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MILD STRATEGIES FOR IVF: FROM THEORY TO PRACTICE

MILDE BEHANDELSTRATEGIEËN VOOR IVF: VAN THEORIE TOT PRAKTIJK

(MET EEN SAMENVATTING IN HET NEDERLANDS)

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CHAPTER 1
INTRODUCTION

Around 10% of couples trying to have a child fail to conceive within one year of regular unprotected intercourse. Twenty-five to thirty percent of these couples are referred for in vitro fertilisation (IVF) treatment once other methods of assisted reproductive technology have failed (Collins and Van Steirteghem, 2004). The current tendency of women to postpone their pregnancy until beyond their most fertile age as more women have pursued higher education and entered the workforce (te Velde and Pearson, 2002; Heffner, 2004) has led to an increasing proportion of couples seeking IVF treatment.

IVF was initially developed for women with tubal disease that could not be surgically corrected. Now IVF treatment is widely accepted as effective treatment for most causes of infertility. Since the birth of the first IVF baby in 1978, hundreds of thousands of pregnancies have been achieved worldwide by this treatment (Edwards, 2007). Annually, about 15,000 IVF and intracytoplasmic sperm injection (ICSI) cycles are started in the Netherlands and 135,000 in all of Europe, with an average success rate of 20 percent per started cycle (Human Fertilisation and Embryology Authority, 2002).

In the last 30 years, IVF treatment itself has evolved in many ways. The first successful IVF attempt was performed in the natural cycle, while today women are routinely treated with ovarian stimulation and luteal phase support. Improved ovarian hyperstimulation regimens have resulted both in increased pregnancy rates per cycle and the introduction of new laboratory techniques such as ICSI and pre-implantation genetic diagnosis have allowed to further extend the treatment possibilities related to IVF treatment.

OVARIAN STIMULATION

When IVF was introduced, clinicians were dependent on the follicular growth in a spontaneous menstrual cycle for the collection of one oocyte. Obtaining the oocyte before it was lost to ovulation required extensive cycle monitoring and careful planning of the oocyte retrieval. Often the attempt had to be abandoned prematurely because of the occurrence of a luteinizing hormone (LH) surge, an unsuccessful oocyte retrieval or fertilization failure of the collected oocyte. As a result the pregnancy rate per cycle was very low.

In order to increase the chance of obtaining embryos of suitable quality for transfer, ovarian stimulation was introduced. The goal of ovarian stimulation is to induce the ongoing development of multiple dominant follicles and to mature many oocytes in order to compensate for inefficiencies of the IVF procedure and enabling the selection of one or more embryos for transfer (Fauser et al., 2005). The use of ovarian stimulation has resulted in a marked increase in pregnancy rates (Trotnov et al., 1995).

PRINCIPLES OF OVARIAN STIMULATION

The initial growth of primordial follicles in the ovaries is thought to be independent of follicle stimulating hormone (FSH) and commences in a random fashion (Peters et al., 1975). The majority of these primordial follicles go into atresia; of the approximately thousand primordial follicles monthly recruited from the resting follicle pool only about ten will happen to be at a more advanced (gonadotrophin dependent) stage of maturation during the inter cycle FSH rise and continue to grow (Gougeon, 1996; McGee and Hsueh, 2000). In the early follicular phase FSH levels rise which is followed by a decrease in FSH levels in the mid to late follicular phase due to the negative feedback of Inhibin B and oestradiol. By this time only the most mature follicle has acquired increased sensitivity for FSH and LH and will continue to grow (van Santbrink et al., 1995; Fauser and van Heusden, 1997; Filicori et al., 2002). By increasing the FSH levels above the FSH threshold for an extended period of time, the process of single dominant follicle selection is overruled and all growing follicles reaching the stage of gonadotrophin dependence during the time FSH levels are above the threshold will continue to grow (Brown, 1978; Schipper et al., 1998).

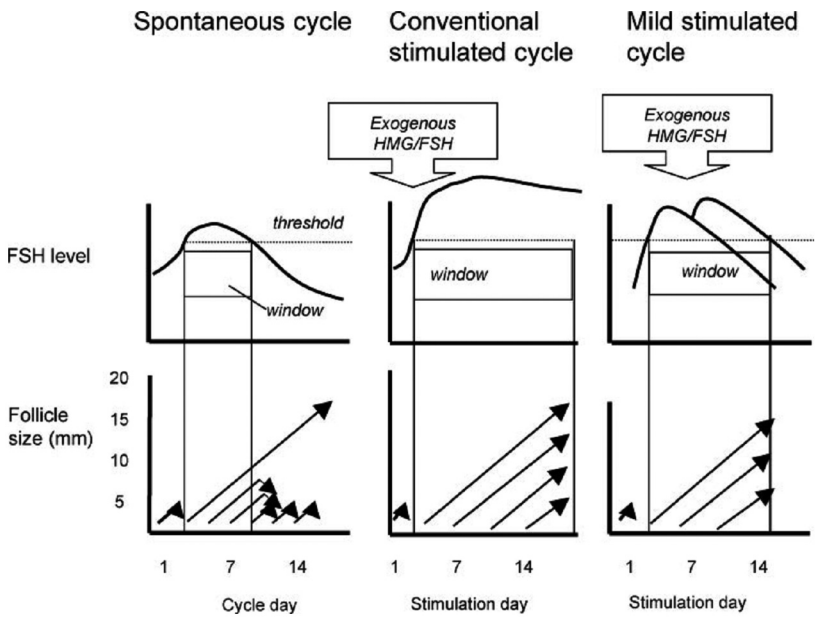

Figure 1.1 The FSH threshold and window concept for monofollicular selection (left panel), as conventionally applied to achieve multifollicular development (middle panel). Each arrow represents a developing follicle. The right panel represents the concept of extending the FSH window by administering exogenous FSH in the midfollicular phase to maintain FSH levels above the threshold allowing multifollicular development to occur. (From: Macklon et al., 2006)

Initially, ovarian stimulation was performed by Clomiphene Citrate (Hoult et al., 1981) which was soon replaced by human menopausal gonadotrophin (hMG) and recombinant gonadotrophin preparations (Fraser and Baird, 1987). In stimulated cycles for IVF, estrogen levels are prematurely elevated, inducing unpredictable but advanced LH rises which have a negative effect on IVF outcome. The introduction of GnRH analogues to prevent untimely LH surges resulted in a further increase of pregnancy rates (Porter et al., 1984). At the present time, a 'long' GnRH agonist protocol, initiated during the midfollicular phase of the preceding cycle, combined with high doses of exogenous FSH administered during the entire follicular phase is the most frequently used stimulation protocol for IVF treatment (FIVNAT 1997; Macklon et al., 2006). As the LH surge is prevented due to the use of GnRH analogues, final oocyte maturation needs to be induced. Because of the low bioactivity of urinary derived LH, an human chorionic gonadotrophin (hCG) bolus, 32 - 36 hours before the oocyte retrieval is used to induce ovulation (Smitz et al., 1988). Because of the detrimental effect of ovarian stimulation on corpus luteum and

endometrial receptivity, luteal phase support is routinely given (Pritts and Atwood, 2002).

The science behind ovarian stimulation is addressed in more detail in chapter 2.2 of this thesis. Table 1 gives an historical overview of the most important developments in the area of ovarian stimulation.

COMPLICATIONS ASSOCIATED WITH IVF TREATMENT

The increase in pregnancy rates due to the use of ovarian stimulation does not come without a price, not only in financial terms but also in terms of medical risks and complications. Currently used medication regimens used for ovarian stimulation are complex and expensive, require weeks of daily injections with intense monitoring and are associated with serious short and long term side effects (Fauser et al., 1999).

The major complication of IVF is the risk of multiple pregnancies. Multiple pregnancies following IVF treatment are the consequence of the practice to transfer of more than one embryo per cycle to improve pregnancy rates. Transfer of several embryos is performed to compensate for the low implantation potential of embryos (Fauser et al., 2005). Multiple pregnancies are associated with a poor perinatal outcome, maternal complications and significant financial consequences (an extensive overview of the complication associated with multiple pregnancies and methods to prevent them is given in chapter 2.1 of this thesis).

Table 1 Historical overview of the most important developments in the area of ovarian stimulation

Year	Author	Event
1910	Crowe et al.	First experimental evidence suggesting that the pituitary has a role in regulating the gonads
1927	Ascheim and Zondek	Isolation of gonadotrophic hormone (later known as human chorionic gonadotropin (hCG)) which was discovered in the urine of pregnant women
1928-1929	Zondek and Ascheim	Isolation of the gonadotrophic hormones of the anterior pituitary and discovery that the pituitary secretes 2 hormones that stimulate the gonads (Prolan A & B)
1930	Zondek	Discovery that blood and urine of postmenopausal women contained gonadotrophins
1930		First animal gonadotrophins available for clinical use
1931	Fevold et al.	First supporting evidence regarding the existence of two separate gonadotrophins
1932		Introduction of Pregnyl (hCG) on the market by Organon
1941		Introduction of the two-step protocol using gonadotrophins and hCG
1945	Hamblen et al.	First pregnancy achieved by two-step protocol

1950		Registration of the first hMG preparation "Pergonal 25 Serono" for clinical use
1957	Borth et al.	Extraction of FSH-like substance of urine of postmenopausal women with gel-electrophoresis
1958	Gemzell	Introduction of human pituitary gonadotrophins (later found to cause Creutzfel-Jakob disease)
1961	Kistner and Smith/ Greenblatt et al.	Discovery that the use of CC led to recommencement of menstrual cycle leading to the discovery of the ovulation inducing capacity of CC.
1962	Gemzell	First pregnancy achieved with human pituitary gonadotrophin
1962	Lunenfeld et al.	First pregnancy achieved with hMG
1971-3	Guillemin Schally	Disclosure of the structure of the decapeptide GnRH and discovery of GnRH agonists and antagonists
1975	Coy et al.	Creation of potent gonadotrophin releasing hormone agonists
1975	Edwards and Steptoe	Collection of preovulatory human eggs for IVF following ovarian stimulation and chorionic gonadotrophin
1978	Brown	Description of the FSH threshold concept
1982	Mettler et al.	Use of human menopausal gonadotrophin for ovarian hyperstimulation in IVF
1984	Porter et al.	Introduction of GnRH agonist for the use premature LH rise suppression
1985	Zeleznik	study on primates showed that mild interference with the decrease in FSH levels in the midfollicular phase was sufficient to override the selection of a single dominant follicle
1987	Baird	Introduction of the FSH gate principle
1988	Smitz	Introduction of luteal phase support following ovarian stimulation
1988		First recombinant human FSH preparation for clinical use by Serono
1988	Keene et al.	First paper on human recombinant FSH
1992	Schoot et al.	First human exposure to recombinant FSH
1992	Germond et al. Devroey et al.	First human IVF pregnancies with recombinant FSH
1992	Donderwinkel et al.	First ovulation induction pregnancy with recombinant FSH
1993	Fauser et al.	Introduction of FSH window principle.
1994	Diedrich et al. Oliveness et al.	First studies on the use of GnRH antagonists on IVF treatment
2000		Introduction of 3rd generation GnRH antagonists by Serono/ Asta Medica and Organon
2001	Boulloux	First report on the use of FSH-CTP in the human
2003	Beckers et al.	First pregnancy with recombinant FSH-CTP

Worldwide, IVF resulted in a 27 percent twin and 3 percent triplet rate in the year 2000 (Adamson FS 2006), assisted reproductive techniques (ART) is estimated to account for 15 percent of all multiple births in the United States (Wright et al., 2005). The most effective way to reduce the number of multiple pregnancies in IVF and ICSI is to limit the number of embryos transferred; (elective) single embryo transfer (SET) was shown to be effective in reducing the number of multiple pregnancies following IVF treatment to a normal range (Pandian et al., 2005).

The main short-term complication in IVF is the ovarian hyperstimulation syndrome (OHSS). OHSS is characterized by increased vascular permeability and simultaneous overexpression of vascular endothelial growth factor in ovarian cells resulting in fluid movement out of the intravascular space and haemoconcentration, which can result in thromboembolism and even death (Elchalal and Schenker, 1997). Currently, up to 10% of IVF cycles result in OHSS, with severe OHSS observed in 0.5-5% of cycles (Devigne and Rozenberg, 2002).

Prevention of OHSS is possible by identifying known risk factors i.e. polycystic ovaries (Tulandi et al., 1984; MacDougall et al., 1992), by an appropriate choice and application of drugs during treatment i.e. using a GnRH-antagonist instead of a GnRH agonist to prevent a LH surge (Al Inany, 2002) or by cancelling the cycle, or cryopreservation of all embryos (D'Angelo and Amso, 2007), coasting (Garcia-Velasco, 2006), the use of 5000 IU hCG for final follicular maturation (Mathur et al., 2007) early unilateral ovarian follicular aspiration (Egbase et al., 1998), administration of glucocorticoids, macromolecules or progesterone (Delvigne and Rozenberg, 2002), the use of gonadotropin-releasing hormone (GnRH) agonist to induce oocyte maturation (Engmann et al., 2007) or prolonging the use of the GnRH antagonist (de Jong, 1998) in case of an excessive ovarian response.

Serious side effects directly related to the use of fertility drugs rarely occur. Local side-effects at the site of injection have been associated with the use of urinary gonadotrophins (Engmann et al., 1998). Elevated levels of symptoms of depression (Eyal et al., 1996), headache (Amir et al., 2005; de Klerk et al., 2006) and muscle pain (de Klerk et al., 2006) have been reported following the use GnRH agonists. Additionally, about 10% of patients will develop functional cysts while on GnRH agonist regimes (Qublan et al., 2006).

Furthermore, concerns regarding the outcome of ART pregnancies have been raised. Higher morbidity and mortality are observed in IVF/ICSI babies (Halliday, 2007). The high incidence of multiple pregnancies has been held widely responsible (Bergh et al., 1999). However, singletons after IVF/ICSI are also with a worse obstetrical and neonatal outcome when compared to spontaneous singletons or to the general population (Schieve et al., 2002, 2004; Ochsenkuhn et al., 2003; Jackson et al., 2004; Helmerhorst et al., 2004). These studies show an increased incidence of (very) low birthweight, preterm delivery, small for gestational age and perinatal mortality. Furthermore, there is evidence suggesting an increased risk of major birth

defects in IVF conceived children (Hansen et al., 2002; Olson et al., 2005). A meta-analysis of twenty-five studies on the prevalence of birth defects in infants conceived following IVF and/or ICSI compared with spontaneously conceived infants showed a 25% or greater increased risk of birth defects in IVF/ICSI infants and suggested a 30-40% increased risk of birth defects associated with ART (Hansen et al., 2005). Long term follow up studies have indicated that IVF children up to 7 years of age are significantly more frequently admitted to hospital than control children (Kallen et al., 2005 and Koivurova et al., 2007). During this period post-neonatal hospital care costs per child were 2.6 fold increased for IVF singletons compared to controls (Koivurova et al., 2007).

These findings have raised questions as to whether IVF/ICSI treatment per se is detrimental or whether the infertile population in itself carries an increased risk of adverse outcome (Pandian et al., 2001; Kapiteijn et al., 2006). Alternatively, the occurrence of vanishing twin has been suggested as the cause for the lower fetal outcome in IVF/ICSI singletons (Pinborg et al., 2005). However, studies on neonatal outcome following single embryo transfer did not support this (Poikkeus et al., 2007; De Neubourg et al., 2006).

Apart from health risks, many investigators have reported high stress levels related to infertility and IVF treatment (Cousineau and Domar, 2007). For many, IVF treatment is the last resort after years of exhausting other avenues to try to have a family. Besides the psychological impact of the chance of treatment failure, IVF treatment itself, with its daily injections, ultrasounds and invasive procedures, such as oocyte retrieval, might be a cause of psychological distress. The stress of infertility treatment has been ranked second to that involving the death of a family member or divorce by couples undergoing this treatment (Freeman et al., 1985; Baram et al., 1988). The negative effect of stress on treatment outcome is twofold; stress appears to have a direct negative effect on the chance of conceiving (Verhaak et al., 2001; Smeenk et al., 2001 and 2005; Cwikel et al., 2004) and stress was found to be the main reason why patients discontinue treatment before they become pregnant (Olivius et al., 2004). Early cessation of treatment deprives couples an optimal cumulative chance of achieving pregnancy, and therefore impacts on the overall success of the IVF program.

AIMS AND OUTLINE OF THE THESIS

Given the above concerns, it is unsurprising that current strategies of ovarian stimulation for IVF and embryo transfer are increasingly being questioned (Edwards et al., 1996; Olivennes and Frydman, 1998; Fauser et al., 1999). In the past few years, fertility practitioners have increasingly acknowledged elective SET as an effective method of reducing the risks and complications associated with multiple pregnancies following IVF treatment (Thurin et al., 2004; De Neubourg and Gerris 2003;

De Sutter et al., 2002; Gardner et al., 2004; Gerris et al., 2004; Tiitinen et al., 2003). Many clinics, especially in Europe, now offer transfer of one embryo as routine clinical care for good prospect patients (Andersen et al., 2007).

Furthermore, mild ovarian stimulation protocols are being developed with the aim to achieve cost effective and more patient friendly regimens in an attempt to optimize the balance between outcomes and side effects/ risks of treatment (Fauser et al., 1999). The introduction of GnRH antagonists into clinical practice and a better understanding of ovarian follicular growth and maturation allow ovarian stimulation to be started in a natural menstrual cycle and resulted in the development of novel, gentler approaches to ovarian stimulation (Macklon et al., 2006). Chapter 2.1 of this thesis provides an overview of the literature regarding various mild stimulation approaches available. Mild ovarian stimulation approaches with GnRH antagonist co-treatment were shown to be effective in reducing the duration of treatment, gonadotrophins needed, side-effects, and oocyte retrieval procedures with reduced chance of OHSS (de Jong et al., 1998; Hohmann et al., 2003).

Despite the clear advantages of mild ovarian stimulation, concerns regarding its efficacy have been raised. It has been argued that mild stimulation leads to a decrease in ovarian response compared to conventional stimulation leading to higher cycle cancellation rates, fewer embryos, and a reduced pregnancy rate per cycle (Gleicher and Barad, 2007). In contrast, consecutive cycles of mild or minimal ovarian stimulation have been shown to result in comparable pregnancy rates to those following standard ovarian stimulation approaches while remaining cost-effective (Nargund et al., 2001; Heijnen et al., 2007). However, both patients and physicians appear reluctant to distance themselves from the traditional paradigms of success such as the pregnancy rate per cycle. The latter can be explained by the fact that in many countries patients have to pay for IVF treatment themselves and market driven fertility practices are dependent on their pregnancy rates per cycle.

Consequently, if mild strategies are to be widely adopted into clinical practice, success rates should be improved. The aim of this thesis is to identify factors which may improve the efficacy and therefore the uptake of milder treatment strategies for IVF.

Chapter 2.1 reviews the existing literature on the contribution of fertility treatment to multiple pregnancies and strategies for reducing multiples in ART and chapter 2.2 reviews the existing literature on milder ovarian stimulation approaches and the evidence to justify their introduction into standard clinical practice.

A combination of mild ovarian stimulation with SET is used to prevent complications associated with ovarian stimulation and multiple pregnancies. However, SET in an unselected population leads to a reduction of pregnancy rates per transfer. In order to improve overall pregnancy rates, and therefore encourage the use of SET, evidence based strategies to identify patients and clinical conditions which qualify

for the use of SET are required. The purpose of the study in chapter 3 was to develop a prognostic model for ongoing pregnancy exclusively in SET cycles following mild ovarian stimulation for IVF.

Mild ovarian stimulation has been proposed as a means of reducing the adverse effects associated with profound ovarian stimulation. As previously mentioned, it has been argued that the decrease in the number of oocytes obtained following mild stimulation causes a decrease in pregnancy rates. However, initial studies applying mild stimulation found the highest pregnancy rate in patients with a moderate number of oocytes. The purpose of the study presented in chapter 4 was to investigate the clinical significance of the retrieval of low numbers of oocytes following mild ovarian stimulation. The embryo implantation rate in relation to number of oocytes obtained was assessed in a combined data set of three previously published randomized trials comparing mild versus conventional ovarian stimulation for IVF.

Even though the retrieval of a low number of oocytes might be related to good outcomes in mild stimulation, cancellations should be prevented to optimise the benefit of mild stimulation. The purpose of the study presented in chapter 5 was to develop a model for the prediction of mild stimulation cycles likely to be cancelled due to insufficient ovarian response. The application of a standard protocol in patients with a high chance of cancellation may result in a decrease in cancellation rate and consequently, the use of such a prediction model may improve the efficacy of the mild stimulation protocol.

High drop-out rates are a considerable problem in IVF treatment. The prognosis of a couple and thereby the effectiveness of the program, in terms of cumulative pregnancy rates, is strongly influenced by selective early cessation. Stress appears to be an important reason for couples to drop-out of IVF treatment before they conceived. Due to the shorter treatment cycle, reduced need of medication and lower incidence of side-effects, mild ovarian stimulation might lead to a decrease of stress during IVF treatment. The aim of the study of chapter 6 was to determine the incidence of drop-out from (reimbursed) IVF treatment and to identity factors that influence the decision to discontinue treatment including the role of the ovarian stimulation protocol applied.

Finally, in chapter 7 the most important conclusions from the conducted studies are summarized and implications for clinical practice and future research are discussed.

CHAPTER 2.1
ART – IATROGENIC MULTIPLE PREGNANCY?

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INTRODUCTION

Subfertility affects one in six to ten couples (Hull et al., 1985; Snick et al., 1997). With the help of assisted reproductive technologies (ART) such as in vitro fertilisation (IVF), intracytoplasmic sperm injection (ICSI) and ovulation induction (OI), many of these couples can be effectively treated. Annually, around 122,000 IVF and 135,000 ICSI cycles are started in Europe alone, with an average success rate of 25 percent per started cycle (Andersen et al., 2006).

Although a number of innovations have improved ART outcomes, these have been accompanied by higher costs, short term side effects such as ovarian hyperstimulation syndrome, possible long term consequences and a high rate of multiple pregnancies. Spontaneous twinning and triplets occur on average in 1 in 90 and 8100 pregnancies respectively (Evans et al., 1998). ART has radically changed these figures. Since the development of IVF and ICSI, the number of twin births increased by 52 percent and the number of higher order multiple births increased by 404 percent (Martin and Park, 1999). The tendency of women to postpone their pregnancy beyond their most fertile age is likely to result in increased application of ART with its associated risks and side effects (Gustafsson, 2001).

Multiple pregnancies are considered to be unfavourable due to the poor neonatal outcome, maternal complications, long term developmental problems and high costs involved. In the past decade the general attitude towards multiple pregnancies has changed. Prevention of multiple pregnancies has become a target of increasing importance for most European IVF centres. The aim of this article is to review the contribution of fertility treatment to multiple pregnancies and strategies for reducing multiples in ART.

CONSEQUENCES OF MULTIPLE PREGNANCIES

Perinatal outcome from multiple gestations is compromised in numerous ways. Multiple pregnancies are associated with increased rates of stillbirths, early neonatal deaths, late neonatal deaths and infant mortality. Compared to singletons, twins (7 fold) and triplets (20 fold) have an increased risk of fetal death (Scher et al., 2002; Doyle, 1996). Multiple gestations also have an increased morbidity rate due to numerous complications associated with the increased risk of prematurity, low birth weight and intrapartum complications. An analysis of a French IVF registry on the neonatal outcome of IVF cycles between 1986-1994 clearly shows the impact of multiplicity on fetal outcome (FIVNAT, 1997). (Table 1)

Table 1 Negative outcomes in the newborn according to the number of fetuses.

	Singletons	Twins	Triplets
Births (*n*)	7448	4822	1176
Total prematurity	9.3	42.9	92.0
Premature <32 weeks	1.2	5.1	23.6
Weight <1500 g	1.6	6.2	27.0
Weight <2500 g	10.7	56.9	94.6
Apgar score 1 min <7	4.4	9.6	21.5
Mortality (percent)			
in utero	6.2	13.5	30.2
neonatal <day 7	5.2	18.9	26.7
neonatal days 7-28	0.8	2.4	6.2
Complications	2.0	4.0	11.6

(Data from: FIVNAT, 1996) Unless otherwise stated, all figures are percentages.

Multiples also have a compromised long term outcome including an increased risk of long term medical and developmental problems such as learning problems, cerebral palsy and adult health risks. Compared to singletons, twins and triplets have post neonatal survivors relative risk (RR) for severe handicaps of 1.7 and 2.9, respectively (Luke and Keith, 1992). Prevalence of child disabilities is up to 50 percent higher in twins and 100 percent in triples (ESHRE Capri Workshop group, 2000; Wennerholm, 2004). Cerebral palsy is one of the most significant long term neurological impairments associated with multiple births; a three- to seven-fold increased risk compared with singletons is observed amongst twins and over ten-fold amongst triplets (Petterson et al., 1993).

Complications associated with multiple pregnancies are not limited to the offspring. Pregnancy-induced maternal complications, hypertension disorders in

particular, are reported to be three to seven times more common in association with multiple than with singleton pregnancies (ESHRE Capri Workshop group, 2000). These complications are likely to be the consequence of an exaggerated physiologic response due to the increased placental and fetal mass. A Canadian retrospective comparison of the obstetric outcome of 44,674 multiple pregnancies matched with 165,188 singleton pregnancies showed significantly increased maternal morbidity and mortality compared with the former group (Walker et al., 2004). (Table 2)

Table 2 Comparison of maternal outcomes between women carrying a multigestational pregnancy (n= 44 674) and women carrying a singleton pregnancy (n= 165 188).

Outcome	Singletons	Multiples	RR (95% CI)
In-hospital death	9 (0.005)	5 (0.0011)	2.05 (0.69-6.13)
Pre-eclampsia	5873 (3.56)	4407 (9.87)	2.78 (2.67-2.88)
Gestational diabetes	5387 (3.26)	1633 (3.66)	1.12 (1.06-1.18)
Heart failure (left ventricular)	2 (0.001)	7 (0.016)	12.94 (2.69-62.30)
Venous thrombembolism	131 (0.08)	94 (0.21)	2.65 (2.04-3.46)
Pulmonary oedema	28 (0.017	54 (0.12)	7.13 (4.52-11.25)
Postpartum haemorrhage	7680 (4.65)	3903 (8.74)	1.88 (1.81-1.95)
Caesarean delivery	34,383 (20.81)	20,106 (45.006)	2.16 (2.13-2.19)
Hysterectomy	97 (0.059)	60 (0.13)	2.29 (1.66-3.16)
> 4 days in hospital	41.242 (24.97)	22,279 (61.062)	2.45 (2.42-2.47)

(Adapted from: Walker et al., 2004). RR, relative risk; CI, confidence interval. Values are presented as n (%).

Multiple pregnancies entail an increased risk for intrapartum complications as a consequence of uterine atony and malpresentations, resulting in high incidences of caesarean sections and postpartum haemorrhage (Table 2). The incidence of postpartum haemorrhage requiring transfusion has been reported to be 12.3 percent for triplets (95% CI 3.8-20.8 percent) and 13 percent for quadruplets (95% CI 5-21 percent) (Albrecht and Tomich, 1996; Collins and Bleyl, 1990). The frequency of caesarean deliveries for multiple pregnancies is approximately two-three times higher than that for singleton pregnancies (Senat et al., 1998; Walker et al., 2004).

