INTRAUTERINE INSEMINATION (IUI) TREATMENT IN SUBFERTILITY

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ABSTRACT

The effectiveness of intrauterine insemination (IUI) combined with controlled ovarian hyperstimulation (COH) in the treatment of subfertility was investigated in the present study. For this purpose the prognostic factors associated with success of clomiphene citrate (CC)/human menopausal gonadotrophin (HMG)/IUI were identified in 811 treatment cycles. Furthermore, a long gonadotrophin-releasing hormone agonist (GnRHa)/HMG stimulation protocol was compared with a standard CC/HMG protocol. In addition, the usefulness of alternative insemination techniques including fallopian tube sperm perfusion (FSP) and intrafollicular insemination (IFI) was investigated. Finally, the obstetric and perinatal outcome of pregnancies after COH/IUI was examined and compared with those of matched spontaneous and in vitro fertilization (IVF) pregnancies.

Female age, duration of infertility, aetiology of infertility, number of large preovulatory follicles and number of the treatment cycle were predictive as regards pregnancy after CC/HMG/IUI. The highest pregnancy rate (PR) was obtained in women of <40 years of age with infertility duration £6 years, who did not suffer from endometriosis. A multifollicular ovarian response to CC/HMG resulted in better treatment success than a monofollicular response, indicating the necessity of COH combined with IUI. A significantly higher PR was achieved in the first treatment cycles compared with the others, and 97% of the pregnancies were obtained in the first four treatment cycles.

The PR per cycle did not differ significantly between a long GnRHa/HMG and a standard CC/HMG protocol, but the average medication expense of GnRHa/HMG stimulation was four times the cost of CC/HMG stimulation. Therefore, the routine use of a long GnRHa/HMG protocol in IUI treatment remains questionable.

The FSP procedure was easy to perform by using a paediatric Foley catheter. The success rate in couples with either FSP or standard IUI did not differ significantly, although there was a trend towards a lower PR in the FSP group. The FSP technique should not replace the simpler and less time-consuming IUI technique in routine use.The IFI technique was also simple to perform and convenient for patients. However, only one normal singleton intrauterine pregnancy resulted in 50 IFI-treated women, indicating that IFI is inefficacious for treating subfertility.

The IUI parturients differed from average Finnish parturients in respect to higher maternal age, more frequent primiparity and a higher incidence of multiple pregnancies. The use of antenatal care services was significantly lower in IUI singleton pregnancies compared with IVF singletons, although there were no more complications in IVF pregnancies. The hospitalization and Caesarean section rates were generally high in all pregnancies. The mean birthweight of IUI singletons was significantly lower than that in spontaneous pregnancies, but comparable to that in IVF pregnancies. However, the incidence of preterm birth, low birth weight and other variables describing the outcome of infants were similar in IUI, IVF and spontaneous pregnancies. In summary, the IUI procedure itself does not seem to affect adversely the obstetric and perinatal outcome of pregnancy, and patient characteristics and multiplicity may be more important in this respect.

Keywords: assisted reproductive technology, clomiphene citrate, human menopausal gonadotrophin, infertility.

Etsivä löytää.

i i s

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Abbreviations

ART	assisted reproduction technology
CC	clomiphene citrate
CI	confidence interval
COH	controlled ovarian hyperstimulation
CS	Caesarean section
DIPI	direct intraperitoneal insemination
ESHRE	European Society for Human Reproduction and Embryology
FSH	follicle-stimulating hormone
FSP	fallopian tube sperm perfusion
GIFT	gamete intrafallopian transfer
GnRH	gonadotrophin-releasing hormone
GnRHa	gonadotrophin-releasing hormone agonist
HCG	human chorionic gonadotrophin
HMG	human menopausal gonadotrophin
ICSI	intracytoplasmic sperm injection
IFI	intrafollicular insemination
IUI	intrauterine insemination
IVF	*in vitro* fertilization
LH	luteinizing hormone
OHSS	ovarian hyperstimulation syndrome
OR	odds ratio
PR	pregnancy rate
SD	standard deviation

List of original publications

The present thesis is based on the following articles, which are referred to in the text by their Roman numerals:

I Nuojua-Huttunen S, Tomás C, Bloigu R, Tuomivaara L & Martikainen H (1999) Intrauterine insemination (IUI) treatment in subfertility: an analysis of factors affecting outcome. Hum Reprod 14: 698-703.

II Nuojua-Huttunen S, Tuomivaara L, Juntunen K, Tomás C & Martikainen H (1997) Long gonadotrophin releasing hormone agonist/human menopausal gonadotrophin protocol for ovarian stimulation in intrauterine insemination treatment. Eur J Obstet Gynecol Reprod Biol 74: 83-87.

III Nuojua-Huttunen S, Tuomivaara L, Juntunen K, Tomás C, Kauppila A & Martikainen H (1995) Intrafollicular insemination for the treatment of infertility. Hum Reprod 10: 91-93.

IV Nuojua-Huttunen S, Tuomivaara L, Juntunen K, Tomás C & Martikainen H (1997) Comparison of fallopian tube sperm perfusion with intrauterine insemination in the treatment of infertility. Fertil Steril 67: 939-942.

V Nuojua-Huttunen S, Gissler M, Martikainen H & Tuomivaara L (1999) Obstetric and perinatal outcome of pregnancies after intrauterine insemination (IUI). Hum Reprod, in press.

1. Introduction

Infertility is defined as the inability to conceive after 1-2 years of regular, unprotected intercourse. The infertile population consists of subfertile couples (78%) and couples with female/male sterility (22%). Female sterility can be a consequence of ovarian failure or bilateral tubal occlusion, and male sterility can be caused by azoospermia or aspermia. (Jaffe & Jewelewicz 1991, ESHRE Capri Workshop 1996.) In subfertile couples the monthly conception rate is decreased but pregnancy can occur spontaneously (Collins *et al.* 1995, Snick *et al.* 1997).

The number of infertile couples throughout the world has been approximated at 60-80 million (ESHRE Capri Workshop 1996). The reported lifetime prevalences of infertility (having experienced a period of infertility at some stage of reproductive life) vary greatly, which is assumed to result partly from varying definitions and measurements of infertility. In industrialised countries the prevalence ranges from 10 to 33%. (Schmidt & Munster 1995, Sundby & Schei 1996, Chandra & Stephen 1998.) In a Finnish population-based study the lifetime prevalence of infertility was 14% (Notkola 1995). In developed countries, 3-7% of the female population who are at the end of reproductive age, are childless because of infertility (Schmidt & Munster 1995, ESHRE Capri Workshop 1996, Sundby & Schei 1996, Buckett & Bentik 1997, Wulff *et al.* 1997).

Infertility can result from disorders in both partners (40%), in the female partner alone (25-30%) or in the male partner alone (20%). No detectable reason for infertility is found in 10-15% of couples. (Thonneau *et al.* 1991, ESHRE Capri Workshop 1996.) The main causes of infertility are ovulation disorders (20-32%), tubal damage (14-26%) and endometriosis (4-6%). There is no female reason for infertility in 26-30% of couples and decreased semen quality is found in 24-42% of male partners. (Hull *et al.* 1985, Thonneau *et al.* 1991, Schmidt *et al.* 1995.)

Approximately 50% of infertile women seek medical help for fertility problems and over a half of these subsequently will have a treatment-independent or treatment-related child (Schmidt *et al.* 1995, Olsen *et al.* 1996, Buckett & Bentik 1997, Wulff *et al.* 1997, Chandra & Stephen 1998). The choice of the most optimal treatment for a subfertile couple can be confusing. Both over-treatment and unnecessary treatments should be avoided, thereby minimizing the total cost of infertility treatment and the possible health risks associated with ovarian stimulation.

Ovulation induction with clomiphene citrate (CC), gonadotrophins or pulsatile gonadotrophin-releasing hormone (GnRH) is a suitable treatment option in anovulatory conditions such as normogonadotrophic anovulation or hypogonadotrophic hypogonadism (ESHRE Capri Workshop 1996). If anovulation is corrected by an appropriate ovulation induction regimen, cumulative conception and live birth rates may be normal. Close monitoring and the high cost associated with the use of gonadotrophins and GnRH and the risk of multiple pregnancy are the disadvantages of ovulation induction treatment. (Balen *et al.* 1994, Tadokoro *et al.* 1997.) Ovulation induction regimens have been used as empirical treatment in unexplained infertility although the reported treatment results are somewhat variable and less successful than in anovulatory infertility (Glazener *et al.* 1990, Simon *et al.* 1991, Fujii *et al.* 1997, Tadokoro *et al.* 1997).

The amount of surgical intervention used in the treatment of subfertility has decreased, and surgery has mainly been replaced by assisted reproduction technology (ART). Currently, conservative laparoscopic surgery and operative hysteroscopy are used for the management of endometriosis, ovarian tumours, adhesions, subserous and submucous fibroids and intrauterine abnormalities.

Nowadays numerous treatment options in ART are widely used for subfertility, and *in vitro* fertilization (IVF) is the most commonly used. The overall pregnancy rate (PR) per cycle after IVF ranges from 23 to 26%, multiple PR from 22 to 36% and the miscarriage rate from 18 to 19%. (Anonymous 1993, Anonymous 1998, Gissler & Tiitinen 1998.)

Despite the high PR associated with IVF, there are some disadvantages, to which attention must be paid. The total treatment cost is high because of the need for highly trained personnel and expensive equipment. For couples, heavy medication and close monitoring are physically demanding and time- and money-consuming. Therefore, it seems reasonable to consider simpler and inexpensive therapies such as controlled ovarian hyperstimulation (COH) combined with intrauterine insemination (IUI) for first-line treatment in subfertility. Overall, ovarian stimulation is not so aggressive, and the IUI procedure is simpler than in IVF, making COH/IUI treatment convenient for patients. Furthermore, in several studies, COH/IUI treatment has been found to be cost-effective for subfertile couples before undergoing invasive ART (Simon *et al.* 1991, Peterson *et al.* 1994, Dawood 1996, van Voorhis *et al.* 1997, Zayed *et al.* 1997).

In our unit COH/IUI is the first-line treatment for subfertility without tubal factor or severe endometriosis/male factor. In the present study, we investigated whether the effectiveness of a standard COH/IUI treatment can be further improved by identifying predictive variables for successful outcome or by using an alternative stimulation protocol or new insemination techniques. In addition, we examined the obstetric and perinatal outcome of pregnancies after IUI treatment.

