV repeated IUI cycles on individuals were included and each woman was considered as her own cluster. In multivariate analysis (Study IV), morphology and head anomalies were entered separately into the model as a result of their high negative correlation ($r_p = -0.98$). In Study II the diagnostic accuracy of HyCoSy was expressed in terms of its concordance with THL, and sensitivity, specificity, negative predictive value and positive predictive value with their respective 95% confidence intervals. In Studies I, II and III all statistical analyses were performed using SPSS for Windows, either version 11.0 or 14.0 (SPSS Inc., Chicago, Illinois, USA). In Study IV all statistical analyses were analysed by using Stata 8.2 for Windows (StataCorp LP, College Station, Texas, USA).

8.4 ETHICAL CONSIDERATIONS

The Ethics Committee of Tampere University Hospital approved the study protocols. All subjects participating in a prospective study gave their informed written consent before entering the study protocol.

9 RESULTS

9.1 The validity of air and saline HyCoSy in tubal patency investigation in relation to IUI success

Bilateral tubal patency was found in 80% of the women investigated by means of laparoscopy, in 83% of those evaluated by HyCoSy and in 91% of those evaluated by HSG.

The clinical pregnancy rates per cycle were 14%, 18% and 18% in the laparoscopic, HyCoSy and HSG groups, with no statistically significant difference between the groups. The numbers of tubal pregnancies were similar in the subgroups. Four tubal pregnancies were found in women investigated by laparoscopy and three tubal pregnancies were diagnosed in women evaluated by HyCoSy (Table 3).

TABLE 3. Results of IUI treatment per cycle

	Laparoscopy	HyCoSy	HSG	Total	p
No. of cycles	n=571	n=494	n=175	n=1240	
Pregnancy % (n)	14 (78)	18 (88)	18 (31)	16 (197)	0.14
Delivery %	73	76	68	74	0.45
Miscarriage %	19	21	32	22	
Tubal pregnancy %	5	3	0	4	
Result unknown %	3	0	0	0	

The cumulative pregnancy rates (mean 2.3 cycles) were 30%, 41% and 38% respectively, with a significant difference between laparoscopy and HyCoSy (Table 4).

TABLE 4. Cumulative results of IUI treatment

	Laparoscopy	HyCoSy	HSG	Total	p
No. of subjects	n=261	n=217	n=81	n=559	
Pregnancy %	30	41	38	35	0.043
Delivery %	22	31	26	26	0.081

In cases of unilateral patency, cumulative pregnancy rates after two cycles were 18% (laparoscopy), 29% (HyCoSy) and 29% (HSG), with no statistically significant difference between the subgroups.

In the laparoscopy group eleven of the pregnancies were multiple (14%); three of them being triplet pregnancies. The twin pregnancy rate in the HyCoSy group was 13% (n=11) and no multiple pregnancies were found in the HSG group.

9.2 THL in verifying the findings in HyCoSy

In HyCoSy the women's compliance was good and no complications occurred. In THL access to the pouch of Douglas failed in five cases (8.9%). In three of them the reason was fixed retroverted uterus and in one case, massive obesity. No reason for failed access was found in one woman. The complication rate was low, 1.8%, consisting of one postoperative haematoma in the fossa Douglas, healing spontaneously. All the other women returned to their professional activities next day. Altogether, 51 subjects and 101 tubes were examined; one woman had only one tube (Table 5). Of the tubes investigated by HyCoSy, 61 were patent and 40 occluded. In THL, 82 of 101 tubes were patent and 19 were occluded. Of the 40 tubal occlusions suspected in HyCoSy, 18 were occluded and 22 were patent in THL. Of 61 tubes found to be patent in HyCoSy, 60 were also patent in THL. The concordance, sensitivity, specificity, positive predictive value and negative predictive value are shown in Table 5. Tubal patency per subject is presented in Table 6.

Table 5. Agreement between HyCoSy and THL in detection of tubal patency in all (n=101) Fallopian tubes in 51 patients

HyCoSy	THL		Total
	Occlusion	Patent	
Occlusion	18	22	40
Patent	1	60	61
Total	19	82	101

Concordance 0.77

Sensitivity 0.95, 95% CI 0.75–0.99

Specificity 0.73, 95% CI 0.63–0.82

Positive predictive value 0.45, 95% CI 0.31–0.60

Negative predictive value 0.98, 95% CI 0.91–1.00

Table 6. Tubal patency in hysterosalpingo-contrast sonography (HyCoSy) and transvaginal hydrolaparoscopy (THL) per subject (n=50)

	HyCoSy	THL
Bilateral patency	24	37
Unilateral patency	13	7
Bilateral occlusion	13	6
Total	50	50

Because of major adhesions and/or sactosalpinx (not visible in ultrasound) in THL, five subjects (9.8%) with confirmed occlusion were referred to laparoscopy. Two of them were treated by means of unilateral salpingectomy and one by means of bilateral salpingectomy. Salpingolysis was performed in one case, resulting in spontaneous pregnancy. One subject had untreatable adhesions.

9.3 Aetiology of infertility in successful IUI treatment

The pregnancy rate/cycle was highest (19.2%) among women with anovulatory infertility and lowest (11.9%) in endometriosis-based infertility. The multiple pregnancy rate varied between 3.6% (male infertility) and 13.2% (anovulatory infertility). The PRs per cycle according to aetiology are presented in Table 7.

In unexplained infertility (PR 14.1%, MPR 8.9%), logistic regression analysis showed that the two positive predictors of pregnancy were the number of preovulatory follicles (OR one/three follicles = 3.26, 95% CI = 1.72–6.20) and the inseminated motile sperm count (OR lowest/highest = 3.44, 95% CI = 0.79–14.98). Infertility duration of more than three years and increased age of the woman affected the IUI results negatively, but not statistically significantly. All the stimulation protocols were equally effective.

In cases of male infertility most of the cycles were performed with a TMC of less than 5 million per mL, with a good pregnancy rate of 16.5%, the total PR in this group being 18.2%. The MPR (3.6%) was the lowest in this subgroup. Stimulation with sequential CC/HMG resulted in the best

PR/cycle (25.0%). In this group, female age, infertility duration and the number of follicles did not influence the outcome of IUI.

TABLE 7. Pregnancy rates (%) per cycle (n) according to aetiology

	unexplained		male		anovulatory		endometriosis		many reasons	
	%	n	%	n	%	n	%	n	%	n
Overall PR	14.1	637	18.2	154	19.2	198	11.9	126	17.9	56
Female age (y)										
<25	25.0	32	0	11	10.0	10	28.6	7	0	4
25-29	16.2	204	18.8	48	22.5	111	11.7	60	18.2	33
30-34	11.9	235	20.3	69	15.4	65	11.1	54	18.8	16
35-39	12.7	166	19.2	26	16.7	12	0	5	33.3	3
Infertility duration (y)										
<2	17.9	78	20.0	20	21.3	61	21.1	19	30.8	13
2 – 3	15.8	283	18.1	83	23.4	77	9.7	72	6.7	15
>3	10.2	263	18.0	50	10.5	57	12.5	32	17.9	28
Type of infertility										
primary	12.8	406	16.3	86	17.3	121	10.9	101	20.8	48
secondary	16.0	231	20.6	68	22.1	77	16.0	25	0	8
Cycle no.										
1	14.9	275	17.3	81	18.7	91	11.9	59	6.9	29
2	13.0	192	22.2	45	17.5	57	10.3	39	23.5	17
3	12.6	119	9.5	21	18.2	33	16.0	25	44.4	9
≥4	17.6	51	28.6	7	29.4	17	0	3	0	1
Follicle number										
1	9.2	218	16.3	49	16.2	68	11.4	35	0	19
2	14.3	273	17.5	63	17.0	94	10.0	60	23.1	26
3	24.2	120	18.9	37	29.6	27	11.5	26	33.3	9
≥4	7.7	26	40.0	5	33.3	9	40.0	5	50.0	2
Stimulation regimen										
CC	15.5	193	16.9	71	15.4	65	21.1	38	7.1	14
HMG	14.0	387	19.1	68	17.9	106	7.5	80	18.8	32
Combination	11.5	52	25.0	12	36.0	25	12.5	8	30.0	10
Sperm count (x10 ⁶ /mL)										
<5	6.7	30	16.5	91	0	6	0	3	18.2	33
5.1–10	11.4	105	25.0	24	28.6	21	4.5	22	11.1	9
10.1–20	11.0	145			20.4	54	5.6	36	12.5	8
20.1–30	11.2	98			24.1	29	11.5	26	0	1
>30	19.8	237			16.7	78	24.3	37	0	2

In anovulatory infertility (PR 19.2%, MPR 13.2%) ovulation induction by means of sequential CC/HMG was significantly associated with the PR (OR = 3.3, 95% CI = 1.14–9.59), this being 36%/cycle. A short duration of infertility and an increased number of follicles also positively affected the pregnancy rate, but the tendency did not reach statistical significance.

In endometriosis-based infertility (PR 11.9%, MPR 6.7%) the pregnancy rate decreased with increasing female age and the best PRs were observed after CC stimulation (21.1%) and with an IMC $> 30 \times 10^6/\text{mL}$ (24.3%), but these variables were not statistically significant.

The number of couples with combined infertility aetiologies was low and therefore the variables did not show significant statistical differences. The highest pregnancy rate was achieved with sequential CC/HMG stimulation and with three follicles (30%), and even with an inseminating motile sperm count of < 5 million per mL the PR was 18.2%.

Intrauterine insemination treatment resulted in 181 pregnancies. The overall PR per cycle was 15.5% and per couple 34.0%. Seventeen multiple pregnancies (9.4%) occurred and three of them were triplet pregnancies (1.7%). Pregnancy ended in miscarriage in 22.2% of cases and 3.6% were ectopic. Ovulatory dysfunction was associated with the highest miscarriage rate (30.0%) and the multiple reasons group had the lowest rate (12.5%).

9.4 Impact of post-wash sperm morphology on IUI outcome

A total of 44 pregnancies (16.4 %) occurred in 268 insemination cycles. In binary logistic regression analysis the percentage of normal forms of sperm in post-wash samples had a significant influence on IUI pregnancy rate ($p=0.026$). When sperm anomalies were detailed, it turned out that this was due to a decreased amount of head anomalies after preparation ($p= 0.008$). The amounts of neck and flagellar anomalies were low and not associated with IUI outcome. Secondary infertility also predicted pregnancy ($p=0.030$) (Table 8).

Teratozoospermia (normal forms $< 30\%$) was found in 253 sperm samples. Isolated teratozoospermia (concentration ≥ 20 million/mL, progressive motility $\geq 50\%$ and normal forms $< 30\%$) was noticed in 125 cases (46.6%). The pregnancy rate (17.6%) in cycles with isolated teratozoospermia was better than that (13.3%) in cycles ($n=128$) with teratozoospermia combined with (an) additional poor semen parameter(s) (concentration < 20 million/mL or progressive motility $< 50\%$). The difference was not statistically significant, probably because of the small number of cycles. In 15 cycles the overall morphology was considered normal ($\geq 30\%$ normal forms), but sperm concentration or motility were subnormal. In isolated teratozoospermia all the

other sperm parameters were better than in the cases with additional male factor infertility (concentration < 20 million/mL and/or progressive motility < 50%).