With regard to maternal mortality a population-based study on data from 13 European countries reported an estimated maternal mortality rate of 5.2 for singleton births versus 14.9 for multiple pregnancies (RR 3.5) (Senat et al., 1998). Causal factors included hypertensive disorders, abruptio placentae, caesarean delivery and post-partum haemorrhage.

FINANCIAL CONSEQUENCES

The financial consequences of multiple pregnancies are substantial for both parents and health care providers. While carrying a multiple pregnancy, women are more likely to generate extra medical costs due to longer periods of bedrest, hospitalization, administration of medication to prevent pre-term labour, surgical procedures such as emergency caesarean section and cervical cerclage. Due to the high rate of premature deliveries, multiple gestations generally result in higher perinatal healthcare costs as well. In an American study, neonatal care increased from an average of 4.6 days for singletons to 8.2 days for each twin and 10.0 days for each higher-order multiple neonate (Callahan et al., 1994). The same study revealed longer maternal admissions following multiple birth. Compared to singletons, obstetric care costs are 2.1-, 4.5- and 7-fold higher for twins, triplets and quadruplets, respectively (Mugford and Henderson, 1995). The estimated costs of care in the year 2000 for all assisted reproductive treatment multiple births in the USA amounted to $640 million (Collins and Graves, 2000).

The economic impact of a multiple pregnancy is not limited to increased costs of maternal hospitalization and obstetric and neonatal (intensive) care. Life time costs for chronic medical care, rehabilitation and special education related to extreme prematurity also have to be taken into account. For a low birth weight child, the average cost of heath care and education up to the age of eight years is 17-fold higher than the costs for a normal birth weight child (Stevenson et al., 1996). Table 3 shows an estimation of costs of hospital admissions in the first five years of life according to fetal number. The authors concluded that multiple births contribute disproportionately to hospital inpatient costs, especially during the child's first year of life (Henderson et al., 2004).

Table 3 Mean cost of hospital inpatient stay by order of multiplicity.

	Singleton pregnancy	Twin pregnancy	Higher order multiple pregnancy	p-value
Initial birth admission	846 (841-850)	1931 (1874-1987)	3678 (3085-4271)	<0.001
Readmissions 1st year	414 (403-427)	1662 (1541-1784)	4157 (3189-5125)	<0.001
Readmissions 2nd year	104 (101-107)	119 (94-145)	54 (1-107)	0.30
Readmissions 3rd year	74 (71-77)	80 (63-97)	504 (47-960)	<0.001
Readmissions 4th year	67 (64-70)	71 (58-85)	30 (3-57)	0.74
Readmissions 5th year	65 (62-68)	49 (41-56)	135 (0-289)	0.088
Entire study period	1532 (1516-1548)	3826 (3724-3929)	8156 (7559-8754)	<0.001

Values are presented as mean costs per child in pounds sterling (95% CI).
(Adapted from: Henderson et al., 2004).

THE CONTRIBUTION OF FERTILITY TREATMENT TO MULTIPLE PREGNANCIES

The high rate of multiple pregnancies in ART is a consequence of ovarian stimulation interfering with the process of single dominant follicle growth. Available data suggest that infertility treatment accounts for around 30-50 percent of twin births and for over 75 percent of higher order multiples. Approximately 30 percent of multiple pregnancies are due to gonadotrophins and ovulation induction medications used for non-IVF or ICSI ART treatment (Toner, 2002; Evans et al., 1995). The exact numbers of multiple pregnancies arising from ovarian stimulation, with or without intra uterine insemination (IUI), are unknown, as there are no national registers that record the outcome of controlled ovarian stimulation (The ESHRE task force on ethics and law, 2003). Fifteen to twenty percent of all multiple births in the USA have been attributable to IVF/ICSI and gamete intrafallopian transfer (GIFT) procedures (15.5 percent of twins and 43.8 percent of triplet and higher order births) (Wright et al., 2005).

Ovulation induction is one of the most common interventions for the treatment of infertility. Classically, patients are treated with antiestrogens first and, if unsuccessful, with gonadotrophins. Antiestrogens such as Clomiphene Citrate (CC) induce ovulation in 70-90 percent of patients (Imani et al., 1998). Multiple pregnancy rates of 2-13 percent have been reported in association with CC use (Messinis, 2005; Schenker et al., 1981; Venn and Lumley, 1994). When CC fails, exogenous gonadotrophins may be used. Both CC and gonadotrophins are associated with a risk of multiple follicle development. When this is combined with *in vivo* methods of fertilization such as timed intercourse or IUI, it is not possible to limit the number of embryos developed. In IUI and OI programmes, the use of gonadotrophins is associated with multiple pregnancy rates of 15-40 percent, depending on the stimulation strategy used (Mitwally et al., 2005; Fauser and van Heusden, 1997; Fauser et al., 2005).

In IVF and ICSI treatment, gonadotrophins are used to develop a large cohort of dominant follicles and to acquire an increased quantity of gametes. This is necessary to compensate for the inefficiency of the in vitro fertilization procedure while maintaining the potential to select the best embryo. Multiple pregnancies in IVF and ICSI result from the transfer of more than one embryo into the uterus. The number of fetuses is directly related to the number of embryos transferred (Jones et al., 1995). Approximately 25 percent of IVF and ICSI pregnancies in Europe and 34 percent in the United States are twins or higher order multiples (ASRM 2000, Andersen et al., 2005).

HOW CAN MULTIPLE PREGNANCIES BE PREVENTED?

To prevent multiples, patients with a good chance of spontaneous pregnancy should not be subjected to unnecessary fertility therapies. As most patients seeking fertility treatment are not sterile but have decreased fertility, many of them will eventually conceive spontaneously. It has been estimated that 54 percent of the moderately

subfertile population will not conceive spontaneously within one year, however the majority of the remaining patients (62%) will conceive spontaneously in the next 12 months (Evers and te Velde, 2001) (Table 4). A number of predictive models have been developed which can aid in advising couples of their chance of conceiving spontaneously within 12 months (Eimers et al., 1994; Hunault et al., 2005). Using those could reduce the number of ART treatments performed and consequently reduce the number of multiple pregnancies in this group of patients.

Table 4 Hypothetical model of cumulative spontaneous pregnancy rates in five categories according to duration of subfertility.

	Cumulative pregnancy rate after:		
Category	**6 months**	**12 monts**	**24 months**
Superfertile	100 %		
Normal fertile	74 %	93 %	100 %
Moderately subfertile	26 %	46 %	71 %
Severly subfertile	6 %	11 %	21 %
Infertile	0 %	0 %	0 %

(Adapted from: Evers and te Velde, 2001)

Lifestyle changes, such as exercise and weight reduction improve the chance of spontaneous conception and should be promoted. Cohort studies have shown that weight loss in obese patients with polycystic ovary syndrome (PCOS) restores normal fertility (Kiddy et al., 1992; Hoeger, 2001; Norman et al., 2004; Clark et al, 1998). Additionally, both alcohol intake and smoking are related to an increased chance of fertility problems and a decreased chance of a live birth in IVF treatment (Tolstrup et al., 2003; Lintsen et al., 2005; Midgette and Baron, 1990).

OVULATION INDUCTION

The majority of multiples in ovulation induction therapies result from the use of gonadotrophins. To prevent multiples in ovulation induction the use of gonadotrophins should be restricted to women who are resistant to first line medication, such as CC or metformin (National Institute for Clinical Excellence, 2003). There is no conclusive evidence that multiples are prevented when CC treatment cycles are monitored by estradiol levels and/ or ultrasound (Fauser and Macklon, 2004). A low starting dose of 50 mg daily is advised (Adashi et al, 2003). A number of adjuvant and alternative therapies may be effective in CC resistant patients, thus avoiding gonadotrophin therapy, or may reduce the risk of multiples when administered together with CC. A frequently used approach in CC resistant patients is the combination of CC with insulin sensitizers or insulin sensitizers alone. A meta-analysis of two

randomized controlled trials showed that metformin combined with CC was significantly more effective in achieving ovulation compared with CC alone in CC resistant patients (Lord et al., 2003). However, a recent multicentre randomized placebo controlled trial showed that metformin should not be used routinely as part of first line treatment for inducing ovulation (Moll et al., 2006). Only one randomized trial was found comparing CC and metformin with gonadotrophins; no significant differences in outcome could be found, probably due to the small number of patients included (George et al., 2003). Exact data on the incidence of multiple pregnancies associated with metformin treatment are lacking. When gonadotrophins are used, low dose step up with low starting doses of 37.5 to 75 IU and slow gradual increase should be combined with frequent monitoring. When multiple follicle growth occurs a step down protocol or conversion to IVF with SET are alternatives for cancellation (Fauser and Macklon, 2004).

Recently, aromatase inhibitors (AI) have been employed for ovulation induction. A recent literature review of the preliminary evidence available concluded that it appeared that AIs are as effective as CC in inducing ovulation with good pregnancy results and a lower incidence of multiple pregnancies (Casper and Mitwally, 2006). Recent data have raised concerns regarding possible teratogeneity (Biljan et al., 2005). Other medications or co-medications such as dexamethasone and opioid antagonist have been introduced with initial enthusiasm but to date convincing evidence in their efficacy is lacking (Messinis, 2005).

Ovarian surgery has been a frequently used approach for women with CC resistant PCOS as an alternative to hormonal medication. Currently, laparoscopic ovarian drilling (LOD) is the most commonly used surgical treatment for this indication. A recent Cochrane review showed that LOD is equivalent to gonadotrophins in terms of ongoing pregnancy rate and live births but with a significant lower multiple pregnancy rate (Farquhar et al., 2005; Bayram et al., 2004). Disadvantages of LOD are the requirement of general anaesthesia, the potential risk of thermal damage to adjacent organs, possible adhesion formation and the unknown long term effects on ovarian function.

INTRA UTERINE INSEMINATION

IUI is used as a first line treatment for ovulatory patients with patent fallopian tubes and no severe male factor infertility. For this patient group, IUI was shown to significantly improve pregnancy rates compared to timed intercourse (Hughes, 1997; National Institute for Clinical Excellence, 2003). IUI in combination with gonadotrophins is used to improve timing, correct subtle cycle disorders and improve pregnancy rates by increasing the number of follicles. However, this method is also associated with a high rate of multiple pregnancies (Cohlen, 2005). To limit the number of multiples, guidelines with strict cancellation criteria based on female age, the number of follicles and estradiol levels have been developed (The ESHRE Capri

Workshop group, 1996 and 2000). Unfortunately, even when strict cancellation criteria are applied, multiple pregnancy rates up to 29 percent may occur (Cohlen, 2005; Gleicher et al., 2000).

IUI in spontaneous cycles could represent a preferable alternative. A meta-analysis comparing the outcome of IUI in the spontaneous cycle with IUI combined with ovarian stimulation for male subfertility showed that there is limited evidence to support the use of gonadotrophins (Cohlen et al., 2000). In other causes of subfertility higher pregnancy rates may be achieved when IUI is combined with gonadotrophins (Cohlen, 2005). However, it is questionable whether the small rise in pregnancy rates merits the additional concomitant risk of multiple pregnancies (Macklon et al., 2005; Fauser et al., 2005; Collins, 2003). A health economics evaluation recommended IUI in the natural cycle as the therapy of first choice in unexplained infertility (Goverde et al., 2000). The Fertility Treatment Guidelines published by the National Institute for Clinical Excellence in the UK made similar recommendations (National Institute for Clinical Excellence, 2003). Large randomized controlled trials comparing natural cycle IUI with stimulated IUI for unexplained subfertility and mild endometriosis are still awaited.

IVF AND ICSI

The most effective way to reduce the number of multiple pregnancies in IVF and ICSI is to limit the number of embryos transferred. A randomized controlled trial comparing outcomes of the transfer of two or three embryos demonstrated that triplet pregnancies can be reduced to a negligible number without a significant reduction in pregnancy rate (Templeton and Morris, 1998). Unfortunately, in this study the number of twin pregnancies also remained unaffected. In order to impact significantly on the multiple pregnancy rate, elective single embryo transfer (SET) should be implemented.

In the last few years, six randomized controlled trials comparing SET with double embryo transfer (DET) have been published. Despite the high pregnancy rates observed after SET in these studies, a higher success rate will be achieved transferring two embryos. For SET to be established as the method of first choice, it must be associated with acceptable pregnancy rates (Table 5).

Table 5 Results from randomized controlled trials concerning elective single embryo transfer (SET) compared with double embryo transfer (DET).

Author, year	n	Ongoing pregnancy rate (Delivery rate) SET (%)	Twin Pregnancy (%)	Ongoing pregnancy rate (Delivery rate) DET (%)	Twin Pregnancy (%)
Gerris et al., 1999	53	38.5 (n.a.)	0.1	74.1 (n.a.)	30.0
Martikainen et al., 2001	144	32.4 (29.7)	0.04	47.1 (40)	33.3
Thurin et al., 2004	661	28.2 (38.8)*	0.01	43.8 (42.9)	33.1
Gardner et al., 2004	48	60.9 (n.a.)	0	76.0 (n.a.)	47.4
Lukassen et al., 2005	107	55.6 (40.7)**	0	47.2 (35.8)	28.0
Montfoort et al., 2006	308	21.4 (n.a.)	0	40.3 (n.a.)	21.0

* Pregnancy rate of cumulative fresh embryo and thawed embryo cycle.
** Pregnancy rate of two consecutive cycles of SET. n.a., not available.

Randomized trials have shown that the decrease in outcome with elective SET can be compensated by increasing the number of treatment cycles either with fresh (Lukassen et al., 2005) or frozen embryos (Thurin et al., 2004). This alternative does not come without negative consequences. Increasing the number of fresh treatment cycles involves recurrent cycles of hormonal stimulus and follicular aspiration, procedures not without risks (Land and Evers, 2003). A large Dutch randomized trial comparing full treatment outcome of three cycles with DET with a maximum of four cycles and mild ovarian hyperstimulation with SET showed that the SET group had equally high cumulative ongoing pregnancy and life birth rates but significantly less multiple pregnancies and was cost-effective (Heijnen et al., 2006). Alternatively, repeated cycles of natural cycle IVF could be performed, but with a low success rate of about 7 percent per cycle (Pelinck et al., 2002), this approach has not been widely adopted. The gain from frozen embryo cycles is limited due to the relatively large losses in the freezing, thawing and selection procedures. Therefore, take home baby rates per frozen embryo usually do not exceed 10-15 percent per frozen embryo (Menezo, 2004).

To improve IVF success rates in SET, reliable methods to select the best embryo for transfer are required. Prolonged embryo culture has been proposed as a method to improve embryo selection and increase pregnancy rates (Gardner et al., 2004). Extended culture may select the most developmentally competent embryos for transfer, thereby avoiding transfer of embryos predestined to arrest (Bavister and Boatman, 1997). Additionally, higher implantation rates might be the result of better synchronization between embryo and uterus, since in the natural situation embryos are not exposed to the uterus until the blastocyst stage (Bongso et al., 1994; Menezo, 1995). Indeed, with blastocyst transfer, implantation rates of 60 percent in a highly

selected group of patients have repeatedly been reported (Gardner et al., 2004; Papanikolaou et al., 2005). A recent randomized controlled trial studying blastocyst vs. cleavage stage SET in women under 36 years old reported significantly higher pregnancy and delivery rates in blastocyst transfer (Papanikolaou et al., 2006). However, others found the merit of blastocyst versus cleavage stage embryo transfer not to exist for the overall population (Emiliani et al., 2003; Pantos et al., 2004). Further concerns about the use of this method are the increased ratio of monozygotic splitting and the greater loss of embryos due to the prolonged culture procedure resulting in fewer embryos to cryopreserve, an altered sex ratio of births and epigenetic effects on the embryo (Behr et al., 2000; Gardner et al., 1998).

Another method to improve embryo selection is by preimplantation genetic screening (PGS) for chromosomal abnormal embryos (Thornhill et al., 2005). It is known that embryonic aneuploidies are likely to be responsible for pregnancy failure and low implantation rates and that morphologically normal embryo's do not necessarily exclude an abnormal chromosomal constitution (Baart et al., 2006; Magli et al., 2001; Twisk et al., 2006). Although preliminary findings were promising, two randomized trials which addressed the effectiveness of PGS in terms of live births, showed a tendency towards higher pregnancy rates in the control group (Stevens et al., 2004; Staessen et al., 2004), nor was it proven to be cost-effective (Platteau et al., 2005). Further developments are required if PGS it to become clinically useful and cost-effective.

We can therefore conclude that in a very good prognostic category, high success rates can be achieved with elective SET and multiple pregnancies can be effectively prevented. However, a higher success rate still will be achieved when two embryos are transferred. The lower success rate may be compensated by increasing the number of treatment cycles.

MULTIFETAL PREGNANCY REDUCTION (MFPR)

Unfortunately, over the past 15 years, MFPR has become a well-established and integral part of infertility therapy and aims to deal with the sequelae of aggressive infertility management. By method of suction, injection of KCl or mechanical disruption selective reduction of one of more embryos per multifetal pregnancy can be performed. MFPR has shown to be an effective method to limit the number of multiple births and their complications. Research has shown that the number of complications in reduced twins and singletons are similar to those of nonreduced twins and singletons conceived by ART (Smith-Levitin et al., 1996; Brambati et al., 2004). Nonetheless, a strong correlation remains between the starting number and the likelihood of poor outcome for both losses and prematurity (Evans et al., 2004). A further downside is the 2-12 percent risk of total pregnancy loss due to the detrimental effect of necrotic tissue left, the development of a [latent] inflammatory response, spilling of KCl beside the fetal heart and acute bleeding (Evans et al., 2004).

Whether achieving pregnancy resulting in a singleton live birth is a justification for MFPR has been a matter of debate (Chervenak et al., 1992; Chervenak et al., 1995; Evans et al., 2003). With the current low risk of fetal loss and the possibility to combine MFPR with chromosomal investigation, MFPR has become acceptable for many but certainly not standard practice in most centres (Evans and Britt, 2005). All this does not take away the obvious moral and ethical issues associated with destroying one or more healthy fetuses and in our opinion the prevention of multi-fetal pregnancies should be preferred.

Practice points to prevent multiple pregnancies in ART treatment:

- In patients with a good chance of spontaneous conception, treatment should be delayed
- Lifestyle changes improving fertility should be promoted
- Alternative options such as Metformin should be considered for CC resistant anovulatory patients
- Natural cycle IUI should be method of first choice for patients with ovulatory patients with patent fallopian tubes and no severe male factor infertility
- IUI and OI treatment cycles with more than two dominant follicles should be cancelled
- elective SET should become the method of first choice in women below 37 years of age undergoing IVF
- MFPR can be considered as a last resort when a multiple pregnancy occurs

ATTITUDES TOWARDS SET

An improvement in success rates might not be sufficient for SET to be established as the method of first choice since many patients perceive multiple pregnancy as a desirable outcome from fertility treatment. In a prospective study with a 97 percent response rate, 20.3 percent of patients listed twin, triplet or quadruplet pregnancies as preferable to a singleton pregnancy as outcome of their IVF treatment (Ryan et al., 2004). However, the wish for a multiple pregnancy was negatively correlated with the awareness of the risks associated with multiples (Ryan et al., 2004; Grobman et al., 2001; Child et al., 2004). These findings imply that, patients should be informed of the risks and costs of multiple pregnancies to become motivated for SET.

The competition for patients, desire for high fertility rates and the need for quick results driving the majority of fertility practices in some countries are factors that could cause resistance by physicians towards SET (D'Alton, 2004). One means of breaking this pattern could be to alter the method of reporting success. Currently, money back guarantees, and the reporting of pregnancy rates per treatment cycle encourage multiple embryo transfer (D'Alton, 2004; Fauser et al., 2005). A universal

agreement of the definition of success would improve the ability to compare the outcome of clinical trials for the professional community and the assessments of clinics for the recipient of care (Davies et al., 2004). Currently, a wide range of outcomes are used. The nominator and denominator in reports of results in IVF have to be consistent. Both the definition of nominator; ongoing pregnancy, (term) (singleton) live birth, singleton term gestation or healthy born baby and the units that should be used to report treatment outcomes (per cycle, per started treatment, per complete treatment or per year) have been the matter of recent debate (Min et al., 2004; Davies et al., 2004; Heijnen et al., 2004; Pinborg et al., 2004; Griesinger et al, 2004; Land and Evers, 2004; Germond et al., 2004). Agreement on this matter would rewards efficacy and penalizes unsafety and result in an expected decrease of multiple pregnancies.

An opportunity for individual governments to influence the rate of multiple pregnancies is to encourage health insurance companies to provide full coverage of fertility treatments so that patients will be more willing to use milder strategies, including SET. In most countries, health insurance do not cover (the full costs) of IVF treatment cycles even though cost-efficiency analyses showed that the total health costs for [repeated] IVF cycles of SET where lower than the total costs of (fewer) cycles of DET due to significantly higher neonatal costs (Lukassen et al., 2005; Heijnen et al., 2006). In July 2003, new legislation allowing six IVF cycles to be reimbursed if the number of embryos transferred is limited, was introduced in Belgium with the aim of reducing the number of multiples. In women under 36, only one embryo is transferred at the first treatment cycle, at the second cycle one or two fresh embryos are transferred and at the third and following cycles a maximum of two fresh embryos is transferred. In women aged 36 - 40 years, a maximum of two fresh embryos are transferred in the first and second cycles and at the third and following cycles a maximum of three fresh embryos are transferred. Women over 39 years old are not subjected to any restrictions on the maximum number of fresh embryos for transfer (Ombelet et al., 2005). A retrospective analysis comparing IVF treatment outcomes of one clinic over the year following the introduction of the new legislation with those over the preceding year showed that the legislation resulted in a threefold reduction of multiple pregnancies without a reduction of the pregnancy rate per embryos transferred (Debrock et al., 2005). It should be noted that the reduction of multiple pregnancies was completely due to a lower rate of multiples in the age group under 36 since there was no reduction in the clinical multiple pregnancy rate in the older age groups (Debrock et al., 2005).

Finally, there is a role for society to discourage the tendency of women to postpone their pregnancy until beyond their most fertile age. This could lead to a reduction in women needing fertility treatment, a reduction in spontaneous multiples due to the strong relation between increasing female age and multiple pregnancy

(Bulmer, 1970) and for women who do require fertility treatment less pressure to use methods associated with a high risk of multiple pregnancy.

SUMMARY

Due to the use of ART, the incidence of multiple pregnancies has increased dramatically. Multiple pregnancies in ART are the result of ovarian hyperstimulation used to improve treatment outcome and the transfer of more than one embryo after in-vitro fertilisation. Methods to prevent multiple pregnancy include being restrictive in using ART for patients with good chance of spontaneous pregnancy, cautious use of gonadotrophins, increased use of natural cycle IUI and elective SET in IVF and ICSI. Besides the maintenance of high success rates, an attitude change in both patients and physicians in needed. By encouraging women to start their families early and by encouraging health insurance companies for full coverage of fertility treatments, surely health providers could play an important role in the prevention of multiple pregnancies.

Practice Points:

- Improve information for physicians and patients about the risks involved in multiple pregnancies
- Transparency of negative outcomes and advertise with rates of healthy born singletons
- Encourage health insurance companies to cover IVF cycles (with SET)
- Motivate women to start their family early

Research Agenda:

- Improve embryo culture, cryopreservation and embryo selection methods
- Studies for interventions to reduce the patient stress involved with IVF treatment and to decrease drop-out rates

CHAPTER 2.2
MILD OVARIAN STIMULATION FOR IN VITRO FERTILISATION – A LITERATURE REVIEW

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(invited for Human Reproduction Update)

INTRODUCTION

Ovarian stimulation has become a key component of assisted reproductive technologies (ART). For twenty-five years, it has been applied with the aim of increasing the number of oocytes in order to compensate for inefficiencies of the in vitro fertilisation (IVF) procedure and enabling the selection of one or more embryos for transfer (Fauser et al., 2005). At the present time, a long gonadotrophin-releasing hormone (GnRH) agonist pituitary suppression combined with high doses of exogenous follicle-stimulating hormone (FSH) remains the most frequently used stimulation protocol (FIVNAT, 1997; Macklon et al., 2006). Gonadotrophin starting doses usually vary between 150 and 450 IU/day, although several randomized trials have failed to demonstrate improvements in outcome when higher doses are employed (van Hooff et al., 1993; Hoomans et al., 1999 and 2002; Out et al., 2000 and 2001; Latin-American Puregon IVF Study Group 2001; Wikland et al., 2001; Yong et al., 2003).

Currently used medication regimens for ovarian stimulation are complex and expensive, may require weeks of daily injections with intense monitoring and are associated with the risk of serious side effects such as ovarian hyperstimulation syndrome (OHSS) (Fauser et al., 1999; Delvigne and Rozenberg, 2002; Aboulghar and Mansour, 2003). Other negative effects associated with ovarian stimulation include emotional stress, high drop-out rates, and abdominal discomfort (Osmanagaoglu et al., 1999). Moreover, uncertainties exist regarding long-term health risks, such as the development of ovarian cancer and an increased incidence of low birth weight and birth defects in the offspring conceived following IVF and ICSI treatment (Hansen et al., 2002; Oliveness et al., 2002; Wang et al., 2005; Kapiteijn et al., 2006).

In 1996 Edwards was the first to express concern with regard to contemporary ovarian stimulation approaches for IVF and called for the use of milder stimulation protocols (Edwards et al., 1996). The aim of mild stimulation is to develop safer and more patient friendly protocols in which the risks of treatment are minimized (Diedrich and Felberbaum, 1998; Olivennes and Frydman, 1998; Oliveness, 2002; Nargund and Frydman, 2007; Pennings and Ombelet, 2007) (Table 1).

Table 1 Considerations for mild ovarian stimulation

Current ovarian stimulation approaches
High numbers of oocytes and embryos
Complex stimulation regimens
Time consuming
High costs
Patient discomfort
Short term complications – OHSS
Long term health consequences
High drop-out rates
Supraphysiological levels of steroid levels
Aims of mild stimulation
Less complex
Less time consuming
Cheaper
Reduced complications/ discomfort/ drop-outs
Effect oocyte quality/ endometrial receptivity

A potential concern regarding the applications of milder stimulation protocols in standard practice is that a decrease in ovarian response following mild stimulation will reduce pregnancy rates. However, increased efficiency of IVF laboratory procedures and the current tendency – in some parts of the world – to limit the number of embryos transferred, has reduced the need for large numbers of oocytes. Although the cryostorage of supernumary embryos is often put forward as a strong argument in favour of profound stimulation, the actual added value in terms of additional pregnancies is modest. Furthermore, supportive evidence regarding a potentially negative effect of supraphysiological steroid levels on endometrial receptivity (Simon et al., 1995; Devroey et al., 2004), corpus luteum function (Fauser and Devroey, 2003; Beckers et al., 2006), oocyte and embryo quality (Valbuena et al., 2001; Baart et al., 2007) indicate that limited ovarian stimulation and response might have a beneficial effect upon implantation potential.