2. Review of the literature

2.1. Intrauterine insemination (IUI) treatment

2.1.1. Historical background

The first human artificial insemination with husband's semen was performed intravaginally in the late 1700s in England. Sophia Kleegman from the United States was one of the pioneers who used artificial insemination with husband's or donor sperm for the treatment of infertility from 1930 on. (Arny & Quagliarello 1987.) There are published reports on the use of artificial insemination with husband's sperm as early as the beginning of the 1950s (Hanson & Rock 1951). Different insemination techniques such as intravaginal, intracervical (ICI), pericervical using a cap, and IUI were used (Allen *et al.* 1985). Intrauterine insemination with raw or defectively washed semen was not preferred because of its questionable effectiveness and risk of uterine cramps and infection (Mastroianni *et al.* 1957, Kremer 1979). Renewed interest in IUI has increased in the last decade when new methods of sperm preparation, employed in IVF, were also applied in IUI treatment. By using these methods, spermatozoa can be separated from the seminal plasma thoroughly; the concentration of highly motile sperm is increased and pregnancy rates improved. (Marrs *et al.* 1983, Kerin *et al.* 1984, Sher *et al.* 1984, Byrd *et al.* 1987.) The removal of seminal plasma is important because it includes elements (decapacitating factors, prostaglandins and microbes), which may result in adverse effects such as inhibition of fertilization, uterine cramps and risk of infection (Kanwar *et al.* 1979, Toth & Lesser 1981, Witkin & Toth 1983). Nowadays sperm samples are regularly washed and prepared for IUI employing modern preparation techniques. In addition to the new techniques for sperm preparation, the combination of COH with IUI has been another important factor as regards the increased use of IUI treatment. It was first described by Sher *et al.* (1984). Previously, IUI was combined mainly with spontaneous or CC-stimulated cycles and insemination timing was based on basal body temperature records, assay of serum luteinizing hormone (LH)/oestradiol or evaluation of cervical mucus (Allen *et al.* 1985). The use of COH requires close monitoring and the exact timing of ovulation, which is possible by transvaginal ultrasonography and by administration of human chorionic gonadotrophin (HCG) for ovulation induction.

2.1.2. Ovarian stimulation with IUI

2.1.2.1. Advantages

It is well documented that IUI in combination with preceding COH is more effective than IUI in spontaneous cycles (Kemmann *et al.* 1987, Serhal *et al.* 1988, Chaffkin *et al.* 1991, Nulsen *et al.* 1993, Arici *et al.* 1994, Guzick *et al.* 1999). Several advantages associated with COH have been speculated to improve the chance of fertilization and implantation in IUI treatment: multiple follicles and subsequently multiple oocytes and increased gonadal steroid secretion can be achieved, more exact time to ovulation and insemination can be determined and subtle ovulatory disorders may be corrected (Dodson *et al.* 1987, Wallach 1991). However, a monofollicular ovarian response after COH has also been seen to result in an acceptable PR per cycle of 13% (Balasch *et al.* 1994), but this has not been confirmed in all studies (Hughes *et al.* 1998). The optimal number of large preovulatory follicles to maintain an acceptable cycle fecundity and minimize the risk of multiple gestation is not known.

2.1.2.2. Disadvantages

The increased number of follicles induced by COH is not always an advantage, and it is also proposed to correlate with the risk of multiple pregnancy (Navot *et al.* 1991a, Farhi *et al.* 1996). In several studies, a correlation between the number of large preovulatory follicles and multiple gestation in COH/IUI treatment has been investigated, and the results are contradictory (Dickey *et al.* 1991, Dodson & Haney 1991, Gagliardi *et al.* 1991, Dickey *et al.* 1992, Valbuena *et al.* 1996, Goldfarb *et al.* 1997, Isaksson & Tiitinen 1997). Nevertheless, when the ovarian response is considered excessive for IUI treatment, the cycle is generally cancelled or converted to IVF, or supernumerary mature follicles are aspirated in order to decrease the possibility of multiple pregnancy. Pregnancy rates achieved after converting IUI to IVF or aspirating excessive follicles have been 40% and 24%, respectively (Nisker *et al.* 1994, de Geyter *et al.* 1996). The serum oestradiol concentration (Gagliardi *et al.* 1991) and the number of inseminated motile spermatozoa (Shelden *et al.* 1988) have also been supposed to predict multiple pregnancy in IUI treatment, but this is not confirmed in all studies (Dickey *et al.* 1991, Dodson & Haney 1991, Valbuena *et al*. 1996, Goldfarb *et al.* 1997, Isaksson & Tiitinen 1997).

Other disadvantages associated with COH are the expense of medication, the need for cycle monitoring, the risk of ovarian hyperstimulation syndrome (OHSS) and possible late health risks. Overall, the average cost of ovarian stimulation is much lower in COH/IUI than in IVF treatment. This is due to the lower ovarian response needed for IUI treatment. In addition, the incidence of severe OHSS has been shown to be rare (0-5%) in COH/IUI treatment (Chaffkin *et al.* 1991, Dodson & Haney 1991, Nulsen *et al.* 1993, Lu *et al.* 1996, Ransom *et al.* 1996, Brzechffa *et al.* 1998). Currently, an association between fertility drug use and ovarian cancer is unproven, but close clinical surveillance of subjects before, during and after treatment of infertility is warranted (Bristow & Karlan 1996, Burmeister & Healy 1998).

To minimize the influence of disadvantages, subjects should undergo a limited number of treatment cycles with proven efficacy. In the literature, the recommended number of COH/IUI cycles is somewhat variable. Cycle fecundity has been reported to be relatively constant for up to the first seven cycles (Dickey *et al.* 1992), but decreasing PRs with an increased number of treatment cycles have also been shown (Burr *et al.* 1996, Tomlinson *et al.* 1996). In several studies, the majority (>90%) of pregnancies have been seen to occur within the first three to five treatment cycles (Remohi *et al.* 1989, Chaffkin *et al.* 1991, Nulsen *et al.* 1993, Plosker *et al.* 1994, Agarwal & Buyalos 1996).

2.1.3. Stimulation protocols

There are currently many different hormonal treatment protocols for COH combined with IUI, but the optimal, most cost-effective method has not yet been determined. The PRs achieved by using the same stimulation model vary greatly between studies and can be surprisingly low or high in some subgroups (Martinez *et al.* 1994). The variation may be partly due to the small size of the study populations and variability in characteristics of the subjects, in study designs and in insemination techniques. Overall, the number of randomized, controlled trials of stimulation regimens is small.

2.1.3.1. Clomiphene citrate (CC) alone

Clomiphene citrate is a widely used and inexpensive drug for ovarian stimulation. It acts as an anti-oestrogen on the central nervous system and increases the secretion of follicle-stimulatign hormone (FSH) and LH, giving a moderate gonadotrophin stimulus to the ovary (Martikainen *et al.* 1991). Hence, some ovulatory disturbances can be overcome and the cohort of follicles reaching ovulation can be increased (Tarlatzis & Grimbizis 1998). The timing for the IUI procedure can be established by a simple and cheap method of urinary LH detection (Deaton *et al.* 1997).

In general, the PR per cycle in CC/IUI treatment varies between 4 to 14% and the miscarriage and multiple gestation rates have been reported to be 14% and 0 to 24%, respectively (Deaton *et al.* 1990, Dickey *et al.* 1992, Arici *et al.* 1994, Balasch *et al.* 1994, Agarwal & Buyalos 1996, van der Westerlaken *et al.* 1998). In randomized controlled studies, CC/IUI treatment has resulted in increased cycle fecundity (9.5%) compared with natural cycles and timed intercourse (3.3%) (Deaton *et al.* 1990), but when comparing CC/IUI with spontaneous cycles/IUI the results have been contradictory (Martinez *et al.* 1990, Arici *et al.* 1994).

2.1.3.2. CC combined with gonadotrophins

The PRs achieved after IUI combined with sequential CC and human menopausal gonadotrophin (HMG) for COH have ranged from 9 to 22% in couples with subfertilty of heterogeneous aetiology. The reported miscarriage and multiple gestation rates have been 5 to 26% and 0 to 33%, respectively. (Fioretti *et al.* 1989, Dickey *et al.* 1993, Lu *et al.* 1996, Ransom *et al.* 1996, Brzechffa *et al.* 1998.) There is only a single randomized study in which the result of CC/HMG/IUI was compared with that of HMG/IUI in subjects, who had been previously treated unsuccessfully with CC. A higher PR (19 vs. 9%) was obtained when HMG was used alone (Ransom *et al.* 1996). In retrospective studies, the PRs have not differed between the stimulation protocols (Dickey *et al.* 1993, Lu *et al.* 1996).

When HMG stimulation is replaced by CC/HMG in COH, the cost, and inconvenience to the subjects are decreased because of a reduced need for monitoring and daily HMG injections. On the other hand, CC acts as an antioestrogenic drug and this effect on the endometrium has been speculated to influence treatment outcome negatively. (Rönnberg *et al.* 1985, Bonhoff *et al.* 1996, Ransom *et al.* 1996.)

2.1.3.3. Gonadotrophins alone

The use of gonadotrophins alone for COH is an established protocol in IUI treatment. The average cycle fecundity has ranged from 9 to 20% according to the results of studies which have included women with various infertility aetiologies. Of the pregnancies, 11 to 33% have ended in miscarriage and 11 to 28% have been multiple (Chaffkin *et al.* 1991, Dickey *et al.* 1991, Dodson & Haney 1991, Nulsen *et al.* 1993, Lu *et al.* 1996, Ransom *et al.* 1996, Brzechffa & Buyalos 1997, Guzick *et al.* 1999). In an unselected subfertile population the mean cycle fecundity has been shown to be significantly higher with HMG/IUI (12-20%) compared with IUI alone (2-3%) (Chaffkin *et al.* 1991, Nulsen *et al.* 1993). To reduce the disadvantages of gonadotrophin stimulation and especially the risk of multiple pregnancy, Balasch *et al.* (1994) tested an alternative stimulation protocol, in which FSH was injected daily at low doses during the late follicular phase. The ovarian response was mainly monofollicular and the PR per cycle was 13%. All pregnancies were singletons.

2.1.3.4. Gonadotrophin-releasing hormone agonist (GnRHa) combined with gonadotrophins

Pituitary suppression with GnRHa and ovarian stimulation with exogenous gonadotrophins is the most commonly used stimulation protocol in IVF. The beneficial effects of the use of GnRHa in IVF include a lower cycle cancellation rate, the prevention of premature luteinization and an increased number of oocytes and embryos. (Antoine *et al.* 1990, Chetkowski *et al.* 1991, Ron-El *et al.* 1991.)

In a few studies, a long GnRHa/HMG protocol has been used for ovarian stimulation in IUI treatment. The reported PRs have varied between 11 and 26%, and the miscarriage and multiple pregnancy rates have been 12 to 33% and 12 to 36%, respectively (Dodson *et al.* 1991, Gagliardi *et al.* 1991, Manzi *et al.* 1995, Tomlinson *et al.* 1996). Results regarding the effectiveness of GnRHa/HMG/IUI in comparison with HMG/IUI have been contradictory and also difficult to compare because of greatly different study populations and study designs (Dodson *et al.* 1991, Gagliardi *et al.* 1991, Manzi *et al.* 1995). Hence the usefulness of this stimulation protocol among unselected subfertile couples remains unproven.

Table 1. A summary of studies on different protocols for COH.

Study	Year	N p/c*	Design	Pregnancy rate/cycle (%)
CC				
Deaton *et al.*	1990	67/148	randomized	9.5
Martinez *et al.*	1990	-/35	randomized	14.3
Dickey *et al.*	1992	849/1974	prospective	7.2
Arici *et al.*	1994	-/49	randomized	14.3
Balash *et al.*	1994	50/98	randomized	4.1
Agarwal & Buyalos	1996	290/664	prospective	10.4
van der Westerlaken *et al.*	1998	566/1763	retrospective	6.9
CC/HMG				
Fioretti *et al.*	1989	41/181	prospective	10.5
Dickey *et al.*	1993	-/119	retrospective	21.8
Lu *et al.*	1996	61/106	retrospective	20.8
Ransom *et al.*	1996	45/110	randomized	9.1
Brzechffa *et al.*	1998	208/416	retrospective	14.2
HMG				
Chaffkin *et al.*	1991	135/357	retrospective	19.6
Dickey *et al.*	1991	424/779	prospective	12.8
Dodson & Haney	1991	371/808	retrospective	15.2
Nulsen *et al.*	1993	-/294	randomized	12.2
Lu *et al.*	1996	183/443	retrospective	20.1
Ransom *et al.*	1996	53/130	randomized	19.2
Brzechffa & Buyalos	1997	184/363	retrospective	12.1
Guzick *et al.*	1999	231/618	randomized	8.7
GnRHa/HMG				
Dodson *et al.*	1991	81/81	randomized	11.0
Gagliardi *et al.*	1991	64/102	retrospective	26.5
Manzi *et al.*	1995	90/90	retrospective	18.9
Tomlinson *et al.*	1996	134/260	retrospective	19.6

*p/c=patients /cycles.