Table 8. Univariate and multivariate logistic regression analysis of the determinants of 268 IUI cycles

Determinant	Non-pregnant	Pregnant	p	Univariate analysis		Multivariate analysis	
	(n=224)	(n=44)		OR	(95% CI)	OR	(95% CI)
Age (years) ^a	30.0	31.0	0.555	0.97	(0.92-1.05)	0.95	(0.88-1.03)
Duration of infertility (years) ^a	2.0	2.0	0.930	0.99	(0.78-1.25)	1.05	(0.80-1.39)
Type of infertility							
Primary (%)	72.8	56.8		1.00	(referent)		
Secondary (%)	27.1	43.2	0.030	2.03	(1.07-3.84)	2.40	(1.20-4.82)
Stimulation regimens per cycles							
Clomifene (%)	21.5	20.5		1.00	(referent)		
Clomifene + gonadotrophin (%)	32.7	34.1	0.847	1.10	(0.43-2.79)	0.77	(0.28-2.16)
Gonadotrophin (%)	45.7	45.5	0.919	1.05	(0.44-2.49)	0.85	(0.32-2.22)
Number of follicles ^a	1.0	1.0	0.463	1.22	(0.72-2.07)	1.14	(0.67-1.96)
Sperm parameters ^a							
Concentration	63	63	0.273	1.00	(1.00-1.01)	1.01	(1.00-1.02)
Motility (a+b) (%)	50	52	0.909	1.00	(0.99-1.02)	1.00	(0.98-1.02)
Post-wash TMC (10 ⁶ /mL)	15	16	0.840	1.00	(0.99-1.02)	1.00	(0.98-1.02)
Morphology ^a							
Pre-wash normal morphology (%)	17	19	0.064	1.04	(1.00-1.09)	1.04	(0.99-1.10)
Post-wash normal morphology (%)	27	31	0.026	1.04	(1.00-1.08)	1.05	(1.01-1.09)
Change in normal morphology (%)	10	11	0.394	1.02	(0.97-1.07)	1.01	(0.94-1.09)
Sperm head anomalies ^a							
Pre-wash head anomalies (%)	81	77.5	0.096	0.97	(0.94-1.00)	0.97	(0.94-1.00)
Post-wash head anomalies (%)	72	68.5	0.008	0.95	(0.92-0.99)	0.94	(0.89-0.99)
Change in head anomalies (%)	-9	-9	0.273	1.02	(0.98-1.06)	1.02	(0.96-1.07)

Morphology and head anomalies were entered separately in multivariate analysis because of high negative correlation ($r_P = -0.98$)

^a median values

10 DISCUSSION

Every sixth Finnish couple has difficulty achieving pregnancy within one year (Tiitinen 2008). Primary investigations of infertile couples include confirming ovulation and tubal patency, as well as sperm analysis. In contrast to the rapid development of assisted reproductive techniques (ARTs) such as IVF and ICSI, intrauterine insemination has remained a low-cost, low-tech approach which is often chosen for first-line treatment of infertile couples in cases of unexplained or male infertility. The current best evidence continues to support the long-established clinical strategy based on progression from low-tech to high-tech treatment (Collins 2003).

In the current work we have made an attempt to find out the best and most cost-effective tubal investigation methods and optimal predictive factors for successful IUI.

In recent years HyCoSy has taken its place as the primary tubal patency investigation method, rather than laparoscopy. Because of this change in our clinical practice we assessed its influence on IUI results. In our study concerning tubal investigations, HyCoSy findings were prospectively compared with the findings in transvaginal hydrolaparoscopy, which is at present a rarely used investigation method in our country.

There are many investigations concerning the impact of infertility aetiology on the success of IVF treatment, but no previous studies have focused on this aspect as regards IUI treatment. In the present retrospective study we analysed the factors having an impact on successful outcome of IUI among different aetiology groups.

Although sperm parameters after semen preparation have been shown to be more predictive as regards IUI success than parameters in raw samples, there are only a few prospective studies concerning the impact of post-wash sperm morphology on the pregnancy rate after IUI.

10.1 Tubal investigations before IUI treatment (Studies I and II)

Although laparoscopy with dye has been considered to be the gold standard in the diagnosis of tubal pathology, non-invasive HyCoSy has become the most popular method in the evaluation of tubal patency in many countries, e.g. in Finland. Laparoscopy is approximately ten times more expensive than HyCoSy with air and saline and there are no resources to offer laparoscopy to every infertile

woman. Furthermore, HyCoSy is a safe and time-saving procedure that can easily be performed even at the first visit to the infertility clinic.

Since 1997, when HyCoSy was first presented in our clinic, we have recorded a decrease in the number of laparoscopic procedures. Today, the percentage of laparoscopic procedures in the evaluation of tubal patency in our clinic is < 10%. One of our aims was to evaluate if this practical change has affected the results of IUI treatment. It could be assumed that by using this non-invasive method, tubal pathologies would be missed. In our first study we compared the results of insemination treatment when tubal patency was evaluated by laparoscopy, HSG or HyCoSy with a mixture of air and saline as a contrast medium. The results showed that the pregnancy rate among women whose tubal patency was confirmed by means of HyCoSy was at least as high as that among women evaluated by means of laparoscopy or HSG. Cumulative pregnancy rates were also satisfactory in cases of unilateral tubal patency. The use of air and saline HyCoSy in subject selection did not seem to increase the rate of ectopic pregnancies.

Our results support the role of HyCoSy as a first-line procedure for the assessment of tubal patency, as has been reported in several studies which have demonstrated a high concordance rate (80–91%) of HyCoSy compared with laparoscopy and HSG or laparoscopy alone (Volpi et al. 1991, Heikkinen et al. 1995, Volpi et al. 1996, Holz et al. 1997, Spalding et al. 1997, Hamilton et al. 1998, Reis et al. 1998, Hauge et al. 2000, Tanawattanachaoren et al. 2000, Exacoustos et al. 2003, Shahid et al. 2005).

Healthy Fallopian tubes are not visible in ultrasonography, and therefore a contrast medium is required. In the majority of studies Echovist[®] has been used as the ultrasonographic contrast medium (Campell et al. 1994, Holz et al. 1997, Hamilton et al. 1998, Reis et al. 1998, Strandell et al. 1999, Dijkman et al. 2000, Hauge et al. 2000, Skinner et al. 2000, Hamilton et al. 2003, Chan et al. 2005, Shahid et al. 2005, Tamasi et al. 2005), but investigations involving the use of air and saline as a contrast medium seem to have concordance rates (vs. laparoscopy or HSG) as high as in studies with positive contrast medium (Heikkinen et al. 1995, Chenia et al. 1997, Spalding et al. 1997, Exacoustos et al. 2003). In studies in which the contrast media used in HyCoSy have been compared, some investigators found a mixture of air and saline to be comparable with Echovist[®] (Deichert et al. 1989, Spalding et al. 1997), whereas in one report saline was found to be worse than a positive contrast agent (Boudghene et al. 2001). Our results agree with the concept that the use of air and saline as a contrast medium gives sufficiently reliable results and is also safe and cost-effective.

It is also important to point out that the use of HyCoSy was not associated with an increase in the rate of tubal pregnancy, as might be assumed when non-invasive methods are used. The rate of

ectopic pregnancy in our study was similar to that in earlier studies (Hamilton et al. 2003). Flushing the Fallopian tubes might also serve as infertility treatment, removing cellular debris from the tubal lumen. A recent review article indicated that tubal flushing with oil-soluble contrast media increases the odds of pregnancy and live birth, versus no intervention (Luttjeboer et al. 2005).

Most studies reporting the results of IUI treatment have included only subjects with bilateral tubal patency. Our study showed a satisfactory pregnancy rate even among women with unilateral tubal patency, as in a recent report by Farhi et al. (2007), where women with unilateral proximal tubal occlusion diagnosed by HSG had a cumulative pregnancy rate of 30.9%. However, a better cumulative PR (29%) among women in the HyCoSy and HSG groups than in the laparoscopy group (18%) might be due to benign unilateral tubal spasm, which was assessed to be very common when using the non-invasive method in this work (Study II). Moreover, this finding justifies the use of IUI among women with unilateral patency.

The reason why the laparoscopy group had a lower pregnancy rate might be a result of subject selection: women with a suspicion of pelvic pathology were more likely to be placed in the laparoscopy group. Although unilateral or bilateral patency among these women was addressed by laparoscopy, mild endometriosis might have had an influence on pregnancy outcome. However, all the laparoscopic procedures were only diagnostic. On the other hand, apparently healthy women (with moderate male factor infertility) were more likely to have undergone HyCoSy than laparoscopy in the evaluation of tubal patency.

Transvaginal hydrolaparoscopy and dye test with optional salpingoscopy and hysteroscopy allows complete evaluation of the reproductive tract. It is an outpatient method that does not require operating theatre facilities. In addition, it can be performed by one doctor with a nurse and it does not necessitate absence from professional activities. Thus, it is a cost-effective method when compared with conventional laparoscopy. It has proven to be a reliable method in investigation of tubal patency (Casa et al. 2002, Watrelot et al. 2003), but it has not been previously used as a confirmatory tool in combination with HyCoSy to assess tubal patency. The method is easy to perform when the learning curve plateau has been reached.

The aim of our second study was to assess the clinical usefulness of THL versus laparoscopy in verifying the findings in HyCoSy. It has been reported earlier that the positive predictive value of ultrasonographic methods has been good as regards tubal patency, but unsatisfactory for tubal occlusion (Deichert et al. 1992). The results of the present study are in line with the concept above and confirm that hysterosalpingo-contrast sonography has an excellent 95% sensitivity in the evaluation of tubal patency. On the other hand, the specificity (73%) and especially the positive

predictive value (45%) of HyCoSy remain low as a result of tubal spasm and difficulty in distinguishing between true occlusion and benign spasm.

In present study the concordance rate between these two methods (HyCoSy and THL) was 77%, which is slightly lower than in studies in which HyCoSy and laparoscopy with dye have been compared (Volpi et al. 1991, Heikkinen et al. 1995, Volpi et al. 1996, Holz et al. 1997, Spalding et al. 1997, Hamilton et al. 1998, Reis et al. 1998, Hauge et al. 2000, Tanawattanachaoren et al. 2000, Exacoustos et al. 2003, Shahid et al. 2005). However, it has been reported that the concordance rate between HyCoSy and laparoscopic chromopertubation decreased from 86% to 67% when it was based on the affected tubes only (Spalding et al. 1997b). In our study the cases were selected on the basis of a history of previous tubal risk factor and this may have attenuated the concordance rate.

It is well known that non-invasive methods are associated with false-positive results in cases of tubal occlusion as a result of tubal spasm caused by manipulation of the pelvic organs. Injected contrast media and pain are the main reasons for tubal spasm. A recent study has shown that warming contrast media to body temperature reduces discomfort and this could also reduce the possibility of tubal spasm (Nirmal et al. 2006). To rule out false occlusion in HyCoSy, waiting for a few minutes, using pain medication or repeating HyCoSy later may be beneficial. In some women visualization of tubal flow by means of Doppler ultrasonography could be helpful. On the basis of our results the possibility of tubal spasm should be kept in mind when tubes appear to be occluded in HyCoSy, even if there is a history of previous tubal risk factors.