This overview will discuss the rationale behind milder ovarian stimulation approaches and the evidence to justify their introduction into standard clinical practice.

RELEVANT PHYSIOLOGY OF FOLLICLE DEVELOPMENT

Complete follicular development takes over 220 days and can be classified into three phases according to the developmental stage and the follicular gonadotrophin dependence (Gougeon, 1996; Fauser and van Heusden 1997; McGee and Hsueh, 2000). First, the initial recruitment of resting primordial follicles, second the development of pre-antral and early antral follicles and finally cyclic recruitment of a cohort of antral follicles followed by the selection of a single dominant follicle during the mid-follicular phase of the menstrual cycle (Figure 1).

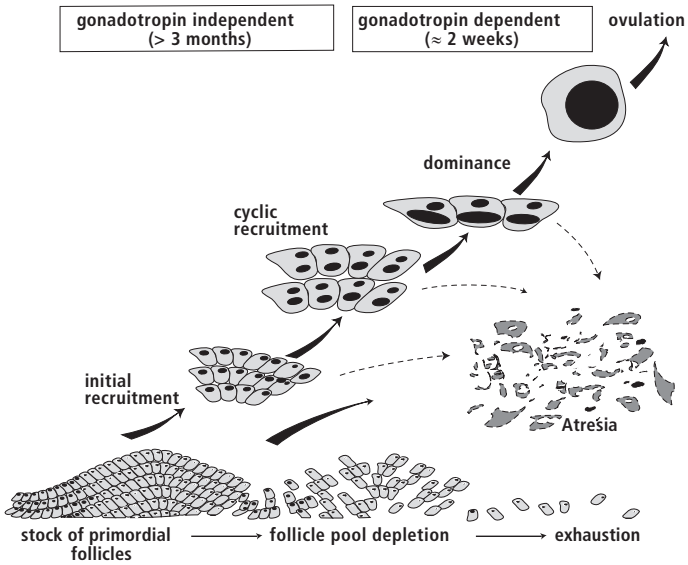

Figure 1 Schematic representation of life history of ovarian follicles: endowment and maintenance, initial recruitment, maturation, atresia or cyclic recruitment, ovulation, and exhaustion (adapted from: McGee and Hsueh, 2000).

In the adult ovary, folliculogenesis starts when follicles leave the pool of resting follicles to enter the growth phase. The size of the follicle pool is determined during fetal life and reaches its maximum of 6 to 7 million by 20 weeks of gestation (Baker, 1963). From this point in time, germ cell content will decrease due to a continuous flow of follicles leaving the primordial follicle pool (initial recruitment). Around 1000 primordial follicles start growing every month. The exact mechanism underlying the initiation of growth is not well understood and appears to be under the control of intra-ovarian autocrine and paracrine factors (Gougeon, 1996; Fortune et al., 2000). The great majority of primordial follicles that enter this development phase undergo atresia before reaching the antral follicle stage, principally though apoptosis (McGee and Hsueh, 2000).

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After initial recruitment, follicles entering the growth phase enlarge, both by proliferation and differentiation of granulosa cells and an increase in the size of the oocyte. The time span of the development from primary recruitment to the early antral follicle stage in humans is unknown but is proposed to be several months. During early pre-antral follicle development, FSH receptors become detectable on granulosa cells. Although at this stage the follicles seem unaffected by the absence of gonadotrophins (i.e. Kallmann syndrome, or after hypophysectomy), growth is stimulated by the presence of FSH (McGee and Hsueh, 2000).

In contrast to the early stages of follicle development, the presence of FSH is an absolute requirement for the development of larger antral follicles. From this point onwards, FSH acts as a survival factor for antral follicles, which are being rescued from atresia by the intercycle rise in serum FSH level (Fauser and van Heusden, 1997). Although each growing follicle may initially have an equal potential to reach full maturation, only those follicles that are at a more advanced stage (2-5 mm) at the time FSH levels surpass the FSH threshold, (during the luteo-follicular transition) continue to grow. The number of follicles available for cyclic recruitment is dependent on the age of the ovary and is believed to be around 11 follicles per ovary on average (Hodgen, 1982; Pache et al., 1990) (Figure 2).

After the initial rise in FSH levels, FSH concentrations plateau during the early follicular phase and finally decrease during the mid to late follicular phase as a consequence of inhibin B and ovarian steroid negative feedback (Zeleznik et al., 1985; Groome et al., 1996; Schipper et al., 1998). The decrease in FSH limits the time that the FSH concentration is above the threshold, which appears to be essential for single dominant follicle selection (van Santbrink et al., 1995). Despite the decline in FSH, the most mature follicle continues its growth due to its increased sensitivity for FSH and acquired responsiveness to luteinizing hormone (LH) (Hillier, 1994; McGee and Hsueh, 2000). All other recruited follicles lack sufficient FSH stimulation and enter atresia. The “FSH gate” (Baird, 1987) or “FSH window” (Fauser et al., 1993) concept adds the element of time to the FSH threshold theory and emphasized the importance of a transient increase of FSH above the threshold level in order to gain single

dominant follicle selection. Ovarian stimulation makes use of the phenomenon that disruption of the decline of FSH levels leads to the development of multiple dominant follicles.

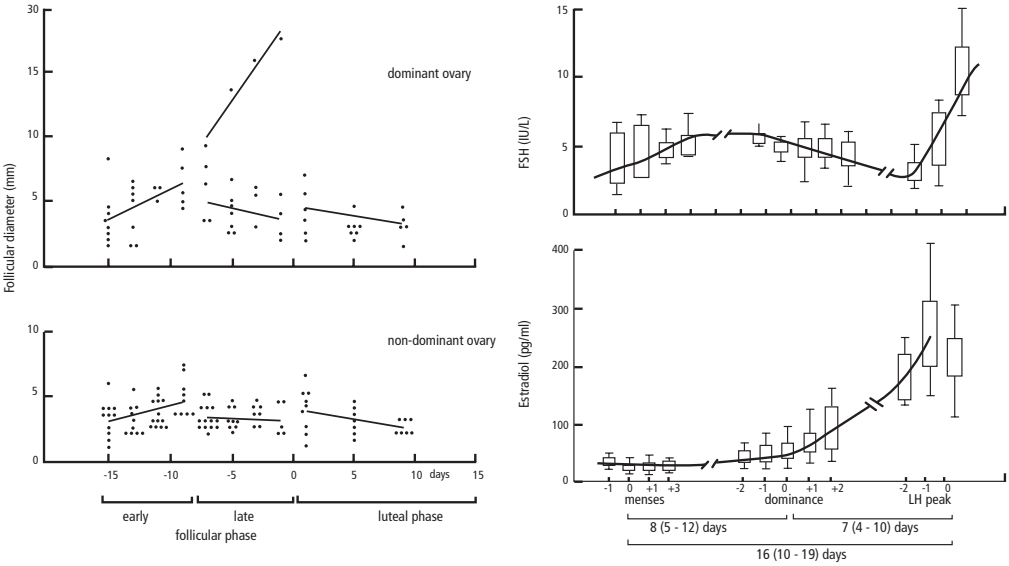

Figure 2 Left: Reflection of follicle diameters during the menstrual cycle of normal cycling women (day 0=LH surge) (Pache et al., 1990). Right: Box and whisker plots representing serum FSH (upper panel) and E2 (lower panel) concentration in 16 regularly menstruating female volunteers, synchronized around the initiation of menses, around the first day of visualization on US, and preceding the serum LH peak (From: van Santbrink et al., 1998)

After gonadotrophins became available, multiple dominant follicles growth was accomplished by the administration of high doses of exogenous gonadotrophins during the entire follicular phase (Hillier et al., 1985) (Figure 3). However, a later study on primates showed that mild interference with the decrease in FSH levels in the midfollicular phase was sufficient to override the selection of a single dominant follicle (Zeleznik et al., 1985). Subsequently, this concept was confirmed in humans; a moderate, but continued, elevation of FSH levels during the mid to late follicular phase (effectively preventing decremental FSH concentrations) was sufficient to interfere with single dominant follicle selection and induces ongoing growth of multiple follicles in normo-ovulatory volunteers (Figure 4) (Schipper et al., 1998).

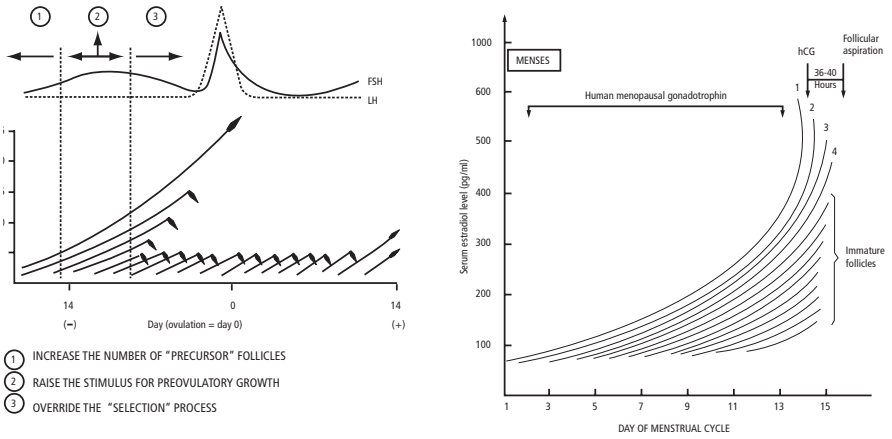

Figure 3 Left: Schematic representation of serum follicle-stimulating hormone levels and number and size of ovarian follicles during ovarian hyperstimulation for in vitro fertilization (Hillier et al., 1985).

Right: Schematic representation of the heterogeneous cohort of recruited and selected follicles in hMG stimulated cycles (From: Oehninger and Hodgen, 1990).

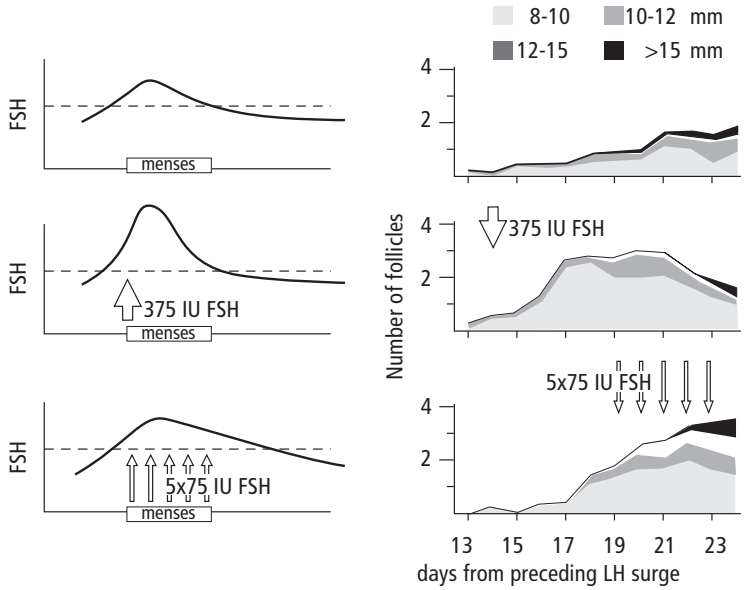

Figure 4 FSH window concept with mild intervention approaches, stressing the significance of the limited duration of FSH elevation above the threshold level rather than the height of the elevation of FSH for single dominant follicle selection.

The left figures show the intervention, and the right figure shows the resulting number of follicles during the follicular phase. Upper: natural cycle with single dominant follicle selection. Middle: Intervention cycle with administration of a single sc injection of 375 IU FSH on Day LH+14. Under: Intervention cycle with five sc injections of 75 IU FSH daily from DayLH+19 until DayLH+23. (Adapted from: Schipper et al., 1998).

THE DEVELOPMENT OF MILDER STIMULATION PROTOCOLS

INTRODUCTION OF GNRH ANTAGONISTS

The introduction of GnRH antagonists into clinical practice has allowed for the introduction of milder stimulation approaches for IVF treatment (Tarlatzis et al., 2006). GnRH antagonists prevent the premature LH rise by competitive blockade of the GnRH receptor. Unlike GnRH agonists, GnRH antagonists do not induce an initial flare of endogenous gonadotrophin release, but cause an immediate and rapid, reversible suppression of gonadotrophin secretion. The use of GnRH antagonist exclusively during the mid to late stimulation phase (the period at risk for a premature rise in LH) therefore allows for the initiation of the IVF treatment cycle in a normal menstrual cycle with an undisturbed early follicular phase recruitment of a cohort of follicles. This enables the endogenous inter-cycle FSH rise to be utilized rather than suppressed resulting in a reduction of medication needed. The use of ovarian stimulation in the normal menstrual cycle also enables more IVF cycles to be carried out in a given period than is possible with a long GnRH agonist stimulation protocol.

Three general approaches for GnRH antagonist co-treatment have emerged. A single subcutaneous injection of a large dose can be administered on approximately the eighth day of stimulation with gonadotrophins. Alternatively, daily injections of small doses are initiated on a fixed day of stimulation (usually day 6) or depending on the size of the dominant follicle or the estradiol level (flexible protocol) and continued until the day that human chorionic gonadotrophin (hCG) for final oocyte maturation is given (For review Huirne and Lambalk, 2001).

As was shown in a meta-analysis of 27 studies, compared with IVF treatment cycles with a standard long GnRH agonist protocol, the use of GnRH antagonist co-treatment leads to a reduction in the number of days analogue treatment is needed (OR -20.90, 95% CI -22.20 to -19.60), the number of days of gonadotrophin treatment (OR -1.54, 95% CI -2.42 to -0.66), the number of gonadotrophin ampoules used (OR -4.27, 95% CI -10.19 to 1.65) and the incidence of severe OHSS (RR 0.61, 95% CI 0.42 to 0.89). Moreover, in contrast to GnRH agonist, the use of GnRH antagonists is not complicated by chances of cyst formation due to the GnRH agonist flare-up effect. Although initial studies suggested a detrimental effect on pregnancy rates following GnRH antagonist compared to agonists (Ludwig et al., 2001; Al-Inany et al., 2006; Tarlatzis et al., 2006) a recent meta-analysis including 22 randomized controlled trials involving 3,176 subjects showed no difference in the probability of live birth (Kolibianakis et al., 2006).

To date, GnRH agonists remain in use in the majority of clinics. This is probably due to the established position of GnRH agonist in standard regimens (Kolibianakis et al., 2005), initial reports on a possible reduction in pregnancy rates (Al-Inany et al., 2006) and the reduced flexibility in the programming of IVF cycles with GnRH antagonist co-treatment (Fauser and Devroey, 2005).

NATURAL CYCLE AND MODIFIED NATURAL CYCLE WITH FSH ADD-BACK

The first successful IVF treatment was performed in an unstimulated menstrual cycle (Steptoe and Edwards, 1978). Soon thereafter IVF in natural cycles was largely replaced by IVF with ovarian stimulation to improve the success rate per cycle (Trounson et al., 1981; Cohen et al., 2005). Natural cycle IVF in its basic form consists of simply monitoring the spontaneous cycle, and retrieving a single oocyte prior to the LH peak. Consequently, the chance for multiple pregnancies and OHSS are minimized. Natural cycle IVF is physically less demanding, requiring no or far less hormonal medication. The per-cycle costs of natural cycle IVF have been calculated to be 20-23% of those of stimulated IVF (Aboulghar et al., 1995; Nargund et al., 2001).

Ongoing pregnancy rates per started natural cycle IVF have been reported to be 7.2% although this may vary according to the population studied (for review Pelinck et al., 2002). In natural cycle IVF, results are hampered by high cancellation rates due to premature LH rises, premature ovulation and low percentages of successful oocyte retrievals (Pelinck et al., 2002). The planning of oocyte retrieval based on a LH rise requires frequent monitoring and almost round-the clock oocyte pick up and laboratory facility. The use of hCG for the triggering of final oocyte maturation allows a degree of planning, and Indomethacin to postpone follicle rupture (Nargund et al., 2001). Flushing of the follicle during oocyte retrieval (Bagtharia and Haloob, 2005) may further increase the efficacy of the procedure.

Only four randomized controlled trials comparing natural cycle IVF with stimulated IVF cycles have been published (Table 2). The outcome of natural cycle IVF was compared with IVF in clomiphene citrate (CC) stimulated cycles (MacDougall et al., 1994; Ingeslev et al., 2001), human menopausal gonadotropin (HMG)/ GnRH agonist long protocol cycles (Levy et al., 1991) and with IVF cycles combining purified FSH ovarian stimulation with a GnRH agonist microdose flare protocol (Morgia et al., 2004). Despite relatively small numbers of patients, and variable numbers of treatment cycles per patient, natural cycle IVF was consistently observed to result in lower pregnancy rates (Table 2).

To improve effectiveness, natural cycle IVF could be offered as a series of treatment cycles, for it is safer, less stressful compared to conventional stimulation and can be offered over consecutive cycles. It has been postulated that after four cycles of natural cycle IVF, the cumulative probability of pregnancy was about 46% with an associated live birth rate of 32% in a selected groups of patients (Nargund et al., 2001). To improve outcomes while preserving the advantages of natural cycle IVF, modifications have been made. In the "modified" natural cycle, the occurrence of a premature LH rise is prevented by the use of a GnRH antagonist during the late follicular phase. The ongoing growth of the dominant follicle is supported by the addition of exogenous gonadotrophins (added "back"). In most studies, GnRH antagonist and gonadotrophins (75 IU - 300 IU/ day) are initiated at a follicle diameter of 12 to 17 mm.

Table 2 Characteristics of randomized controlled trials involving natural cycle IVF treatment

Study	Inclusion criteria	Study protocol	Control stimulation protocol	Main outcome
Levy et al., 1991 (abstract)	Patients with regular ovulatory menstrual cycles and no male factor.	Natural cycle IVF with hCG when the leading follicle was ≥ 16 mm and E2 ≥ 160 pg/ml. (22 cycles)	Long GnRH agonist protocol with HMG. (26 cycles)	Cancellation rate 27% vs 4%. Ongoing pregnancy rate 0% vs 23% ($p < 0.01$).
MacDougall et al., 1994	Patients ≤ 38 years with >1 year of infertility, spontaneous ovulatory regular cycles and normal semen analysis.	Natural cycle IVF with hCG when the leading follicle was 17 mm (n=14).	CC 100 mg, from days 2 to 6, hCG when the leading follicle was 17 mm (n=16)	Cancellation rate 71% vs. 0%. Ongoing pregnancy rate 0% vs. 13% (NS).
Ingerslev et al., 2001	Couples with no previous IVF attempts under 35 years with ICSI indication, tubal factor or idiopatic infertility	Natural cycle IVF with hCG when the leading follicle was ≥ 17 mm. (64 patients, 114 cycles).	CC 100 mg, from days 3 to 7 and hCG when the leading follicle was ≥ 20 mm. (68 patients, 111 cycles).	Cycles resulting in embryo transfer 25.4% vs. 53.2%. Ongoing pregnancy rate (per cycle) 3.5% vs. 18.0% ($p < 0.001$).
Morgia et al., 2004	Poor-responding patients (< 4 follicles in a previous IVF attempt) with a regular menstrual cycle. ICSI was performed in all cycles.	Natural cycle IVF with hCG when the leading follicle was ≥ 16 mm. (59 patients, 114 cycles).	GnRH analog flare protocol with 0.05 mg Buserelin twice daily from day 1 and 600 IU purified FSH/ day from day 3. (70 patients, 101 cycles)	Cycles resulting in embryo transfer 41.2% vs. 68.3%. Ongoing pregnancy rate (per cycle) 6.1% vs. 6.9% (NS).

Up to the present, no randomized controlled trials studying the efficacy of modified natural cycle IVF have been published. Most studies on modified natural cycle IVF include patients with a poor response following conventional ovarian stimulation IVF. In this population, success rates between 0 and 14 percent per started cycle have been reported in non-randomized studies (Elizur et al., 2002; Castelo-Branco et al., 2004; Kolibianakis et al., 2004; Weghofer et al., 2004; Hur et al., 2005). One large cohort study analyzed the cumulative pregnancy rate after three modified natural IVF cycles in good prognosis patients (Pelinck et al., 2006). 844 treatment cycles in 350 patients of less than 36 years of age with no previous IVF treatment were included. The ongoing pregnancy rate per cycle was 8.3% and 20.8% after up to three cycles. The number of cancelled cycles related to a rise in LH or ovulation in this study was 13% per started cycle, compared to an average of 20% reported following natural cycle IVF.

Higher pregnancy rates have been reported in young couples with severe male infertility as the only fertility compromising factor might benefit from the use of modified natural cycle IVF. In this category of patients, the success rate per started cycle was 13.3% (Zhioua et al., 2004) and cumulative pregnancy rates of 43.8% after six successive cycles (Vogel et al., 2003) have been reported.

These studies show that (modified) natural cycle is a safe and patient friendly treatment option. Despite the advantages of this approach, relatively low efficacy has restricted the widespread use. The use of (modified) natural cycle IVF in a series of consecutive cycles is likely to result in improved effectiveness in a selected population.

CLOMIPHENE CITRATE

The anti-oestrogen CC was one of the first preparations used for ovarian stimulation in IVF (Trounson et al., 1981; Quigley et al., 1984; Cohen et al., 2005). CC has now been largely replaced by more effective HMG/FSH protocols in combination with GnRH analogue co-treatment (Fraser and Baird, 1987). Important advantages of CC compared to gonadotrophins remain however, and include its oral administration, low price and availability. CC acts to increase pituitary FSH secretion by reducing negative oestrogen mediated feedback. The combined synergistic effects of CC and gonadotrophins, could lead to a reduction in the amount of gonadotrophin required, for ovarian stimulation. Because gonadotrophins counteract the undesired anti-oestrogenic effects of the CC on the endometrium (Markiewicz et al., 1988; Nelson et al., 1990), which has been held responsible for the relatively low embryo implantation rates observed following successful ovarian stimulation, this combination might lead to improved pregnancy rates compared to CC alone.

Two randomized trials have compared the outcome of CC/gonadotrophin treatment cycles with a standard long GnRH agonist ovarian stimulation protocol. In one study, significantly higher cycle cancellation rates and lower pregnancy rates per cycle were observed following a CC protocol in combination with 150 IU HMG (p-value 0.002) (Dhont et al., 1995). However, in a more recent study with similar patient numbers, a stimulation regimen that combined CC with 225 IU FSH and 75 IU of recombinant LH (rLH) on alternate days resulted in comparable cancellation and ongoing pregnancy rates per cycle to those following a standard long GnRH agonist protocol (Weigert et al., 2002). The characteristics of the randomized controlled trials on the various CC protocols for IVF treatment have been summarized in Table 3.

The recent availability of GnRH antagonists has allowed for the prevention of premature LH rises in combination with CC. One randomized controlled study showed that a CC/gonadotrophin regimen with GnRH antagonist co-treatment resulted in comparable pregnancy outcomes with a standard long GnRH agonist stimulation protocol while significantly reducing the number of ampoules HMG used, number of treatment days and oocytes retrieved (Lin et al., 2006). This study

confirmed the findings of two earlier retrospective analyses which concluded that equally high pregnancy rates could be obtained with a CC/gonadotrophin protocol with GnRH antagonist co-treatment, compared to standard ovarian stimulation, with a significant reduction in the total dose of gonadotrophins needed (Williams et al., 2002; Fiedler and Ludwig, 2003). In contrast, a non-randomized comparative study observed significantly lower pregnancy rates following ovarian stimulation with a CC/ HMG protocol with GnRH antagonist co-treatment compared to a long GnRH agonist protocol (Mansour et al., 2003). Whether the addition of GnRH antagonist to the CC/gonadotrophin protocol improves outcomes remains unclear. A randomized controlled study observed similar ongoing pregnancy rates with and without the use of GnRH antagonist (Fiedler et al., 2001). The need for pituitary suppression in combination with CC is probably dependent on the dosage of medication used and individual ovarian responses.

In most studies, gonadotrophins are combined with CC in a dose of 100 mg/day for five days during the early follicular phase. However, there exists a high rate of heterogeneity in studies concerning the optimal use of the other components of this stimulation approach. It has been debated whether HMG or FSH with or without rLH supplementation should be used (Engel et al., 2002; Weigert et al., 2002). Additionally, there is no evidence regarding the most optimal gonadotrophin regimen; most studies vary in the starting dose, day of initiation, daily injections or on alternate days or as a single shot of long acting FSH (Corfman et al., 1993; Obruca et al., 1993; Tavaniotou et al., 2003; Engel et al., 2002; D'Amato et al., 2004; Kawachiya et al., 2006).

In conclusion, more studies are required to optimize the CC/gonadotrophin stimulation protocol. The heterogeneity in the outcome of studies thus far published prevents from drawing conclusions regarding the possible benefits of CC in ovarian stimulation for IVF. However, given its low cost, CC may have a place in cost-effective mild ovarian stimulation treatments.

AROMATASE INHIBITORS

Aromatase inhibitors selectively inhibit the conversion of androgens to oestrogens leading to a reduced amount of oestrogen negative feedback at the pituitary and increased endogenous stimulation of gonadotrophin secretion. By administering aromatase inhibitors in the early follicular phase the need for exogenous gonadotrophins is likely to be reduced. Aromatase inhibitors may therefore serve a similar purpose as CC. Like CC, aromatase inhibitors are orally taken and are relatively cheap. However, compared to CC they offer the potential advantage of not causing depletion of oestrogen receptors (Mitwally and Casper 2001 and 2003) and are more rapidly cleared from the body because of their shorter half-life (≈45 hours instead of a few weeks). Aromatase inhibitors have been in clinical use for more than 20 years, primarily in the treatment of postmenopausal patients with advanced breast cancer

Table 3 Characteristics of randomized controlled trials involving ovarian stimulation with Clomiphene Citrate for IVF treatment

Study	Inclusion criteria	Study protocol	Control stimulation protocol	Main outcome
MacDougall et al., 1994	Patients ≤ 38 years with >1 year of infertility, spontaneous ovulatory regular cycles and normal semen analysis.	CC 100 mg, from days 2 to 6, hCG when the leading follicle was 17 mm (n=16)	Natural cycle IVF with hCG when the leading follicle was 17 mm (n=14).	Cancellation rate 0% vs. 71%. Ongoing pregnancy rate 13 % vs. 0% (NS).
Dhont et al., 1995	Patients with no previous IVF attempts. Treatment included IVF-ET, ZIFT and GIFT	OAC pre-treatment, CC 100 mg for 5 days and (150) subsequent HMG. (n=151)	OAC pre-treatment, long acting GnRH agonist and (300 IU) HMG. (n=152)	Cancellation rate 20.5% vs. 2.6%. Ongoing pregnancy rate 24.5% vs. 36.8% (p 0.02).
Ingerslev et al., 2001	Couples with no previous IVF attempts under 35 years with ICSI indication, tubal factor or idiopathic infertility	CC 100 mg, from days 3 to 7 and hCG when the leading follicle was ≥ 20 mm. (68 patients, 111 cycles).	Natural cycle IVF with hCG when the leading follicle was ≥ 17 mm. (64 patients, 114 cycles).	Cycles resulting in embryo transfer 53.2% vs. 25.4%. Ongoing pregnancy rate (per cycle) 18.0% vs. 3.5%% (p < 0.001).
Fiedler et al., 2001 (abstract)	Random selected normal cycling women.	100 mg CC CD 5-9, from day 9 additional 150 IU HMG or FSH. GnRH antagonist from day 10. (n=295)	100 mg CC CD 5-9, from day 9 additional 150 IU HMG or FSH. (n=291)	Ongoing pregnancy rate 23% vs. 21% (NS).
Weigert et al., 2002	Women with no previous IVF cycles, between 20-39 years, with normal ovulatory cycles with tubal, male factor or unexplained infertility	OAC pre-treatment. CC 100 mg for 5 days in combination with 225 IU of rFSH and 75 IU of rLH on alternate days. (n=154)	Long GnRH suppression and 150 IU rFSH. (n=140)	Ongoing pregnancy rate 35% vs. 29% (NS).
Engel et al., 2003	Healthy female partners of infertile couples, between 18-39 years, with regular cycle length. No more than 3 previous IVF cycles or basal FSH > 10 IU/L	Single dose GnRH antagonist protocol. CC 100 mg CD2-6 of 3-7, CD 6 start 150 IU rFSH. (n=5)	Single dose GnRH antagonist protocol. CC 100 mg CD2-6 of 3-7, CD 6 start 150 IU HMG. (n=5)	Live birth rate 40% vs. 20% (NS).
Lin et al., 2006	Couples with male-factor infertility who were about to undergo their first ICSI cycle	CC/HMG. Cetrorelix protocol. (n=60)	Buserelin long protocol. (n=60)	Pregnancy rate 41.7% vs. 40% (NS).