2.1.4. Insemination techniques

The rationale in all insemination techniques is to deposit motile sperm as close to the oocytes as possible. After the use of new sperm preparation methods and COH, IUI replaced other insemination techniques and became the most widely used method for insemination (Allen *et al.* 1985). During the last decade, some new insemination techniques such as direct intraperitoneal insemination (DIPI), intrafollicular insemination (IFI) and fallopian tube sperm perfusion (FSP) have been introduced. The effectiveness of these new methods has been mainly compared with a standard IUI technique.

Infectious complications and formation of antisperm antibodies in women undergoing insemination are the potential risks of all kinds of insemination procedures. However, according to the literature, infections following insemination have been very rare (Dodson & Haney 1991, Sacks & Simon 1991, Nulsen *et al.* 1993) and there has been only a small (4.8-10.8%) risk of developing antisperm antibodies (Horvath *et al.* 1989, Goldberg *et al.* 1990, Friedman *et al.* 1991, Kahn *et al.* 1993a).

2.1.4.1. Intrauterine insemination

In the IUI procedure, washed spermatozoa in a small volume are injected through the cervical canal into the uterine cavity using a thin catheter. A high number of motile spermatozoa near the fertilization site together with a multifollicular ovarian response to COH is thought to improve the chance of conceiving. In many randomized controlled studies, the value of the IUI procedure has been evaluated by comparing it with timed intercourse after COH. The results are contradictory and in about half of the studies IUI resulted in higher PRs (20-26%) than timed intercourse (6-9%), suggesting that IUI itself has a therapeutic benefit in addition to COH (Martinez *et al.* 1990, Chaffkin *et al.* 1991, Chung *et al.* 1995, Gregoriou *et al.* 1996). On the other hand, IUI has failed to demonstrate any advantage over timed intercourse (PR: 7.5-19% for IUI vs. 5.5-15% for intercourse) in many reports (Martinez *et al.* 1991, Karlström *et al.* 1993, Zikopoulos *et al.* 1993, Melis *et al.* 1995).

2.1.4.2. Direct intraperitoneal insemination (DIPI)

Direct intraperitoneal insemination has described as early as 1986. Washed sperm were injected through the posterior vaginal fornix into the pouch of Douglas after COH and a high PR of 30% was achieved (Forrler *et al.* 1986). In later reports, cycle fecundity has been lower and has varied between 7 to 18% and the pregnancy loss rate has ranged from 19 to 39% (Hovatta *et al.* 1990, Turhan *et al.* 1992, Gregoriou *et al.* 1993, Ragni *et al.* 1997, Tiemessen *et al.* 1997). According to the results of randomized studies, PRs have not been higher after using DIPI (7-16%) compared with IUI (10-24%) in stimulated cycles (Hovatta *et al.* 1990, Gregoriou *et al.* 1993, Tiemessen *et al.* 1997). Because DIPI is not more effective than IUI and is more expensive and invasive, it has not become a widely used method in the treatment of non-tubal infertility.

2.1.4.3. Intrafollicular insemination (IFI)

A new and intriguing insemination method, IFI, has been described in two case reports. A small volume of medium with motile spermatozoa is injected directly into large preovulatory follicles after COH. The oocyte is speculated to become fertilized inside the follicle before ovulation. The hypothetical benefits of IFI are environmental stability and the presence of follicular fluid factors, which may improve sperm fertilization capacity. (Lucena *et al.* 1991, Zbella *et al.* 1992.) Encouraging results from 14 procedures resulting in four pregnancies (Lucena *et al.* 1991) need to be confirmed in larger study populations.

2.1.4.4. Fallopian tube sperm perfusion (FSP)

To increase the number of motile spermatozoa in the fallopian tube ipsilateral to the ovary with large follicles, a method of direct tubal insemination was developed. The PR per cycle achieved with this method (7%) was not higher than that with standard IUI (7%) in a randomized study carried out by Oei *et al.* (1992), and because of possible disadvantages (vasovagal episodes, trauma, perforation) associated with tubal cannulation, the technique is not recommended for routine use. The same principle but without tubal cannulation was demonstrated in the FSP technique, in which a large volume of sperm suspension (4 ml) was injected into the uterine cavity. The volume perfused the fallopian tubes and even reached the pouch of Douglas. To prevent reflux of fluid to the vagina, an Allis clamp on the cervix was used. (Kahn *et al.* 1992.) The PR in women with unexplained infertility has been reported to be significantly higher with COH/FSP (27%) than with COH/IUI (10%) (Kahn *et al.* 1993b). A simpler modification of the same method was described in a case report in which a paediatric Foley catheter was inserted into the uterine cavity and an inflated balloon was pressed on the internal os of the cervix to prevent reflux (Li 1993). However, the effectiveness of this method had to be confirmed in larger study population with a heterogeneous aetiology of infertility.

In recent randomized studies, the standard IUI technique has been compared with different modifications of FSP. The results have been conflicting and the PRs per cycle have varied from 11 to 20% in IUI and from 11 to 40% in FSP treatment (Fanchin *et al.* 1995, Gregoriou *et al.* 1995, Karande *et al.* 1995, Mamas 1996).

2.1.5. Other factors affecting the outcome of IUI treatment

The stimulation protocol used for COH and the insemination technique may affect the outcome of COH/IUI treatment. In addition, patient characteristics such as female age, duration of infertility and diagnostic category may have an effect on the likelihood of pregnancy. All these factors should be taken into account when treatment success is estimated for individual couples and the results of different studies are compared with each other.

2.1.5.1. Female age

The decrease in female reproductive capacity with advancing age is widely recognized (Menken *et al.* 1986, Collins & Rowe 1989, Collins *et al.* 1995, Scott *et al.* 1995). The ovarian reserve diminishes with advancing age, but there is great variability in the timing of the onset of impaired reproductive potential for individual women. A high basal FSH concentration early in the proliferative phase (cycle day 3) or an ultrasonographic image of the ovaries with a low number of small follicles before stimulation are useful markers of poor ovarian responsiveness. (Tomás *et al.* 1997, Sharara *et al.* 1998.)

In studies involving donor sperm with IUI, fertility has been found to be impaired as early as by the age of 30 (Schwartz & Mayaux 1982, van Noord-Zaadstra *et al.* 1991) or 35 years (Kang & Wu 1996, Pittrof *et al.* 1996). The success rate of IVF has also been reported to decrease with advancing female age (Templeton *et al.* 1996). Even some studies of IVF with donated oocytes have demonstrated an age-related decline in PRs, suggesting that fertility does not depend merely on oocyte age and quality (Navot *et al.* 1991b), but also on uterine age (Flamigni *et al.* 1993, Cano *et al.* 1995, Borini *et al.* 1996).

Furthermore, the age-related decrease in fertility has been found in IUI treatment with partner's sperm although there has been variability in the results. Many investigators have confirmed a significantly lowered PR per cycle in women over the age of 35 years (7 to 10%) compared with younger ones (15 to 23%) (Dickey *et al.* 1991, Agarwal & Buyalos 1996, Brzechffa & Buyalos 1997, Brzechffa *et al.* 1998). In some studies, success has not become reduced significantly until after the age of 40, and the reported PRs in women younger than 40 and in older women have been 6 to 18% and 2 to 7%, respectively (Frederick *et al.* 1994, Plosker *et al.* 1994, Campana *et al.* 1996, Corsan *et al.* 1996, Tomlinson *et al.* 1996). On the other hand, an advanced female age has not been found to be predictive of treatment outcome when taking into account other factors contributing to the decreased PR in COH/IUI teatment (Mathieu *et al.* 1995).

2.1.5.2. Duration of infertility

The duration of infertility has been shown to be a prognostic factor for live births among untreated subfertile couples. After 2 to 4 years' infertility the likelihood of a live birth begins to decrease. (Dunphy *et al.* 1989, Collins *et al.* 1995, Snick *et al.* 1997.) In COH/IUI therapy, some authors have found the treatment outcome to be significantly impaired after 3 to 8 years' infertility (Nulsen *et al.* 1993, Crosignani & Walters 1994, Mathieu *et al.* 1995, Tomlinson *et al.* 1996), but there are also studies in which the duration of infertility has not been shown to affect the PR (Deaton *et al.* 1990, Dodson & Haney 1991).

2.1.5.3. Aetiology of infertility

Controlled ovarian hyperstimulation together with IUI is widely used for the treatment of subfertility, particularly for couples with unexplained infertility, male factor infertility or endometriosis (Chaffkin *et al.* 1991, Dodson & Haney 1991, Nulsen *et al.* 1993, Brzechffa *et al.* 1998). However, randomized trials comparing COH/IUI with a non-treated control group in different categories of subfertility are rare.

Unexplained infertility. A diagnosis of unexplained infertility is assigned to couples for whom the results of a standard infertility evaluation (history, physical examination, semen analysis, laboratory assessment of ovulation and tubal patency) are normal (ESHRE Capri Workshop 1996).

The PRs per cycle achieved in cases of unexplained infertility, using different stimulation protocols (CC, CC/HMG or HMG) and standard IUI are mainly good, varying between 11 to 20%. (Sunde *et al.* 1988, Dodson & Haney 1991, Aboulghar *et al.* 1993, Nulsen *et al.* 1993, Melis *et al.* 1995, Brzechffa *et al.* 1998.) In a randomized multicentre study in Europe, the effectiveness of five methods (superovulation alone or combined with IUI, DIPI, gamete intrafallopian transfer [GIFT] or IVF) for the treatment of unexplained infertility was evaluated. The PR achieved after superovulation alone appeared to be inferior to that achieved with superovulation together with one of the methods of assisted procreation. The PR per cycle for superovulation combined with IUI was 23%. With each of these methods the PR was much better than without any treatment. (Crosignani *et al.* 1991.)

Male factor infertility. There are many studies on the treatment of male factor infertility by using COH/IUI, but there is still controversy concerning the effectiveness of this treatment procedure. One of the major reasons for inconsistency of the results may be differences in the use of criteria for male factor infertility. The World Health Organization (1992) has defined normal values of semen variables (Table 2), but criticism has been presented concerning the true value of this classification (Ombelet *et al*. 1997a, Bonde *et al*. 1998).

Cycle fecundity has varied between 4 to 15% when male factor infertility is treated by using COH/IUI in randomized studies comparing COH/IUI with COH/timed intercourse (Ho *et al.* 1992, Nan *et al.* 1994, Melis *et al.* 1995, Gregoriou *et al.* 1996), or comparing COH/IUI with natural cycle/IUI (Nulsen *et al.* 1993, Arici *et al.* 1994, Cohlen *et al.* 1998). The same variation in PRs has also been found in other studies (Sunde *et al.* 1988, Chaffkin *et al.* 1991, Dodson & Haney 1991, Plosker *et al.* 1994). In a prospective randomized multicentre trial on the value of four different assisted reproduction methods in male infertility treatment, firm evidence was found that IUI (PR per cycle 13%), IVF and GIFT in conjuction with ovarian stimulation were beneficial when compared with DIPI or ovarian stimulation alone (Crosignani & Walters 1994).