In the literature the complication rate associated with THL has been low: in a total of 4,232 procedures there were 26 bowel injuries (0.61%). In a recent study involving 177 women, the incidence of rectal perforation was 1.1% (Shibihara et al. 2007). In our work the complication rate was 1.8% (one haematoma in the fossa Douglas) with no bowel injuries. It has also been shown that with increasing experience, the prevalence of bowel injury can be lowered significantly (Gordts et al. 2001), which is in line with our experience. In addition, according to our experience, the most important aspect of successful THL is appropriate selection of subjects.

The combination of HyCoSy and transvaginal hydrolaparoscopy is inexpensive and more time-saving for the subject than HyCoSy followed by laparoscopy, or laparoscopy alone. Our study showed that in every second case a false-positive tubal occlusion in HyCoSy was a result of benign tubal spasm. Thus, most women do not need conventional laparoscopy and final diagnosis can be verified by THL.

We can conclude that HyCoSy with a mixture of air and saline as a contrast medium is a reliable method in selecting women for insemination treatment, which is also justified in cases of unilateral tubal patency. Our results show that the increased use of this inexpensive outpatient

investigation method does not impair the IUI pregnancy rate and should be used as a first-line tubal investigation method. In cases of suspected tubal occlusion in HyCoSy, transvaginal hydrolaparoscopy is a safe, practical and inexpensive procedure to verify the findings in carefully selected subjects. Laparoscopy can be reserved for those women with pelvic pain or suspicion of pelvic pathology in gynaecological status or ultrasonographic examination.

10.2 Role of aetiology in successful IUI

Intrauterine insemination is a simple and relatively non-invasive first-line treatment in most cases of subfertility. Furthermore, ovulation induction and IUI have been shown to be cost-effective given the four- to five-fold lower cost compared with IVF treatment (Collins et al. 2003, Hughes, 2003). The most important findings in our second study were the significance of the aetiology of infertility in the prognosis of insemination treatment and the differences in factors affecting the pregnancy and multiple pregnancy rates. To our knowledge there have been no earlier reports on the impact of various independent variables within infertility aetiology groups.

In unexplained infertility and IUI treatment, ovulation induction seems to be the most crucial factor. Our study showed that in the subgroup of unexplained infertility more than one follicle at the time of ovulation was identified as a statistically significant positive prognostic factor and the MPR was 8.9% even with two to three leading follicles. The pregnancy rate with $> 30 \times 10^6$ motile spermatozoa per mL was almost three times higher (19.8% vs. 6.7%) than that in the lowest sperm count category. According to our results, IUI treatment in cases of unexplained infertility can be recommended to couples with a young female (< 30 years), a short duration of infertility (< 3 years), 2–3 preovulatory follicles and a high inseminated motile sperm count ($> 30 \times 10^6$ /mL).

It is well documented that IUI is the best first-line treatment in cases of mild and moderate male factor infertility (Ombelet et al. 2003, Cohlen et al. 2005). In our data, female age up to 39 years, duration of infertility and the number of follicles did not influence the outcome of IUI. Use of sequential CC/HMG in ovarian induction showed the best result and most of the pregnancies resulted from the first two IUI cycles. The lowest sperm count resulting in pregnancy was 0.6 million per mL. Our results (PR of 16.5% with TMC $< 5 \times 10^6$ /mL) are in agreement with those in a review article by Ombelet et al. (2003) confirming that IUI treatment is very successful in couples having an TMC of less than 5×10^6 /mL, a fact disputed in some previous studies (Stone et al. 1999, Khalil et al. 2001, Iberico et al. 2004). Total motile sperm count is a well-known predictor of IUI

treatment, but an exact cut-off level has not been determined owing to a lack of standardization of semen analysis and other variables affecting IUI success (Ombelet et al. 2003).

In cases of anovulatory infertility the PR was highest (19.2%) of all subgroups, as was the MPR (13.2%). This subgroup also had the highest rate of miscarriages (30.0%), which could be due to obesity and polycystic ovarian syndrome, which are known risk factors of miscarriage. Ovarian induction with sequential CC/HMG led to a significantly higher PR per cycle (36.0%) when compared with HMG (17.9%) or CC (15.4%) alone, which is in accordance with earlier findings (Dickey et al. 1993, Ryan et al. 2005). Three or more follicles at the time of insemination resulted in very high PRs, but also high MPRs. Two of three sets of triplets in the study were in this subgroup. According to our results, stimulation of an anovulatory woman, resulting in three or more follicles, should be avoided in order to minimize the MPR rate.

A diagnosis of endometriosis reduces the success of IUI treatment. A meta-analysis (Hughes et al. 2003) showed that in infertility associated with endometriosis the effectiveness of ovarian stimulation and IUI was reduced by approximately half in cases of persistent infertility, but the results of a recent study (Werbrouck et al. 2006) suggested that ovarian stimulation and IUI in women with surgically treated mild endometriosis is as effective as in women with unexplained infertility. The PR of 11.9% per cycle in our study was acceptable and slightly better than in previous studies (Nuojuua-Huttunen et al. 1999). Intrauterine insemination can therefore be recommended as a worthwhile first treatment for women with mild or moderate endometriosis, especially when female age (< 25 years), duration of infertility (< 2 years) and motile sperm count ($> 30 \times 10^6/\text{mL}$) are favourable.

Most of the couples in the multiple reasons subgroup had mixed diagnoses of male factor-, anovulatory-, tubal- and endometriosis-based infertility. Because the number of cycles in this subgroup was low ($n = 56$) conclusions cannot be drawn. Nevertheless, it seems that IUI in cases of moderate male infertility ($\text{IMC} < 5 \times 10^6/\text{mL}$) combined with some other infertility problem can be successful, the PR/cycle being 18.2%.

Female age has been found to be a predictive determinant of the likelihood of pregnancy in many studies (Tomlinson et al. 1996, Nuojuua-Huttunen et al. 1999, Stone et al. 1999, Dickey et al. 2002, Ghosh et al. 2003), but not in all (Khalil et al. 2001, Iberico et al. 2004) and in our study group increasing female age was associated with a decrease in pregnancy rate, although this correlation was not significant, probably as a result of the age limit of < 40 years. In our study series IUI treatment was also effective in women aged 35–39 years, with the PR of 13.7% being similar to that in other studies (Iberico et al. 2004). However, it seems that there is a negative linear

association between a woman's age and pregnancy rate after IUI, although PRs seem acceptable up to the age of 40 (Nuojuua-Huttunen et al. 1999, Goverde et al. 2000, Wainer et al. 2004).

With the exception of the infertility, we found that a decreased PR was associated with a longer duration of infertility. As regards other causes of infertility, a duration of infertility of more than three years should be considered when choosing between IUI and IVF treatment, as has been reported in earlier studies (Tomlinson et al. 1996, Iberico et al. 2004).

Stimulation with sequential CC and HMG resulted in a higher PR in cases of male-type, anovulatory and multifactorial infertility. The MPR in women stimulated by means of this protocol (9.1%) was lower than in those stimulated with HMG alone (13.3%), but considerably higher than in those in the CC group (3.3%), which is in agreement with the results of an earlier study (Ryan et al. 2005), confirming that stimulation with CC + gonadotrophins reduces the risk of MPR when compared with gonadotrophins alone. In the whole study group the multiple pregnancy rate was 9.4% and three pregnancies of 181 (1.7%) were trigemini (two in the anovulatory group and one in the 'unexplained' group). As our results show, in IUI treatment the cause of infertility must be considered in ovulation induction. Because the risk of multiple pregnancy is lowest with male-type and endometriosis-based infertility, two to three follicles can be favourable. In cases of unexplained infertility two follicles could be ideal, although three follicles result in the best pregnancy rates. However, in anovulatory women only one follicle is acceptable.

There have been earlier reports indicating that endometrial pattern on the day of insemination may predict IUI success (Hock et al. 1997, Tsai et al. 2000) and conflicting results concerning endometrial thickness (Tomlinson et al. 1996, Hock et al. 1997, Nuojuua-Huttunen et al. 1999, Tsai et al. 2000, Kolibianakis et al. 2004, Esmailzadeh et al. 2007). In the present study endometrial thickness was not a prognostic factor as regards IUI outcome, nor was the use of luteal support.

In planning individual treatments the role of aetiology must be considered, because in different infertility subgroups the variables influencing the probability of pregnancy have different impacts, as our study has shown. The future of IUI will depend on our ability to maintain an acceptable MPR (< 10%) without affecting the overall PR (> 10%). A change in the nature of stimulation can also be seen in our study material, with a decreasing tendency in the number of follicles during the study years (1996–2003). Hence, when aiming at single embryo pregnancies with IUI, it must be remembered that the number of follicles, the stimulation protocol and the necessary sperm count are not identical as regards the different causes of infertility.

10.3 Impact of post-wash sperm morphology on IUI outcome

Although intrauterine insemination is one of the most common assisted reproductive technology methods, the relative influence of various semen characteristics on the likelihood of a successful outcome is controversial. In this prospective study we evaluated the prognostic value of sperm morphology before and after sperm preparation in connection with the success rate in cases of intrauterine insemination with male infertility. The increase in the proportion of normal forms after sperm preparation predicted conception and this was a result of the decreased percentage of spermatozoa with head anomalies after sperm washing. Moreover, isolated teratozoospermia seemed not to influence the success rate of IUI.

Studies on the impact of sperm morphology, before and after preparation, as regards IUI outcome have led to divergent results (Francavilla et al. 1990, Matorras et al. 1995, Burr et al. 1996, Karabinus and Gelety, 1997, Dickey et al. 1999, Hauser et al. 2001). In earlier studies a correlation between sperm morphology and fertilization rate in IVF treatment has seemed clear (Kruger et al. 1986, Ombelet et al. 1994, Coetzee et al. 1998, Liu et al. 2002), but the recent studies have shown opposite results (Keegan et al. 2008, Chen et al. 2009). In IUI a meta-analysis of six studies showed that the tendency to achieve pregnancy was significantly decreased when the percentage of morphologically normal spermatozoa was < 4%, as evaluated by strict criteria (van Waart et al. 2001). Using WHO morphological evaluation (1987, 1992), no differences have been detected in pregnancy rates when using 30% as a morphological cut-off point (Burr et al. 1996, Tomlinson et al. 1996). In a study by Wainer et al. (2004), the results showed that a minimum of 5×10^6 motile spermatozoa per mL should be inseminated when the normal morphology of the sperm after preparation is < 30%, meaning that the quantity compensates at least in part for the defective quality, but results considering sperm morphology only, before or after preparation, did not help to predict IUI results. Several other investigators have reported the same conclusions (Matorras et al. 1995, Karabinus and Gelety, 1997, Dickey et al. 1999), but opposite findings have also been published (Francavilla et al. 1990, Burr et al. 1996, Hauser et al. 2001). The threshold for the percentage of spermatozoa with normal morphology below which IVF is recommended thus varies according to team and technique from 4% to 50% (Wainer et al. 2004).

Semen preparation may modify sperm characteristics considerably, and the number of motile spermatozoa and the morphological criteria should logically be assessed after semen preparation. In most cases overall sperm morphology improves after preparation and thus the proportion of normal forms might exceed the threshold needed for pregnancy. In our study, using WHO 1992 criteria, even a modest increase in the percentage of sperm with normal morphology in post-wash samples

had a significant influence in IUI pregnancy rate. However, taking into account the fact that the mean total count of spermatozoa in the sample was over 200 million, a one percent increase after preparation means an increase of two million spermatozoa with normal features.

In a study by Hauser et al. (2001), the extent of change in overall sperm morphology after preparation was significantly greater in their pregnant group than in the non-pregnant group. This tendency was also demonstrated in our study, but it was not statistically significant.