(Winer et al., 2002). They have only recently been introduced in infertility treatment, namely for ovulation induction (Casper and Mitwally, 2006) and as a mild and safe ovarian stimulation method for IVF treatment in patients with breast cancer (Oktay et al., 2003 and 2005). Recent data have raised concerns regarding possible teratogeneity of aromatase inhibitors (Biljan et al., 2005), although these findings were not confirmed in a larger group of patients (Tulandi et al., 2006).

There are limited clinical data available concerning the use of aromatase inhibitors in IVF treatment. One preliminary uncontrolled study observed an ongoing pregnancy rate of 27% following the use of aromatase inhibitors as a cheap treatment alternative in 22 good prognosis patients with limited financial means (Grabia et al., 2006). In this study HMG was initiated on CD 7 after five days of Letrozole (2.5 mg CD 3-7) with GnRH antagonist co-treatment.

To date, only three randomized controlled trials have studied the use of aromatase inhibitors in IVF. However, in all three, aromatase inhibitors were administered in combination with a standard rather than mild ovarian stimulation protocol. In two trials, aromatase inhibitors were added to a standard treatment schedule using high doses of gonadotrophins in patients with a poor response in a previous treatment cycle (Goswami et al., 2004; Kahraman et al., 2005). Both studies showed no benefit although the study groups were too small to draw meaningful conclusions. The third study randomized twenty good prognosis patients for the use of 150 IU rFSH from CD 2 with or without the addition of 2.5 mg letrozole and GnRH antagonist co-treatment from CD 6 (Verpoest et al., 2006). The use of aromatase inhibitors resulted higher numbers of oocytes and a tendency towards higher clinical pregnancy rate per started cycle in the Letrozole group although not significant. In conclusion, more sufficiently powered studies are needed to assess the true benefit of aromatase inhibitors in IVF treatment.

EXOGENOUS GONADOTROPHINS

Mild ovarian stimulation in which low dose gonadotropin (FSH/HMG) administration is delayed until the mid follicular phase is based on the FSH-window concept (Fauser et al., 1993). Exogenous FSH administration is limited to the mid to late follicular phase with the aim of preventing a decrease of FSH levels and thus inducing multi-follicular development (Schipper et al 1998; De Jong et al., 2000). The availability of GnRH antagonists for acute suppression of a premature LH rise enabled this concept to be introduced into IVF (Macklon and Fauser, 2002). A pilot study showed that multiple dominant follicles could even be induced when the initiation of FSH was postponed until cycle day 7. However, there was a tendency toward a lower percentage of women presenting with multiple dominant follicle development compared to patients started on cycle day 3 or 5 (Figure 5) (Hohmann et al., 2001). A fixed daily dose of 150 IU rFSH compared to 100 IU/d was found to be more effective in inducing multiple follicular growth when ovarian stimulation was initiated on cycle day 5 (de Jong et al., 2000).

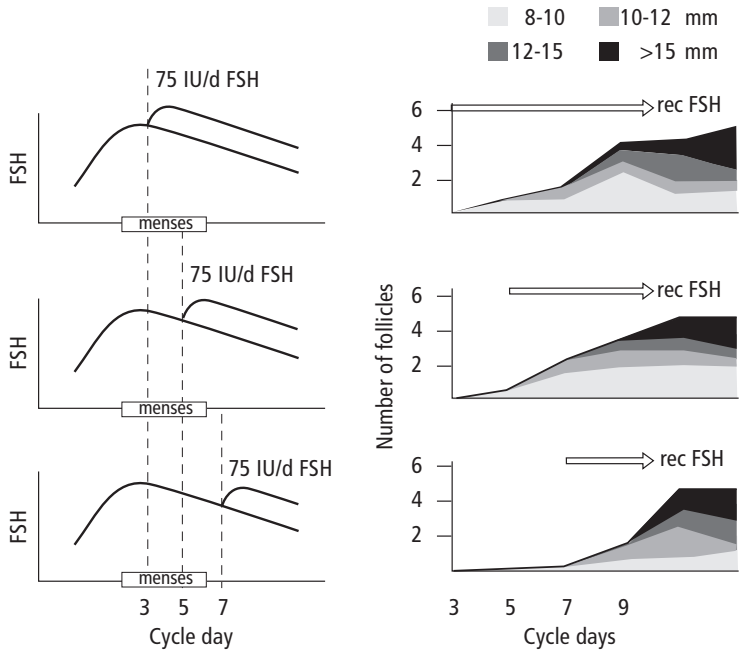

Figure 5 Multifollicular development in normo-ovulatory women receiving fixed low daily doses (75 IU) of exogenous FSH (starting on either cycle day 3, 5 or 7). The left figures show the intervention, and the right figure shows the resulting number of follicles during the follicular phase (Adapted from: Hohmann et al., 2001).

In a prospective randomized study of 142 patients, the efficacy of a stimulation protocol initiating ovarian stimulation (150 IU/ day) on cycle day 5 with GnRH antagonist co-treatment from a follicle size of 14 mm (CD 5 protocol) was compared to a conventional long GnRH agonist protocol and a standard GnRH antagonist protocol with an early follicular start of FSH (Hohmann et al., 2003). This study showed that the mild protocol resulted in pregnancy rates per started cycle comparable to those observed following convention ovarian stimulation with GnRH agonist co-treatment (p-value 0.98) despite a shorter stimulation and a marked reduction in exogenous FSH needed, and higher cancellation rates (p-value < 0.001 and 0.02 respectively).

To confirm the efficacy of this mild stimulation protocol in standard practice, a large randomized study was performed to analyse whether a mild strategy in IVF (combining mild ovarian stimulation with single embryo transfer (SET)) would lead to a similar overall outcome while reducing patients' discomfort, multiple pregnancies, and costs compared to a standard treatment involving conventional stimulation and the transfer of two embryos (Heijnen et al., 2007). The study included a total of 404 patients (almost 800 consecutive IVF cycles) and observed that due to the

shorter duration of treatment per cycle, less medication needed and a reduction in twin pregnancies, the mild approach resulted in an equal cumulative chance of term live birth after a year of treatment while reducing the total costs (Figure 6). Table 4 shows the characteristics of three randomized controlled trials regarding a mild "late start, low-dose" ovarian stimulation.

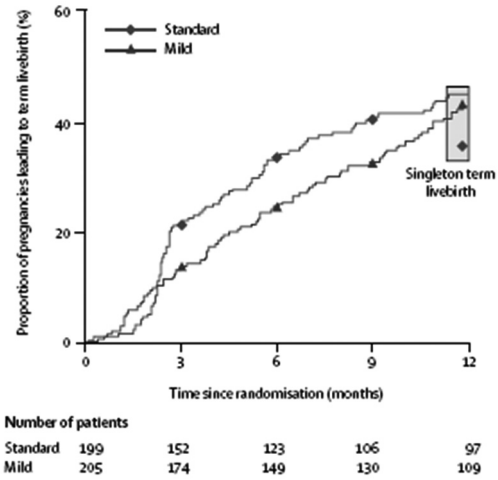

Figure 6 Proportions of cumulative term livebirth rate within 12 months after starting IVF. Mild: mild ovarian stimulation with GnRH antagonist and single embryo transfer. Standard: standard ovarian stimulation with GnRH antagonist and dual embryo transfer. The shaded area represents the singleton livebirth rate after 12 months (From: Heijnen et al., 2007).

LATE FOLLICULAR PHASE HCG/ LH

A stimulation protocol with late follicular phase replacement of FSH administration by LH stimulation has recently been proposed as an alternative mild stimulation approach. The replacement of FSH by LH is based on the acquired LH responsiveness of granulosa cells in dominant follicles (Hillier, 1994). In sheep, LH administration maintained elevated ovulatory rates despite FSH withdrawal (Campbell et al., 1999) while in humans, the administration of rLH (300-750 IU/day) activity was found be sufficient to maintain follicular growth in the late follicular phase after initial ovarian stimulation with exogenous gonadotrophins (Sullivan et al., 1999). Besides the expected reduction of gonadotrophin usage, this ovarian stimulation approach might also reduce the number of small, less mature follicles, conceivably reducing the chance of OHSS, because smaller ovarian follicles are not responsive to LH (Filicori et al., 1999).

Three randomized trials comparing the late follicular phase hCG/LH protocol with the outcome of conventional stimulation protocols in good prospect patients

Table 4 Characteristics of randomized controlled trials involving mild ovarian stimulation for IVF treatment

Study	Inclusion criteria	Study protocol	Control stimulation protocol	Main outcome
De Jong et al., 2000	Normo-ovulatory patients with a regular indication for IVF.	From cycle day 5 ovarian stimulation with 100 IU/d FSH. GnRH antagonist from cycle day 8 or from leading foll 13 mm. No luteal support was provided. (n=8)	From cycle day 5 ovarian stimulation with 150 IU/d FSH. GnRH antagonist from cycle day 8 or from leading foll 13 mm. No luteal support was provided. (n=7)	Multiple follicle development 63% vs. 100%. Ongoing pregnancy rate 25% vs. 14%. (NS)
Hohmann et al., 2003	Normo-ovulatory patients with an indication for IVF (or IVF/ICSI)	Fixed FSH doses 150 IU/ day from CD 5, GnRH antagonist from leading foll 14 mm. (n=45).	1. Fixed FSH doses 150 IU/ day from CD 2, GnRH antagonist from leading foll 14 mm (n= 48). 2. Long GnRH agonist protocol, fixed FSH doses after 2 weeks 150 IU/ day. (n=49).	Ongoing pregnancy rate 16% vs. 17% (1.) vs. 18% (2.) (NS).
Heijnen et al., 2007	Regular cycling patients, below 38 years, BMI 19-29.	Fixed FSH doses 150 IU/ day from CD 5, GnRH antagonist from leading foll 14 mm. Combined with single embryo transfer. (205 patients, 444 cycles)	Long GnRH agonist protocol, fixed FSH doses after 2 weeks 150 IU/ day. (199 patients, 325 cycles)	Ongoing pregnancy rate per year of treatment 47% vs. 51% (NS)
Baart et al., 2007	Regular cycling patients, below 38 years, BMI 19-29. Sperm count > 5 mln/ml. First cycles.	Fixed FSH doses 150 IU/ day from CD 5, GnRH antagonist from leading foll 14 mm. (n=55)	Long GnRH agonist protocol, fixed FSH doses after 2 weeks 225 IU/ day. (n=40)	Proportionally less chromosomal abnormal embryos were obtained after mild ovarian stimulation.

were identified. In a large randomized controlled trial in 323 IVF patients the outcomes of a stimulation protocol with regular ovarian stimulation until CD 6, followed by a combination of 75 IU FSH and 200 IU hCG with GnRH antagonist co-treatment until oocyte retrieval were compared to a standard GnRH antagonist and a long GnRH agonist stimulation protocol (Serafini et al., 2006). The hCG protocol resulted in a significant reduction in rFSH needed, and no difference in the number of (mature) oocytes obtained, ongoing pregnancy rates and incidence of OHSS. A

similar design was applied in a study involving 109 patients, with the only exception that in the hCG group, hCG was initiated when the largest follicle was 14 mm and a fixed FSH dosage was applied (Koichi et al., 2006). This study also observed similar pregnancy rates between the three groups, no difference in the number of oocytes or incidence of severe OHSS. However, a significant decrease in the total dose of gonadotrophins needed and small follicles at the time of final oocyte maturation was observed in the hCG group. Finally, the efficacy of a stimulation protocol with complete replacement of FSH with hCG from a follicular size of 12 mm in combination with a long GnRH agonist downregulation protocol was studied (Filicori et al., 2005). This approach resulted in a significant reduction in FSH needed and small follicles at final oocyte maturation in the hCG protocol without compromising the pregnancy rate compared to a standard long GnRH agonist protocol. None of the studies reported untimely increments of follicular phase progesterone secretion or premature LH surges in the hCG/LH protocol.

These findings confirm that, in an selected group of patients, an ovarian stimulation protocol with late follicular phase hCG/ LH stimulation leads to a reduced need of exogenous FSH and good pregnancy results. However, despite the reported reduction in the number of small follicles, high estradiol levels were found and a reduced incidence of OHSS could not be established as yet. Additional studies are needed to determine the critical threshold for FSH replacement by LH stimulation, the most appropriate dosage of LH or hCG and confirm the clinical benefit.

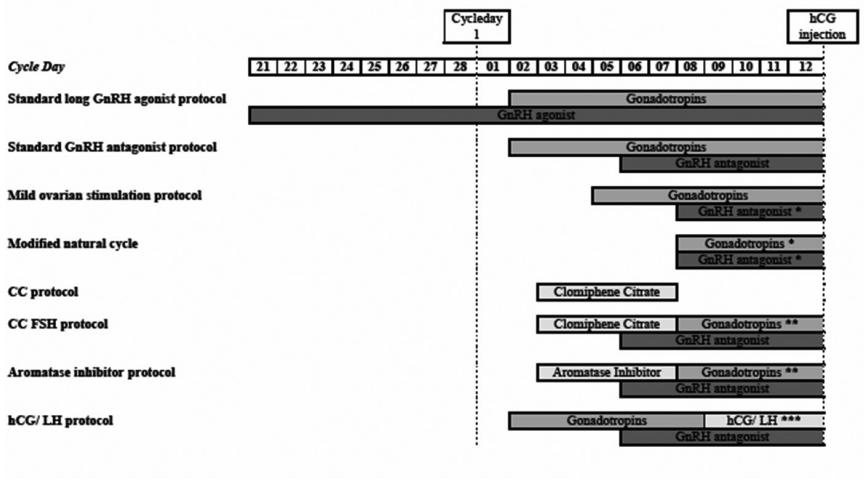

Figure 7 Schematic representation of the number of days of medication needed in different ovarian stimulation strategies for IVF.

* Fixed start from CD 5 or 6 or flexible start depending on the size of the follicle

** Studies exact initiation of Gonadotrophins varies between studies.

*** Starting day varies from CD 9-13.

IMPLICATIONS OF MILD OVARIAN STIMULATION

EMBRYO QUALITY

There are indications that conventional ovarian stimulation affects the embryo quality as assessed by morphology as well as the chromosomal constitution of the embryos (Munne et al., 1997; Katz-Jaffe et al., 2005; Baart et al., 2007). This phenomenon could be the result of interference with the natural selection of good quality oocytes or the exposure of growing follicles to the potentially negative effects of ovarian stimulation.

Supportive evidence regarding the potentially negative effects of ovarian stimulation comes from several human and animal studies reporting detrimental effects of ovarian stimulation on oocyte and embryo quality. Increased incidences of morphology and chromosomal abnormalities have been observed in oocytes after exposure to high doses of gonadotropins during *in vitro* maturation of oocytes (Eppig et al., 1998; van Blerkom and Davis, 2001; Roberts et al., 2005). Ovarian hyperstimulation and concurrent high estradiol levels were shown to have a negative impact on the developmental and implantation potential of embryos (Valbuena et al., 1999; Ertzeid and Storeng, 2001; Van der Auwera and D'Hooghe, 2001) as well as the chromosomal constitution of embryos (Katz-Jaffe et al., 2005). Moreover, ovarian stimulation might disrupt mechanisms involved in maintaining accurate chromosome segregation (Munne et al., 1997; Hodges et al., 2002). A randomized trial concerning the chromosomal analysis of human embryos following mild ovarian stimulation for IVF showed a significantly higher proportion of euploid embryos compared to conventional ovarian stimulation, suggesting that through maximal stimulation the surplus of obtained oocytes and embryos are of lower quality (Baart et al., 2007) (Figure 8).

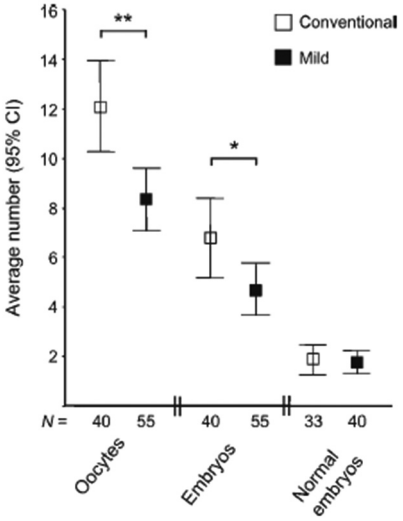

Figure 8 Oocyte and embryo yield and embryos successfully biopsied and diagnosed by fluorescent in-situ hybridization (FISH) as chromosomally normal on the basis of FISH results form one cell following conventional and mild stimulation (From: Baart et al., 2007).

These findings appear to conflict with the general assumption that an increased quantity of oocytes leads to better outcomes (Devreker et al., 1999). However, this effect is mainly caused by the poor outcomes observed in patients with a poor response following standard stimulation. In fact, in most of the studies investigating the relationship between oocyte numbers and pregnancy rates, the positive effect on pregnancy rates with a growing number of oocytes eventually levels off (Devreker et al., 1999; Melie et al., 2003; Kok et al., 2006) or falls (Van der Gaast et al., 2006).

Potential disadvantage of the development of lower numbers of oocytes might be the reduction of supernumerary embryos for cryopreservation to transfer in subsequent (unstimulated) cycles. However, the true added value of cryopreservation programmes is still under debate (Jones et al., 1997) and the resulting increase of patient specific pregnancy rates (i.e. increased chance of an ongoing pregnancy from a frozen transfer after a failed fresh transfer) may be less than generally perceived. In view of the many legal and ethical issues relating to cryopreserved embryos, the possibility of cryopreserved supernumerary oocytes rather than embryos has been proposed (Jain and Paulson, 2006).

LUTEAL FUNCTION AND ENDOMETRIAL RECEPTIVITY

Ovarian stimulation affects luteal phase function and alters endometrial receptivity. This negative effect of ovarian stimulation has largely been held responsible for the impaired embryo implantation compared to natural cycles utilized in ovum dona-

tion (Paulson et al., 1990). The exact pathophysiology remains unclear although supraphysiological steroid levels are widely held responsible (Beckers et al., 2003) (for review Fauser and Devroey 2003; Strowitzki et al., 2006). A negative influence of supraphysiological estradiol levels has been clearly demonstrated; estradiol levels > 3,000 pg/ml on the day of hCG administration resulted in reduced implantation rates independent of embryo quality (Simon et al., 1995). Mild stimulation approaches, aiming at a more physiological response, might therefore improve embryo implantation rates (Devroey et al., 2004). Indeed, increased pregnancy rates have been observed following a FSH step-down regimen for high response patients when estradiol levels were decreased during the pre-implantation period (Simon et al., 1998).

HEALTH ECONOMICS CONSIDERATIONS

Due to the limited use of ovarian stimulating medication and the prevention of complications such as OHSS, the per cycle costs of mild stimulation IVF will be substantially lower than conventional stimulation approaches. It was calculated that the mean cost for the treatment of OHSS ranged from 400 to 553 dollar per day depending to the treatment strategy applied, and over six thousand dollar when the cycle was cancelled (Wittenberger et al., 2005). However, in order to analyse the cost effectiveness of mild stimulation, the total cost per live birth should be calculated. Besides the costs for medication, medical consultations and visits, laboratory charges (general, hormone and embryology), ultrasound procedures, IVF procedures (oocyte retrieval and embryo transfer), hospital charges, nurse coordinator costs, administrative charges, fees for anaesthesia, costs for complications, travel expenses and lost wages should all be taken into account (Collins, 2002). Depending on the embryo transfer policy applied perinatal and neonatal costs might also contribute significantly to the total costs per treatment (Fiddelers et al., 2007).

Up to the present, there are limited numbers of studies that (properly) analyse the cost effectivity of various mild stimulation approaches. Studies evaluating natural versus stimulated IVF showed that natural cycle IVF was more cost-effective than stimulated cycles per live birth (Daya et al., 1995; Nargund et al., 2001). However, it is not clear what aspects were included in these cost estimates. Costs per patient after up to three cycles of modified natural cycle IVF were found to be higher than one cycle of conventional stimulation (Pelinck et al., 2005). In this analysis, costs of cryopreservation and OHSS were not taken into account and data for the conventional stimulation protocol were derived from the literature. CC stimulated cycles with GnRH antagonist co-treatment was not found to be cost effective compared to a GnRH agonist flare protocol (Kovacs et al., 2004) or a long GnRH agonist protocol (Mansour et al., 2003). However, the first study only included medication costs and although the latter included medical and treatment costs, potential additional costs were excluded. In a prospective randomized trial on the efficacy IVF with mild ovarian stimulation in combination with SET with a long GnRH agonist co-treatment

conventional stimulation protocol in combination with double embryo transfer the costs and clinical outcome after 12 months of treatment were compared (Heijnen et al., 2007). This study showed that the costs for IVF per year of treatment were comparable between both stimulation protocols applied, while the costs for the pregnancy and neonatal period were significantly lower following mild stimulation and SET, which will be mostly the consequence of the reduction of twin pregnancies.

PSYCHOLOGICAL BURDEN

Apart from health risks, emotional stress should be considered an important negative side-effect associated with IVF treatment. The stress of infertility treatment has been ranked second to that involving the death of a family member or divorce by couples undergoing IVF treatment (Freeman et al., 1985; Baram et al., 1988). Conflicting evidence has been reporting the effect of IVF treatment on marital relationships. Although some studies observe a high incidence of marital stress and divorces (Wang et al., 2007), other studies have not confirmed this (Pinborg et al., 2003; Holter et al., 2006; Repokari et al., 2007). Mild ovarian stimulation, aiming to provide a shorter and more patient friendly treatment with a reduction in complications, might decrease IVF treatment related stress. Following minimal ovarian stimulation IVF (unstimulated cycle or CC), patients reported fewer side effects and stress related to hormone treatment and cycle cancellation compared with conventional stimulation (Hojgaard et al., 2001). Furthermore, mild ovarian stimulation was found to lead to a significant reduction in drop-out rate per cycle despite a similar level of overall discomfort compared to a standard treatment strategy (Heijnen et al., 2007). Especially in women with high stress levels before IVF, treatment related stress was significantly reduced during mild stimulation (de Klerk et al., 2007).

Besides a direct negative effect on the chance of conceiving (Verhaak et al., 2001; Smeenk et al., 2001 and 2005; Cwikel et al., 2004), treatment related stress was found to be the most important reason for patients dropping out of IVF treatment (Olivius et al., 2004). The early drop-out from treatment deprives the couple an optimal cumulative chance of achieving pregnancy, and therefore also impacts on the success of the respective IVF program. Average drop-out rates well above 50% have frequently been reported in literature (Callan et al., 1988; Tan et al., 1992; Olivius et al., 2002; Schroder et al., 2004; Land et al., 1997). Mild stimulation might therefore have a positive impact on cumulative treatment success rates as it positively affects the chance patients are willing to continue treatment following a failed attempt.

CURRENT STATUS AND FUTURE DEVELOPMENTS

Up to now, studies on alternative (milder) stimulation protocols have been limited by the relative small numbers of patients included, poor methodological quality (there are few randomized studies) and the use of surrogate endpoints such as the number

of oocytes or embryos. Furthermore, many studies on alternative stimulation strategies have involved poor prognosis patients, while it is mainly the young patients with no signs of ovarian aging that have the highest risk of complications of ovarian stimulation. Therefore, especially young women should be included in studies evaluating the possible benefits of mild stimulation. Even so, most studies in older patients failed to show a benefit of a high dose stimulation regimen over milder forms of ovarian stimulation. As most of these patients will fail to respond well to any type of ovarian stimulation, a mild stimulation protocol seems to be preferred for economical reasons and the assumed beneficial effect on embryo and endometrial quality.

Increased awareness among patients and their physicians of the complications associated with ovarian stimulation will facilitate the acceptance of milder stimulation (Edwards, 2007; Nargund and Frydman, 2007; Nargund et al., 2007; Pennings and Ombelet, 2007; Ubaldi et al., 2007). Crucial to the success of implementing mild ovarian stimulation strategies will be achieving a consensus as to how complications of IVF treatment are reported in literature. By reporting the incidence and severity of complications, the number of treatment days, medication used, costs, patient discomfort and drop-outs, awareness of the price paid for currently applied stimulation protocols will increase. Furthermore, a reappraisal of the current paradigm of maximizing treatment outcomes per cycle at all costs is needed. The competition for patients, desire for high fertility rates and the need for quick results driving the majority of fertility practices in some countries, are factors that could cause resistance by physicians towards the use of mild ovarian stimulation, while in the long term (cumulative) mild stimulation cycles might lead to a safer and more cost-effective treatment outcome.

Eventually, ovarian stimulation might be replaced by in-vitro maturation of oocytes. This technique aims at the in vitro culture of follicles after retrieval immature oocytes from unstimulated or minimally stimulated cycles. Consequently, it does not require the use of (large doses of) gonadotrophins for in vivo follicular growth and oocyte maturation (Barnes et al., 1995 and 1996; Oktay et al., 1998). However, series published to date are small and even with the help of limited ovarian stimulation and hCG for oocyte maturation, pregnancy rates of 30% have only been obtained by transplanting multiple embryos, because implantation rates remain 10-15%. Insufficient data are currently available from follow-up studies to assess the safety of this technique for offspring (Chian et al., 1999 and 2000; Rao and Tan, 2005).