Cut-off values of sperm parameters which would be predictive of COH/IUI success have been sought. In several studies, treatment outcome has been shown to be more successful if the number of inseminated motile sperm is above 0.25 to 1 x 10^6 (Horvath *et al.* 1989, Dodson & Haney 1991, Mathieu *et al*. 1995, Campana *et al.* 1996, Berg *et al*. 1997) or even above 10 x 10^6 (van der Westerlaken *et al*. 1998). In addition, an amount of morphologically normal sperm under 10% has been found to be predictive of poor treatment outcome (Comhaire *et al*. 1995, Toner *et al*. 1995, Burr *et al*. 1996), although this has not been confirmed in all studies (Karabinus & Gelety 1997, Ombelet *et al*. 1997b). Couples with severe male factor infertility or azoospermia can now be treated successfully with intracytoplasmic sperm injection (ICSI) (Aytoz *et al*. 1998), and the value of COH/IUI treatment should be evaluated only in mild to moderate male factor infertility.

Table 2. Normal values of semen variables.

Standard tests	Value
Volume	≥2.0 ml
pH	7.2-8.0
Sperm concentration	≥20 x 10^6 spermatozoa/ml
Total sperm count	≥40 x 10^6 spermatozoa/ejaculate
Motility	≥50% with forward progression (categories A and B) or ≥25% with rapid progression (category A) within 60 minutes of ejaculation
Morphology	≥30% with normal forms
Vitality	≥75% live, i.e. excluding dye
White blood cells	<1 x 10^6/ml
Immunobead test	<20% spermatozoa with adherent particles
Mixed antiglobulin reaction	<10% spermatozoa with adherent particles

Endometriosis-related infertility. Although the aetiology and pathophysiology of endometriosis have remained controversial, its negative impact on fertility has been documented (Collins *et al.* 1995). The exact correlation between fertility and severity of endometriosis remains less clear, particularly in respect to minimal or mild endometriosis (Prentice & Ingamells 1996). Ovarian stimulation with IUI has been used as an empirical treatment for infertility in women with endometriosis, mainly classified as minimal or mild. There is only one randomized study where PRs were compared between treated (superovulation with FSH combined with IUI) and untreated women with minimal or mild endometriosis. Live birth resulted in 11% of treatment cycles and only in 2% of non-treatment cycles indicating the superiority of COH/IUI over expectant management in the treatment of infertility in women suffering from endometriosis. (Tummon *et al.* 1997.) Generally, the reported PRs obtained among endometriosis patients with COH/IUI have ranged from 5 to 16% per cycle (Chaffkin *et al.* 1991, Dickey *et al.* 1991, Dodson & Haney 1991, Dickey *et al.* 1992, Nulsen *et al.* 1993, Plosker *et al.* 1994, Brzechffa *et al.* 1998). Some studies have demonstrated poorer treatment outcome in endometriosis groups compared with other subfertility categories (Dickey *et al.* 1991, Dickey *et al.* 1992, Crosignani & Walters 1994, Omland *et al.* 1998). In addition, in a recent meta-analysis, which included only randomized controlled trials of the use of COH/IUI for persistent infertility, the independent effects of different variables on success rate were examined. A diagnosis of endometriosis reduced treatment effectiveness by approximately half. (Hughes 1997.)

2.2. Obstetric and perinatal outcome of pregnancies after ART

The widespread use of ART has raised the question of the safety of infertility treatments for newborns, the goal of every assisted reproductive technique being a healthy infant. In previous descriptive studies, a high incidence of preterm delivery (gestational age at birth <37 weeks) and low birth weight (<2500 g) has been found in IVF pregnancies, including singletons (Wennerholm *et al.* 1991, Doyle *et al.* 1992, Gissler *et al.* 1995). In addition, pregnancy complications such as pregnancy-induced hypertension and placenta previa, and Caesarean sections (CSs) have been found to be more frequent in IVF than in spontaneous pregnancies in some matched studies (Tan *et al.* 1992, Tanbo *et al.* 1995). According to the results of recent, more strictly matched studies, singleton IVF pregnancies and pregnancies after transfer of cryopreserved embryos did not carry an increased risk of prematurity, low birth weight or other complications of newborns compared with controls (spontaneous pregnancies). Nevertheless, the CS rate was higher in ART pregnancies. (Reubinoff *et al.* 1997, Wennerholm *et al.* 1997.) In a matched case-control study, Dhont *et al.* (1997) found no differences in CS rate and perinatal outcome of IVF/ ICSI and spontaneous pregnancies apart from ART twin pregnancies, which showed a higher incidence of preterm delivery and needed more neonatal intensive care. In contrast, Minakami *et al.* (1998) reported that twins conceived by ART (ovulation induction, GIFT, IVF and ICSI) had a lower risk of adverse outcome compared with those spontaneously conceived, obviously because of a lower frequency of monochorionic placentas in ART pregnancies.

In singleton ICSI pregnancies, the rates of preterm delivery, low birthweight and perinatal mortality have been found to be higher than normal, but similar to those in IVF pregnancies (Wisanto *et al.* 1995, Aytoz *et al.* 1998). However, in a Swedish study including singleton and multiple ICSI pregnancies, the incidences of multiple birth, preterm birth, low birth weight infants and congenital malformations were lower in ICSI than in IVF pregnancies (Wennerholm *et al.* 1996).

So far, results on the outcome of IUI (with partner's sperm) pregnancies and infants have not been published. In a large French study on pregnancies after IUI with frozen donor semen, the mean birth weight, incidence of preterm delivery and low birth weight, and number of malformations did not differ from those in normal pregnancies (Lansac *et al.* 1997).

In ART pregnancies, the major problem is the high incidence of multiple gestation (25-30%) with its related obstetric and perinatal complications (Tan *et al.* 1992, Gissler *et al.* 1995, Wisanto *et al.* 1995). By lowering the number of multiple pregnancies the general outcome of ART pregnancies will improve. In addition, other reasons such as advanced maternal age, primiparity, infertility itself and ovarian stimulation have been suggested to partly explain complications in ART pregnancies (Doyle *et al.* 1992, Tan *et al.* 1992, Olivennes *et al.* 1993, Wang *et al.* 1994).

3. Purpose of the present study

The purpose of this study was to evaluate the clinical value of COH/IUI treatment. We characterized the prognostic factors affecting the outcome of COH/IUI which would make a correct patient selection for IUI and IVF treatment possible, and thereby would increase the cost-effectiveness of infertility therapy in general. Furthermore, we investigated alternative protocols for COH and alternative insemination techniques in order to discover, whether the treatment results could be further improved by such modifications. Finally, the outcome of IUI pregnancies and newborns was examined. The specific aims were:

1. to identify the prognostic variables of the subject and cycle characteristics for successful outcome of CC/HMG/IUI treatment
2. to compare the PRs achieved after a GnRHa/HMG protocol and a standard protocol involving CC/HMG in combination with IUI
3. to investigate the usefulness of IFI in the treatment of subfertility and to compare the PRs achieved after FSP and after a standard IUI technique in CC/HMG-stimulated cycles
4. to investigate the obstetric and perinatal outcome of pregnancies after COH/IUI treatment compared with spontaneous and IVF pregnancies

4. Materials and methods

4.1. Subjects

Subfertile couples suitable for IUI treatment were recruited to prospective studies II-IV at the Family Federation of Finland in Oulu and the Department of Obstetrics and Gynaecology of Oulu University Hospital during 1992-1995. The retrospective studies (I, V) included couples whose IUI cycles were performed between 1992 and 1996 (study I), and couples who conceived after IUI during 1991-1996, giving a live birth (study V) at the Family Federation of Finland in Oulu. The controls in study V were chosen from the Finnish Medical Birth Register (MBR).

All the treated couples had at least 1 year of infertility, and had undergone basic infertility evaluation consisting of history, assay of mid-luteal progesterone, prolactin and thyroid hormones, and semen analysis. Tubal patency was confirmed by laparoscopy, hysterosalpingography or salpingosonography. Women with abnormal tubal function were excluded from studies I-IV and women aged ≥40 years were excluded from studies II and IV.

The main categories of infertility aetiology were unexplained infertility, male factor, minimal to mild endometriosis and ovulatory dysfunction. Study V also included women with tubal factor infertility. Male factor infertility was defined as a sperm count <20 x 10^6/ml, normal morphology <30% or progressive motility (grade A+B) <40% (studies I, V) or <50% (studies II-IV) before sperm preparation. If the progressively motile sperm count after preparation was <1 x 10^6/ml in pretreatment sperm analysis, the couple was not enrolled in IUI therapy. Endometriosis was diagnosed by laparoscopy and classified in accordance with the revised classification of the American Fertility Society (1985). Patients with polycystic ovarian disease were excluded from studies I-IV.

Subject characteristics are summarized in Table 3.

Table 3. Summary of subject characteristics in studies I-V.

	I	II	III	IV	V
Number of subjects	388	148	50	100	111
Age					
mean (years)	33	32	32	31	32
(range)	(20-46)	(24-39)	(22-41)	(22-39)	(20-42)
Infertility duration					
median (years)	3	4	5	3	3
(range)	(1-15)	(1-15)	(1-14)	(1-11)	(1-13)
Primary infertility (%)	56	71	58	70	58
Infertility aetiology (%)					
Unexplained	51	49	38	52	60
Male factor	28	24	38	9	21
Endometriosis	17	20	16	34	6
Ovarian dysfunction	4	7	8	5	9
Tubal abnormalities	0	0	0	0	4

4.2. Study designs

Study I. This retrospective study included 811 IUI cycles in which a CC/HMG stimulation protocol and standard IUI with partner's sperm were used. The data of each treatment cycle were carefully collected and analyzed.

Logistic regression analysis was used to identify significant variables that affect the outcome (pregnancy: yes or no) of COH/IUI treatment. The following variables were chosen for the initial analysis: age (<40 or ≥40 years), duration of infertility (≤6 or >6 years), aetiology (unexplained infertility, male factor, minimal to mild endometriosis or ovarian dysfunction) and type (primary or secondary) of infertility, sperm concentration (<5, 5-10 or >10 x 10^6/ml) and progressive motility (<40 or ≥40%) after preparation, number of follicles (1, 2, 3 or 4; more than four follicles was recorded as four), thickness of the endometrium (<6, 6-10 or >10 mm) and number of the treatment cycle (1, 2, 3, 4 or 5; more than five treatments was recorded as five). Only statistically significant variables which affect IUI success were included in the final model.

Study II. This prospective study was undertaken to examine the usefulness of a long GnRHa/HMG protocol in IUI treatment and to compare its effectiveness with that of a standard CC/HMG protocol. Both of these stimulation protocols were presented to couples with a heterogeneous infertility aetiology, who were eligible for the study. The couples were informed about the high medication cost (approximately four times the cost of the CC/HMG protocol) and the risk of a strong ovarian response (over 6 follicles >16 mm in diameter on the ovulation induction day, in which case IVF would be performed instead of IUI) associated with the study protocol. A total of 75 couples accepted the GnRHa/HMG protocol. The control group consisted of 88 women undergoing CC/HMG stimulation. Only the first treatment cycle per subject was taken into the statistical analysis. We compared ovarian responses and PRs between the groups.