Nowadays, when teratozoospermia is a very common finding in sperm samples (using strict criteria), many doctors and especially patients experience it as a very confusing situation. In IVF treatment isolated teratozoospermia seems not to affect the outcome (Keegan et al. 2007), but contrasting results have also been reported (Kruger et al. 1986). In recent retrospective studies (Spiessens et al. 2003, Grigoriou et al. 2005), isolated teratozoospermia (normal count and motility and < 10% normal forms, using strict criteria) predicted a reduced cumulative live birth rate after four IUI cycles compared with normospermia. An adverse impact of isolated teratozoospermia on IUI success became clearly evident after the first IUI failure, the situation being similar to that associated with astenozoospermia and oligozoospermia. In our study, the PR was 17.6% among couples with isolated teratozoospermia, which was better than the PR (13.3%) among couples with teratozoospermia combined with (an) additional sperm defect(s) and similar to the PR among couples with normospermia in previous studies (Nuojua-Huttunen et al. 1999, Khalil et al. 2001). The knowledge that teratozoospermia affects IUI pregnancy rate only when it is combined with (an) additional sperm defect(s) makes the interpretation of semen analysis easier.

It has been clearly demonstrated that aneuploidy is increased in the spermatozoa of infertile men with poor semen quality and teratozoospermia (Templado et al. 2002, Harkonen et al. 2001). Flagellar anomalies are associated with good prognosis, but anomalies affecting sperm chromatin and the neck region are associated with an increased chance of failure (Chemes et al. 2003). It has also been shown that severe sperm head abnormalities are associated with a lower chance of establishing successful pregnancies, even though fertilization may be achieved (Nikolettos et al. 1999). In our study the amount of head anomalies in post-wash sperm was significantly lower in the pregnant group than in the non-pregnant group.

Even a small increase in the percentage of sperm with normal morphology in post-wash samples, when characterized by a decrease in the amount of head anomalies, is a prognostic factor as regards pregnancy after IUI. It should be remembered that an increase in the amount of normal forms of only a few percent means millions more normal spermatozoa in a sperm sample. Sperm morphology seems to be more crucial in IUI than in IVF/ICSI treatment, but isolated teratozoospermia seems not to affect the success rate of IUI.

11 SUMMARY AND CONCLUSIONS

The primary goal of every clinician is to choose the most appropriate treatment with the least invasive technology for an individual couple. Insemination as a low-cost and patient-friendly method still has a definitive role in the field of infertility treatment. The present study was designed to find out the most favourable factors for IUI success as well as criteria for appropriate selection of subjects.

The main findings and conclusions of the study were:

1. Subjects whose tubes were evaluated by means of a less invasive method – hysterosalpingo-contrast sonography (HyCoSy) with air and saline as a contrast medium – achieved pregnancy results in IUI as good as those among subjects investigated by means of laparoscopy or HSG. Compared with laparoscopy, HyCoSy is a safe, inexpensive and time-saving procedure that can be performed in outpatient clinics. Changing clinical practice from laparoscopy to HyCoSy as a first-line tubal investigation method has not impaired the results of IUI as regards the cumulative live birth rate. Intrauterine insemination seems acceptable even in cases of unilateral tubal patency. On the basis of our results, HyCoSy should be considered as a first-line tubal investigation method.
2. Hysterosalpingo-contrast sonography seems to be a very reliable procedure when tubal patency is demonstrated. In every second case tubal spasm turned out to be the cause of false-positive tubal occlusion in HyCoSy, even in women with tubal risk factors in their history. Transvaginal hydrolaparoscopy (THL) is a safe, inexpensive and practical alternative to confirm HyCoSy findings. This is the first investigation in Finland concerning THL and our results suggest that its introduction could decrease the need of laparoscopic procedures in our country.
3. Factors affecting the pregnancy rate such as the number of follicles, the stimulation protocol and sperm count are not identical as regards the different aetiologies of infertility. Women with infertility due to ovulatory disorders had the best prognosis (19.1%) in IUI treatment, but they were also at the highest risk as regards multiple gestation (13.2%). The prognosis of women with endometriosis was the worst (11.9%). In cases of unexplained infertility, three follicles and a sperm count $> 30 \times 10^6/\text{mL}$ were statistically significant positive prognostic factors and increasing female age showed a negative trend in relation to IUI success. In cases of male infertility, female age up to 39 years was not a prognostic factor and IUI treatment is worth performing even in cases of low sperm count ($< 5 \times 10^6/\text{mL}$). Stimulation with sequential clomifene citrate and human menopausal

gonadotrophin resulted in the best PRs in cases of anovulatory and male-factor infertility, whereas clomifene citrate stimulation was the most successful in endometriosis-based infertility.

Consideration of the aetiology helps us to achieve one child at a time without affecting the prognosis of pregnancy.

4. Analysis of post-wash semen parameters demonstrated that even a small increase in the percentage of sperm with normal morphology after preparation, when characterized by a decrease in head anomalies, was a significant predictive factor as regards pregnancy after IUI. Our results also showed that isolated teratozoospermia did not affect the success of IUI.

Nowadays, when the costs of health care are limited, IUI can hold its place as a low-cost method of infertility treatment. With careful selection of subjects, and appropriate ovarian stimulation and sperm preparation, good pregnancy results with a low multiple pregnancy rate can be achieved.

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The validity of air and saline hysterosalpingo-contrast sonography in tubal patency investigation before insemination treatment

Katja Ahinko-Hakamaa ^{a,*}, Heini Huhtala ^{b,c}, Helena Tinkanen ^a

^a Department of Obstetrics and Gynecology, Tampere University Hospital, PL 2000, 33521 Tampere, Finland

^b School of Public Health, University of Tampere, 33014 Tampere, Finland

^c Research Unit, Tampere University Hospital, Finland

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Abstract

Objective: To compare the prognostic significance of tubal patency investigation by means of laparoscopy, hysterosalpingo-contrast sonography (HyCoSy) with air and saline as a contrast medium, and hysterosalpingography (HSG) in relation to the outcome of intrauterine insemination (IUI) treatment.

Study design: A retrospective study of 559 consecutive women attending the university hospital infertility clinic for infertility treatment in 1996–2003. Tubal patency was evaluated by laparoscopy in 261 women, by HyCoSy in 217 and by HSG in 81 women before insemination treatment. Altogether, 1240 insemination cycles were evaluated and the results were compared in the three study groups.

Results: The clinical pregnancy rates per cycle were 14%, 18% and 18% in the laparoscopic, HyCoSy and HSG groups, with no statistically significant difference between the groups. The cumulative pregnancy rates (mean 2.3 cycles) were 30%, 41% and 38%, respectively, with a significant difference between the study groups. In cases of unilateral patency, cumulative pregnancy rates after two cycles were 18% (laparoscopy), 29% (HyCoSy) and 29% (HSG). The numbers of tubal pregnancies were similar in the subgroups.

Conclusions: Hysterosalpingo-contrast sonography with air and saline as a contrast medium is a very cost-effective tubal investigation method as regards selection of subjects for insemination.

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Keywords: HyCoSy; Intrauterine insemination treatment; Tubal patency

1. Introduction

Tubal disorder is the most frequent female factor associated with infertility, occurring in up to 35% of infertile women. Traditionally, X-ray hysterosalpingography (HSG) and laparoscopy with dye (gold standard) have been used in the diagnosis of tubal pathology. Hysterosalpingo-contrast sonography (HyCoSy), also called sonosalpingohysterography (SSHG), was introduced over 15 years ago and has proved to be a reliable method in the evaluation of tubal patency. It is thought to have several advantages over conventional methods; it is a non-invasive, rapid and inexpensive outpatient procedure. Echovist[®] (Schering AG, Berlin), which contains

galactose microbubbles in galactose solution, has been used as a contrast medium in most investigations, but a mixture of air and saline is an inexpensive alternative. Questions on the clinical usefulness of these three methods should also incorporate the capacity of the methods to predict future fertility.

In the literature, three to six intrauterine insemination treatments (IUI) are recommended for infertile couples when tubal patency has been addressed and there are no severe male factor parameters. Fulfilling these criteria, the pregnancy rate after IUI treatment has varied between 9 and 21% per cycle [1–4] and the proportion of ectopic pregnancies has been 5–6% in IUI treatment [1].

It could be assumed that the results of insemination would be impaired when tubal patency is investigated by means of a method that does not reveal the peritubal adhesions, the

* Corresponding author. Tel.: +358 3 3116 4418; fax: +358 3 3116 4360.
E-mail address: katja.ahinko-hakamaa@pshp.fi (K. Ahinko-Hakamaa).

condition of the fimbriae, and mild endometriosis. The main purpose of this study was to evaluate if the method used in investigation of tubal patency affects the results of IUI treatment.

2. Materials and methods

In a retrospective study, 559 consecutive infertile women attending Tampere University Hospital Infertility Clinic were investigated, the time period being from January 1996 to August 2003. Tubal patency was evaluated by means of laparoscopy and dye in 261 cases, by HyCoSy in 217 and by HSG in 81 cases. If at least one fallopian tube was patent and the sperm was normal or slightly subnormal, one to five (median 2.0) insemination treatments were carried out for each couple. Male infertility was considered when the total motile sperm count (swim-up technique) was <5 million. Ovulatory disorder was diagnosed when menstrual cycle was not regular (21–35 days) and/or mid-luteal serum progesterone concentration was <10 nmol/l. The diagnosis of mild or moderate endometriosis was based on laparoscopy findings, observation of rectovaginal endometriosis in pelvic examination or endometrioma(s) in ultrasound scanning.

Altogether, 1240 insemination cycles were evaluated and three study groups were formed: 571 cycles among women investigated by means of laparoscopy (46%), 494 cycles among women investigated by HyCoSy (40%) and 175 cycles among women investigated by means of HSG (14%). Table 1 presents the demographic data on the subjects with no statistically significant differences between the groups.

Table 1
Demographic data of the patients, used stimulation regimes and cause of infertility

	Laparoscopy (n = 261)	SSHG (n = 217)	HSG (n = 81)
Age (years) ^a	30	30	31
Length of infertility (years) ^a	2	2	2
Primary infertility (%)	66	63	63
IUI cycles (n) ^a	2.0	2.0	2.0
Sperm swim up (million) ^a	18	17	18
Stimulation regimes per cycle (n = 1233)			
Clomiphene citrate (%)	25	43	32
Gonadotrophin (%)	65	43	60
Clomiphene citrate + gonadotrophin (%)	9	13	7
Natural cycle (%)	1	1	1
AID (donor sperm) (%)	3	7	5
Cause of infertility (n = 556)			
Unexplained (%)	49	48	53
Male (%)	11	27	15 ^b
Anovulatory (%)	17	15	20
Endometriosis (%)	18	4	6 ^b
Many reasons (%)	5	6	6

^a Median.

^b *p*-Value < 0.001.

The number of women investigated by hysterosalpingo-contrast sonography increased from 1997 when the first HyCoSy procedure was performed to 73% in the 2003. In contrast, the number of women investigated by means of laparoscopy decreased from 79% in 1996 to 24% in 2003. The numbers of women undergoing HSG were low during these years, with a decreasing tendency, and in 2003 HSG procedures were carried out in only 3% of our study subjects.