CONCLUSIONS

Evidence in favour of mild ovarian stimulation for IVF is accumulated in recent literature. However, the data discussed in this review do not allow concluding what is the most optimal mild ovarian stimulation protocol. Increased understanding of the physiology of follicle development has lead to more individualized stimulation approaches. The development of at least more than one dominant follicle appears to be necessary to produce competing treatment outcomes compared to standard stimulation protocol. However, a reduction in medication is feasible when the initiation of exogenous FSH is postponed until the end of the FSH window, or when FSH is being replaced by hCG of LH when ongoing follicle growth is no longer dependent on FSH. Furthermore, by suppressing endogenous negative feedback mechanisms for the natural selection of a single dominant follicle by CC or aromatase inhibitors, the use of gonadotrophins can be limited. A combination of these strategies might lead to an even further reduction in medication (days) needed, while it may assist in the selection of a homogenous cohort of good quality and mature oocytes and achieving a more physiological response. The way to the implementation of mild stimulation into standard clinical practice appears to be open; however, more studies are needed to further elaborate the various mild stimulation approaches.

CHAPTER 3
PREDICTION OF ONGOING PREGNANCY FOLLOWING SINGLE EMBRYO TRANSFER

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INTRODUCTION

In vitro fertilisation (IVF) and intracytoplasmatic sperm injection (ICSI) are widely accepted as effective treatments for most causes of infertility. With improving success rates, attention has shifted more towards the adverse effects of IVF treatment, which are mainly associated with profound ovarian stimulation and multiple pregnancies (Fauser et al., 2005; Macklon et al., 2006). Milder stimulation protocols and elective single embryo transfer (SET) have been proposed as a means of reducing such complications (Fauser et al., 2005). The challenge is to include these modifications in routine IVF without compromising pregnancy rates.

The introduction of gonadotropin-releasing hormone (GnRH) antagonists into clinical practice along with a greater understanding of the process of follicle recruitment and dominant follicle selection in normo-ovulatory cycles led to new opportunities for developing mild ovarian stimulation protocols (Fauser and van Heusden, 1997). The application of a GnRH antagonists to prevent a premature luteinizing hormone (LH) rise allows to restrict its administration to the mid to late follicular phase only. Under those circumstances the endogenous follicle-stimulating hormone (FSH) rise can be utilized in concert with exogenous FSH administration (Fauser et al., 1999).

In spite of a potentially increased risk for cancellation due to low response, mild stimulation protocols are likely to reduce patient discomfort associated with pituitary down regulation, lengthy stimulation and follicle aspiration procedures (Hohmann et al., 2003). A recent large randomized trial performed by our group showed that mild ovarian stimulation together with SET in IVF results in similar cumulative term live birth rates over 1 year of treatment compared to standard stimulation with

two embryo transfer, while significantly reducing multiple pregnancy rates, patient discomfort, and overall costs (Heijnen et al., 2007).

However, SET in an unselected population leads to a reduction of pregnancy rates per transfer (van Montfoort et al., 2006; Lukassen et al., 2005). In order to improve overall pregnancy rates, evidence based strategies to identify patients and clinical conditions which qualify for the use of SET are required. In line with this, the American Society for Reproductive Medicine recently published new guidelines on how a more individualized embryo transfer policy could help to reduce the incidence of higher-order multiple gestation (The Practice Committee of the American Society for Reproductive Medicine, 2006).

Most available evidence regarding when to perform SET is derived from retrospective studies and focus on the identification of risk factors for multiple pregnancy when more than one embryo is transferred (Templeton and Morris, 1998; Hunault et al., 2002; Roseboom et al., 1995; van Royen et al., 1999; Lee et al., 2006; Templeton et al., 1996). Important predictors for multiple pregnancy have been shown to include embryo quality (van Royen et al., 1999 and 2001; Hunault et al., 2002; Thurin et al., 2005; Staessen et al., 1992; Giorgetti et al., 1995; Ziebe et al., 1997; De Neubourg et al., 2004; Lee et al., 2006; Rooseboom et al., 1995) and female age (Templeton and Morris, 1996; Strandell et al., 2000; Hunault et al., 2002; De Neubourg et al., 2004; Rooseboom et al., 1995). Other variables such as first treatment cycle (Thurin et al., 2005; Templeton and Morris, 1996; Strandell et al., 2000), endometrial thickness (Richter et al., 2007), ovarian reserve test outcome (Hendriks et al., 2005), body weight (Nichols et al., 2002), cause of infertility (Rooseboom et al., 1995) and IVF as the method of fertilization (Thurin et al., 2005) have also been proposed.

There is accumulating evidence that the use of a mild stimulation protocol per se might affect the process of implantation. High estradiol levels, as a consequence of ovarian stimulation, appear to have detrimental effects on the endometrium, corpus luteum function and embryo quality (Devroey et al., 2004; Tarlatzis et al., 2006). Mild ovarian stimulation might reduce these adverse effects and improve implantation chances. The purpose of this study was to develop a prognostic model for ongoing pregnancy exclusively in SET cycles following mild ovarian stimulation for IVF.

MATERIALS AND METHODS

STUDY DESIGN

Data were derived from the mild stimulation arm of a randomized controlled trial (RCT) on effectiveness of IVF treatment strategies (Heijnen et al., 2007). The study was approved by the local ethics review board of both participating centers. In this study, infertile patients with a regular indication for IVF or intracytoplasmic

sperm injection (ICSI) who attended the Erasmus Medical Center (Rotterdam, the Netherlands) or the University Medical Center Utrecht (Utrecht, the Netherlands) were invited to participate. Participants were less than 38 years of age, had a regular menstrual cycle (25-35 days) and a body mass index (BMI) between 18-28 kg/m^2. Only couples with no previous IVF treatment or a healthy born child after a previous IVF treatment were included. Study design and clinical outcomes of this RCT been have reported recently (Heijnen et al., 2007, Eijkemans et al., 2006). Data of the first (fresh) treatment cycle that resulted in an embryo transfer were included in the present study.

Patients in the mild stimulation arm were treated with a fixed daily dose of 150 IU recombinant FSH (rFSH) (Gonal-F®: Serono Benelux B.V., Amsterdam, the Netherlands; or Puregon®: N.V. Organon, Oss, the Netherlands) s.c., initiated on cycle day five (CD 5 protocol). GnRH antagonist (ganirelix, Orgalutran®: N.V. Organon, 0.25 mg/day; or cetrorelix, Cetrotide®: Serono Benelux, 0.25 mg/day) was administered s.c. from the day at least one follicle with a diameter ≥ 14 mm was observed. Human chorionic gonadotropin (hCG) (Profasi®: Serono Benelux B.V.; or Pregnyl®: N.V. Organon) 10,000 IU s.c. was administered as a single bolus injection to induce final oocyte maturation, when the largest follicle had reached at least 18 mm in diameter and at least one additional follicle > 15 mm had been observed. Oocyte retrieval and fertilization "in vitro" was performed according to standard procedures as described previously (Kastrop et al., 1999; Huisman et al., 2000).

In determining which embryos were suitable for day 3 transfer, parameters such as fragmentation and the number of blastomeres were taken into consideration, whereas for day 4 transfer, compaction, cavitation, and expansion were taken into consideration. The embryo that demonstrated the most advanced developmental stage and best morphology was selected for transfer. Well-developed supernumerary embryos were cryopreserved. Standard luteal phase support in the form of intra-vaginal progesterone (Progestan®: N.V. Organon) 600 mg/day was given from the day of oocyte retrieval until a urine pregnancy test was performed 18 days later.

For patients with less than five oocytes at follicle aspiration the stimulation protocol was adjusted in a subsequent treatment cycle so that exogenous FSH was initiated on cycle day two (CD 2 protocol) while the FSH dosage remained unchanged.

DATA ANALYSIS

The primary endpoint of this study was ongoing pregnancy defined as the presence of fetal cardiac activity on ultrasonography at 9 weeks gestational age.

In order to identify factors associated with ongoing pregnancy in elective SET cycles, patient characteristics, treatment and embryo quality related variables were studied. Only patients with at least two embryos suitable for transfer were included in the analysis. Patient characteristics that were considered as potential predictive

factors included female age, previous pregnancy, cause of infertility, cycle length and BMI. Variables related to the treatment that were considered as potential predictive factors included fertilization method (IVF or ICSI), number of oocytes retrieved, proportion of fertilized oocytes, duration of stimulation, amount of rFSH per retrieved oocyte and endometrial thickness at the day of hCG injection. Factors related to embryo quality were day of transfer, grade of fragmentation (grade 1 (less than 10 %), grade 2 (10-50%) and grade 3 (more than 50 %)) whether there were supernumerary embryos available for cryopreservation and whether there was a top quality embryo transferred. Top quality embryos were defined as having less than 10 percent fragmentation and either a Morula stage embryo on day 4 or at least 7 cells on day 3 after follicle aspiration.

Multivariate logistic regression analysis was performed with a backward elimination procedure. A p-value < 0.15 was used as a criterion for exclusion. The predictive ability of the model was assessed by determining the area under the receiver-operating characteristics (ROC) curve (AUC). Missing data were imputed, when necessary.

To assess the amount of overfitting of the created model, internal validation was performed with bootstrapping, a statistical technique to create comparable populations. We bootstrapped 200 times. In each of these 200 new data sets, the same multivariate logistic regression analysis with backward elimination was performed, and the resulting model was tested on the original data. In this way the amount of overfitting can be assessed, expressed as a shrinkage factor. The shrinkage factor should be taken into account when applying the model in clinical practice (Van Houwelingen and le Cessie, 1990; Harrell Jr et al., 1996).

To asses the feasibility for clinical application, the model was applied on our own dataset to estimate the number of couples with a significantly reduced chance of ongoing pregnancy per elective SET. The cut-off chance was arbitrarily set at twenty percent as this is about half of the average ongoing pregnancy rate per embryo transfer after SET in a selected group of patients (Gerris et al., 1999) and double embryo transfer in an unselected population. At this cut-off the model was tested against the observed outcomes and classical test characteristics were calculated. Finally, calculations were made to estimate the overall improvement of pregnancy rates by a change in embryo transfer policy for patients with a predicted chance of ongoing pregnancy under the cut-off value.

Comparisons of outcome measures between subgroups were performed using the One-Way ANOVA for continuous data and the $\chi 2$ -test for binary variables unless stated otherwise. The analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA, 1999) and S-plus 2000 (Insightful Corp., Seattle, WA, USA).

RESULTS

Of the 201 women who were randomized to mild ovarian stimulation, 195 started treatment, 6 were excluded from analysis owing to violation of the protocol (no mild stimulation protocol or two embryo transfer instead of SET), 177 resulted in embryo transfer and 152 women, with an elective SET, could be included in the analysis. Forty two (28%) embryo transfers resulted in an ongoing pregnancy of which 40 (95%) resulted in a live birth (Figure 1).

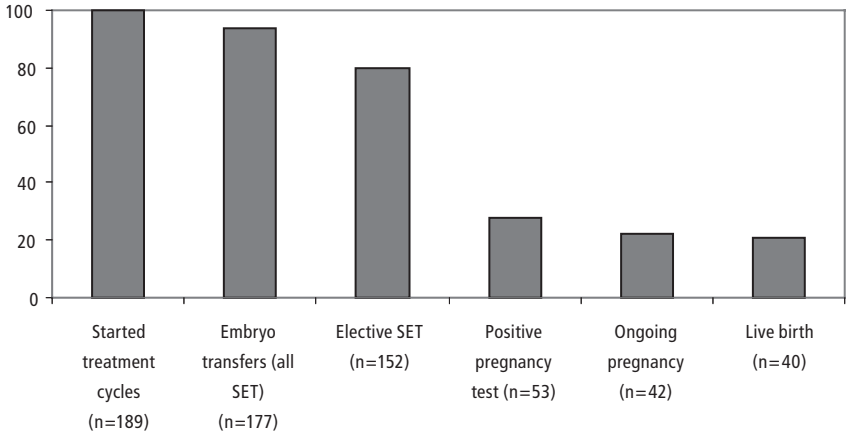

Figure 1 Cumulative allocation of included patients.
SET = single-embryo transfer.

One hundred and six patients were included in their first treatment cycle, 33 in their second, eleven in their third treatment cycle and two patients in their fourth cycle. Hundred and nineteen patients were treated with the CD 5 protocol and 33 (22%) with the CD 2 protocol. In violation of the protocol, the rFSH dosage was adjusted in ten of the included patients. Six patients were treated with less than 150 IU per day (75-112,5 IU) and four patients were treated with an increased dosage (range 200-225 IU). Univariable analysis of patient and stimulation cycle characteristics, including age, BMI, duration of stimulation, number of oocytes retrieved and embryo quality related variables showed no significant differences ($p < 0.05$) between patients with and without ongoing pregnancy.

Table 1 Multivariate analysis for predictors of ongoing pregnancy in the mild ovarian stimulation/elective single-embryo transfer protocol, showing the odds ratio of the included predictors and the discriminatory ability of the model expressed by the area under the ROC curve (AUC). The threshold level of significance for inclusion in the prediction model was p<0.15.

	Odds Ratio (95% Confidence Interval)[b]	Cumulative AUC	p-value
BMI	0.89 (0.76, 1.03)	0.59	0.108
Total amount of rFSH used[a]	0.92 (0.83, 1.03)	0.63	0.146
Number of oocytes	0.93 (0.85, 1.01)	0.67	0.077
Top quality embryo availability	2.18 (0.93, 5.09)	0.68	0.072

[a] Calculated per units of 75 IU

[b] Formula of the model:
*1 / (1 + Exp [- (3.16 - 0.12*BMI - 0.081*[# Ampoules recFSH] - 0.075*[# Oocytes] + 0.78*[Top embryo (yes=1/no=0))]*
Formula after correction:
*1 / (1 + Exp [- (1.25 - 0.064*BMI - 0.043*[# Ampoules recFSH] - 0.040*[# Oocytes] + 0.41*[Top embryo (yes=1/no=0))]*

Following multivariate analysis, predictors of ongoing pregnancy were BMI, total rFSH used, the number of retrieved oocytes and the availability of a top quality embryo for transfer (Table 1). These features were therefore included in the predictive model for ongoing pregnancy. The predictive ability of the model measured by the area under the ROC curve was 0.68. (Figure 2) After correction for overfitting and optimism by bootstrapping the area under the ROC curve of the model was 0.60.

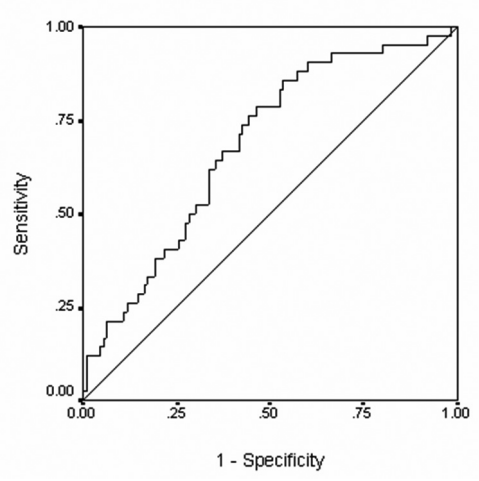

Figure 2 Receiver operating characteristic (ROC) curve of the prediction model for the occurrence of ongoing pregnancy after elective single-embryo transfer following mild ovarian stimulation for IVF. The area under the final ROC curve is 0.68.

The model's clinical usefulness was tested by the ability of the prediction model to correctly identify women with no ongoing pregnancy after elective SET. At the arbitrary probability cut-off of 20%, the test performance in predicting patients with no ongoing pregnancy on our own data showed a sensitivity of 37% and specificity of 90%. In our study population, 41 patients would have been correctly identified by the model as having a low chance of an ongoing pregnancy, four patients with an ongoing pregnancy would have been wrongly identified as having a low chance of success. By such testing, 37% of all non pregnant cases would be identified, while less than 10% of the ongoing pregnant cases would be wrongly assigned by the model to have a low pregnancy chance and hence would unnecessarily receive 2 embryos.

The 45 patients with a predicted chance under the cut-off value had an average chance of ongoing pregnancy of 14% according to the model. Assuming that the chance of implantation for both embryos is independent, the average pregnancy rate for this group after double embryo transfer would be 26%, $(2*(0.14 \times 0.86) + (0.14)^2 = 0.26)$. This would mean 11 extra pregnancies, with a low multiple pregnancy rate $(0.14^2 = 2\%)$.

When the inclusion of patients was restricted to first cycle data, the variable "top quality embryo availability' was replaced by "availability of an embryo for cryopreservation". The other factors in the model did not change and the area under the ROC curve remained 0.68. However, due to the reduction of patients the correction factor for overfitting was higher than for the presented model (data not shown).

DISCUSSION

To our knowledge this is the first published study predicting ongoing pregnancy in mild stimulation IVF exclusively on SET data. The use of SET data allows the fate of a single embryo, and its role in determining successful implantation to be studied. When more than one embryo is transferred assumptions must be made as to which embryo implanted and regarding possible interactions between embryos.

In contrast to most previous studies, implantation was not chosen as the outcome parameter of the model. The purpose of the prediction model was to provide an evidence based strategy to select patients with good pregnancy prospects after SET. Because the transfer of more than one embryo could partly compensate for early pregnancy losses, the prediction of ongoing pregnancy is therefore clinically more relevant. In our opinion, this should therefore be the endpoint for models used to predict which patients will not be optimally treated by SET.

In this study, ongoing pregnancy could be predicted on the basis of a combination of the woman's BMI, the number of days with ovarian stimulation (or units of rFSH needed), the number of retrieved oocytes and the availability of a top quality embryo for transfer. Conflicting data have been published regarding relationship between BMI and the chance of ongoing pregnancy following fertility treatment (Lashen et

al., 1999; Wang et al., 2000; Nichols et al., 2003; Fedorcsak et al., 2004; Dokras et al., 2006; Dechaud et al., 2006). Although the prevalence of obesity in infertile women is high, there is no conclusive evidence that extremes of weight are associated with a low rate of pregnancy in women receiving assisted reproduction treatment. Mechanisms through which body mass may affect reproduction that have been cited include menstrual disturbance and anovulation, but these problems can be overcome through assisted reproduction treatment. Alternatively, a high BMI could cause negative effects on steroid metabolism and altered secretion and action of various hormones such as insulin, leptin, resistin, ghrelin or adiponectin. These alterations might affect follicle growth, embryo development, placentation and implantation (Poretsky et al., 1999; Moschos et al., 2002; Pasquali et al., 2003).

The inverse relationship between total gonadotrophin dose used and IVF success has previously been observed in studies predicting the chance of pregnancy following IVF (Stadtmauer et al., 1994; Strandell et al., 2000). In the study of Strandell et al. (2000) the total FSH dosage needed was negatively correlated with the chance of pregnancy and strongly associated with age. This finding suggests an association between ovarian aging and total FSH dose required. It would thereby explain the negative association with the chance of ongoing pregnancy and the absence of female age as predictor in our model. However, others found that the association between higher total dose of FSH required per cycle and lower pregnancy rates was independent of age or the number of eggs retrieved (Stadtmauer et al., 1994).

The finding of a relationship between low numbers of oocytes and a good chance of ongoing pregnancy is a consistent observation in mild ovarian stimulation where exogenous FSH is initiated on cycle day 5 (Hohmann et al., 2003; Baart et al., 2007). This is in sharp contrast to the well known relationship between a poor response and a poor clinical outcome in conventional stimulation protocols. This has led to the hypothesis that in mild stimulation a low number of oocytes is not associated with poor outcomes but aids in selecting the better oocytes in the FSH sensitive cohort (Hohmann et al., 2003; Baart et al., 2007). The findings of the present study are consistent with this hypothesis.

Finally, the availability of a top quality embryo was also positively correlated with an ongoing pregnancy. The finding of a relationship between embryo quality and pregnancy is in accordance with most previous studies on the prediction of pregnancy. However, these studies show inconsistency regarding the method to score the embryo. Conclusive evidence is lacking on the best method to select the embryo. Our scoring method (van Kooij et al., 1996) resembles the scoring method that had the most predictive ability in three robust studies on the relationship between embryo quality and implantation except for the scoring of multinucleated blastomeres (van Royen et al., 1999 and 2001; Desai et al., 2000). The predictive value and reproducibility of the blastomeres scoring has been debated by others (Hnida et al., 2005; Arce et al., 2006).

In contrast to many previous studies, neither age nor a history of a previous successful pregnancy were found to be independent predictors of ongoing pregnancy, and were therefore not included in the model. The absence of female age from the prediction model could be the result of the appearance of total FSH in the model and might be partly the result of the age restriction in the inclusion criteria for the study. Additionally, inclusion in the model was restricted to patients with at least two embryos available for transfer after mild stimulation. This might already have led to the selection of women without signs of ovarian aging. Although the sample size was too small to show a significant difference between women included in the analysis and those who did not meet the criteria for inclusion, there was indeed a tendency towards a higher age in the latter (data not shown). We have no straight-forward explanation for the absence of previous successful pregnancy in our model but it is likely to be the result of the small sample size.

As was previously mentioned, the use of a mild stimulation protocol is likely to affect the process of implantation. Besides the possibility of improved oocyte selection, lower estradiol levels could improve endometrial receptivity and corpus luteum function (Devroey et al, 2004). In addition, the chromosomal constitution of embryos may also be affected by ovarian stimulation regimens (Munne et al., 2003). In a recent randomized study, mild stimulation was found to be associated with a significantly higher proportion of chromosomally normal embryos compared to conventional ovarian stimulation (Baart et al., 2007).

Due to the high specificity of the model the number of patients that will be unnecessarily exposed to double embryo transfer is limited; only four patients would unnecessarily receive two embryos in our population. Consequently, the model is, in our opinion, clinically useful although the area under the ROC curve is modest. By transferring two instead of one embryo in the 45 patients with a predicted chance of ongoing pregnancy below 20% per ET, the ongoing pregnancy rate would nearly double from 14% to 26%. In other words, adjusting the embryo transfer according to our model could have lead to 11 extra pregnancies at the cost of few twin pregnancies in our population.

Before the model can be used as a standard method to select patients for elective SET in mild stimulation IVF treatment, the model should be corrected for overfitting and optimism and external validation should be performed (Mol et al., 2003). Although external validation of some prediction models in the field of subfertility have resulted in a lower predictive performance (Altman and Royston, 2000; Stolwijk et al., 1998), others have demonstrated a good predictive potential in other populations (Hunault et al., 2007).

In conclusion, the developed prediction model for ongoing pregnancy provides an evidence based strategy for guidance for when to perform SET in a mild stimulation protocol. After correction for overfitting and optimism and external validation, our model can be used in counseling couples for single or double embryo transfer.

Whether the model is useful for standard stimulation protocols needs to be established.

CHAPTER 4
A LOW NUMBER OF OOCYTES RETRIEVED FOLLOWING MILD OVARIAN STIMULATION FOR IVF IS NOT A POOR RESPONSE

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(Submitted)

INTRODUCTION

Ovarian stimulation is a key component of assisted reproductive treatment. Since the early days of in vitro fertilisation (IVF) ovarian stimulation has been applied to compensate for inefficiencies in the IVF procedure by increasing the number of oocytes retrieved enabling the selection of the best quality embryos for transfer (Fauser et al., 2005). Currently, long gonadotropin-releasing hormone (GnRH) agonist pituitary suppression combined with high doses of exogenous follicle-stimulating hormone (FSH) is the most frequently used stimulation protocol (Macklon et al., 2006). This conventional ovarian stimulation regimen is expensive, complex and associated with significant side-effects and stress. There is increasing interest in the application of milder stimulation protocols which aim to render IVF treatment more patient friendly, reduce the chance for complication (especially ovarian hyperstimulation syndrome) and lower cost (Fauser et al., 1999) The availability of GnRH antagonists has allowed for the clinical development of milder ovarian stimulation protocols involving subtle interference with single dominant follicle selection (Fauser and van Heusden, 1997; Tarlatzis et al., 2006).

Following conventional ovarian stimulation, a low number of oocytes after follicle aspiration is associated with a poor clinical outcome and is believed to represent ovarian aging (Beckers et al., 2002; Tarlatzis et al., 2003). Despite the lack of consensus regarding the exact definition of poor response (Klinkert et al., 2004), the relationship between the number of oocytes retrieved and success rates is well established in conventional IVF (Keay et al., 1997). In contrast to the poor outcomes observed following the retrieval of a low number of oocytes in conventional ovarian stimulation, recent studies suggest that a similar ovarian response following mild

stimulation is associated with a distinctly higher chance of conceiving (Hohmann et al., 2003). This has led to the contention that a low number of oocytes obtained following mild stimulation represents a physiological response to the subtle interference with single dominant follicle selection and not a pathological reduction in ovarian response associated with ovarian aging. The clinical implications of low oocyte numbers following mild stimulation may therefore be quite different from the poor ovarian response observed in conventional GnRH agonist suppression cycles. A mild treatment strategy in IVF has recently been shown to result in similar term live birth rates as conventional treatment within one year of treatment (Heijnen et al., 2007).

The purpose of the present study was to investigate the clinical significance of the retrieval of low numbers of oocytes following mild stimulation of IVF. The embryo implantation rate in relation to retrieved oocyte numbers was studied in a combined data set of three recently published randomized trials comparing mild versus conventional ovarian stimulation for IVF (Hohmann et al., 2003; Heijnen et al., 2007; Baart et al., 2007).

MATERIALS AND METHODS

SUBJECTS

This study analyses outcome data from three previously published randomized trials comparing the efficacy of a novel mild ovarian stimulation regimen (involving late follicular phase administration of the GnRH antagonist) for IVF with a conventional long GnRH agonist co-treatment stimulation protocol (Hohmann et al., 2003; Heijnen et al., 2007; Baart et al., 2007). Detailed information on the design and clinical outcomes of these individual studies has been described previously (Heijnen et al., 2007). All studies were approved by the local ethics review boards of participating centers and written informed consent was obtained from each participant.

Inclusion criteria for patient characteristics were comparable for the three studies. All studies included infertile patients with a regular indication for IVF or intracytoplasmic sperm injection (ICSI), age below 38 years, regular menstrual cycles (mean 25-35 days), body mass index (BMI) between 18-29 kg/m^2 and no relevant systemic disease, severe endometriosis and uterine or ovarian abnormalities. One study focussing on clinical outcome excluded patients with a partner with a sperm count ≤ 5 million progressively motile sperm/ ml (Baart et al., 2007).

STUDY DESIGN

In all three studies, the mild stimulation protocol consisted of a low dose of recombinant (r)FSH (Gonal-F®: Serono Benelux B.V., Amsterdam, the Netherlands; or Puregon®: N.V. Organon, Oss, the Netherlands) s.c. initiated on cycle day 5,

combined with a GnRH antagonist (ganirelix, Orgalutran®: N.V. Organon, 0.25 mg/day; or Cetrorelix, Cetrotide®: Serono Benelux, 0.25 mg/day) s.c. initiated when at least 1 follicle ≥ 14 mm was observed. The conventional stimulation protocol consisted of a standard long suppression protocol with a GnRH agonist (Leuproline, Lucrin®: Abbott B.V., Amstelveen, the Netherlands, 0.2 mg/day; or triptoreline, Decapeptyl®: Ferring B.V., Hoofddorp, the Netherlands, 0.1 mg/day) s.c. for approximately two weeks starting during the mid-luteal phase of the pre-treatment cycle.