Study III. In this pilot study, the effectiveness of IFI treatment in subfertility was investigated. A total of 50 subfertile women with open tubes were recruited to the study. The women were on the waiting list for IVF. The experimental nature of the procedure was explained to all of the couples.

Ovarian stimulation was carried out by using a CC/HMG protocol. A Percoll gradient technique was used for sperm preparation and washed spermatozoa were injected into one to three large follicles via vaginal puncture. The IFI procedure was performed 30 h after HCG administration in the first 29 cases and after 12 h in the remaining 21 cases, according to the methods of two previous case reports (Lucena *et al.* 1991, Zbella *et al.* 1992). Natural progesterone was given for luteal support. Each subject underwent only one IFI cycle.

Study IV. To obtain information on the usefulness of the FSP technique in the treatment of non-tubal infertility, the efficacy of the technique was compared with that of a standard IUI technique in this prospective, randomized study.

A total of 100 couples were enrolled in the study and were randomized on the day of HCG administration to either an FSP group (n=50) or an IUI group (n=50). In contrast to studies I-III and V the inclusion criteria regarding sperm parameters were more strict and only mild male factor was included in study IV. The CC/HMG protocol was used for ovarian stimulation in both of the groups. The first treatment cycle per couple was used in the analysis. Pregnancy rates and outcomes after FSP and IUI were compared.

Study V. In our retrospective, matched case-control study the obstetric and perinatal outcomes of pregnancies after ovarian stimulation and IUI were compared with those of spontaneous and IVF pregnancies. Ovarian stimulation was carried out using CC alone (11%) or together with gonadotrophins (73%). In the rest of the cycles gonadotrophins with or without GnRHa were used. The controls were matched according to year of delivery, number of fetuses, number of previous deliveries, maternal age, residence of mothers, maternal smoking and socio-economic class. Because of the high number of multiple births (17%), we were not able to match the IUI pregnancies with spontaneous and IVF controls in all aspects. The difference between cases and controls was statistically significant as regards residence and socio-economic class and this could have caused a possible source of bias. However, social status has been found to be a minor risk factor as regards adverse perinatal outcome in a recent Finnish study (Sipilä *et al.* 1994). In the final analysis the IUI group included 111 pregnancies (92 singletons, 16 twins and 3 triplets) and 133 newborns. Both of the control groups consisted of 333 pregnancies and 399 infants. Data on the obsteric and perinatal outcome of all pregnancies was obtained from the MBR. Intensity of use of antenatal care was measured by means of a relative index (Gissler *et al.* 1995).

4.3. Methods

4.3.1. Controlled ovarian stimulation and monitoring

4.3.1.1. CC/HMG/HCG protocol

Ovarian stimulation was mainly carried out by using a sequential CC (Clomifen; Leiras, Tampere, Finland) plus HMG (Humegon; Organon, Oss, The Netherlands; or Pergonal; Serono, Aubonne, Switzerland) plus HCG (Pregnyl; Organon or Profasi; Serono) protocol. The women were given a dose of 50 or 100 mg of CC on cycle days 3 to 7 followed by 75 to 150 IU of HMG daily. Ovarian and endometrial responses were monitored by vaginal ultrasonography starting on cycle day 9 and 5 000-10 000 IU of HCG was administered when at least one follicle was >16 mm in mean diameter. Insemination was performed 36 h after administration of HCG. Luteal support was not given except in study III, where the subjects received natural progesterone (Lugesteron; Leiras, Turku, Finland), 200 mg daily for 2 weeks.

4.3.1.2. Long GnRHa/HMG/HCG protocol

A long GnRHa (Suprefact; Hoechst, Frankfurt am Main, German)/HMG/HCG protocol was employed for ovarian stimulation in studies II and V. Buserelin nasal spray was started (200 µg x 6/day) on cycle day 23. Once ovarian suppression was verified by vaginal ultrasonography (absence of functioning follicles) and by determining the oestradiol concentration (<0.05 nmol/l), HMG (150-225 IU) was injected daily until at least one preovulatory follicle (>16 mm in diameter) was recorded in ultrasonography. Ovulation was induced with HCG and insemination was carried out 36 hours later. The luteal phase was supported with 400 mg natural progesterone daily for 2 weeks.

4.3.1.3. CC alone or HMG/HCG protocol

In study V, CC or HMG alone was also used for ovarian stimulation. A daily dose of 50-100 mg of CC was given on cycle days 3 to 7. The size of the follicles was measured by vaginal ultrasonography on cycle days 9 to 11 and urinary LH tests were started 2 to 3 days before the expected day of ovulation. Insemination was performed on the day after the LH test result was positive. Patients with polycystic ovarian disease used HMG alone for COH. Injections were started on cycle day 2 and the initial HMG dose was 75 IU once a day for 6 days. Thereafter, the doses were adjusted according to the ovarian response which was monitored by vaginal ultrasonography. The time of HCG administration and insemination was determined as in the CC/HMG protocol.

All pregnancies resulting from COH/IUI treatment were confirmed by ultrasonography.

4.3.2. Sperm preparation

Semen was collected by masturbation into a sterile jar after 2-4 days of sexual abstinence. After liquefaction and initial sperm analysis the standard swim-up or Percoll gradient technique was used for preparation employing Earle's balanced salt solution or Medi-Cult IVF medium (Medi-Cult a/s, Copenhagen, Denmark), both supplemented with human serum albumin. In the swim-up technique, the sperm sample was centrifuged at 400 g for 15 minutes. The supernatant was discarded, the pellet was suspended in 2.5 ml of medium and thereafter centrifuged once more. After removing the supernatant the pellet was gently over-layered with medium and incubated for 1 h at 37 °C. After incubation, the medium layer containing motile sperm was carefully collected and used for insemination. In the Percoll technique, semen was layered onto a Percoll gradient (40%, 90%; Pharmacia, Bio Process Technology AB, Uppsala, Sweden) containing Medi-Cult medium and centrifuged at 500 g for 20 minutes. The 90% bottom fraction was then suspended in 6 ml of medium and centrifuged (500 g for 10 minutes). The sperm pellet was suspended most commonly in 0.5 or 1 ml of medium and used for insemination.

4.3.3. Insemination techniques

4.3.3.1. Intrauterine insemination

Standard IUI was performed using an intrauterine catheter (Kremer Delafontaine; Prodimed, Neuilly-en-Thelle, France) with a 1 or 2 ml syringe. First a bivalve speculum was placed in the vagina. The catheter was then gently passed through the cervical canal and the sperm suspension was expelled into the uterine cavity. Insemination volumes ranged from 0.5 to 2 ml. The subjects remained in a supine position for 10 to 15 minutes after IUI.

4.3.3.2. Fallopian tube sperm perfusion

The FSP procedure was performed using a paediatric Foley catheter of size 8 or 10 (Rüsch, Kernen, Germany). After a bivalve speculum had been placed in the vagina and the cervix rinsed with physiological saline solution, the catheter was inserted into the uterine cavity and the balloon was inflated and pressed gently against the internal os of the cervix. Using a 5 ml syringe, 3.5-4 ml of sperm suspension was infused slowly for approximately 3 minutes. After this, the balloon was deflated and the catheter withdrawn. The subjects remained in a supine position for 10 to 15 minutes.

4.3.3.3. Intrafollicular insemination

Intrafollicular insemination was carried out under vaginal ultrasonographic guidance. The women were given analgesics before the procedure. The vaginal pouch was cleaned with sterile water and the largest pre-ovulatory follicles (18-20 mm in diameter) were identified. One to three follicles were punctured using a 30 cm 17 gauge ovum retrieval needle (Cook, Queensland, Australia), and 50-200 µl of sperm suspension were injected into each of the preovulatory follicles.

4.4. Statistical analysis

Statistical analysis in studies I-IV was performed using a Statview II software package (Abacus Concepts, Inc., Berkeley, CA, USA). The results are described in terms of mean and SD or range or by median and range in all studies. Student's *t* test was used to determine the significance of differences between the studied groups as regards continuous data. Categorized data were presented using their frequency distribution. The Chi-square test was used to test the significance of differences between the groups.

Logistic regression analysis in study I was performed using the PC version of SPSS Inc. Professional Statistics, Release 6.1 (Chicago, IL, USA) and the results are presented as odds ratios (ORs) and 95% confidence intervals (CIs).

Statistical analysis in study V was performed using the unix version of SAS Proprietary Software Release 6.12 (SAS Institute, Cary, NC, USA). Differences between the groups were assessed by using Student's *t* test and the Chi-square test.

The chosen level of significance was $p<0.05$ in all studies.

5. Results

5.1. Factors affecting the outcome of CC/HMG/IUI treatment

5.1.1. Overall clinical results

The average PR per cycle was 12.6% (102/811) in our material. Of the 102 pregnancies, 70.6% (72) were viable, 23.5% (24) resulted in spontaneous abortion and 5.9% (6) were ectopic. The multiple PR was 13.7% (12 pairs of twins and 2 sets of triplets).

A female age of <40 years and an infertility duration of ≤6 years was associated with a significantly better PR compared with an older age or a longer duration of infertility (Table 4). No pregnancies were achieved among women >42 years old. The live birth rate was 3.1% (3/98) per cycle in women ≥40 years old. As regards the diagnosis of infertility the highest PR (15.3%) was achieved in women with unexplained infertility and the lowest (6.5%) in women suffering from endometriosis. Infertility type (primary or secondary) and sperm parameters after preparation did not significantly affect the outcome of IUI treatment.

Table 4. Pregnancy rate according to female characteristics in IUI treatment.

	Pregnancies/cycle (%)
Age (years)*	
<40	98/713 (13.7)
≥40	4/98 (4.1)
Infertility duration (years)**	
≤6	92/646 (14.2)
>6	10/165 (6.1)
Infertility aetiology†	
Unexplained	63/413 (15.3)
Male factor	27/229 (11.8)
Endometriosis	9/138 (6.6)
Ovarian dysfunction	3/31 (9.7)

*p=0.007. **p=0.005. †p=0.05.

In cycles with a single preovulatory follicle (>16 mm in diameter on the HCG day) the PR (5.7%) was significantly lower than in cycles with more follicles. The highest PR (16.3%) in this regard was observed with three preovulatory follicles. There was no correlation between the number of follicles and multiple PR. The thickness of the endometrium was not related to treatment outcome.

The best PR per cycle (18%) was achieved in the first treatment cycle, and no pregnancies were obtained in the sixth and seventh cycles. Almost all of the pregnancies, 99/102 (97%), occurred within the first four treatment cycles (Table 5).

Table 5. Pregnancy rate according to the number of preovulatory follicles and the number of the treatment cycle in IUI treatment.

	Pregnancies/cycle (%)
Number of follicles*	
1	10/177 (5.7)
2	36/265 (13.6)
3	32/196 (16.3)
≥4	24/173 (13.9)
Number of treatment cycle**	
1	51/283 (18.0)
2	26/228 (11.4)
3	15/160 (9.4)
4	7/73 (9.6)
≥5	3/67 (4.5)

*p=0.013. **p=0.007.