The HyCoSy procedures were performed between days 5 and 10 of the menstrual cycle. The patients were advised to take analgetic drugs one hour before the procedure. The cervix was cleansed with sterile saline and a catheter was inserted into the uterine cavity. The balloon was inflated 1–2 ml of saline and positioned at the internal os of the cervical canal. A mixture of air and physiological saline with 2–5 ml at a time was injected through the catheter during continuous transvaginal ultrasound scanning. A total amount of saline used during the procedure was between 10–15 ml. The uterine cavity was evaluated first and after that the field of view on ultrasound examination was adjusted so that the area between uterine horn and ovary was kept under observation. When flow through the fallopian tubes emerging into the abdominal cavity was demonstrated, the tubes were considered patent and the woman was included in the study. If patency of the fallopian tubes was uncertain, laparoscopy was performed and a woman was included in the laparoscopy group. Only six women had to be laparoscoped after non-diagnostic HyCoSy and three of them had unilateral tubal patency. At the end of procedure the balloon was deflated and catheter removed. The use of prophylactic antibiotic treatment was not routine.

The gynecologists at the same clinic carried out the laparoscopies and those were mainly diagnostic, but in cases of peritoneal endometriosis, coagulation or ablation were done. If adhesions were found; only those patients with insignificant adhesions, not disturbing tubal function were included in the study. Serious adhesions were programmed to another operation or to IVF treatment. The HSG procedures were carried out at the department of radiology.

Superovulation was carried out with clomiphene citrate (34%), HMG (56%) or both (10%). The regimens used in the groups are shown in Table 1. Clomiphene citrate (50–150 mg/d; Clomifen; Leiras, Turku, Finland) was given between days 3 and 7 of the menstrual cycle. HMG (Menogon; Ferring, Copenhagen, Denmark; or Humegon; Organon, Oss, the Netherlands) was started with 75–150 IU on cycle day 5 and the dose was adjusted according to ultrasound monitor. Ovarian and endometrial responses were monitored by vaginal ultrasound between cycle days 10 and 12. When leading follicle reached 18 mm, 5000–10,000 IU of HCG (Profasi; Serono Laboratories, Rome, Italy) was administered and after 39 h intrauterine insemination was performed with 1.0 ml of partner's washed sperm. The procedure was carried

out by using intrauterine catheter (Gynetic, Gynetics Medical products; Hamont-Achel, Belgium) with a 2 ml syringe. With the patient in the lithotomy position, the IUI catheter was gently directed into the uterine lumen and the sperm suspension then slowly infused. Women were allowed to stand up and leave immediately. Luteal support was used in case of irregular bleeding in luteal phase.

In cases of azoospermia, 27 women underwent AID (donor sperm insemination) in 69 cycles. All sperm preparations (swim-up technique) were carried out by a single medical laboratory technologist during the study period. The results of the IUI cycles in the three study groups were calculated and compared.

The causes of infertility are presented in Table 1. There were significantly more women with endometriosis in the laparoscopy group than in the HyCoSy and HSG groups. On the other hand, male infertility was statistically more common in the HyCoSy group than in the other study groups.

Categorical variables between the groups were compared using the Chi square test. All statistical analyses were performed using SPSS for Windows, version 11.0 (SPSS inc., Chicago, IL, USA). The Ethics Committee of Tampere University Hospital approved the study.

3. Results

Bilateral tubal patency was found in 80% of the women investigated by means of laparoscopy in 83% of those evaluated by HyCoSy and in 91% of those evaluated by HSG.

The clinical pregnancy rate per insemination cycle was 14% in the laparoscopy group, 18% in the HyCoSy group and 18% in the HSG group. There was no statistically significant difference between the groups in this regard. The number of ectopic pregnancies did not differ among those assessed by HyCoSy versus laparoscopy. Four tubal pregnancies were found in women investigated by laparoscopy and three tubal pregnancies were diagnosed in women evaluated by HyCoSy (Table 2).

The HyCoSy group showed the best cumulative pregnancy rate (41%) after 2.3 insemination cycles, but

Table 2
Results of IUI treatments per cycle

	Laparoscopy (n = 571)	SSHG (n = 494)	HSG (n = 175)	Total (n = 1240)	p-Value
Pregnancy % (n)	14(78)	18(88)	18(31)	16(197)	0.14
Delivery %	73	76	68	74	0.45
Miscarriage %	19	21	32	22	
Tubal pregnancy %	5	3	0	4	
Result unknown %	3	0	0	0	

n is the number of cycles.

Table 3
Cumulative results of IUI treatments

	Laparoscopy (n = 261)	SSHG (n = 217)	HSG (n = 81)	Total (n = 559)	p-Value
Pregnancy %	30	41	38	35	0.043
Delivery %	22	31	26	26	0.081

n is the number of patients.

there was no statistically significant difference in the delivery rate between the groups (Table 3). The cumulative pregnancy rate among women with unilateral patency after two IUI cycles was 18% (laparoscopy), 29% (HyCoSy) and 29% (HSG).

In the laparoscopy group eleven of the pregnancies were multiple (14%); three of them trigemines. The twin pregnancy rate in the HyCoSy group was 13% (n = 11) and no multiple pregnancies were found in the HSG group.

4. Discussion

In this study we compare the results of insemination treatment when tubal patency was evaluated by laparoscopy, HSG and HyCoSy with a mixture of air and saline as a contrast medium. The data from 1240 insemination cycles showed that hysterosalpingo-contrast sonography with a mixture of air and saline was associated with a completely satisfactory accuracy compared with the other techniques in selection of subjects for insemination treatment.

In the last decade, several investigators have addressed the use of hysterosalpingo-contrast sonography for evaluation of infertility. Many studies have demonstrated a high concordance rate of HyCoSy versus laparoscopy in the diagnosis of tubal pathology [5–13] and in a meta-analysis of 1007 women a concordance rate of 83% was found when HyCoSy was compared with either HSG or laparoscopy with dye [14]. Compared with laparoscopy, HyCoSy has been shown to have a sensitivity of 92.8%, a specificity of 96.2% and a positive predictive value of 92.8% and a negative predictive value of 98.1% [12]. Similar rates have been reported for hysterosalpingography [10,11,15], but some authors have reported the limits of HSG especially diagnosing distal tubal obstruction and hydrosalpinx [16,17].

Most of the HyCoSy studies have been performed using Echovist[®] as a contrast medium, but investigations involving use of saline as a contrast medium have concordance rates as high as in studies with positive contrast media [6,11]. Studies on the contrast medium used in HyCoSy have shown conflicting reports. Some investigators found a mixture of air and saline to be comparable with Echovist[®] [7,18] whereas in one report saline was found to be worse than a positive contrast agent [19].

One previous study [1] has shown that intrauterine insemination results are not affected if HyCoSy with Echovist[®] is used as the sole test of tubal patency. In that

study the pregnancy rates per cycle were 9% in the laparoscopy group, 9% in the HyCoSy group and 11% in the HSG group. The cumulative pregnancy rates after three cycles were 17%, 17% and 15% respectively. There were no differences in the rates of ectopic pregnancy between those assessed by HyCoSy and by laparoscopy [1].

Since 1997, when hysterosalpingo-contrast sonography was first presented in our clinic, we have recorded a decreased in the number of laparoscopic procedures. One of our aims was to evaluate if this practical change has affected the results of IUI treatments. It could be assumed that by using this non-invasive, safe and inexpensive method tubal pathologies would be missed. Our data show that the pregnancy rate among women investigated by means of HyCoSy was at least as high as that among women evaluated by laparoscopy or HSG. The reason why the laparoscopy group had a lower pregnancy rate might be caused by subject selection: women with a suspicion of pelvic pathology were more likely to be placed in the laparoscopy group. Although unilateral or bilateral patency among these women was addressed by laparoscopy, mild endometriosis might have had an influence on pregnancy outcome. On the other hand, apparently healthy women (with moderate male factor infertility) were more likely to have undergone HyCoSy than laparoscopy in the evaluation of tubal patency.

It is also very important to point out that the use of HyCoSy was not associated with an increase in the rate of tubal pregnancy, as might be assumed, and the rate of ectopic pregnancy in our study was similar to that in other studies [1].

Most studies concerning the results of IUI treatments have included only subjects with bilateral tubal patency. Our study showed a satisfactory pregnancy rate even among women with unilateral tubal patency, and this finding justifies performing IUI also with these subjects.

It has been suggested that it is no longer justifiable to carry out laparoscopy in all infertile women, because this expensive procedure carries anesthetic and operative risks and reveals a normal pelvic condition or mild endometriosis in 40–70% of women investigated [20], without a proven effect on fertility [21,22]. The cost of HyCoSy with a mixture of saline and air as a contrast medium is cheaper than with Echovist[®], and in our hospital it is less than 10% of the cost of diagnostic laparoscopy. In this study period, using HyCoSy in the investigation of infertility has led to significant reductions in both time and costs. In public hospitals, where all women have public funding for ART and evidence-based medicine is required, hysterosalpingo-contrast sonography should be considered as a first-line investigation method.

It is well known, that non-invasive methods are associated with false positive results of tubal occlusion in relationship with tubal spasm. Therefore, laparoscopy or fertiloscopy should be considered if HyCoSy or HSG shows bilateral tubal occlusion. In our study six women needed laparoscopy after HyCoSy and three of them had normal tubes. It is also reasonable to perform laparoscopy if there is

evidence of pelvic pathology or with complains of pelvic pain.

Fertiloscopy, the combination of transvaginal hydro-pelviscopy, dye-test, optional salpingoscopy, and hysteroscopy, allows complete evaluation of the reproductive tract. It has shown a good correlation with conventional laparoscopy [23,24]. Fertiloscopy has several advantages over laparoscopy: it is a safe, reliable and minimally invasive procedure which can be performed with local anaesthesia. If detailed diagnostic information is required, fertiloscopy is an alternative to diagnostic laparoscopy in women without evidence of pelvic disease.

Recent studies have shown that three-dimensional hysterosalpingo-contrast sonography using Echovist[®] as a contrast medium seems to have advantages over the conventional HyCoSy technique [25,26], especially in terms of tubal visualization, and in the future continuously developing ultrasonographic techniques will favor HyCoSy.

Hysterosalpingo-contrast sonography is an easy, safe and non-invasive way to investigate tubal patency. Our study is the first to show that HyCoSy with a mixture of air and saline as a contrast medium is reliable and cost-effective in selecting women for insemination treatment and IUI treatment is justified also in cases of unilateral tubal patency.

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ORIGINAL ARTICLE

Confirmation of tubal patency in hysterosalpingo-contrast sonography by transvaginal hydrolaparoscopy

KATJA MAARIT AHINKO-HAKAMAA¹, HEINI HUHTALA^{2,3} & HELENA TINKANEN¹

¹Department of Obstetrics and Gynaecology, Tampere University Hospital, Tampere, Finland, ²School of Public Health, University of Tampere, Tampere, Finland, and ³Research Unit, Tampere University Hospital, Tampere, Finland

Abstract

Objective. To confirm tubal patency in hysterosalpingo-contrast sonography (HyCoSy) by transvaginal hydrolaparoscopy (THL). **Design.** A prospective study. **Setting.** Infertility clinic at a university hospital. **Population.** Fifty-six infertile women with a history of tubal risk factor(s) who underwent HyCoSy and THL between 2003 and 2006. **Methods.** After performing HyCoSy, the tubal patency was verified by THL. **Results.** Altogether, 51 women and 101 tubes could be examined. Using HyCoSy, 61 of the tubes were considered patent and 40 occluded. THL confirmed 82 tubes patent and 19 occluded. Of 61 tubes found to be patent in HyCoSy, 60 were also patent in THL. In contrast, of 40 tubes occluded in HyCoSy only 18 tubes were confirmed to be occluded by THL. The concordance was 77%. The sensitivity of HyCoSy was 95%, the specificity 73%, the negative predictive value 98% and the positive predictive value 45%. **Conclusions.** HyCoSy is a reliable method when tubal patency is demonstrated. In cases of suspected tubal occlusion, THL is an inexpensive and safe outpatient procedure to exclude benign tubal spasm and confirm the presence of true occlusion.