All patients in the mild stimulation group were treated with a fixed daily s.c. dose of 150 IU rFSH. In the conventional stimulation group, a fixed daily dose of 150-225 IU rFSH s.c. was administered. In cycles resulting in the development of less than 2 (Heijnen et al., 2007, Baart et al., 2007) or 4 (Hohmann et al., 2003) pre-ovulatory follicles no oocyte retrieval was performed. When the largest follicle had reached at least 18 mm in diameter and at least one additional follicle > 15 mm had been observed, human chorionic gonadotropin (hCG) (Profasi®: Serono Benelux B.V.; or Pregnyl®: N.V. Organon) 10,000 IU s.c. was administered to induce final oocyte maturation. Oocyte retrieval was performed 35-36h thereafter. Oocyte retrieval and fertilization in-vitro was performed according to standard procedures as described previously (Kastrop et al., 1999; Huisman et al., 2000). Embryos were classified according to their developmental stage (advanced, appropriate or retarded) and morphology (extent of fragmentation). Luteal phase support in the form of intravaginal progesterone (Progestan®: N.V. Organon) 600 mg/day was given from the day of oocyte retrieval until a urine pregnancy test was performed 18 days later. When good quality excess embryos were available, they were cryopreserved and transferred in a subsequent unstimulated cycle, according to standard procedures (Heijnen et al., 2007).

Embryos were transferred on day 3 or 4 (Heijnen et al., 2007), day 3, 4 or 5 (Hohmann et al., 2003) or day 4 (Baart et al., 2007) after oocyte retrieval. In one study the number of embryos to be transferred was related to the ovarian simulation protocol used (i.e. in the conventional stimulation protocol two embryos were transferred, in the mild protocol only one embryo was transferred) (Heijnen et al., 2007). In the other two studies a maximum of two embryos was transferred independent of the ovarian stimulation protocol used.

In one study preimplantation genetic screening (PGS) of embryos was also performed (Baart et al., 2007). Embryos were biopsied on day three when at least six blastomeres were present. One or two cells were removed for a fluorescence in situ hybridization (FISH) procedure. Detailed information on the process of biopsy, fixation and PGS has been described previously (Baart et al., 2005 and 2007).

OUTCOME MEASURES

The primary outcome measure was ongoing pregnancy rate per embryo transferred (ET), defined as the number of ongoing pregnancies divided by the number of

embryos transferred. This endpoint was elected as it compensates for differences between the studies regarding the number of embryos transferred. Ongoing pregnancy was defined as the presence of fetal cardiac activity on ultrasonography at 9 weeks gestational age.

DATA ANALYSIS

Comparisons of baseline characteristics and outcomes were performed using the t-test for continuous variables and chi-square test for categorical variables. A nonparametric Mann-Whitney-Wilcoxon test was used to compare the distribution of oocytes retrieved following mild or conventional stimulation (Wilcoxon, 1945).

The ongoing pregnancy rate per ET was studied as a function of the number of retrieved oocytes by logistic regression with a flexible 4-knot spline curve. Separate curves were fitted for the mild protocol and for the conventional stimulation protocol. For this analysis, first treatment cycle data were derived from all patients with an embryo transfer. A likelihood ratio test was used to assess the interaction between protocol and the function defining the spline curve.

Multivariate analysis was performed in order to adjust for potential confounding factors such as age, BMI, duration of infertility and previous pregnancy. A robust correction for statistical dependence between embryos when more than one embryo was transferred, was performed with a generalized estimating equation (GEE) approach (Zegers et al., 1988).

P values are two-sided, and $P < 0.05$ was considered the limit of statistical significance. Analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA, 1999) and SAS 9.1 (SAS institute, Cary, NC).

RESULTS

SUBJECTS AND OOCYTE DISTRIBUTION

A total of 592 patients started treatment. Table 1 shows the number of patients in whom oocyte retrieval and embryo transfer were performed in the first treatment cycle. Baseline clinical characteristics of patients with cycles resulting in an oocyte retrieval procedure are summarized in Table 2, and no significant differences according to treatment protocol were documented.

Table 1 Number of first IVF treatment cycles (total = 592) and progress to embryo transfer for three independent trials comparing mild versus conventional ovarian stimulation.

		Started treatments	Retrieval procedures	Embryo transfers
Hohmann et al., 2003	Mild	49	32	28
	Conventional	45	38	26
Baart et al., 2007	Mild	63	56	41
	Conventional	41	40	33
Heijnen et al., 2007	Mild	201	147	124
	Conventional	193	176	160
Total	Mild	313	235	193
	Conventional	279	254	219

Data represent absolute numbers of patients

The range of the number of retrieved oocytes per patient was similar for both protocols (range 0-28). However, the distribution of oocyte numbers was significantly different following mild and conventional ovarian stimulation (median oocyte number (25-75 percentile), 6 (3-10) and 9 (6-12), respectively ($p < 0.001$)). Figure 1 shows a bar chart of the distribution of retrieved oocytes per stimulation protocol.

Table 2 Baseline characteristics of included couples (reaching oocyte retrieval) according to the ovarian stimulation protocol used for IVF (combined data from three randomized comparative studies)

Characteristics	Mild stimulation group (n=235)	Conventional stimulation group (n=254)
Age (years)	32.7 ± 3.3	32.8 ± 3.2
BMI (kg/m^2)	22.0 ± 5.5	22.5 ± 4.8
Duration of infertility (years)	3.6 ± 2.0	3.7 ± 2.2
Primary infertility	71.2 %	75.2 %
ICSI	24.3 %	23.0 %

There were no significant differences between the two groups

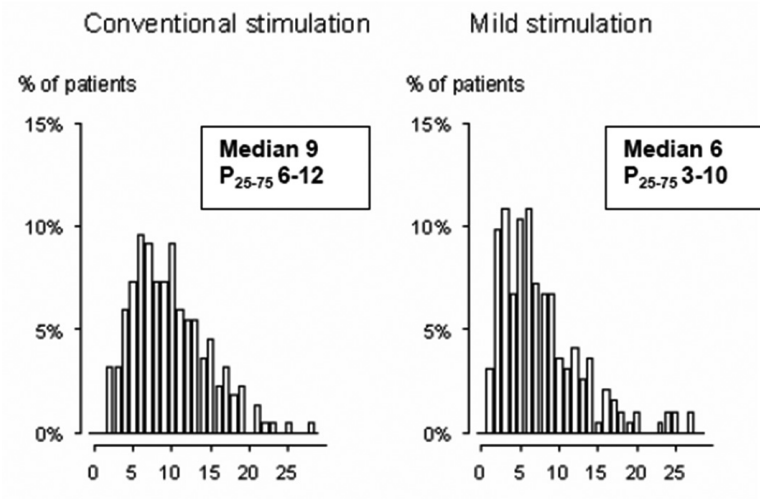

Figure 1 Distribution of the number of retrieved oocytes per patient following mild (n=235) or conventional (n=254) ovarian stimulation for IVF.
The number of patients is expressed as a percentage of total.

IMPLANTATION RATE PER RETRIEVED NUMBER OF OOCYTES

Figure 2 shows the fitted curves for the ongoing pregnancy rate per ET as a function of the number of retrieved oocytes for each stimulation protocol. This figure shows that following mild ovarian stimulation, the retrieval of a low number of oocytes is associated with the highest chance of ongoing pregnancy per ET (30.7%). Optimal outcomes following mild stimulation are observed in those oocyte retrievals where 5 oocytes were obtained (median value). The retrieval of more than five oocytes following mild stimulation is associated with a sharp decrease in implantation rates. In the conventional ovarian stimulation protocol, the highest ongoing pregnancy rate per ET (28.5%) is associated with a median of 10 oocytes. In contrast to the mild stimulation protocol, low numbers of oocytes are associated with a poor outcome. The difference in pattern of the ongoing pregnancy rate per ET as a function of the number of retrieved oocytes between the two stimulation regimens was statistically significant (p= 0.045) (Figure 2).

Correction for clustering of embryos when more than one embryo was transferred and correction for potential confounding factors including age, BMI, duration of infertility and previous pregnancy, did not change the observed difference in the relationship between oocyte number and ongoing pregnancy rate per ET for both protocols (p-value 0.047).

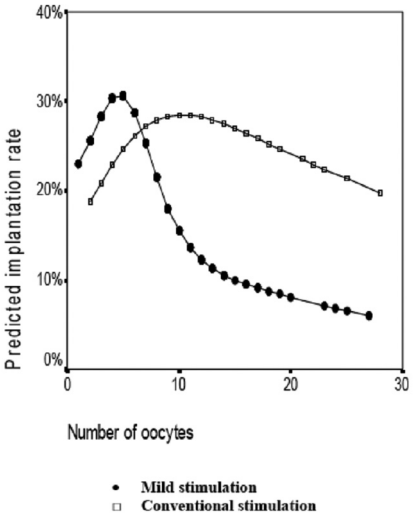

Figure 2 Ongoing pregnancy rate per embryo transferred (implantation rate) according to the number of oocytes retrieved following mild or conventional ovarian stimulation for IVF ($p = 0.045$). Curves were fitted to the observed implantation rates using a flexible 4 knotted spline function.
Black dots: Mild ovarian stimulation, Open squares: Conventional ovarian stimulation.

DISCUSSION

This study, based on the combined analysis of three randomized controlled trials, is the first to establish that the optimal number of oocytes retrieved in IVF is dependent on the stimulation regimen used. The retrieval of a modest number of oocytes following mild ovarian stimulation is associated with the optimal chance of achieving a pregnancy. These findings are in striking contrast to the well established relationship between a poor ovarian response and poor clinical outcome related to ovarian aging (Tarlatzis et al., 2003). Our current findings imply that the fear of obtaining low numbers of oocytes, which drives current practice in ovarian stimulation, is unjustified.

The observed relationship between low oocyte numbers and a high chance of achieving an ongoing pregnancy following mild stimulation suggests that when few oocytes are obtained, they are likely to represent a homogenous group of good quality oocytes. This could be the result of the subtle interference with the natural selection of good quality oocytes or the minimized exposure of growing follicles to the potentially negative effects of ovarian stimulation. In contrast to the conventional stimulation protocol with GnRH agonist co-treatment resulting in pituitary desensitisation, ovarian stimulation with GnRH antagonist co-treatment enables the

endogenous intercycle FSH rise to occur (Tarlatzis et al., 2006). Therefore, cyclic follicle recruitment and initial stages of gonadotropin-dependent growth of the recruited cohort of follicles can proceed undisturbed (Fauser and van Heusden, 1997; McGee and Hsueh, 2000). By postponing the initiation of ovarian stimulation to the mid-follicular phase, exogenous FSH may only stimulate the most mature follicles to ongoing development up to the Graafian stage giving rise to the best quality oocytes (Keay et al., 1997).

Supportive evidence regarding the potentially negative effects of ovarian stimulation comes from several human and animal studies reporting detrimental effects of ovarian stimulation on oocyte and embryo quality. Increased incidences of morphological and chromosomal abnormalities have been observed in oocytes after exposure to high doses of gonadotropins during *in vitro* maturation of oocytes (Eppig et al., 1998; van Blerkom and Davis, 2001; Roberts et al., 2005). Ovarian hyper stimulation and concurrent high estradiol levels were shown to have a negative impact on the developmental and implantation potential of embryos (Valbuena et al., 1999; Ertzeid and Storeng, 2001; Van der Auwera and D'Hooghe, 2001) as well as the chromosomal constitution of embryos (Katz-Jaffe et al., 2005). Moreover, ovarian stimulation might disrupt mechanisms involved in maintaining accurate chromosome segregation (Munne et al., 1997; Hodges et al., 2002). A randomized trial concerning the chromosomal analysis of human embryos following mild ovarian stimulation for IVF showed a significantly higher proportion of euploid embryos compared to conventional ovarian stimulation, suggesting that through maximal stimulation the surplus of obtained oocytes and embryos are of lower quality (Baart et al., 2007).

Following conventional stimulation, a low number of oocytes was associated with a poor outcome. Optimal outcomes were observed when 10 oocytes were obtained. These findings are consistent with most previous studies on this subject. A low number of oocytes following conventional stimulation is related to ovarian aging caused by the depletion of the primordial follicle pool, leading to a decreased number of developing follicles and diminished oocyte quality (Tarlatzis et al., 2003; Broekmans et al., 2007). Few studies have addressed the issue of the optimal number of oocytes in a conventional stimulation protocol reported (Timeva et al., 2006; van der Gaast et al., 2006). Timeva et al. (2006) report an optimum between five and 15 and van der Gaast et al. (2006) found an optimum median number of oocytes of 13. Indeed, most previous studies on this subject report a steady increase of pregnancy rates with increasing numbers of oocytes which eventually levels off (Devreker et al., 1999; Sharma et al., 2002). This confirms the concept that in conventional stimulation increasing oocyte numbers augment the ability to select the best embryo (s) for transfer. Beyond a certain point however, pregnancy rates decrease due to the aforementioned detrimental effects of the development of large quantities of follicles (and concomitant supraphysiological hormone levels) on oocyte and embryo quality (Ertzeid and Storeng, 2001; Simon et al., 2005; Pena et al., 2002) as well as endo-

metrial receptivity (Devroey et al., 2004; Kodaman and Taylor, 2004). In the mild stimulation group this effect was clearly demonstrated when more than nine oocytes were obtained. Alternatively, the impaired pregnancy rate in patients with a more pronounced ovarian response in the mild stimulation protocol group could be related to the occurrence of premature LH rises (The ganirelix dose-finding study group, 1998; Born et al., 2000). The occurrence of an untimely LH rise (a consequence of the high estradiol levels developed early during ovarian stimulation) has a negative impact on the chance of achieving an ongoing pregnancy (Humaidan et al., 2002). In the mild stimulation protocols used in the three studies a flexible GnRH antagonist protocol was applied to limit the number of days of usage (initiating the GnRH antagonist dependent on the size of the follicle) (Al-Inany et al., 2005).

Under conditions of a profound ovarian response with an early rise in estradiol and subsequent elevated LH levels, the ultrasound criteria used to initiate GnRH antagonist co-treatment may no longer be valid (The ganirelix dose-finding study group, 1998; Born et al., 2000; Albano et al., 1997). This observation might explain the observed lower efficacy of the flexible protocol compared to a fixed protocol (OR 0.70 (95% CI 0.47-1.05)) in a meta-analysis on four studies (Tarlatzis et al., 2006). For patients with a profound ovarian response, early initiation of the GnRH antagonist is worth evaluation (Kolibianakis et al., 2006). An important weakness of the present study is the lack of endocrine data to confirm the occurrence of LH rises or surges in our patient group.

A relative shortcoming of the present study is the heterogeneity regarding the number of embryos transferred per patient in the different studies. This was overcome by using the implantation rate (ongoing pregnancy rate per embryo transferred) as the primary outcome measure of this study. Even though the number of transferred embryos was independent on the ovarian response (unless only one embryo was available) the use of implantation rates introduces a new potential bias. When embryo implantation is used, outcomes are clustered within each woman. Thus, variability in implantation status of individual embryos comes from two sources, within-women variation and between-woman variation (Hogan and Blazar, 2000). Consequently, a statistical method is required to compensate for the clustering of data. In this study a validated method for correction for statistical dependence between embryos was performed (GEE approach) (Eppig et al., 1998). Because the difference between outcomes in both protocols remained statistically significant after this analysis, it was assumed that the clustering of data could not be held responsible for the observed effect.

A second potential weakness of the study is the fact that the current analysis is based on data derived from three different studies. However, the great similarity of the three studies allowed for the pooling of data. Additionally, data of individual studies all pointed in the same direction and the fact that patients were treated in two different treatment centres renders the findings more robust. The current observa-

tions in the conventional protocol are in accordance with the existing literature which confirms the authenticity of the observed findings.

In conclusion, this study shows that the optimal number of oocytes obtained is dependent on the ovarian stimulation regimen used and confirms that (in contrast to conventional stimulation) the retrieval of low numbers of oocytes following mild stimulation is associated with favourable pregnancy chances. These data further substantiate that clinicians should not fear a relatively low ovarian response to mild stimulation and that current criteria for low response or cycle cancellation do not apply under those circumstances. Indeed, obtaining low oocyte numbers in the context of a mild stimulation protocol is clearly associated with good outcomes, and may aid in the selection of embryos for transfer (Baart et al., 2007). The lower implantation rates in high responders in the mild stimulation protocol warrants further study as to its cause.

CHAPTER 5
PREDICTION OF INSUFFICIENT OVARIAN RESPONSE FOLLOWING MILD OVARIAN STIMULATION FOR IVF

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INTRODUCTION

As assisted reproductive science progresses, a shift in the focus of in vitro fertilization (IVF) is occurring from striving for maximizing instant success 'at all costs' to developing safer and more patient friendly protocols in which the risks of treatment are minimized while optimizing the chance of a singleton live birth (Edwards et al., 1996; Gerris, 2005). Ovarian hyperstimulation is applied in IVF to generate multiple follicle growth in order to obtain an increased quantity of oocytes to compensate for inefficiency of the IVF procedure while maintaining the potential to select the best embryo (Fleming et al., 1990). Currently this goal is usually achieved by a long gonadotrophin-releasing hormone (GnRH) agonist suppression protocol, in association with ovarian stimulation with high doses of exogenous follicle-stimulating hormone (FSH). Disadvantages of this approach are the high cost, complex stimulation protocols which take several weeks, physical and emotional discomfort, chances for complications and the essentially uncontrollable degree of ovarian response. The current trend of limiting the number of embryos to be transferred reduces the need for large numbers of oocytes. Moreover, there is increasing evidence of the detrimental effects of ovarian hyperstimulation on corpus luteum function, endometrial receptivity and embryo quality (Valbuena et al., 2001; Bourgain and Devroey, 2003). As a consequence, mild ovarian stimulation protocols are being developed to minimise the adverse treatment effects of ovarian hyperstimulation (Fauser et al., 1999). The introduction of GnRH antagonists into clinical practice and a greater understanding of the process of follicle recruitment and dominant follicle selection have led to new opportunities for developing mild stimulation protocols.

It has been shown that by interfering with the physiological decrease in FSH levels during the follicular phase, it is possible to override the selection of a single

dominant follicle (van Santbrink et al., 1995; Hohmann et al, 2001). Both the degree and the duration of the FSH elevation will lead to an extension of the so called "FSH window" which enables the development of several rather than just a single dominant follicle (Zeleznik et al., 1985; Schipper et al., 1998; de Jong et al., 2000). Indeed a slight but extended, elevation of FSH levels during the mid to late follicular phase has been shown to be sufficient for growth of a modest number of dominant follicles (Schipper et al., 1998; Hohmann et al., 2001). Yet, there appears to be an individual variability in the optimal moment of initiating exogenous FSH supplementation. Hohmann et al. (2001) observed no difference in multifollicular growth when daily FSH administration was started on day 3 or day 5, although a tendency towards lower numbers of dominant follicles was seen when cycle day 7 was chosen to initiate FSH injections.

With the use of GnRH antagonists to prevent a premature luteinizing hormone (LH) rise, the IVF treatment cycle can commence at a point in the course of a spontaneous menstrual cycle where the recruitment of a cohort of antral follicles has already been established (Fauser and van Heusden, 1997; Macklon and Fauser, 2000). This approach enables limiting the use of exogenous FSH in order to extend the FSH window, allowing multiple dominant follicle development to take place. As a consequence the number of treatment days and the total amount of exogenous FSH required is substantially reduced (de Jong et al., 2000; Hohmann et al., 2003; Heijnen et al., 2007). A potential drawback of mild stimulation is a decrease in ovarian response compared to conventional stimulation leading to higher cancellation rates (Fauser et al., 1999; Hohmann et al., 2003). Although low numbers of oocytes appear to be related to good outcomes in mild stimulation (Hohmann et al., 2003), cancellations should be prevented to optimise the benefit of mild stimulation. The purpose of this study was the prediction of mild stimulation cycles likely to be cancelled due to insufficient ovarian response. The development of methods to identify women who may benefit from an earlier start of exogenous FSH may reduce the number of cancelled cycles and improve the efficacy of the mild stimulation protocol.

MATERIALS AND METHODS

STUDY DESIGN

Data were derived from the mild stimulation arm of a randomized controlled trial on effectiveness of IVF treatment strategies (Heijnen et al., 2007). The study was approved by the local ethics review board of both participating centers. In this study, infertile patients with a regular indication for IVF or intracytoplasmic sperm injection (ICSI) who attended the Erasmus Medical Centre (Rotterdam, the Netherlands) and the University Medical Centre Utrecht (Utrecht, the Netherlands) were invited to participate. Participants were less than 38 years of age, had a regular menstrual cycle

(25-35 days) and a body mass index (BMI) between 18-28 kg/m^2. Couples who had been previously treated with IVF were excluded. Study design and clinical outcomes of the RCT have been reported recently (Heijnen et al., 2007).

Patients in the mild stimulation arm were treated with a fixed daily starting dose of 150 IU rFSH (Gonal-F®:Serono Benelux B.V., Amsterdam, the Netherlands; or Puregon®: N.V. Organon, Oss, the Netherlands) s.c., initiated on the fifth cycle day (CD 5 protocol). The dose of exogenous FSH was not adjusted during the stimulation. GnRH antagonist (ganirelix, Orgalutran®: N.V. Organon, 0.25 mg/day; or cetrorelix, Cetrotide®: Serono Benelux, 0.25 mg/day) was administered s.c. from the day that at least one follicle attained a diameter ≥ 14 mm (Hohmann et al., 2003). Human chorionic gonadotrophin (hCG) (Profasi®: Serono Benelux B.V.; or Pregnyl®: N.V. Organon) 10,000 IU s.c. was administered as a single bolus injection to induce final oocyte maturation, when the largest follicle had reached at least 18 mm in diameter and at least one additional follicle ≥ 15 mm was observed. Oocyte retrieval and fertilization "in vitro" was performed according to standard procedures as described previously (Kastrop et al., 1999; Huisman et al., 2000). Single embryo transfer of the resulting best quality embryo was performed on day 3 or 4 after oocyte retrieval. Standard luteal phase support in the form of intravaginal progesterone (Progestan®: N.V. Organon) 600 mg/day was given from the day of oocyte retrieval until a urine pregnancy test was performed 18 days later.

Insufficient ovarian response resulting in cancellation of the cycle was defined as the development of less than three follicles > 12 mm. In these patients, exogenous FSH was initiated on cycle day 2 (CD 2 protocol) in a subsequent treatment cycle while the daily dosage remained unchanged. Cycles at risk for ovarian hyperstimulation syndrome (OHSS), defined as more than 20 follicles with a diameter > 10 mm or estradiol concentrations > 15.400 pmol/l were also cancelled before hCG injection.

DATA ANALYSIS

In order to identify "a priori" predictors of cancellation cycles due to insufficient response in a mild stimulation protocol, female age, previous pregnancy, previous childbirth, cause of infertility, menstrual cycle length, IVF or ICSI treatment and BMI were compared between patients where ovarian stimulation was cancelled and patients who had a sufficient ovarian response to proceed to oocyte pick-up in their first treatment cycle. For this analysis, cycles cancelled due to increased risk of OHSS were considered as good responders and included in the analysis. Cycles cancelled due to premature luteinisation were excluded from the analysis. Multivariable logistic regression analysis was performed with a backward elimination procedure, a p-value < 0.3 was used as a criterion for exclusion. The predictive ability of the model was assessed by determining the area under the receiver-operating characteristics (ROC) curve (AUC).

To assess the amount of overfitting of the created model, internal validation was performed with bootstrapping, a statistical technique to create comparable populations. We bootstrapped 200 times. In each of these 200 new data sets, the same multivariable logistic regression analysis with backward elimination was performed, and the resulting model was tested on the original data. In this way the amount of overfitting can be assessed, expressed as a shrinkage factor. The shrinkage factor should be taken into account when applying the model in clinical practice (Van Houwelingen and le Cessie, 1990; Harrell Jr. et al., 1996).

To study whether patients who were cancelled due to insufficient ovarian response in their first treatment cycle with the CD 5 protocol presented with improved ovarian response when stimulation in the subsequent cycle was started at CD 2, a "within patient comparison" was performed. In this analysis the ovarian response of a second treatment is compared to the response in the first treatment cycle of the same individual. To compare both cycles, duration of stimulation, total rFSH needed and number of developed follicles and oocytes of patients who were cancelled for insufficient ovarian response in their first treatment cycle were included in the analysis.

Comparisons of outcome measures between the groups were performed using the t-test for continuous data and the χ^2 -test for binary variables unless stated otherwise. Within patient comparison was done by a paired t-test. Analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA, 1999) and S-plus 2000 (Insightful Corp., Seattle, WA, USA).

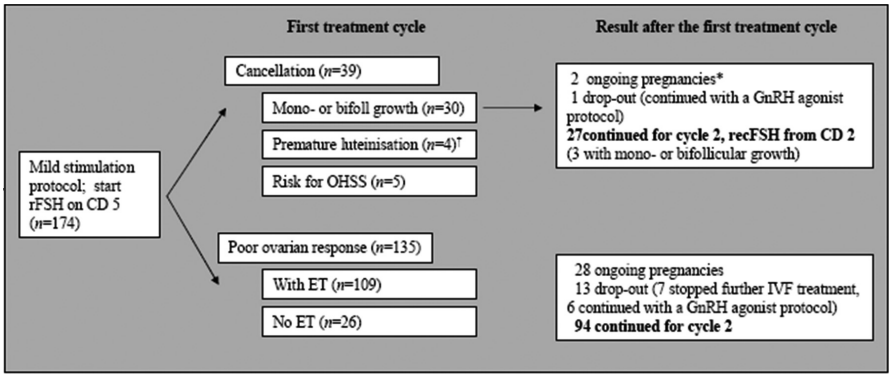

Figure 1 Flow chart showing the number of patients divided according to the degree of ovarian response in the mild CD 5 stimulation protocol and follow up in the second treatment cycle.

† Premature luteinisation was defined as a LH serum level above 15, which was measured on clinical suspicion.

* 1 spontaneous pregnancy, 1 pregnancy after escape IUI

RESULTS

Of the 174 first cycles started, 39 (22%) ended in a cancellation, 30 (17%) due to an insufficient response and 9 (5%) for other reasons (Figure 1). Univariable analysis of patient characteristics is shown in Table 1. A significantly shorter menstrual cycle length (the number of days of an average menstrual cycle in the previous year as indicated by the patient) (28.2 vs 27.5 days; p= 0.045) and longer duration of infertility (4.4 vs 3.6 years; p= 0.022) were observed in patients with an insufficient response. The number of treatment days and medication used were also compared between cancellation for insufficient ovarian response and patients who did continue for follicle aspiration. As expected, there was a significant difference in treatment days and medication used in these cycles (p-values all < 0.001) (Table 2).