5.1.2. Logistic regression

Logistic regression analysis revealed five predictive variables for CC/HMG/IUI success. These were the number of the treatment cycle (p=0.009), duration of infertility (p=0.017), age (p=0.028), number of follicles (p=0.031) and aetiology of infertility (p=0.045). The results of the final model are presented as ORs and 95% CIs in Table 6. We also performed the analysis including only cycles in women <40 years old (n=713), and found that age did not affect the outcome of IUI treatment, while the other predictive variables remained significant. Our data fitted logistic regression analysis well, as indicated by the Hosmer and Lemeshow goodness-of-fit test (p=0.57).

Table 6. Logistic regression model for predicting the success of IUI treatment.

Variable	OR*	CI**	p
Age (years)†			0.028
<40 (years)	3.24	1.14, 9.23	
Infertility duration†			0.017
≤6 (years)	2.33	1.16, 4.66	
Infertility aetiology†			0.045
unexplained	2.79	1.33, 5.87	
Number of follicles†			0.031
2	2.45	1.16, 5.18	
3	3.18	1.48, 6.81	
≥4	2.51	1.13, 5.55	
Number of treatment cycle††			0.009
2	0.57	0.34, 0.96	
3	0.44	0.24, 0.83	
4	0.43	0.19, 1.03	
≥5	0.22	0.07, 0.75	

Values are presented as *odds ratio (OR) and **95% confidence interval (CI). †Odds ratio in contrast with the poorest category. ††Odds ratio in contrast with the best category.

5.2. Comparison between GnRHa/HMG and CC/HMG protocols

Fifteen (20%) women had more than six preovulatory follicles on the day of HCG administration in the GnRHa/HMG regimen and IVF was performed instead of IUI. After exclusion of these cycles, a total of 60 cycles in the GnRHa group and 88 in the CC group (CC/HMG protocol) were analyzed.

Subject characteristics including mean female age, median duration of infertility and distribution of infertility diagnoses and primary infertility were similar in the GnRHa and CC groups. In the GnRHa group, HMG requirements were significantly higher than in the CC group (mean 21.5 vs. 8.1 ampoules). Otherwise, the response to ovarian stimulation (number of preovulatory follicles >16 mm on the HCG day, endometrial thickness) and sperm parameters after preparation did not differ between the groups (Table 7). No cases of severe OHSS developed.

Data on the pregnancy rates are presented in Table 7. The PR of 20% per cycle in the GnRHa group was higher, but not significantly, than that of 12.5% in the CC group. The highest PRs were obtained among women with unexplained infertility, 30% in the GnRHa group and 16.3% in the CC group, but the number of pregnancies did not differ significantly between the treatment groups in relation to the diagnostic categories. There were three multiple pregnancies (2 pairs of twins, 1 set of triplets) in the GnRHa group and four (4 pairs of twins) in the CC group, and five miscarriages in both groups. The number of follicles was similar in singleton and multiple gestations (mean 3.1 [SD 1.9] vs. 3.9 [1.3], respectively).

Of the fifteen women shifted from IUI treatment to IVF, five (33.3%) became pregnant. Four of these women had a singleton pregnancy and one had twins.

Table 7. Cycle characteristics, and pregnancy rates.

	GnRHa group	CC group
Number of cycles	60	88
Number of HMG ampoules/cycle*	21.5 (5.1)	8.1 (3.1)**
Number of follicles*	3.0 (1.7)	2.8 (1.4)
Endometrial thickness (mm)*	9.4 (2.1)	8.8 (2.2)
Sperm count (x 10^6/ml)*†	64.2 (50.1)	51.5 (48.2)
Progressive motility (grade A+B, %)*†	74.5 (16.0)	79.4 (15.2)
Pregnancies/cycle	12/60	11/88
Pregnancies/infertility diagnosis		
Unexplained	9/30	7/43
Male factor	1/13	2/22
Endometriosis	1/10	2/19
Ovarian dysfunction	1/7	0/4

*Values are expressed as means (SD). **P=0.0001. †After sperm preparation.

5.3. IFI in the treatment of subfertility

After CC/HMG stimulation the mean (range) number of preovulatory follicles (>16 mm on the HCG day) was 3.2 (1-7) and the endometrial thickness was 8 mm (5.5-12). The sperm count after preparation on the day of insemination was 22.1 x 10^6/ml (0.2-110) and progressive motility was 71.7% (6-100).

The IFI procedure was simple to perform and convenient for the subjects. During injection the flow of sperm suspension into the follicles could be seen in ultrasonography, and no rupture of the follicles took place.

One normal singleton pregnancy resulted. There were no biochemical or ectopic pregnancies or complications related to the procedure.

5.4. Comparison between FSP and a standard IUI technique

The IUI and FSP groups were similar as regards mean female age, proportion of cases of primary infertility, duration of infertility and distribution of infertility diagnoses. The current cycle was the first infertility treatment for 60% of the women in the IUI group and for 62% in the FSP group. Approximately 40% of the women had undergone ovulation induction with CC as an empirical treatment for infertility.

The characteristics of the stimulation cycles did not differ between the groups. The mean (SD) number of preovulatory follicles was 2.5 (1.0) in the IUI group and 2.4 (1.1) in the FSP group and the endometrial thickness was 8.1 (1.5) and 8.0 mm (1.5), respectively. The progressively motile sperm count in inseminates was comparable between the treatment groups (Table 8).

The FSP procedure was easy to perform using a paediatric Foley catheter. There were no difficulties passing it through the cervical canal, and the inflated balloon prevented sperm reflux effectively. A few patients felt mild pelvic discomfort and only seldom was any reflux seen after removing the Foley catheter.

The PR per cycle was 20% in the IUI group and 8% in the FSP group, a difference that was not significant (p=0.08). Similarly, no significant difference was found in the PRs of different infertility categories between groups (Table 8). There were two miscarriages in both groups and one twin pregnancy in the IUI group. No cases of infection or OHSS were observed.

Table 8. The number of progressively motile sperm in inseminates, and pregnancy rates.

	IUI group	FSP group
Number of cycles	50	50
Inseminated progressively motile sperm count (x 10^6)*	48 (39.6)	63 (51.5)
Pregnancies/cycle	10/50	4/50
Pregnancies/infertility diagnosis		
Unexplained	6/26	2/26
Endometriosis	2/17	1/17
Male factor	2/6	1/3
Ovarian dysfunction	0/1	0/4

*Values are means (SD).

5.5. Obstetric and perinatal outcome of IUI pregnancies compared with spontaneous and IVF pregnancies

The mean (SD) maternal age of IUI parturients was 31.8 (4.5) years; 81% were primiparous and the mean (SD) number of previous deliveries was 0.24 (0.6). The use of antenatal care services was generally high among all studied women. However, in singleton pregnancies the IUI group had significantly fewer visits to maternity care units than the IVF group (Table 9). Mother's hospitalization during pregnancy was also frequent in all groups. In the IUI group 28% of women with singleton pregnancy and 74% with multiple pregnancy were hospitalized, but the groups did not differ in this respect (Table 9). The exact distribution of the reasons for hospitalization was not available from the MBR. The CS rate was 25% in singleton and 58% in multiple IUI preganacies and approximately half of the CSs were classified as emergency. There was no difference in CS rates between the groups. Pregnancies after IUI did not carry any increased risk of placenta praevia, placental abruption or eclampsia compared with spontaneous and IVF pregnancies.

Table 9. Use of antenatal care and mother's hospitalization during pregnancy, by plurality.

	Singletons			Multiple			Total		
	IUI	Sp.	IVF	IUI	Sp.	IVF	IUI	Sp.	IVF
Number of births	92	276	276	19	57	57	111	333	333
Number of antenatal visits	16.2 (4.8)	15.8 (1.4)	18.1** (6.1)	17.8 (5.3)	19.4 (10.6)	17.2 (5.7)	16.6 (4.9)	16.6 (6.6)	18.0* (6.2)
Intensity of antenatal care††	1.5 (0.4)	1.4 (0.5)	1.7† (0.5)	2.1 (0.4)	2.4 (1.3)	2.2 (0.6)	1.6 (0.5)	1.6 (0.8)	1.8** (0.6)
Number of ambulatory visits	2.4 (2.4)	2.5 (2.4)	3.8† (2.6)	8.4 (3.0)	6.8* (2.5)	6.0** (2.8)	3.4 (3.3)	3.2 (2.9)	4.2* (2.8)
Mother's hospitalization during pregnancy	28.3	27.9	34.1	73.7	61.4	70.2	36.0	33.6	40.2
Caesarean section	25.0	25.0	25.4	57.9	61.4	64.9	30.6	31.2	32.1
Emergency section	12.0	15.9	11.2	36.8	36.8	31.6	16.2	19.5	14.7

Sp.=spontaneous. Values are presented as means (SD) and percentages. *P<0.05, **P<0.01. †P<0.001. ††A score of 1.0 indicates the norm.

The mean birthweight (3285 g) of IUI singletons was significantly lower than that (3448 g) in the spontaneous group, but comparable to that (3363 g) in the IVF group. However, the number of infants with low birthweight (<2500 g) and the incidence of preterm births (<37 weeks gestation) were similar in all groups. Otherwise, the outcome of newborns (number of infants with an Apgar score of 0-6 at 1 min, need for treatment in neonatal care units, number of malformations and perinatal mortality) did not differ between ART and spontaneous pregnancies (Table 10). One major malformation (Potter syndrome) and two perinatal deaths occurred in the IUI group. The singleton with Potter syndrome died soon after birth and one triplet with severe growth retardation of unknown reason died in utero at 30 weeks of gestation.

Table 10. Outcome of newborns by plurality.

	Singletons			Multiples			Total		
	IUI	Sp.	IVF	IUI	Sp.	IVF	IUI	Sp.	IVF
Number of infants	92	276	276	41	123	123	133	399	399
Gestation length (weeks)	39.5 (1.8)	39.8 (1.8)	39.4 (2.2)	36.0 (2.8)	35.6 (2.7)	35.4 (3.3)	38.6 (2.6)	38.7 (2.8)	38.3 (3.1)
<37 weeks (%)	8.7	5.1	7.6	36.8	47.4	57.9	13.5	12.3	16.2
Birthweight (g)	3285 (575)	3448* (600)	3363 (611)	2449 (678)	2298 (548)	2369 (648)	3064 (706)	3145 (775)	3101 (760)
<2500 g (%)	8.7	6.2	6.9	46.3	56.9	52.0	20.3	21.8	20.8

Sp.=spontaneous. Values are presented as means (SD) and percentages. *P<0.05.

5.6. The cost-effectiveness of CC/HMG/IUI treatment compared with IVF

In study I, there were 811 IUI cycles and 72 of them resulted in a live birth. We calculated the average cost of live birth for CC/HMG/IUI and for IVF (using a long GnRHa/HMG stimulation protocol) conducted during the same time period. The cost included medication, monitoring (ultrasonographic examinations) and IUI or IVF procedures. In our unit the average cost per live birth was 19 000 FIM for CC/HMG/IUI and over two-fold, 40 000 FIM, for IVF treatment.