Key words: HyCoSy, transvaginal hydrolaparoscopy, tubal patency, tubal spasm

Introduction

The assessment of tubal patency plays an important role in the diagnosis of female infertility. Hysterosalpingography (HSG) and laparoscopy with dye have traditionally been used in the diagnosis of tubal pathology. Hysterosalpingo-contrast sonography (HyCoSy) with Echovist[®] or air and saline as contrast media has proved to be a reliable method and it has many advantages over conventional methods; it is a non-invasive, rapid and inexpensive outpatient procedure (1–3). However, non-invasive methods are associated with false-positive results as regards occlusion related to tubal spasm. Therefore, an invasive method is needed if tubal pathology is suspected. Laparoscopy with dye is still considered to be the gold standard for evaluating tubal pathology, but it requires general anesthesia, operating theatre facilities and absence from professional activities. It also presents some potentially serious risks and very seldom reveals any pathological conditions (4).

Transvaginal hydrolaparoscopy (THL) and dye test with optional salpingoscopy and hysteroscopy allows complete evaluation of the reproductive tract. It is an outpatient procedure and can be performed under local anesthesia. The results of THL have shown good correlation with those of conventional laparoscopy (5,6). The failure rate as regards entrance and the complication rate have been 3.5 and 0.9%, respectively (7). There are no reports on the use of THL to evaluate the value of HyCoSy in cases of suspected tubal occlusion.

The purpose of our study was to estimate the clinical usefulness of THL instead of laparoscopy in verifying the HyCoSy findings.

Material and methods

The study series consisted of consecutive 56 infertile women referred to the infertility clinic of Tampere University Hospital between October 2003 and

Correspondence: Katja Maarit Ahinko-Hakamaa, Department of Obstetrics and Gynecology, Tampere University Hospital, PL 2000, Tampere 33521, Finland. E-mail: katja.ahinko-hakamaa@pshp.fi

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September 2006. We included subjects who had previous known tubal risk factors such as pelvic inflammatory disease (PID), tubal pregnancy or pelvic operation (52%) or who had a history of minor genital infections or abdominal pain (48%), but no pathological ultrasonographic findings when entering the study. Women with no tubal risk factor in their past, without any clinical evidence of tubal pathology and with bilateral patent tubes in HyCoSy were excluded due to potential complication risk associated with invasive investigation methods (7,8). Subjects with endometriosis or sactosalpinx suspected in pelvic examination or in transvaginal ultrasonographic scanning were also excluded from the study and laparoscopy was carried out to make operative treatment possible. The other contraindications of THL were acute situations (bleeding or infection), obliterated cul-de-sac and fixed retroverted uterus.

Tubal investigation was started with HyCoSy (H.T) using air and saline as a contrast medium and within one month, THL with a dye test was carried out by another gynecologist (K.M.A-H) to confirm the findings. All THLs were performed without general anesthesia in the operating room of outpatient clinic. There were no withdrawals among the subjects.

The mean age of the women was 31 (20–39) years and 67.7% of the subjects were nulliparous. All the couples had suffered from infertility for at least one year. Sperm quality was evaluated and women, whose partners had normal or mildly subnormal sperm were included in the study.

HyCoSy was performed between days 5 and 10 of the menstrual cycle by very experienced gynecologist (H.T). The women were advised to take analgesic drugs one hour before the procedure. The cervix was cleansed with sterile saline and a catheter was inserted into the uterine cavity. The balloon was inflated with 1–2 ml of saline and positioned either in the cervix or in the lower part of the uterine cavity. A mixture of air and physiological saline 2–5 ml at a time was injected through the catheter during continuous transvaginal ultrasonographic scanning. The total amount of saline used during the procedure was 5–15 ml. The uterine cavity was evaluated first, and after that the field of view in ultrasonography was adjusted so that the area between the uterine horn and the ovary was kept under observation. When flow through the Fallopian tubes into the abdominal cavity was demonstrated, the tubes were considered patent.

THL was also performed in the proliferative phase of the menstrual cycle after the bleeding had ended. All women received 1.5 g cefuroxime (Kefurion®)

intravenously before the procedure. All the THL procedures were carried out by one gynecologist (K.M.A-H) with one nurse as an assistant.

All procedures were performed using the technique described previously by Watrelot et al. (9). With the subject in a dorsal decubitus position, a Colin's speculum was inserted and the vagina was cleansed with sterile saline. Local anesthesia was carried out by means of 5–10 ml of lidocaine applied about 1–1.5 cm below the cervix. The posterior lip of the cervix was then grasped with a tenaculum. For chromopertubation the first balloon introducer (FT 1–29 Soprane SA, France) was inserted into the uterine cavity and balloon inflated with 3 ml of air. Before entering to the peritoneal cavity, 0.5–1.0 ml alfentanil (Rapifen®) was injected intravenously and analgesia was repeated at a later stage of the procedure, if needed. A Veress needle was inserted into the pouch of Douglas and 200–400 ml of prewarmed saline solution (37°C) with lidocaine was freely injected into the abdominal cavity. The Veress needle was removed and the second balloon introducer with a dilatator trocar (4 mm) was inserted into the pouch of Douglas. The balloon maintaining the introducer in the abdominal cavity was inflated with 5 ml of air. A 2.9 mm scope with a 30° lens and a flow channel (Karl Storz SA, Germany) was put through the trocar and a camera was attached to the scope. To allow free movement of the scope, the tenaculum and the speculum were removed and saline solution was allowed to flow freely through the channel. The total amount of saline solution needed in the procedure varied from 500 to 700 ml. Evaluation started from the posterior wall of the uterus moving toward the adnexae. The tubes were followed and every tubal part and the ovaries were examined. Methylene blue was then injected through the intrauterine introducer and chromopertubation was performed. A flow of blue dye at the fimbriae was considered as evidence of tubal patency. After examination of the pelvic cavity, as much of the fluid as possible was removed through the trocar. The vaginal posterior fornix was left to heal and the women were advised to avoid intercourse and tampons for five days. The women were discharged 2–3 hours after the procedure. All the findings were recorded.

Statistical analysis

The results of these two examination methods were compared. The diagnostic accuracy of HyCoSy was expressed in terms of its concordance between HyCoSy and THL, sensitivity, specificity, negative predictive value and positive predictive value with

their respective 95% confidence intervals. All statistical analyses were performed using SPSS for Windows, version 14.0 (SPSS Inc., Chicago, IL, USA) and confidence interval analysis (CIA) for Windows, version 2.0.

The Ethics Committee of Tampere University Hospital approved the study. Approval and informed consent from the patients was obtained before the study.

Results

In HyCoSy, the women's compliance was good and no complications occurred. In THL, access to the pouch of Douglas failed in five cases (8.9%). In three of them, the reason was fixed retroverted uterus and in one case, massive obesity. No reason for failing access was found in one woman. The complication rate was low, 1.8% consisting of one postoperative hematoma in the fossa Douglas, healing spontaneously. All the other women returned to their professional activities next day.

Altogether, 51 patients and 101 tubes were examined; one woman had only one tube. Of the tubes investigated by HyCoSy, 61 were patent and 40 occluded. In THL, 82 of 101 tubes were patent and 19 were occluded. Of the 40 tubal occlusions suspected in HyCoSy, 18 were occluded and 22 were patent in THL. Of 61 tubes found to be patent in HyCoSy, 60 were also patent in THL. The concordance, sensitivity, specificity, positive predictive value and negative predictive value are shown in Table I. Tubal patency per subject is presented in Table II.

We also analyzed the results in individual subjects based on the presence of tubal patency or tubal occlusion (Table III). First, the problematic group of 13 women with unilateral patency/occlusion were grouped with patients with bilateral patency and compared to those with bilateral occlusion. Sec-

Table II. Tubal patency in hysterosalpingo-contrast sonography (HyCoSy) and transvaginal hydrolaparoscopy (THL) per subject ($n = 50$).

	HyCoSy	THL
Bilateral patency	24	37
Unilateral patency	13	7
Bilateral occlusion	13	6
Total	50	50

ondly, women with unilateral patency were grouped with women showing bilateral occlusion and compared to subjects with bilateral patency. The concordance, sensitivity and specificity of the first model were better compared to the second one.

Because of major adhesions and/or sactosalpinx in THL, five of the women (9.8%) with confirmed occlusion were referred to laparoscopy. Two of them were treated with unilateral salpingectomy and one with bilateral salpingectomy. A salpingolysis was performed for one woman resulting in spontaneous pregnancy. One subject had untreatable adhesions.

Discussion

The results of this study confirm that HyCoSy has an excellent sensitivity of 95% in the evaluation of tubal patency. On the other hand, the specificity of HyCoSy remains lower as a result of the difficulty to distinguish between true occlusion and benign spasm. Therefore, an invasive method should always be used to confirm the nature of any tubal occlusion found in HyCoSy.

HyCoSy is a simple and cost-effective method of investigation, which has proved to be very reliable in the evaluation of tubal patency with either Echovist[®] or air and saline as contrast media (2,10–12). In meta-analysis, when compared with laparoscopy, the concordance rate was 83% (13). In a study involving 282 tubes (3), HyCoSy showed an overall concordance

Table I. Agreement between hysterosalpingo-contrast sonography (HyCoSy) and transvaginal hydrolaparoscopy (THL) in detection of tubal patency in all ($n = 101$) Fallopian tubes in 51 patients.

HyCoSy	THL		
	Occluded	Patent	Total
Occluded	18	22	40
Patent	1	60	61
Total	19	82	101
Concordance 0.77			
Sensitivity 0.95, 95% CI (0.75–0.99)			
Specificity 0.73, 95% CI (0.63–0.82)			
Positive predictive value 0.45, 95% CI (0.31–0.60)			
Negative predictive value 0.98, 95% CI (0.91–1.00)			

Table III. Agreement between hysterosalpingo-contrast sonography (HyCoSy) and transvaginal hydrolaparoscopy (THL) in detection of tubal patency per subject ($n=50$) classified by presence of either tubal patency or tubal occlusion.