Table 1 Univariable analysis of patient characteristics

	Cancellation due to insufficient ovarian response (n=30)	Sufficient ovarian response (n=140)†	*Difference (95% Confidence Interval)*
Female age (years)	33.1 ± 3.0	32.7 ± 3.1	0.45 (-1.68, 0.77)
Primary infertility (%)	70%	82 %	-
Cause of infertility (%)	Tubal 10 % Male 43 % Unexplained 37 % Other 10 %	Tubal 16 % Male 54 % Unexplained 26 % Other 4 %	-
Duration of infertility (years)	4.4 ± 2.4	3.6 ± 1.7	0.85 (-1.57, -0.13)*
Cycle length (days)	27.5 ± 1.5	28.2 ± 1.9	0.74 (0.15, 1.47)*
BMI (kg/m^2)	23.5 ± 3.0	23.0 ± 2.5	0.46 (-1.49, 0.58)

Values are means ± SD

† including five cycles that were cancelled as a consequence of increased risk for OHSS

* Differences are statistically significant (P-value < 0.05)

In the multivariable analysis, the variables duration of infertility, menstrual cycle length, primary or secondary infertility and BMI were selected into the prediction model for cancellation during the mild stimulation protocol. A longer duration of infertility, short menstrual cycle length, secondary infertility and higher BMI were found to be associated with an insufficient ovarian response. The predictive ability of the model measured by the area under the ROC curve was 0.69 (95% Confidence Interval 0.58-0.79) (Table 3). Internal validation by bootstrapping showed a shrinkage factor of 0.58. This means that the final model is overfitted to our data and the predictive probabilities of the model on external data will be less. The model will give the most reliable predictions when all regression coefficients are on average 42% smaller in absolute size. A probability cut-off for cancellation due to insufficient response of

0.3 yielded an expected sensitivity of 33%, specificity of 92%, and positive predictive value of 48% on our own data. A probability cut-off of 0.15 yielded an expected sensitivity of 77%, specificity of 54%, and positive predictive value of 26%. Table 4 shows the validity of the model with a range of cut-of values chosen in the area of the AUC curve of the model with the most discriminative power.

Table 2 Univariable analysis of cycle characteristics

	Cancellation due to insufficient ovarian response† (*n*=30)	Sufficient ovarian response (*n*=135)	Difference (95% Confidence Interval)
Duration of stimulation (days)	5.5 ± 1.7	8.0 ± 1.6	2.47 (1.84, 3.01)*
Total rFSH used (IU)	830 ± 248	1200 ± 256	370 (268, 472)*
GnRH antagonist administered (days)	1.4 ± 1.2	3.7 ± 1.4	2.29 (1.72, 2.84)*

Values are means (± SD)

† Insufficient ovarian response was defined as the development of less than three dominant follicles (diameter > 12 mm).

* Differences are statistically significant (P-value < 0.05)

As expected, the within patient comparison between the first and second treatment cycle of patients who were cancelled for insufficient response in their first treatment cycle showed that a change in treatment protocol from CD 5 to CD 2 resulted in a clear improvement in ovarian response. Three of the 27 patients (11%) whose first treatment cycle was cancelled and proceeded with a changed protocol in the second cycle had to be cancelled again.

Table 3 Multivariable analysis for cancellations due to poor response in the mild CD 5 stimulation protocol, the ability of the model measured by the area under the ROC curve was 0.69 (95% confidence interval: 0.58-0.79).

	P-value	Odds Ratio (95% Confidence Interval)**	Cumulative AUC
Duration of infertility	0.033	1.24 (1.02, 1.50)	0.60
Menstrual cycle length	0.034	0.75 (0.59, 0.98)	0.67
Secondary infertility	0.13	2.08 (0.82, 5.27)	0.68
BMI (kg/m^2)	0.26	1.10 (0.93, 1.29)	0.69

** Predicted probability of cancellation due to insufficient response:
*1/(1 + Exp[-(0.608 + 0.126*Duration – 0.158*[cycle length] + 0.423*[secondary infertility (yes=1, no = 0)] + 0.055*BMI)])*

Table 4 Clinical value of the model for cancel prediction with test characteristics at several probability cut-offs

Cut-off value for the probability of cancel	**0.10**	**0.15**	**0.20**	**0.25**	**0.30**
Sensitivity	87	77	43	37	33
Specificity	29	54	74	84	92
PPV	21	26	27	33	48
NPV	91	92	86	89	87
% of patients that will change protocol	89	62	29	19	12
Number of cancels unpredicted (n (%))	4 (13%)	7 (23%)	17 (57%)	19 (63%)	20 (67%)

Values are percentages unless stated otherwise
PPV, positive predictive value; NPV, negative predictive value

DISCUSSION

The aim of the present study was to explore whether it is possible to identify a subgroup of patients at risk for cancellation due to insufficient ovarian response in mild ovarian stimulation for IVF, starting exogenous FSH on cycle day 5. Our study confirmed the previous finding of a relatively high cancellation rate (17%) in the mild stimulation protocol (Hohmann et al., 2003). To increase the benefit of mild stimulation we analysed characteristics of patients with an insufficient ovarian response. According to pre-treatment variables we developed a prognostic model to identify patients at risk for cancellation prior to the start of ovarian stimulation. The predictors in the model were a longer duration of infertility, a shorter menstrual cycle length, secondary infertility and a higher BMI.

The finding of a correlation between a shorter cycle length and insufficient ovarian response may be the consequence of a shortened follicular phase in these patients where dominant follicle selection may have occurred prior to the start of the exogenous FSH (Klein et al., 2002; van Zonneveld et al., 2003). As a consequence involvement of the non dominant follicles in ongoing growth by exogenous FSH becomes impossible. Also, cycle shortening may be a subtle first sign of advanced ovarian ageing and as such be related to a small cohort size. As only patients with a regular cycle length and below 38 years of age were included for this study, this phenomenon may become even more evident in the general population.

A negative association between BMI and ovarian response has been observed previously (Wittemer et al., 2000; Nichols et al., 2003; Fedorcsak et al., 2004). Obese patients usually require significantly higher doses of gonadotrophin and a longer duration of stimulation (Dechaud et al., 1998). However, in a prospective study comparing predictive factors of ovarian response in IVF, BMI did not correlate with the number of follicles or the number of retrieved oocytes (Popovic-Todorovic et al., 2003).

Longer duration of infertility has previously been recognized as an important negative prognostic factor affecting the chance of natural conception, particularly in unexplained infertility (Hull et al., 1985). This might be the consequence of subtle, undiagnosed, disorders related to a diminished ovarian reserve. These disorders might normally be overcome by fierce stimulation in more conventional stimulation protocols and become noticed in natural conception and mild stimulation.

The observed correlation between ovarian response and secondary infertility has not been described previously. In our opinion there is no straight-forward biological explanation for this relationship. Its appearance in the model could be the result of the relatively small sample size used to build the model and might explain the relatively large shrinkage factor that was found in the internal validity analysis.

The only previous study regarding the current mild stimulation protocol with a similar analysis showed a significant difference in age and baseline FSH between cancelled patients and those who met the criteria for oocyte retrieval (Hohmann

et al., 2003). The current study could not confirm the finding of female age as an important predictor in spite of the larger sample size. This might be partly the result of the age restriction in the inclusion criteria for the study. Additionally, both shorter cycle length and longer duration of infertility appearing in the current model might be related to ovarian aging resulting in age being removed in multivariable analysis. Unfortunately data from measurement of baseline FSH or other hormonal markers related to ovarian aging such as Inhibin B or anti-Müllerian hormone were not available.

Analysis of ovarian response in a subsequent treatment cycle with the CD 2 protocol for patients who previously presented with an insufficient ovarian response showed that these were likely to meet the criteria for oocyte retrieval. These results support the hypothesis that in a mild stimulation protocol, insufficient ovarian response is a consequence of suboptimal ovarian stimulation for a specific group of patients which can be overcome by the early commencement of exogenous stimulation. However, it is likely that at least part of the improved ovarian response is the result of the principle of regression towards the mean. This is a principle stating that of related measurements, and selecting those where the first measurement is either higher or lower than the average, the expected value of the second is closer to the mean than the observed value of the first (Davis, 1976). Prospective randomized studies are needed to establish which part of the improved ovarian response should be allocated to the change in the hormonal stimulation schedule.

These data indicate that the CD 2 protocol is likely to improve chances of patients that would be cancelled in the CD 5 protocol due to insufficient ovarian response. Because the prediction model is based on "a priori" parameters, patients at risk for cancellation can be identified prior to the start of the treatment. The overall cancellation rate for insufficient response is therefore likely to be reduced if these patients are treated with the CD 2 protocol instead of the CD 5 protocol.

Although the area under the ROC curve was modest, the model has, in our opinion, the ability to predict sufficient numbers of cancellations to reduce the remaining number of cancellations to an acceptable level. At a cut-off level of 0.30 the model will predict 10 cancellations correctly (sensitivity 33% (95% CI 16.5-50.2)). According to our CD 2 started second cycles, 1 out of these 10 would still be cancelled (11%). This means that with a model based treatment adaptation the percentage cancels would be lowered from 17% to 12% (21/174). This reduction would equal the proportional difference in cancellation rates between the day 2 and day 5 arm of the study by Hohmann (Hohmann et al., 2003), and as such may be considered clinically useful. Due to the high specificity at the cut-off level (92% (95 % CI 87.7 - 96.6)), the number of patients that will be unnecessarily treated with the CD 2 protocol is limited, so that the majority of cases can therefore still benefit of the advantages of the mild stimulation day 5 protocol. Still, one should be aware that the model is devel-

oped on a modest number of patients leading to the relative broad confidence interval for sensitivity (16.5 - 50.2 %).

Overall, the clinical use of the prediction model may render mild ovarian stimulation more patient tailored. The concept of "cycle cancellation" in this novel mild should be viewed in the context of just a few days of medication in the current mild stimulation approach compared to the conventional approach. In such stimulation protocols (GnRH agonist long protocol, sometimes proceeded by oral contraceptives, and followed by extended ovarian stimulation) the time elapsed before an insufficient response can be identified is much longer, resulting in an increased waste of medication and the delay of at least two menstrual cycles. The cancellation rate during mild stimulation should also be balanced against the extended gain (for multiple cycles, if needed) of a later start of stimulation in the great majority of women resulting in fewer injections and reduced patient discomfort and cost.

In conclusion, midfollicular initiation of rFSH in combination with a GnRH antagonist leads to a mild ovarian stimulation protocol but yields a distinct risk of cancellation due to insufficient response. We developed a model that could predict 33% of the cancellations (sensitivity) with a false positive rate of 8% on our own data and so equalise the cancellation rate for insufficient response to that observed in a standard GnRH antagonist ovarian stimulation protocol for IVF. Insufficient response in mild stimulation may be related to ovarian aging and increased BMI. After external validation, the model might be used to identify patients at risk for insufficient ovarian response previous to the start of the treatment cycle. Treatment with ovarian stimulation initiated in the early follicular phase in these patients may reduce the number of cancelled cycles and therefore improve the efficacy of the mild stimulation protocol. Further prospective randomized trial should evaluate the clinical use of adjusting the starting day of ovarian stimulation based on the current prediction model.

CHAPTER 6
WHY DO COUPLES DROP-OUT OF IVF TREATMENT? A PROSPECTIVE COHORT STUDY

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(Submitted)

INTRODUCTION

In vitro fertilisation (IVF) and intracytoplasmatic sperm injection (ICSI) are widely applied treatments for most causes of infertility. However, the ovarian stimulation regimens required to obtain multiple oocytes for fertilisation are expensive, complex and associated with significant side-effects and stress (Fauser et al., 2005; Macklon et al., 2006). High rates of drop-out are therefore frequently encountered in IVF and ICSI treatment. Drop-out from IVF treatment should be considered an adverse treatment outcome since early cessation of treatment deprives the couple an optimal cumulative chance of achieving pregnancy, and therefore impacts on the overall success of the IVF program. The importance of drop-out from IVF treatment has been highlighted by the increasing trend towards reporting IVF outcomes in terms of cumulative success rates per complete treatment or per period of time (instead of per cycle) (Heijnen et al., 2004; Johnson et al., 2003). Average drop-out rates well above 50% have frequently been reported in literature (Callan et al., 1988; Tan et al., 1992; Land et al., 1992; Olivius et al., 2002; Schroder et al., 2004).

It is often assumed that patients withdraw from IVF treatment for only two reasons: the withholding of further treatment because of a poor prognosis (active censoring) and inability to pay for further treatment (Cousineau and Domar, 2007). Although some studies observe a high incidence of patients with a poor prognostic profile among those who elect to discontinue treatment (Stolwijk et al., 1996; Sharma et al., 2002) other studies have not confirmed this (Haan et al., 1991; Roest et al., 1998; de Vries et al., 1999). Furthermore, studies from countries where the costs of IVF are reimbursed indicate that actively censored patients constitute a minority of drop-outs (Land et al., 1997; Olivius et al., 2004). An important reason why many

patients drop-out of IVF treatment before they have received all reimbursed treatment cycles appears to be psychological distress associated with IVF treatment (Olivius et al., 2004). As previously published, additional factors involved in drop-out include the age of the male partner, previously successful IVF treatment, parity of the female partner (Johnson et al., 2003) and pre-existing levels of anxiety and depression (Smeenk et al., 2004).

There are also indications that the nature of the ovarian stimulation protocol applied might determine the extent of psychological stress and drop-out rates from IVF treatment. A study by Højgaard et al. (2001) showed that patients receiving minimal ovarian stimulation IVF (unstimulated or Clomiphene Citrate stimulated cycles) reported fewer side effects and stress related to hormone treatment and cycle cancellation compared with conventional stimulation. A recent publication by our group showed that the drop-out rate following two cycles of a mild ovarian treatment strategy was significantly lower than following a similar number of cycles using a conventional approach (Heijnen et al., 2007).

Insight into the factors that influence the decision of couples to discontinue treatment and their reasons for dropping out may allow early identification of women at risk and the tailored interventions to improve treatment compliance, and as a result, improve cumulative pregnancy rates and the cost-effectiveness of IVF programs. The aim of this study was to prospectively assess the incidence of drop-out from fully reimbursed IVF treatment and to identity factors that influence the decision to discontinue treatment.

MATERIALS AND METHODS

SUBJECTS AND STUDY DESIGN

The subjects of this study were participants in a randomized controlled trial comparing the efficacy of a mild treatment strategy with a conventional treatment strategy (Heijnen et al., 2007). Detailed information on the design and clinical outcomes and preliminary findings regarding the effect of each protocol on the drop-out rate of this study has been described previously (de Klerk et al., 2006; Heijnen et al., 2007). The study was approved by the local ethics review boards of both participating centers and written informed consent was obtained from each participant.

Infertile patients with a regular indication for IVF or ICSI who attended the Erasmus Medical Centre (Rotterdam, the Netherlands) and the University Medical Centre Utrecht (Utrecht, the Netherlands) were invited to participate. Participants were less than 38 years of age, had a regular menstrual cycle (25-35 days) and a body mass index (BMI) between 18-28 kg/m^2. Only couples with no previous IVF treatment or a healthy born child after a previous IVF treatment were included. Data of all patients that actually started treatment were included in the present study.

IVF TREATMENT PROTOCOL

The mild treatment strategy consisted of ovarian stimulation with a fixed daily dose of 150 IU recombinant follicle stimulating hormone (rFSH) (Gonal-F®: Serono Benelux B.V., Amsterdam, the Netherlands; or Puregon®: N.V. Organon, Oss, the Netherlands) subcutaneously (s.c.) per day, initiated on cycle day 5 as previously published (Heijnen et al., 2007). In order to prevent premature luteinization, a gonadotrophin-releasing hormone (GnRH) antagonist (ganirelix, Orgalutran®: N.V. Organon, 0.25 mg/day; or cetrorelix, Cetrotide®: Serono Benelux, 0.25 mg/day) was administered s.c. when at least 1 follicle ≥ 14 mm was observed. In cycles resulting in the development of less than 3 pre-ovulatory follicles no oocyte retrieval was performed. Human chorionic gonadotrophin (hCG) (Profasi®: Serono Benelux B.V.; or Pregnyl®: N.V. Organon) 10,000 IU s.c. was administered for the triggering of final oocyte maturation. Oocyte retrieval and fertilization were performed according to standard procedures, as described previously (Kastrop et al., 1999; Huisman et al., 2000). The single best quality embryo was transferred. The luteal phase was supported with progesterone, 600 mg/day, intravaginally. Excess embryos of sufficient quality were cryopreserved and transferred in a subsequent unstimulated cycle, according to standard procedures. The maximum planned number of mild strategy IVF cycles was four.

The conventional IVF approach included pituitary down-regulation with a GnRH agonist initiated during the mid-luteal phase of the pre-treatment cycle (Leuproline, Lucrin®: Abbott B.V., Amstelveen, the Netherlands, 0.2 mg/day s.c.; or triptoreline, Decapeptyl®: Ferring B.V., Hoofddorp, the Netherlands, 0.1 mg/day s.c.). After two weeks of GnRH agonist administration, ovarian stimulation was started with a daily-dose of 150 IU/day rFSH s.c. Similar criteria applied for cancellation, hCG, oocyte retrieval and fertilization procedures as in the mild strategy group. In the conventional approach a maximum of two embryos were transferred. Luteal phase support and criteria to cryopreserve embryos were applied as in the mild strategy. The maximum planned number of conventional strategy IVF cycles was three. In both arms of the study, the costs of all treatment cycles were reimbursed to the patient. For further information regarding the details of the two treatment strategies, see (Heijnen et al., 2007).

Patients were considered as treatment drop-outs when they did not return for a further IVF cycle after failure of the previous cycle, before they had completed the planned number of treatment cycles. The drop out cycle was defined as the treatment cycle preceding discontinuation of treatment. A cycle not resulting in an ongoing pregnancy was considered to be a failed cycle. Patients who did not complete the total number of cycles offered because they became pregnant (either spontaneously or following treatment) were not considered drop-outs. Due to the difference in the maximum number of treatment cycles offered per treatment protocol, patients treated by the conventional strategy could drop-out following the first or second treat-

ment cycle while patients in the mild strategy arm of the study could also drop-out following a third treatment cycle.

Non cycle-related explanatory variables analysed for drop-out included the age of the male and female partner, cause and duration of infertility, delay before the start of the first treatment cycle, previous pregnancy and previous live birth. In addition, previous fertility treatment (intra-uterine insemination or ovulation induction) prior to the IVF treatment and the education level of the female partner were considered as potential factors. In addition, pre-existing symptoms of depression and anxiety as were measured on the Hospital Anxiety and Depression Scale (HADS) were considered. The HADS was developed as a screening tool to measure symptoms of anxiety (HADA) and depression (HADD) experienced by medical patients in the week prior to screening (Zigmond and Snaith, 1983). Both subscales (range 0-21) of the HADS consist of seven items, which are scored on a 4-point-Likert scale from 0 to 3. Higher scores imply the presence of more symptoms. Cut-off scores for possible and probable depressive and anxiety disorder are 7/8 and 10/11, respectively. The Dutch version of the HADS has shown good test-retest reliability, homogeneity and internal consistency (Spinhoven et al., 1997).

In addition to non-cycle related factors, the role of events occurring during the drop-out cycle was also investigated. This analysis included voluntary delay before the start of the cycle, the number of treatment days, whether or not the cycle was cancelled, the number of oocytes retrieved and, whether the cycle resulted in an embryo transfer and the quality of the embryo (s) transferred. In addition, the incidence of complications such as ovarian hyperstimulation syndrome (OHSS) or infection, whether the treatment included transfer of cryopreserved embryos, the occurrence of early pregnancy loss and the number of visits patients paid to a physician after the failed treatment were analysed.

STATISTICAL METHODS

To compare the drop-out rate between the two treatment strategies applied, Kaplan-Meier survival analysis was performed with the discrete time-variable being the cycle number and censoring (losses from the sample before the final outcome is observed) for patients who achieved an ongoing pregnancy during the period of the study (both spontaneous and following embryo transfer).

To analyse the influence of other potential risk factors on the chance of drop-out, Cox's proportional hazards analysis was performed with correction for the treatment strategy employed. Hazard ratios (HR) for the risk of dropping out of further IVF treatment were calculated (including 95% confidence intervals (CI)). Logistic regression with correction for the treatment strategy applied was used to evaluate the effect of cycle characteristics on the cycle-specific chance of drop-out. To allow for the pooling of data from different treatment cycles within the same patients, logistic regression was performed with correction for cycle number.

A p-value of < 0.05 was considered to be statistically significant. Analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA, 1999) and S-plus 7.0 (Insightful Corp., Seattle, WA, USA).

RESULTS

SUBJECTS AND DISTRIBUTION OF DROP-OUTS

A total of 384 patients started treatment. Figure 1 shows the number of patients that started treatment and the final outcome after completing the treatment. Thirty-five patients elected to discontinue treatment following mild stimulation; eight (23%) after the first cycle, 12 (34%) after the second cycle and 15 (43%) following the third cycle. In those undergoing conventional stimulation, 30 patients dropped-out; 13 (43%) following cycle 1 and 17 (57%) following cycle 2. Although the cumulative number of drop-outs was similar in both treatment groups, the drop-out rate per cycle was lower in the mild strategy group (7.7% vs 10.0%); cox analysis of the difference in cumulative drop-out rates between the two treatment strategies applied showed a significant difference (HR 0.55 (95% CI 0.31-0.96); p = 0.034) in favour of patients treated in the mild stimulation arm of the study (Figure 2).

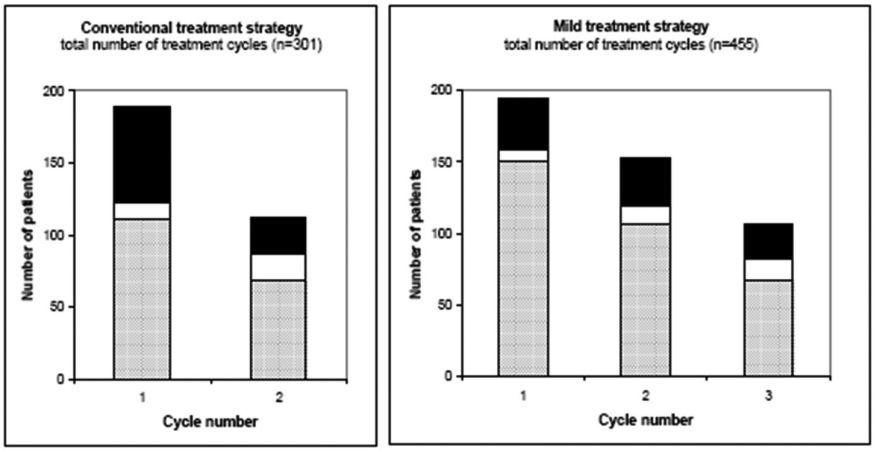

Figure 1 Outcome per treatment cycle according to treatment strategy
Legend: Stippled area, not pregnant; Black area, ongoing pregnancy; White area, drop-out

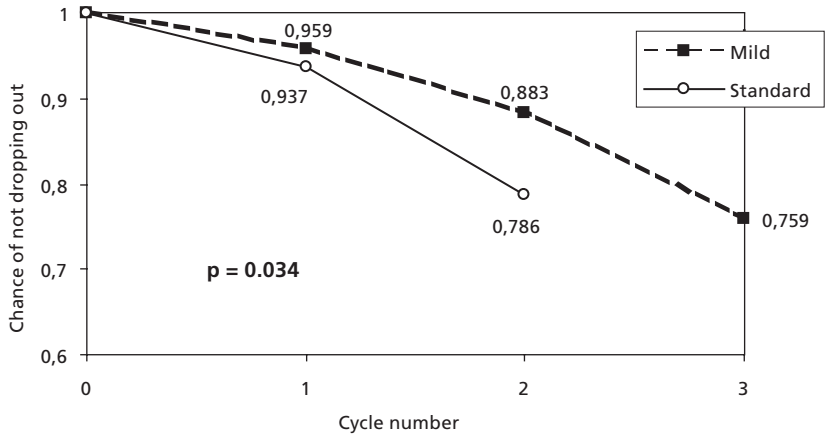

Figure 2 Kaplan-Meier survival curve showing the drop-out rate per cycle compared between patients treated with a mild and conventional treatment strategy with censoring for couples who achieved an ongoing pregnancy.

The causes for drop-out are summarized in figure 3; the principal reason for dropping out was the physical or psychological burden of treatment (28%). Twenty-five percent of patients did not provide a reason for not returning for treatment. In 14% of drop-out patients the primary reason for stopping treatment was a poor prognosis identified by a physician (actively censored). 76% of the drop-outs were voluntary (passively censored).

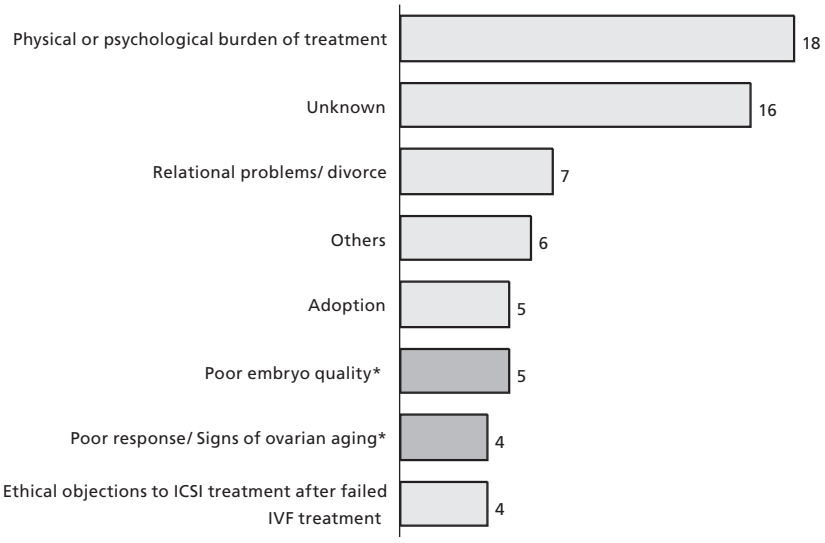

Figure 3 Causes for drop-out from IVF treatment in 384 couples. Dark: actively censored.

CHARACTERISTICS OF DROP-OUT PATIENTS

The HR and 95% CI for cycle independent risk factors after correction for the treatment strategy applied are depicted in Table 1. The indication for IVF was significantly related to the chance of discontinuing treatment (p-value 0.040). Patients with severe male subfertility (and therefore treated by ICSI) had a significantly higher risk of dropping-out of treatment (HR 4.80 (95% CI 1.63 -14.13). Additionally, there was a tendency towards a significant effect for female age (HR 0.94 (95% CI 0.87-1.01), indicating a higher chance of discontinuing treatment with lower female age at the start of the treatment.

Of the risk factors associated with events during the drop-out cycle only the failure to achieve embryo transfer was found to be significantly related to the chance of discontinuing treatment (Odds Ratio (OR) 0.41 (95% CI 0.24-0.72), p 0.002) (Table 2).

Table 1 Hazard ratios for the chance of dropping-out of IVF treatment after correction for treatment strategy applied (384 couples with 756 treatment cycles).