5.7. IVF outcome in women with three or more unsuccessful CC/HMG/IUI cycles

During the time period from March 1991 to December 1996, 103 couples out of 143 shifted to IVF treatment after three or more unsuccessful (no pregnancy) CC/HMG/IUI cycles. The mean (range) age of these women was 33 years (23-47) and the median (range) infertility duration 4 years (1-14). The infertility was primary in 70% of the women and the diagnostic categories were unexplained infertility (46%), endometriosis (25%), male factor (18%), tubal abnormalities (7%) and ovarian dysfunction (4%). After one to five treatment attempts (IVF, ICSI and transfer of cryopreserved embryos) 51 couples achieved a live birth (37 IVF, 7 ICSI and 7 cryopreserved embryo pregnancies). The mean (SD) age of the women with live birth was significantly lower than that of the non-conceiving women (31 [4] vs. 34 years [5], p=0.0003), but the duration of infertility did not differ between subjects (4 [2] vs. 5 years [3], respectively). Of the couples with primary infertility, 53%, and of the couples with secondary infertility, 39% achieved a live birth. Approximately half of the women in each diagnostic category conceived.

6. Discussion

6.1. Factors affecting the outcome of CC/HMG/IUI treatment

6.1.1. Female age

In the present study, the PR per cycle of COH/IUI was reduced significantly in women aged ≥40 years, which is in agreement with the results of several other studies (Plosker *et al.* 1994, Tomlinson *et al.* 1996). However, in some studies an age-related decline in success rate has already been found over the age of 35 years (Agarwal & Buyalos 1996, Brzechffa *et al.* 1998). In our study population, age was not predictive of IUI success in women <40 years old. The reported live-birth rates per COH/IUI cycle in women aged ≥40 years have been very low and have varied from 1.2 to 5.2%, which is in line with the results of the present study (3.1%). A high miscarriage rate, 34% to 73%, has been primarily responsible for the low live-birth rate in this age category. (Pearlstone *et al.* 1992, Frederick *et al.* 1994.)

Overall, the age-related decline in female fecundity has been well documented, particularly in women undergoing IUI with donor sperm (van Noord-Zaadstra *et al.* 1991, Kang & Wu 1996). Accordingly, success rates of IVF and ICSI have been reported to decrease with advancing female age, indicating that the negative impact of age can be overcome only partly by ART (Devroey *et al.* 1996, Templeton *et al.* 1996). This decline in female fertility has been suggested to be a result of decreased oocyte quality (Navot *et al.* 1991b) and/or reduced uterine receptivity (Cano *et al.* 1995). Nowadays, the trend to postpone child bearing to later female life may increase the risk of infertility.

Put together, all these results indicate that IUI is a poor treatment option for women over 40 years of age, and they should be offered ART after their ovarian responsiveness has been documented. Since poor oocyte quality has been implicated as a main cause of the age-related decline in fertility, oocyte donation should be considered in poor responders.

6.1.2. Duration of infertility

Our results demonstrated a significant decrease in the PR in women with infertility duration of over 6 years (6%) compared with the PR in women with shorter infertility duration (14%). There was no difference in PRs between groups of infertility duration <3, or 3 to 6 years. This is in accordance with the results of Tomlinson *et al.* (1996), but in some studies a significant decline in success rate has been shown as soon as after 3 to 4 years' infertility (Nulsen *et al.* 1993, Mathieu *et al.* 1995). Apparently, with an increase in the duration of infertility, less obvious factors become more important in decreasing reproductive potential. On the other hand, some previous studies have demonstrated no correlation between increasing duration of infertility and the likelihood of pregnancy (Deaton *et al.* 1990, Dodson & Haney 1991). Regardless of variability in the published data, IUI treatment cannot be recommended to women with a long-standing duration of infertility.

6.1.3. Aetiology of infertility

In the present study, the highest PR (15.3%) was obtained in the unexplained infertility group in respect to the aetiology of infertility. Our result is in agreement with that of a meta-analysis published by Peterson *et al.* (1994), in which the average PR/cycle in unexplained infertility using HMG/IUI was 18%. In a multicentre trial published by Crosignani *et al.* (1991) the pregnancy rate per cycle was 23% using superovulation with IUI in unexplained infertility. The mechanisms by which COH/IUI improves cycle fecundity in subfertility without any specific reason remain speculative. For example, occult ovulatory dysfunction (Tummon *et al.* 1988) and/or a decreased fertilization rate (Templeton *et al.* 1996) have been suggested to be causes of failure to conceive among women with unexplained infertility. These defects can possibly be overcome by superovulation therapy, which is associated with an increased number of fertilizable oocytes. Nevertheless, other factors may also be operative, since in some reports the combination of COH with IUI has been shown to improve the PR compared with COH alone (Hughes 1997).

We found significantly lower cycle fecundity in endometriosis patients compared with the unexplained infertility category. The PR of 6.5% per cycle among women with endometriosis was lower than those previously reported, which have varied from 11 to 13% (Dodson & Haney 1991, Nulsen *et al.* 1993). The adverse effect of endometriosis on COH/IUI success has also been shown in other studies (Isaksson & Tiitinen 1997, Omland *et al.* 1998), and Hughes (1997) concluded in his meta-analysis that the independent effect of endometriosis reduced the likelihood of pregnancy to approximately half in the treatment of persistent infertility with COH/IUI, which is in accordance with the present data. The causal relationship between reduced fertility and endometriosis without tubal involvement is not clear. Extensive investigations suggest a multifactorial aetiology for endometriosis-associated infertility, which includes, for example, an altered follicular environment (Harlow *et al.* 1996), impaired oocyte quality (Pellicer *et al.* 1995) and reduced implantation rate (Arici *et al.* 1996, Pellicer *et al.* 1998). Immunological alterations observed in women with endometriosis are also thought to interfere with fertility via a direct cytotoxic effect on the gametes and embryo (Oral *et al.* 1996, Martinez-Roman *et al.* 1997, Edelstam *et al.* 1998).

The information available at present indicates that COH/IUI should be considered as a first approach prior to the more expensive and invasive procedure of IVF in women with unexplained infertility. In contrast, the present data and previously published IVF results (PR per embryo transfer 15 to 40%; Geber *et al.* 1995, Olivennes *et al.* 1995, Arici *et al.* 1996), suggest that IVF would be a more favourable treatment than IUI in endometriosis-related infertility.

6.1.4. Number of follicles

The number of follicles was predictive of treatment success according to the present results. In cycles with a single preovulatory follicle the PR (5.7%) was significantly lower than in other cycles. The highest PR (16.3%) was seen in cycles with three follicles. Multifollicular development may result in an increased number of fertilizable oocytes and a better quality endometrium and luteal phase, thereby improving fertilization and implantation rates. The poor outcome in cycles with only one preovulatory follicle, also confirmed in other studies (Tomlinson *et al.* 1996, Hughes *et al.* 1998), indicates the necessity of a multifollicular response to COH in IUI treatment.

The multiple pregnancy rate and its possible correlation to the number of follicles is another important aspect in IUI treatment. The overall multiple PR was 13.7% in our study, which is in agreement with the results of other studies (7-25%) (Dodson & Haney 1991, Brzechffa *et al.* 1998). Less than 2% of the pregnancies were triplets. The multiple PR was lower than that of 25 to 35% generally reported in IVF treatment (Gissler *et al.* 1995, Anonymous 1998). These results emphasize the clinical value of IUI treatment compared with IVF, since the total costs associated with multifetal pregnancies are considerably lower in IUI treatment. There was no correlation between the number of large preovulatory follicles and multiple gestation in the present study. This is in accordance with the results of Dickey *et al.* (1992) and Goldfarb *et al.* (1997), but contradictory results have also been published (Gagliardi *et al.* 1991). Although the ideal number of preovulatory follicles to maintain optimal cycle fecundity and minimize the number of multiple gestations is not known, IUI cycles with more than three to four large follicles should be cancelled or converted to IVF, or supernumerary large follicles should be aspirated in order to decrease the possibility of multiple pregnancy.

6.1.5. Number of the treatment cycle, and IVF after unsuccessful CC/HMG/IUI treatment

In the present study, the PR per cycle was highest in the first treatment cycle (18%) and thereafter it remained at about 10% up to the fourth cycle. Beyond four cycles the PR was poor. In the literature, cycle fecundity has been shown to be relatively constant for the first three to seven cycles (Chaffkin *et al.* 1991, Dickey *et al.* 1992), but decreasing PRs with an increased number of treatment cycles have also been reported (Burr *et al.* 1996, Tomlinson *et al.* 1996). In our study and in some previous studies (Remohi *et al.* 1989, Nulsen *et al.* 1993), most of the pregnancies occurred within the first four treatment cycles, favouring a maximum of four COH/IUI cycles before IVF. In addition, Peterson *et al.* (1994) concluded in their prospective cohort study and meta-analysis that cost-benefit analysis comparing HMG/IUI, IVF and no therapy in infertile patients may favour four cycles of HMG/IUI as the first line treatment before IVF.

Our data indicate that after three or more unsuccessful CC/HMG/IUI cycles, IVF/ICSI is still an effective treatment modality for these couples, as also shown in other studies (Ranieri *et al.* 1995, Ruiz *et al.* 1997).

6.2. Usefulness of a GnRHa/HMG regimen in IUI treatment

The use of GnRHa/HMG for ovarian stimulation in IVF treatment has been found to have obvious clinical advantages. These include, for example, prevention of premature luteinization and an increased number of oocytes recovered (Antoine *et al.* 1990). Theoretically, the beneficial effects of the analogues would also be operative in COH/IUI programmes.

In the present study, the PR per cycle was higher in women who received GnRHa/HMG for COH (20%) than in women with CC/HMG stimulation (12.5%), but the difference was not significant. Gagliardi *et al.* (1991) and Manzi *et al.* (1995) found a clear increase in cycle fecundity when incorporating GnRHa into HMG/IUI cycles. The PR increased from 16 to 26% in the former study and from 11 to 19% in the latter. In these retrospective reports, the study population was selected either on the basis of having several unsuccessful previous HMG/IUI cycles (Gagliardi *et al.*) or by demonstrating premature luteinization in the preceding HMG/IUI treatment (Manzi *et al.*). Manzi *et al.* proposed that the main reason for the improved treatment outcome in both of these studies was the elimination of premature luteinization. However, according to recent studies the significance of premature luteinization is somewhat contradictory in respect to treatment outcome (Huang *et al.* 1996, Moffitt et *al.* 1997).

In a randomized study, Dodson *et al.* (1991) demonstrated no significant difference in PR between GnRHa/HMG (11%) and HMG (22%) stimulated cycles among unselected infertile women with no earlier IUI cycles. The difference between their study and the present one was a trend towards a higher PR in the GnRHa/HMG group in the present study.

In our study, the HMG requirements were significantly higher when GnRHa was used for ovarian stimulation compared with CC. The mean medication expense in women with GnRHa/HMG stimulation was approximately four times that of CC/HMG stimulation. More intensive monitoring was also required when using GnRHa/HMG than when using CC/HMG.

As the PR did not improve significantly, but the cost per cycle increased notably, and many cycles (15%) needed to be converted to IVF, the routine use of GnRHa/HMG stimulation in IUI treatment among unselected patients does not appear to be cost-effective. However, on the basis of our results IUI should be performed in cases of low ovarian response to GnRHa/HMG in IVF treatment, instead of cycle cancellation.

6.3. Effectiveness of IFI and FSP techniques in the treatment of subfertility

6.3.1. Intrafollicular insemination

The rationale of IFI is that by injecting spermatozoa directly into the follicle, fertilization can be induced more easily than after IUI, for example, and hence IFI could become a new alternative for treatment of subfertility.

In the present study, we found the IFI procedure simple to perform and convenient for the women. However, the PR achieved by this technique was poor (one pregnancy in 50 treatment cycles) and would even indicate reduced fecundity in the treatment cycle, because ovarian stimulation alone has been shown to result in a higher PR of 7.4% per cycle (Glazener *et al.* 1990).