HyCoSy	THL	
	Bilateral or unilateral patency	Bilateral occlusion
Any patency		
Bilateral or unilateral patency	37	0
Bilateral occlusion	7	6
Total	44	6
	Concordance 0.86	
	Sensitivity 1.00, 95% CI (0.61–1.00)	
	Specificity 0.84, 95% CI (0.71–0.92)	
	Positive predictive value 0.46, 95% CI (0.23–0.71)	
	Negative predictive value 1.00, 95% CI (0.91–1.00)	
Any occlusion		
Bilateral or unilateral occlusion	Bilateral or unilateral occlusion	Bilateral patency
Bilateral patency	12	14
Total	1	23
	13	37
	Concordance 0.70	
	Sensitivity 0.92, 95% CI (0.67–0.99)	
	Specificity 0.62, 95% CI (0.46–0.76)	
	Positive predictive value 0.46, 95% CI (0.29–0.65)	
	Negative predictive value 0.96, 95% CI (0.80–0.99)	

rate of 85.8%, sensitivity of 90.4%, specificity of 70.3%, a positive predictive value of 91.2% and a negative predictive value of 68.2% in comparison with laparoscopy. In our study, the sensitivity and specificity were comparable with this study, but the negative predictive value was better (98%) and the positive predictive value (45%) lower as a result of benign tubal spasms. The concordance rate was slightly lower (77%) than in studies discussed above. It has also been reported (14) that the concordance between HyCoSy and laparoscopic chromopertubation decreased from 86 to 67% when it was based on the affected tubes only. In our study, the cases were selected on the basis of the history of previous tubal risk factor and this may have attenuated the concordance.

The Fallopian tubes are very sensitive to spasm caused by manipulation of the pelvic organs, injected contrast media and pain, in addition to individual tendency as the main reason for it. With a non-invasive method, it is impossible to determine whether an occlusion is a true tubal obstruction or a benign tubal spasm (2,10). A recent study (15) showed that warming the contrast media to body temperature can reduce discomfort and also the possibility of tubal spasm. In the case where tube(s) are found to be occluded in HyCoSy, waiting for a few minutes, pain medication or repeating HyCoSy later may rule out the false occlusion. Also visualization of tubal flow with Doppler Ultrasound can be helpful. Anyway, the possibility of tubal spasm should be kept in mind when tubes appear to be

occluded in HyCoSy even with a history of previous tubal risk factors.

THL is an outpatient method that does not require operating theatre facilities. It can be performed by one doctor with a nurse and it does not cause absence from professional activities. Thus, it is a very cost-effective method when compared with conventional laparoscopy. It has proven to be a reliable method in investigation of tubal patency (5,6), but it has not been previously used as a confirmatory tool in combination with HyCoSy to assess tubal patency. The method is easy to perform when the learning curve plateau has been reached and the complication rate has been low. In the literature, a total of 4,232 procedures had 26 bowel injuries (0.61%), but in the recent study of Shibahara ($n=177$), the incidence of rectum perforation was 1.1% (8). The use of ultrasound seems to minimize complications and increases the rate of successful access to the pouch of Douglas (16). Rectum perforation is usually extraperitoneal and is treated conservatively with antibiotics. However, the procedure has limitations because in women with a fixed retroverted uterus, it is impossible to access to the pouch of Douglas and the most important aspect of successful THL is appropriate patient selection.

In the study of Jonsdottir et al. (17), 15 out of 120 (12.5%) subjects with unexplained infertility and without obvious pelvic pathology needed operative laparoscopy. This agrees with our study where five women out of 51 (9.8%) required conventional laparoscopy after THL because of major adhesions

and sactosalpinx. No case of sactosalpinx was visible in ultrasonographic examination before HyCoSy, or during it.

The combination of HyCoSy and THL is inexpensive and more convenient for the woman than HyCoSy with laparoscopy or laparoscopy alone. However, subjects with pathological findings and endometriosis should undergo laparoscopy, and they also were excluded from our study.

We have presented our results both for individual tubes and for individual women. Tubal approach is a better way when comparing the sensitivity and specificity of these two diagnostic procedures. However, if the end point is clinical pregnancy, the analysis concerning individual women is more valuable. The main problem is how we deal with a woman with unilateral patency. In our opinion, woman's age affects decision: a young fertile woman with at least one patent tube has a remarkable better prognosis compared to an older woman with one open tube but harboring other fertility decreasing factors.

The drawback of our study is that subject selection is somewhat biased because of exclusion of subjects with bilateral patent tubes in HyCoSy having no tubal risk factor in their past. Their participation in the study would have been ethically inappropriate due to potential complications.

In conclusion, our study shows that in every second case benign tubal spasm is a cause of false-positive tubal occlusion in HyCoSy. THL is a safe, practical and inexpensive procedure to verify HyCoSy findings in carefully selected subjects. On the other hand, HyCoSy is a reliable method if the examination reveals tubal patency.

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ORIGINAL ARTICLE

Success in intrauterine insemination: the role of etiology

KATJA AHINKO-HAKAMAA¹, HEINI HUHTALA^{2,3} & HELENA TINKANEN¹

¹Department of Obstetrics and Gynaecology, Tampere University Hospital, ²School of Public Health, University of Tampere, and ³Research Unit, Tampere University Hospital, Tampere, Finland

Abstract

Background. We aimed to identify the prognostic factors for the highest pregnancy rate and lowest multiple pregnancy rate in different infertility etiology groups among women undergoing insemination treatment. **Methods.** A total of 1,171 cycles among 532 infertile couples were retrospectively studied and the impact of different prognostic factors on pregnancy rate in five different etiology subgroups was analyzed. **Results.** The pregnancy rate/cycle was highest (19.2%) among women with anovulatory infertility and lowest (11.9%) in endometriosis based infertility. Multiple pregnancy rate varied between 3.6% (male infertility) and 13.2% (anovulatory infertility). In unexplained infertility ovarian stimulation resulting in three follicles (pregnancy rate 24.2%) and inseminated motile sperm count $>30 \times 10^6$ (pregnancy rate 19.8%) were significant prognostic factors. In anovulatory infertility stimulation with sequential clomiphene citrate and human menopausal gonadotrophin was a positive predictive factor, with a pregnancy rate of 36%. In male infertility stimulation with sequential clomiphene citrate/human menopausal gonadotrophin resulted in the best pregnancy rate (25.0%). In endometriosis-based infertility the pregnancy rate was best with clomiphene citrate stimulation (21.1%) and inseminated motile sperm count $>30 \times 10^6$ (24.3%). In combined infertility the highest pregnancy rate was with sequential clomiphene citrate/human menopausal gonadotrophin stimulation and with three follicles (30%), and even 18.2% with inseminated motile sperm count $<5.0 \times 10^6$. **Conclusions.** The etiology of the infertility is important when optimal insemination treatment is planned. The impact of the woman's age, sperm count, stimulation protocol, and the follicle number on the pregnancy rate and multiple pregnancy rate is associated with the etiology of the infertility.

Key words: IUI, etiology, prognostic factor, pregnancy rate

Abbreviations: PR: pregnancy rate, MPR: multiple pregnancy rate, IUI: intrauterine insemination, CC: clomiphene citrate, HMG: human menopausal gonadotrophin, IMC: inseminated motile sperm count, FSH: follicle-stimulating hormone

Intrauterine insemination (IUI) has remained a popular treatment option for couples with unexplained infertility, mild to moderate male infertility, ovulatory dysfunction, mild endometriosis, and cervical factors. The benefit of this therapy is based on the assumption that the conception rate will increase if multiple oocytes can be induced, and if with accurate timing an adequate concentration of mobile spermatozoa can be introduced directly into the uterine cavity.

In planning the treatment policy for subfertility, the cost-effectiveness of the treatment and multiple pregnancy rates (MPRs) should be considered. Nowadays, when IVF and ICSI are widely available, overtreatment should also be avoided and

IUI appears to be a more cost-effective option than immediate IVF (1). It has also been demonstrated that three cycles of IUI result in the same cumulative pregnancy rate (PR) as IVF, and IUI is more cost-effective as regards unexplained infertility and moderate male factor infertility (2,3).

The PR after IUI treatment has varied between 9 and 21% per cycle after three to six cycles (4–7). Prognostic variables in IUI treatment include maternal age, history of prior pregnancy, duration of infertility, number of follicles, size of follicles, motile spermatozoa count, and endometrial thickness (5–11). It has been shown that ovulation induction with gonadotrophins significantly improves the PR

in IUI treatment in couples with unexplained infertility (12), but hyperstimulation with gonadotrophins has led to a high MPR, which is the most important concern as regards IUI nowadays.

When the prognostic factors of the success of IUI treatment have been investigated, the etiology has seldom been taken into consideration. In the study of Nuojua-Huttunen et al. (7) the best results were achieved in the unexplained infertility group and the worst in the endometriosis group. The important prognostic factors for successful IUI treatment might not be the same in different infertility etiology groups and to our knowledge there have been no investigations on the impact of different independent variables within infertility etiology groups.

The object of this study was to identify the individual factors that affect the success of IUI treatment in different infertility etiology groups. These findings might be used to help in planning individual infertility treatments.

Material and methods

Subjects

This study is a retrospective analysis of consecutive artificial insemination of homologous sperm (AIH) cycles carried out at the infertility clinic of Tampere University Hospital between January 1996 and August 2003. Altogether, 532 couples and 1,171 insemination cycles were included in this study and for each couple one to five (mean 2.2) insemination cycles were carried out.

All the study couples had experienced at least 1 year of infertility (median 2.0 years). The median age was 30.0 years; 65.2% of the couples suffered from primary infertility. All couples underwent standard infertility investigation consisting of anamnesis, assay of follicle-stimulating hormone (FSH), prolactin, thyroid hormone and mid-luteal progesterone concentrations, and sperm analysis. Tubal patency was evaluated by laparoscopy (47%), hysterosalpingo-contrast sonography (39%), or hysterosalpingography (14%). If at least one tube was patent, the couple was included in the study under the endometriosis subgroup or the multiple reasons subgroup. There were not statistically significant differences in baseline characteristics of the women in the various subgroups.

Five subgroups were formed according to the etiology of infertility: unexplained (54.4%), male factor (13.2%), ovulatory disorders (16.8%), endometriosis (10.8%), and multiple reasons (4.8%). Infertility was considered male if the inseminated motile sperm count (IMC) was $\leq 5 \times 10^6$ /ml in at

least 50% of the IUI cycles. An ovulatory disorder was diagnosed when the menstrual cycle was not regular (21–35 days) and/or the mid-luteal serum progesterone concentration was < 10 nmol/l. A diagnosis of mild or moderate endometriosis was based on findings of laparoscopy, observation of rectovaginal endometriosis in pelvic examination, or ovarian endometrioma(s) in ultrasonographic scanning.

Controlled ovarian stimulation regimen

Ovarian stimulation was accomplished with clomiphene citrate (CC) (34%), human menopausal gonadotrophin (HMG) (56%), or a sequential CC/HMG treatment (10%). The first cycle of many patients was treated with CC (50–150 mg/day; Clomifen; Leiras, Turku, Finland) given between days 3 and 7 of the menstrual cycle. If the antiestrogenic effect of CC caused unsatisfactory endometrial response or the growth of the follicle was not optimal or the patient suffered from antiestrogenic side effects, HMG (Menogon; Ferring, Copenhagen, Denmark; or Humegon; Organon, Oss, the Netherlands) was used in the same or next cycle sequentially with CC or only gonadotrophin was used in the next cycle.

In sequential CC/HMG the gonadotrophin was initiated after ultrasound examination on days 10–12. When only HMG was used, it was started on day 5 at 75–150 IU and the dose was adjusted according to the results of ultrasonographic monitoring. When the leading follicle reached 18 mm, 5,000–10,000 IU of HCG (Profasi; Serono Laboratories, Rome, Italy) was administered and after 39 h IUI was performed with 1.0 ml of the partner's washed sperm. Luteal support was used in cases of irregular bleeding in the luteal phase.

Sperm preparation

Semen samples were collected by masturbation after 3–5 days of sexual abstinence. Sperm were prepared for swim-up after 30–60 min of room temperature liquefaction. After liquefaction each sample was analyzed using WHO guidelines. The sample was then centrifuged at $500 \times g$ for 10 min, and the seminal plasma was carefully removed and the pellet diluted in 2–3 ml of IVF-medium (MediCult/Universal IVF-Medium with Phenol Red; Copenhagen, Denmark). After re-centrifugation at $500 \times g$ for 5 min the supernatant was discarded, 1 ml of IVF medium was added and the sperm suspended. The same protocol was repeated once and the final pellet was gently covered with 1 ml of medium and incubated for 30–60 min at 37°C in a leaning

position. After incubation, swim-up was very carefully separated and analyzed. All sperm preparations were carried out by a single laboratory technician during the study period.

Insemination

Before insemination treatment, endometrial thickness and the presence of ovulation were assessed by transvaginal ultrasonography. The procedure was carried out using an intrauterine catheter (Gynetic, Gynetics Medical products; Hamont-Achel, Belgium) with a 2-ml syringe. With the woman in the lithotomy position, the IUI catheter was gently directed into the uterine lumen and the sperm suspension slowly infused. The women were allowed to stand up and leave immediately. They were advised to perform a urinary pregnancy test 15 days after insemination. Ultrasonographic scanning was carried out in all instances of pregnancy.

Statistical analysis

The variables selected for analysis were female age, previous pregnancies, duration of infertility, number of cycles, stimulation protocol, number of preovulatory follicles, endometrial thickness, presence of ovulation, luteal support, and sperm count. Association between PR and independent variables was assessed by means of binary logistic regression analysis. The cycle was used as an observation (instead of the couple), because the variation (e.g. stimulation protocol, number of follicles) between the consecutive cycles of a couple was often larger than between the couples. Categorical variables were compared using the χ^2 test. All statistical analyses were performed using SPSS for Windows software, version 11.0 (SPSS Inc., Chicago, IL, USA). The Ethics Committee of Tampere University Hospital approved the study protocol.

Results

The PRs according to different etiology groups and different variables are shown in Table I. In the unexplained infertility group the PR per cycle was 14.1% and the MPR was 8.9%.

Logistic regression analysis showed that the two positive predictors of PR were the number of preovulatory follicles (OR one/three follicles = 3.26, 95% confidence interval (CI) = 1.72, 6.20) and the IMC (OR lowest/highest = 3.44, 95% CI = 0.79, 14.98). Infertility duration of more than three years and increased age of woman affected the IUI results

negatively, but not statistically significantly. All the stimulation protocols were equally effective.

In cases of male factor infertility the PR/cycle was 18.2% and the MPR 3.6%. Female age, infertility duration, and the number of follicles did not influence the outcome of IUI. A stimulation with sequential CC/HMG protocol resulted in the best PR/cycle (25.0%).

In the ovulatory dysfunction group the PR/cycle was 19.2% and the MPR was 13.2%. Ovulation induction with sequential CC/HMG was significantly associated with the PR (OR = 3.3, 95% CI = 1.14, 9.59), this being 36%/cycle. A short duration of infertility and an increased number of follicles also positively affected the PR but the tendency did not reach statistical significance.

In women with endometriosis the PR/cycle was 11.9% and the MPR was 6.7%. The PR decreased with increasing female age and the best PRs were observed after CC stimulation (21.1%) and with IMC $> 30 \times 10^6$ (24.3%), but these variables were not statistically significant.

The number of couples with combined infertility etiologies was low and therefore the variables did not show significant statistical differences. The PR/cycle was 17.9%, the pregnancy probability being highest with CC/HMG stimulation and ≥ 3 follicles.

IUI treatment resulted in 181 pregnancies. The overall PR per cycle was 15.5% and per couple 34.0%. Pregnancy ended in miscarriage in 22.2% of cases and 3.6% were ectopic. Ovulatory dysfunction was associated with the highest miscarriage rate (30.0%) and the multiple reasons group had the lowest rate (12.5%). Seventeen multiple pregnancies (9.4%) occurred and three of them were trigeminous (1.7%).

Discussion

The most important findings in our study were the significance of the etiology of infertility in the prognosis of insemination treatment and the differences in factors affecting the pregnancy and especially the MPRs.

In unexplained infertility, ovarian induction and IUI have been shown to be cost-effective given the four- to five-fold lower cost compared with IVF treatment (13,14). In our study, in this subgroup more than one follicle at the time of ovulation was identified as a statistically significant positive prognostic factor and the MPR was 8.9% even with two to three leading follicles. The PR with $> 30 \times 10^6$ motile spermatozoa was almost three times higher (19.8% versus 6.7%) than that in the lowest sperm count category. According to our results,

Table I. Pregnancy rates (%) per cycle (*n*) according to etiology.

	Unexplained		Male		Anovulatory		Endometriosis		Many reasons	
	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>
Overall PR	14.1	637	18.2	154	19.2	198	11.9	126	17.9	56
Female age (years)										
<25	25.0	32	0	11	10.0	10	28.6	7	0	4
25–29	16.2	204	18.8	48	22.5	111	11.7	60	18.2	33
30–34	11.9	235	20.3	69	15.4	65	11.1	54	18.8	16
35–39	12.7	166	19.2	26	16.7	12	0	5	33.3	3
Infertility duration (years)										
<2	17.9	78	20.0	20	21.3	61	21.1	19	30.8	13
2–3	15.8	283	18.1	83	23.4	77	9.7	72	6.7	15
>3	10.2	263	18.0	50	10.5	57	12.5	32	17.9	28
Type of infertility										
Primary	12.8	406	16.3	86	17.3	121	10.9	101	20.8	48
Secondary	16.0	231	20.6	68	22.1	77	16.0	25	0	8
Cycle no.										
1	14.9	275	17.3	81	18.7	91	11.9	59	6.9	29
2	13.0	192	22.2	45	17.5	57	10.3	39	23.5	17
3	12.6	119	9.5	21	18.2	33	16.0	25	44.4	9
≥4	17.6	51	28.6	7	29.4	17	0	3	0	1
Follicle number										
1	9.2	218	16.3	49	16.2	68	11.4	35	0	19
2	14.3	273	17.5	63	17.0	94	10.0	60	23.1	26
3	24.2	120	18.9	37	29.6	27	11.5	26	33.3	9
≥4	7.7	26	40.0	5	33.3	9	40.0	5	50.0	2
Stimulation regimen										
Clomiphene citrate	15.5	193	16.9	71	15.4	65	21.1	38	7.1	14
HMG	14.0	387	19.1	68	17.9	106	7.5	80	18.8	32
Combination	11.5	52	25.0	12	36.0	25	12.5	8	30.0	10
Sperm count ($\times 10^6$)										
<5	6.7	30	16.5	91	0	6	0	3	18.2	33
5.1–10	11.4	105	25.0	24	28.6	21	4.5	22	11.1	9
10.1–20	11.0	145			20.4	54	5.6	36	12.5	8
20.1–30	11.2	98			24.1	29	11.5	26	0	1
>30	19.8	237			16.7	78	24.3	37	0	2

IUI treatment in cases of unexplained infertility can be recommended to couples with a young female (<30 years), a short duration of infertility (<3 years), 2–3 preovulatory follicles, and a high IMC ($>30 \times 10^6$).

It is well documented that IUI is the best first-line treatment in cases of mild and moderate male factor infertility. In our data, female age up to 39 years, duration of infertility, and the numbers of male factor infertility follicles did not influence the outcome of IUI. The sequential CC/HMG in ovarian induction showed the best result and most of the pregnancies resulted from the two first IUI cycles. The lowest sperm count resulting in pregnancy was 0.6 million. Our results are in agreement with those in a review study by Ombelet et al. (15) confirming that IUI treatment is very successful in couples having an IMC of less than 5×10^6 , the fact being argued in some previous studies (5,6,16).

Total motile sperm count is a well known predictor of IUI treatment, but an exact cut-off level has not been determined owing to a lack of standardization of semen analysis and other variables affecting IUI success.

In the anovulatory infertility subgroup, ovarian induction with sequential CC/HMG led to a significantly higher PR per cycle (36.0%) when compared to HMG (17.9%) or CC (15.4%) alone, which is in concordance with earlier findings (17,18). Minimal stimulation with recombinant FSH in IUI cycles may also reduce the rate of twins (<10%) and high-order multiple pregnancies (<0.5%) while maintaining acceptable PRs (19). According to our results, stimulation of an anovulatory woman resulting in three or more follicles should be avoided in order to minimize the MPR rate.

A diagnosis of endometriosis reduces the success of IUI treatment. A meta-analysis (14) showed that

in infertility associated with endometriosis the effectiveness of ovarian stimulation and IUI was reduced by approximately half in cases of persistent infertility, but the results of a recent study (20) suggested that ovarian stimulation and IUI in women with surgically treated mild endometriosis is as effective as in women with unexplained infertility. The PR of 11.9% per cycle in our study was acceptable, especially when some of our endometriosis patients had unilateral tubal patency or moderate endometriosis. IUI can therefore be recommended as a worthwhile first treatment for women with mild or moderate endometriosis, especially when female age (<25 years), duration of infertility (<2 years), and motile sperm count ($>30 \times 10^6$) are favorable.

Most of the couples in the multiple reasons subgroup had mixed diagnosis of male factor-, anovulatory-, tubal-, and endometriosis-based infertility. Because the number of cycles in this subgroup was low ($n=56$), conclusions cannot be drawn. Nevertheless, it seems that IUI in cases of moderate male infertility ($IMC < 5 \times 10^6$) combined with some other infertility factor IUI can be successful, the PR/cycle being 18.2%.

With the exception of the male infertility subgroup we found that a decreased PR was associated with a longer duration of infertility. In other infertility etiologies a duration of infertility more than three years should be remembered when choosing between IUI and IVF treatment (5,7,8).

Female age has been found to be a predictive determinant of the likelihood of pregnancy in many studies (6–9,21), but not in all (5,16), and in our study group increasing female age was associated with a decrease in PR, although this correlation was not significant, probably as a result of the age limit of <40 years in our study. In our study material IUI treatment is effective in women aged 35–39 years with a PR of 13.7%.

Stimulation with sequential CC and HMG resulted in a higher PR in male type, anovulatory, and multifactorial infertility and MPR in women stimulated with this protocol (9.1%) was lower than in those stimulated with HMG alone (13.3%), but considerably higher than in those in the CC group (3.3%). In the whole study group MPR was 9.4% and three pregnancies of 181 (1.7%) were trigeminous. As our results show, in IUI treatment the etiology of infertility must be considered in ovulation induction. Because the risk for multiple pregnancies is lowest with male-type and endometriosis-based infertility, two to three follicles can be favorable. With unexplained infertility, two follicles could be ideal even though three follicles result in the

best PRs. In anovulatory patients only one follicle is acceptable.

In conclusion, IUI is a simple, relatively non-invasive, and cost-effective first-line treatment in most cases of subfertility. In planning individual treatments the role of etiology must be considered, because in different infertility subgroups the variables influencing the response to stimulation and probability of pregnancy have different impacts, as our study has shown. The future of IUI will depend on our ability to maintain an acceptable MPR without affecting the overall PR. Therefore, when aiming at single embryo pregnancies with IUI, it must be remembered that the number of follicles, the stimulation protocol and the necessary sperm count are not identical as regards the different etiologies of infertility.

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