It is unlikely that the low cycle fecundity was related to the CC/HMG protocol, because using the same ovarian stimulation and sperm preparation methods in our IUI programme, a PR of 7-15% was achieved. We think that the insemination procedure itself was the most critical step leading to failure of the treatment.

We injected 200 000-800 000 spermatozoa into each follicle in a volume of 50-200 µl. Assuming the volume of a preovulatory follicle to be 4-7 ml, the final sperm concentration must have been about 30 000-200 000 spermatozoa/ml, which is very similar to that used in IVF. One would expect an even lower number of sperm to fertilize the ovum, because follicular fluid is known to promote capacitation and acrosome reactions (Mbizvo *et al.* 1990, Mendoza & Tesarik 1990).

Sperm injection took place 30 hours after administration of HCG (the first 29 women), according to information published by Zbella *et al.* (1992), or 12 hours after HCG (21 patients), according to data published by Lucena *et al.* (1991). The change of timing was done because no pregnancies resulted in the first 29 cases and only one pregnancy was achieved in the latter group. Hence, it is not likely that the success rate would be significantly improved by modification of insemination time in relation to HCG.

We believe that the most likely reason for poor IFI outcome was an unfavourable environment for fertilization inside the preovulatory follicle. Hypothetically, there may be substances in follicular fluid which could interfere with sperm-ovum interaction. In addition, puncturing of the follicle or the presence of sperm may disturb the ovulation process and/or maturation of the ovum. The present results indicate that IFI is inefficacious for treating subfertility.

6.3.2. Fallopian tube sperm perfusion

In the present randomized study, the PR per cycle achieved by using the FSP technique (8%) was lower, although not significantly, than that obtained by using the standard IUI technique (20%), which was in contrast to our preliminary hypothesis based on a study by Kahn *et al.* (1993b). Overall, results concerning the efficacy of FSP compared with IUI are controversial (Kahn *et al.* 1993b, Fanchin *et al.* 1995, Karande *et al.* 1995) and the studies are difficult to compare because of different stimulation protocols, study populations and FSP techniques.

In agreement with our results, Karande *et al.* (1995) did not find FSP to be more beneficial than IUI in the treatment of an unselected infertility population. Cycle fecundity was 11% in FSP and IUI cycles. They performed both of these insemination procedures by using a catheter without a cervical seal.

In contrast, Kahn *et al.* (1993b) found a significantly better PR with FSP (27%) in comparison with IUI (10%). They applied the same ovarian stimulation protocol as we did, but their study population included only women with unexplained infertility. In our study over half of the patients suffered idiopathic infertility, but in this subgroup also, FSP offered no advantage over IUI. Other authors have also reported contradictory results concerning the effectiveness of FSP and IUI treatment in unexplained infertility (Gregoriou *et al.* 1995, Mamas 1996). In the study of Kahn *et al.* (1993b), the number of motile sperm in the inseminates was significantly higher in the FSP cycles than in the IUI cycles, which difference might have contributed to the higher PR in the couples with FSP. The total motile sperm count in the inseminates did not differ between FSP and IUI treatment in our study. Fanchin *et al.* (1995) reported a remarkably high PR associated with FSP (40%) compared with IUI (20%) in a group of women with heterogeneous infertility aetiology. They used various stimulation protocols for COH and included in their study women with partial tubal damage (37% of the subjects), which would indicate a preference for FSP over IUI, because the pressure of the inseminate of 4 ml may open some tubal obstructions. In our study, women with abnormal tubes were excluded.

The position of the cervical seal was different in our study than in previous studies by Kahn *et al.* (1993b) and Fanchin *et al.* (1995). In our study, the inflated balloon inside the uterine cavity might have had some adverse effect on the endometrium and hence on the PR, which may be avoided by using a pericervical mechanical seal, as in the previous studies.

In summary, we found no advantage in using FSP with a Foley catheter over IUI, and FSP should not replace the simpler and less time-consuming IUI technique in routine use.

6.4. Obstetric and perinatal outcome of pregnancies after COH/IUI treatment, and the cost-effectiveness of CC/HMG/IUI

The characteristics of IUI parturients (mean age 32 years, 81% primiparous and mean number of previous deliveries 0.24) differed clearly from those of average Finnish parturients (30 years, 39% and 1.1, respectively) (Gissler *et al.* 1996), but were comparable with those observed in ART pregnancies (Dhont *et al.* 1997, Wennerholm *et al.* 1997). In addition, the number of multiple gestations (17%) was higher in the study group than that of 1.4% in general (Gissler *et al.* 1996), but was lower than in IVF pregnancies (25%) (Gissler *et al.* 1995).

The total use of antenatal care services in all groups was high, which might be partly due to the characteristics of the studied women. Advanced maternal age, primiparity and multiple pregnancies have been shown to be predictive of obstetric risks (Hartikainen-Sorri *et al.* 1990, Cnattingius *et al.* 1992, Sipilä *et al.* 1994) and they usually increase the need for close follow-up. The restructuring policy in the health care system in our country might also be reflected in the frequent use of maternal care. Generally, a trend towards an increasing number of antenatal care visits has been seen in the last few years in Finland (Gissler *et al.* 1996). This raises the guestion of, whether all the visits to antenatal care units were really indicated. In IVF singleton pregnancies in particular, the use of maternal care was high (significantly higher than in the IUI and spontaneous groups), although there were no more complications in IVF pregnancies. However, this could partly be explained by inaccurate recommendations and IVF mothers' own desire for close follow-up. Optimal and cost-effective follow-up of ART pregnancies should be based on clear recommendations and the individual needs of the mothers. To avoid excessive use of maternal health care services, education of the staff of maternal care units is needed.

The hospitalization and CS rates were comparable in the IUI, spontaneous and IVF groups, but they were higher than general (21% and 16%, respectively) among Finnish parturients (Gissler *et al.* 1996). The diagnoses for hospitalization included haemorrhage, hypertension, threatened premature delivery and other reasons. The distribution of these diagnoses was not available. In IVF pregnancies, the CS rate has been shown to be increased (36-42%) compared with that (15-16%) of spontaneous controls (Gissler *et al.* 1995, Reubinoff *et al.* 1997), though results showing no difference have also been found (Dhont *et al.* 1997). Because the present hospitalization and CS rates were similar in all groups, the ART techniques as such did not appear to affect the outcome of pregnancy, but individual characteristics and plurality were the most important factors in this respect.

The mean birthweight of IUI singletons was significantly lower than that in the spontaneous group, but comparable to that of the IVF group. The reason for this remains unknown, but it could be related to infertility itself (Ghazi *et al.* 1991), especially unexplained infertility (Isaksson & Tiitinen 1998), which was the main indication (60%) for IUI treatment in our study. However, this finding might not be clinically important, because the incidence of low birthweight (<2500g) and premature delivery (<37 weeks) did not differ between ART and spontaneous pregnancies. The results of studies comparing birthweights of IVF and spontaneous infants are somewhat contradictory (Gissler *et al.* 1995, Wennerholm *et al.* 1997). The IUI group did not differ from the control groups in respect to mean gestation length, which was comparable with other ART pregnancies (Wisanto *et al.* 1995, Dhont *et al.* 1997, Wennerholm *et al.* 1997). Neither was the number of other complications in IUI newborns increased compared with the spontaneous and IVF groups.

The outcome of pregnancies and newborns after ART is important when the cost-effectiveness of the treatment model is evaluated. However, data collection and follow-up of the pregnancies may be difficult because infertility treatment and maternal care is often carried out in different units (private, public). Furthermore, the costs and results of different treatment modes can be variable between clinics, making it difficult to carry out generally applicable calculations. We estimated the average cost per live birth for CC/HMG/IUI and for IVF (using a long GnRHa/HMG stimulation protocol), and it was over two-fold for IVF in our unit (study I). The cost included medication, monitoring of ovarian stimulation and luteal support, and IUI or IVF procedures. The cost-effectiveness of IUI treatment whould be more favourable if the longer time off work and higher multiple pregnancy rates resulting in added costs during pregnancy, delivery and the neonatal period after IVF treatment are taken in account in these calculations. The present result is in accordance with that in a recent study by van Voorhis *et al.* (1998), who found IUI treatment to be a more cost-effective approach before resorting to IVF. In future, more careful calculations concerning the cost-effectiveness of different treatment options will be needed to strengthen clinical decision-making and to aid in the development of public policy.

7. Conclusions

The following conclusions can be drawn from the results of the present study:

1. Logistic regression analysis revealed five predictive variables for CC/HMG/IUI success. These were age, duration of infertility, aetiology of infertility, number of follicles and the number of the treatment cycle. The PR per cycle was significantly higher in women aged <40 years compared with older women. In addition, women with infertility duration ≤6 years had a significantly better cycle fecundity than those with longer duration. A significantly lower PR was achieved among women with endometriosis compared with those with unexplained infertility. In cycles with a single preovulatory follicle, cycle fecundity was significantly lower than in cycles with more follicles. A significantly higher PR per cycle was found in the first treatment cycles. The present results indicate that CC/HMG/IUI is a useful and cost-effective treatment option in women <40 years of age with infertility duration ≤6 years, who do not suffer from endometriosis. A multifollicular response results in better treatment outcome than a monofollicular response, indicating the necessity of COH combined with IUI. Most pregnancies (97%) occur during a course of four CC/HMG/IUI cycles. The risks of delayed childbearing and the negative impact of increasing age should be emphasized in general counselling.
2. The PR per cycle was higher with the use of a long GnRHa/HMG protocol compared with a CC/HMG protocol, but the difference was not significant. The average medication expense of GnRHa/HMG stimulation was four times the cost of CC/HMG stimulation. The risk of a high ovarian response associated with GnRHa/HMG stimulation required IVF availability. For these reasons, routine use of a long GnRHa/HMG protocol in IUI treatment for unselected subfertile couples is not recommened.
3. The IFI procedure was simple to perform and convenient for the women. A good ovarian response was obtained by using a CC/HMG protocol for ovarian stimulation. One normal singleton intrauterine pregnancy resulted among 50 IFI-treated women. The present results indicate that IFI is inefficacious for treating subfertility.
4. The FSP procedure was easy to perform using a paediatric Foley catheter, and the inflated balloon pressed on the uterine os internum prevented the reflux of sperm suspension effectively. Cycle fecundities between the FSP and IUI group did not differ significantly, although there was a trend towards a lower PR in the FSP group. Hence,

the FSP method should not replace the simpler and less time-consuming IUI technique in routine use.

5. The IUI parturients differed from average parturients in respect to higher maternal age, more frequent primiparity and a higher incidence of multiple pregnancies. The use of antenatal care services was significantly lower in IUI singleton pregnancies compared with IVF singletons. The hospitalization and CS rates were high in the IUI, spontaneous and IVF groups. The mean birthweight of IUI singletons was significantly lower than that in the spontaneous group, but comparable to that in the IVF group. Otherwise, the outcome of infants was not worse in ART pregnancies than in spontaneous pregnancies.

   The IUI procedure itself does not appear to affect adversely the obstetric and perinatal outcome of pregnancies, and subject characteristics and multiplicity of pregnancies may be more important in this respect. Optimal follow-up of ART pregnancies should be based on the individual characteristics of pregnancies and mothers. Efforts to lower the high incidence of multiple pregnancy after ART are essential to improve the outcome of ART pregnancies.

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