	Hazard Ratio (95% CI)	P-value
Age woman	0.94 (0.87-1.01)	0.088
Age man	1.00 (0.95-1.05)	1.0
Duration of infertility	1.01 (0.90-1.13)	0.8
Category of infertility		0.041
Endometriosis	0.82 (0.11-6.39)	0.8
Male	0.94 (0.46-1.94)	0.9
Severe Male	4.81 (1.63-14.14)	0.004
Unknown	1.32 (0.60-2.89)	0.5
Immunological	1.34 (0.29-6.14)	0.7
Tubal*	1	
Delay before the initiation of the first treatment cycle	1.00 (1.00-1.01)	0.4
Previous pregnancy	0.94 (0.49-1.80)	0.9
Previous childbirth	1.19 (0.70-2.01)	0.5
Previous fertility treatment[1]	0.78 (0.48-1.27)	0.3
Pre-existing symptoms of depression[2]	1.06 (0.95-1.17)	0.3
Pre-existing symptoms of anxiety[2]	1.05 (0.97-1.14)	0.21
Educational level of the woman	**	0.8

[1] Intra-uterine insemination or ovulation induction
[2] Measured on the Hospital Anxiety and Depression Scale (HADS)
* Reference category
** Hazard Ratios for the individual education levels were not given because there was no significant correlation with the chance of drop-out.

The association between the baseline score on HADA and the chance of drop-out was found to be significantly dependent on the treatment strategy applied (HR 1.38 versus 1.16 per HADA scale point in the conventional and mild group respectively, relative reduction in hazard: 0.84 (95% CI 0.72-0.99)). In other words, given the same increase in the level of measured symptoms of anxiety, couples undergoing the mild strategy were half as likely to discontinue treatment as those undergoing conventional stimulation. None of the other studied risk factors showed significant interaction with the stimulation protocol applied.

Table 2 Odds ratios for the chance of dropping out of IVF treatment after correction for treatment strategy applied (384 women with 756 treatment cycles).

	Odds Ratio (95% CI)	P-value
Delay before the start of the cycle	1.00 (0.99-1.00)	0.21
Duration of treatment (days)	1.05 (0.94-1.18)	0.4
Cancelled cycle	1.48 (0.71-3.08)	0.3
Ovarian response (number of oocytes)	0.98 (0.93-1.04)	0.5
Availability of an embryo for transfer	0.41 (0.24-0.72)	0.002
Availability of a top quality embryo for transfer	0.64 (0.37-1.09)	0.10
Cryo preserved embryo transfer cycle	1.23 (0.58-2.60)	0.6
Early pregnancy loss	1.65 (0.65-4.18)	0.3
Complications	0.93 (0.27-3.14)	0.9
Number visits to the physician	0.90 (0.58-1.41)	0.7

DISCUSSION

The aim of the present study was to prospectively assess the rate of drop-out after a complete course of IVF treatment and identify the role of the treatment strategy applied and potential other factors which increase the risk of a couple electing to discontinue reimbursed IVF treatment. The use of a mild treatment strategy was found to be associated with a reduction in drop-out rate. In particular, patients considered to be at an increased risk of giving up treatment due to high pre-existing levels of anxiety symptoms were found to be less prone to drop-out when a mild stimulation protocol was applied. After correction for the treatment strategy applied, the chance of drop-out was observed to be significantly increased when severe male subfertility was the treatment indication and when embryo transfer had not been performed.

The positive effect of a mild treatment strategy on the chance of discontinuing treatment was previously observed in a subgroup of the current studied population

(Heijnen et al., 2007). In our previous preliminary analysis (restricted to data from the first two cycles) the drop-out rate per cycle was also significantly lower in the mild treatment group compared to the conventional group (OR 0.53, 95% CI 0.28-0.98). The positive effect of the mild treatment strategy appears to be related to a reduction of anxiety and treatment related stress, as mild ovarian stimulation provides a shorter and more patient friendly treatment and reduces the risk of complications (Fauser et al., 1999; Hohmann et al., 2001). Consistent with this, a recent study concluded that failure of IVF treatment after a mild treatment strategy resulted in fewer symptoms of depression as compared to failure after a conventional treatment strategy (de Klerk et al., 2007). Taken together, these data suggest that a mild treatment strategy constitutes a more patient friendly treatment protocol and the decrease in drop-out rate can itself compensate for a slightly lower pregnancy rate per cycle observed with the mild approach.

The chance of dropping-out was observed to be increased when severe male subfertility was the indication for IVF treatment. It has been previously shown that male infertility is associated with greater levels of psychological morbidity than other indications for IVF (Connolly et al., 1992), although recently others did not confirm this finding (Holter et al., 2007). Additionally, in our study population couples with severe male subfertility were less likely to have had pre-treatment with IUI or ovulation induction ($p = 0.006$) compared to those undergoing IVF for other indications. Having not previously faced the disappointment of treatment failure, this group may have relatively high expectations with regard to their chance of conceiving following IVF treatment. Consequently, these patients might be less able to cope with the disappointment of a failed IVF cycle, and may be more likely to drop-out of treatment.

Patients whose treatment did not result in embryo transfer were also found to be more prone to discontinue treatment. This could be the result of distress caused by disappointment and loss of hope in a successful outcome. Previous studies have shown that a perceived poor prognosis was strongly associated with stress and was an important reason for drop-out (Sharma et al., 2002; Malcolm and Cumming, 2004; Rajkhowa et al., 2006). Couples were found to have a poor understanding of cumulative live birth rates with three or more attempts despite good awareness of the chance of success per IVF treatment cycles (Rajkhowa et al., 2006). These findings highlight the importance of managing expectations in IVF treatment. If couples are counselled to focus less on the chance of an individual cycle resulting in pregnancy, and encouraged to think in terms of a course of treatment aimed at achieving a live birth, they may be less likely to drop-out of treatment (Fauser et al., 2005).

There is an increasing trend towards the use of milder stimulation approaches for IVF, aiming to achieve a cost-effective and more patient friendly regimen (Nargund and Frydman, 2007). The efficacy of such treatment programs is dependent on the outcome following successive treatment cycles instead of the pregnancy rate per cycle (Fauser et al., 2005, Heijnen et al., 2007). Crucial to the success of these

programs is a reduction in drop-out rates. Until now, most research in this area has focussed on psychological interventions to reduce treatment related stress (Boivin, 2003). However, the beneficial effect on drop-out rates has not been convincingly demonstrated and many patient couples consider such counselling unnecessary (de Klerk et al., 2005). Additionally, randomized studies of counselling interventions on treatment related stress have shown no significant effect (de Klerk et al., 2005). This study shows that the use of a mild strategy reduces drop-out rates, and that patients at increased risk of discontinuing treatment can be specifically identified. Recognition of the factors which cause couples to drop out of IVF treatment allows interventions to be designed aimed at reducing drop-out rates and further improve the efficacy and cost-effectiveness of IVF treatment.

CHAPTER 7
GENERAL DISCUSSION

Over the last 25 years, ovarian stimulation has gradually become more complex, time consuming and expensive. Recently, the downsides of ovarian stimulation have attracted increasing attention. Currently, a shift in the focus of IVF is occurring from striving for maximizing instant success 'at all costs' to developing safer and more patient friendly protocols in which the risks of treatment are minimized while optimizing the chance of a singleton live birth (Edwards et al., 1996; Gerris, 2005).

As a consequence of the use of ovarian stimulation, the possibility arose to increase pregnancy rates by transferring more than one embryo per cycle. The transfer of multiple embryos to compensate for the low implantation rate following IVF treatment has become common practice. While about 80% of started IVF treatment cycles result in an embryo transfer, only 30-50% of these patients will become pregnant. Although the transfer of more than one embryo was found to be effective in increasing the ongoing pregnancy rate per cycle, this resulted in an epidemic of multiple pregnancies. As was shown in the literature review on multiple pregnancies in this thesis, multiple pregnancies are unfavourable compared to singleton pregnancies due to the poor neonatal outcome, maternal complications, long term developmental problems and high costs involved. SET proved to be effective in the prevention of multiple pregnancies following IVF treatment and is cost-effective when neonatal health care costs are taken into consideration. However, SET in an unselected population leads to a reduction of the pregnancy rate per transfer.

Increasing knowledge regarding the physiology of ovarian follicle development and dominant follicle selection, together with the clinical availability of new compounds have resulted in milder ovarian stimulation approaches. The availability of GnRH antagonist allowed for the development of more physiological ovarian stimulation approaches making use of the endogenous intercycle FSH rise (Fauser et al., 1999). The literature review on milder ovarian stimulation strategies in this thesis discusses the evidence regarding various mild ovarian stimulation regimens.

Milder stimulation protocols aim to reduce the number of treatment days, total amount of exogenous FSH required, costs and side-effects of treatment, with the risk of reducing the ovarian response. Additionally, considerable evidence indicates that supraphysiological steroid levels associated with standard ovarian stimulation have detrimental effects on endometrial receptivity, corpus luteum function, oocyte and embryo quality. Consequently, a more physiological approach might have a beneficial effect upon implantation. However, insufficient evidence on most approaches is available to confirm the efficacy in terms of pregnancy rates and cost-effectivity.

A study to which this thesis is related showed that the application of a mild treatment strategy (mild stimulation in combination with SET) in women under 38 does not reduce the chance of achieving a term live birth within 1 year of treatment compared to a standard treatment strategy, provided they undergo more "mild treatment" cycles in the same period of time (Heijnen et al., 2007). However, an important concern regarding the use of a mild treatment strategy remains the reduction in the per cycle chance of pregnancy. The principle aim of this thesis was to identify means of improving the efficacy and therefore the uptake of milder treatment strategies for IVF such as mild ovarian stimulation and SET.

Most previous studies on evidence based strategies to reduce multiple pregnancies have aimed to select patients with a high risk of twins after double transfer. Consequently, SET should be performed in these patients. It can be argued that the opposite should be the case; SET should be the treatment method of choice and DET only applied to patients with a negative risk profile. In this patient group the risk of multiple pregnancy will remain low due to the relatively low chance of implantation of a single embryo. Consistent with this approach, the development of a risk profile of patients with a very low risk of success following SET is described in this thesis.

An additional benefit of studying pregnancy following SET is the opportunity it provides to analyse the fate of a single embryo, and its role in determining successful implantation. When more than one embryo is transferred assumptions must be made as to which embryo implanted and regarding possible interactions between embryos. The multi-variate analysis of factors associated with ongoing pregnancy showed that BMI and number of oocytes retrieved were negatively correlated, while ovarian sensitivity to stimulation and the availability of a top quality embryo were positively correlated with the chance of ongoing pregnancy after SET. Calculations showed that (assuming the chance of implantation for each embryo is independent) the average pregnancy rate for patients with a negative risk profile would nearly be doubled after DET transfer while the multiple pregnancy rate would be limited to just approximately 2%. Consequently, after external validation the use of such a prediction model is likely to improve the efficacy of the mild treatment strategy.

In contrast to most previous studies, implantation was not chosen as the outcome parameter of the model. Because the transfer of more than one embryo could partly compensate for early pregnancy losses, the prediction of ongoing pregnancy is therefore clinically more relevant and should be the endpoint for models used to predict which patients might be better treated with DET.

As a consequence of the significant increase in pregnancy rates following the introduction of ovarian stimulation in IVF treatment, a high number of oocytes obtained following ovarian stimulation have become a surrogate marker of success; ovarian stimulation protocols has been graded according to the number of oocytes they resulted in. This tendency was supported by the fact that patients with a low number of oocytes despite "optimal" treatment were found to have a poor treatment outcome.

Mild ovarian stimulation leads to a reduction in ovarian response. Despite the reduced need for high numbers of oocytes due to the tendency to reduce the number of embryos transferred (Macklon et al., 2006), fear of retrieving a low number of oocytes appears to underly the reluctance of physicians to use mild ovarian stimulation for IVF. However, little is known regarding the optimal number of oocytes following ovarian stimulation. To investigate whether this fear of obtaining a reduced number of oocytes is justified, the clinical significance of low numbers of oocytes obtained following mild ovarian stimulation with regard to the pregnancy rate was studied.

This analysis showed that the optimal number of oocytes retrieved in IVF is dependent on the stimulation regimen used. Following conventional stimulation, we observed that the highest pregnancy rate was associated with the retrieval of 10 oocytes. In contrast, following mild stimulation, the best outcomes were observed following the retrieval of 5 oocytes. The higher success rates of the milder stimulation protocol with limited number of oocytes compared to a standard stimulation regimen could be the result of the subtle interference with the natural selection of good quality oocytes or the minimized exposure of growing follicles to the potentially negative effects of ovarian stimulation. This indicates that the fear associated with obtaining a reduced number of oocytes is unjustified, and should not be considered a negative side-effect of mild stimulation. A poor response should therefore no longer be defined regarding the absolute number of oocytes that is obtained; it is the ability to respond to a conventional maximal stimulation protocol that is associated with a poor outcome.

Although a modest number of oocytes following mild stimulation are sufficient to achieve good pregnancy results, a minimal response of two or three follicles is still required in order to compensate for the losses during stimulation, retrieval and oocyte pick up. Consequently, a second concern regarding the reduction in ovarian response following mild stimulation is the increased chance of cancellation because

of mono- or bifollicular growth. Although cycle cancellation following mild stimulation should be viewed in the context of the loss of just a few days of medication compared to the conventional approach in which the time elapsed and the amount of medication administered before an insufficient response is identified is much longer, a reduction in the number of cancellations would improve the efficacy and uptake of the mild approach.

The multivariate analysis of patient related characteristics associated with an insufficient response following mild ovarian stimulation in this thesis showed that a longer duration of infertility, short menstrual cycle length, secondary infertility and higher BMI were associated with an insufficient ovarian response. Using these parameters it was possible to develop a prediction model to identify patients at risk for cycle cancellation. The application of a standard GnRH antagonist stimulation protocol with an early start of FSH in these patients is likely to reduce their risk of cancellation to an average level. With the use of the model it was possible to predict patients at risk to the extent that the overall cancellation risk was similar to a standard stimulation protocol. Consequently, the use of such a prediction model may improve the efficacy of the mild stimulation protocol. Further prospective randomized trial should evaluate the clinical use of adjusting the starting day of ovarian stimulation based on the current prediction model.

As previously described, equally high pregnancy rates per time unit can be obtained when a mild treatment strategy is applied as long as patients are willing to undergo more mild treatment cycles in the same period of time (Heijnen et al., 2007).

A concern regarding the need for multiple cycles to obtain maximal treatment outcomes is the high rate of drop-outs frequently encountered in IVF and ICSI treatment. However, an important reason why many patients drop-out of IVF treatment before they have received all reimbursed treatment cycles appears to be psychological distress associated with IVF treatment (Olivius et al., 2004). As one of the aims of mild stimulation is to develop a patient friendly treatment modality, it is to be expected that the number of patients dropping out of a mild treatment will be lower than following conventional treatment. Indeed, analysis of factors influencing the decision of couples to discontinue treatment showed that the use of a mild treatment strategy had a positive effect on the drop-out rate. This effect was most evident in patients with high pre-existing levels of anxiety symptoms. The fact that the drop-out rate per cycle was lower in the mild strategy group, shows that patients are indeed willing to undergo more mild treatment cycles as long as a mild treatment strategy is applied.

Furthermore, the chance of drop-out was observed to be significantly increased when severe male subfertility was the treatment indication and when embryo transfer had not been performed. These findings highlight the importance of managing expectations in IVF treatment. If couples are counselled to focus less on the chance

of an individual cycle resulting in pregnancy, and encouraged to think in terms of a course of treatment aimed at achieving a live birth, they may be less likely to drop-out of treatment.

IMPLICATIONS FOR CLINICAL PRACTICE AND FUTURE RESEARCH

The most effective way to reduce the complications associated with IVF treatment is to limit the number of patients undergoing IVF treatment. There is a role for society to discourage the tendency of women to postpone their pregnancy until beyond their most fertile age. This could lead to a reduction in women needing fertility treatment. Furthermore, young women who do require fertility treatment have a better chance of becoming pregnant than older women (Lintsen et al., 2007) and will feel less pressure to use methods associated with a high risk of complications such as multiple pregnancy.

With regard to IVF treatment, if the mild approach is to be adapted into daily practice at least two conditions should be met. The first condition is that success from IVF treatment should be considered in terms of a complete treatment or treatment period instead of in terms of ongoing pregnancy rate per cycle, while also taking the risks, complications and patients discomfort into account. A second prior condition is the availability of (reimbursed) IVF treatment. As long as IVF is not easily accessible or patients have to pay for each cycle themselves it is likely that treatment strategies with the highest pregnancy rates (and most complications) will be preferred. Since society will bear a large proportion of the costs associated with the complications from IVF treatment, there is a role for individual governments to assist in the uptake of mild strategies for IVF by increasing the accessibility of IVF in the public sector and encourage health insurance companies to provide full coverage of fertility treatments so that patients will be more willing to use milder strategies.

However, in the short term, the most effective way to improve the further uptake of mild strategies in standard practice is to improve its efficacy. In this thesis it was shown that fear regarding the use of mild stimulation for a reduction in ovarian response or an increase of drop-outs (when the number of treatment cycles needed is increased) is unjustified. Indeed, the retrieval of a modest number of oocytes following mild ovarian stimulation was, in contrast to following conventional ovarian stimulation, associated with optimal pregnancy outcomes. Additionally, patients were found to be willing to undergo an increased number of treatment cycles as long as a mild ovarian stimulation protocol was applied. Furthermore, prediction models were developed to reduce the chance of cancellation following mild stimulation and to provide an evidence based method to identify women who may qualify for the transfer of a single versus two embryos. After external validation the first model should be able to reduce the chance of cancellation following mild stimulation to an

average level, the second should lead to an increase the overall pregnancy rate per transfer while maintaining a low number of multiple pregnancies. These interventions could increase the efficacy and implementation of mild treatment strategies.

A possible disadvantage of the development of lower numbers of oocytes might be the reduction of supernumerary embryos for cryopreservation to transfer in subsequent (unstimulated) cycles. However, the real added value of cryopreservation programmes is still under debate (Jones et al., 1997, de Jong et al., 2002) and the resulting increase of patient specific pregnancy rates (i.e. increased chance of an ongoing pregnancy from a frozen transfer after a failed fresh transfer) may be less than generally perceived. Until the efficacy of embryo cryopreservation programmes is improved, increasing embryo numbers solely to enhance the possibility of cryopreservation is not a strong argument in favour of aggressive ovarian stimulation. The recent introduction of vitrification as a new technique for cryopreservation of embryos (and oocytes) might change this. This method appears to be more efficient and reliable than slow freezing and leads to improved survival rates and clinical outcomes (Vajta and Nagy, 2006). However, safety validation in view of the high cryoprotectant concentrations used is required. Nevertheless, evidence suggests that the number of chromosomally normal embryos per stimulated cycle is limited and equally high following mild of conventional ovarian stimulation (Baart et al., 2007). This finding suggests that despite the increase in absolute numbers of embryos, aggressive stimulation is unlikely to increase the number of embryos with a high implantation potential and will therefore not improve the overall pregnancy rate per stimulated cycle.

The studies presented in this thesis have also generated a number of new research questions. For instance, it was observed that a profound ovarian response following mild stimulation was associated with a relatively poor chance of pregnancy. A plausible explanation for this finding may be a greater incidence of premature LH rises in the presence of large number of follicles (The ganirelix dose-finding study group, 1998; Born et al., 2000). As a flexible GnRH antagonist protocol was applied in the studies presented in this thesis, an earlier start of GnRH antagonist co-treatment might prevent this and result in a further improvement of mild stimulation. This phenomenon may also partly explain the overall slight reduction in pregnancy rates observed in IVF cycles with GnRH antagonist co-treatment compared to GnRH agonist cycles (Al-Inany et al., 2006) although this finding was not confirmed in another study (Kolibianakis et al., 2006). Alternatively, in high response patients more oocytes of lower quality are stimulated to develop, while the associated supraphysiological steroid levels negatively affect the endometrium resulting in impaired implantation.

The studies presented in this thesis focused on IVF patients below 38 years of age with a regular menstrual cycle. However, it is mainly patients with PCOS that encounter the highest risk of complications of ovarian stimulation such as OHSS.

Consequently, this patient group may benefit most from milder treatment strategies. Furthermore, even though it is predominantly the young patients with the highest risks of complications, poor response patients with signs of ovarian aging might also benefit from a more physiological stimulation approach with less detrimental effects on endometrium and oocytes. As most of these patients will fail to respond well to any type of ovarian response, a mild stimulation protocol might prove to be cost-effective. Consequently, future studies of the application of mild strategies should also be directed at these patients groups.

Besides this, further improvement following SET is to be expected when embryo selection techniques and embryo quality are improved. Methods such as extended embryo culture could improve the outcome per cycle, even when SET is applied (Quea et al., 2007).

Eventually, ovarian stimulation might be replaced by in-vitro maturation (IVM) of oocytes. This technique aims at the in vitro culture of follicles after retrieval of immature oocytes from unstimulated or minimally stimulated cycles. Consequently, it does not require the use of large doses of gonadotrophins for in vivo follicular growth and oocyte maturation (Barnes et al., 1995 and 1996; Oktay et al., 1998). Over the next few years the role of IVF on in vitro matured oocytes will be defined. At the present time, over 400 babies have been born following this procedure (Nargund and Frydman, 2007). It appears to be suitable for younger women with polycystic of multifollicular ovarian syndrome who are at high risk of developing sever OHSS (Mikkelsen et al., 2001). However, recent data have raised concerns regarding the safety of IVM, deleterious effects on the organization of the meiotic spindle and chromosome alignment of human oocytes and epigenesis have been suggested (Young et al., 2001; Kerjean et al., 2003; Li et al., 2006). The safety of the procedure should be demonstrated before it can be implemented in standard practice.

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SUMMARY

CHAPTER 1

In the introduction of this thesis the advantages and disadvantages of current strategies for IVF treatment are discussed as are the aims and outline of this thesis.

Main concerns regarding the current treatment strategies for IVF are the high costs, high rate of multiple pregnancies, the physical and psychological burden on patients concerns regarding the neonatal outcome of offspring conceived by IVF. Milder forms of ovarian stimulation and SET have been introduced as means of reducing these problems. However, physicians have shown reluctance towards adapting these treatment strategies in general practice fearing to reduce the efficacy of the treatment. This thesis addresses these arguments and search for factors which may improve the efficacy and therefore the uptake of milder treatment strategies for IVF.

CHAPTER 2.1

This chapter reviews the literature on the contribution of fertility treatment to multiple pregnancies and strategies for reducing multiples in assisted reproductive technologies (ART).

Multiple pregnancies are considered to be unfavourable due to the poor neonatal outcome, maternal complications, long term developmental problems and high costs involved. Multiple pregnancies in ART are the result of ovarian hyperstimulation used to improve treatment outcome and the transfer of more than one embryo after in-vitro fertilisation. Methods to prevent multiple pregnancies include restrictive use of ART in couples with good chance of spontaneous pregnancy, cautious use of gonadotrophins, increased use of natural cycle intra uterine insemination and elective SET in IVF and ICSI treatment. The challenge is to include these modifications in routine practice despite a potential decrease in the pregnancy rate per treatment cycle. One way of improving the acceptance to alter the method of reporting success;

reporting outcomes per completed treatment instead of per cycle including the complications and overall costs will emphasize the benefits of these treatment strategies.

CHAPTER 2.2

This chapter provides an overview of the literature with respect to mild ovarian stimulation approaches for IVF.

Mild ovarian stimulation for IVF aims at achieving a cost effective, patient friendly regimen in which the balance between outcome and discomfort and risks of treatment are optimized. The introduction of GnRH antagonists into clinical practice and a greater understanding of the process of follicle recruitment and dominant follicle selection have led to the development of milder, more physiological ovarian stimulation approaches. Currently applied mild stimulation approaches include the limited use of gonadotrophins or the use of alternative ovarian stimulating medication such as anti-estrogens, aromatase inhibitors and late follicular phase luteinizing hormone or human chorionic gonadotrophin administration.

Besides a reduction in costs and side effects, there is an increasing amount of evidence indicating that mild ovarian stimulation has a positive effect on embryo quality and endometrial receptivity. In general, the size and methodological quality of the studies on the efficacy of the newly introduced mild stimulation protocols are poor. Furthermore, structured reporting of the incidence and severity of complications, the number of treatment days, medication used, costs, patient discomfort and number of patient drop-outs in studies on IVF strategies is needed to increase the awareness of the need to apply mild ovarian stimulation.

CHAPTER 3

The purpose of this chapter was to develop a model for the prediction of ongoing pregnancy after SET following mild stimulation for IVF in women below 38 years of age.

As part of a randomized controlled study, 195 normo-ovulatory women, below 38 years of age, with a regular indication for IVF/ ICSI were treated with a mild ovarian stimulation protocol including cycle day five start with a low fixed dose (150 IU/day) gonadotrophins and late follicular phase GnRH antagonist co-treatment and SET. Only women with an elective SET (n=152) were included in the analysis. The ongoing pregnancy rate per elective SET in these patients was 28% (42/152). Multivariate logistic regression was used to develop a prediction model for ongoing pregnancy. BMI, number of oocytes retrieved, ovarian sensitivity to stimulation and the availability of a top quality embryo were all correlated with ongoing pregnancy and were included in the model. The overall predictive ability of the model was

0.68 (area under the ROC curve). At a probability cut-off level of 0.20, the model showed a sensitivity of 36% and a specificity of 90%. The presented model provides an evidence based guidance for conditions under which conditions SET may be performed. After external validation, application of the model may help to improve overall singleton pregnancy rates.

CHAPTER 4

This chapter addresses the clinical significance of the retrieval of a low number of oocytes following mild ovarian stimulation for IVF.

The relationship between oocytes number and the chance of conceiving after mild and conventional stimulation were compared. Data from three randomized controlled trials in woman < 38 years of age comparing mild stimulation with GnRH antagonist co-treatment (313 patients) with a conventional GnRH agonist co-treatment stimulation protocol (279 patients) were analysed. The mild stimulation protocol resulted in lower numbers of oocytes compared to conventional ovarian stimulation (median 6 vs. 9 respectively, $p < 0.001$). Optimal embryo implantation rates were observed with five oocytes retrieved following mild stimulation (31%) versus ten oocytes following conventional stimulation (29%) ($p = 0.045$). This study shows that the optimal number of oocytes is dependent on the stimulation regimen used and confirms that in contrast to the case following conventional stimulation, the retrieval of low numbers of oocytes following mild stimulation is associated with better chances of pregnancy. This observation suggests that when few oocytes are obtained following mild stimulation they are likely to represent a homogenous group of good quality oocytes. The relatively poorer outcome in high responders in the mild stimulation protocol compared to the conventional protocol warrants further study as to its cause. One possible explanation is the occurrence of untimely luteinizing hormone rises as a consequence of the use of a flexible GnRH antagonist downregulation protocol.

CHAPTER 5

The aim of this chapter was to develop a prognostic model for the prediction of cycle cancellation due to insufficient response to mild ovarian stimulation for IVF.

Multivariable logistic regression analysis was performed on data of 174 IVF patients aged < 38 years and with a BMI < 28 treated with mild ovarian stimulation and SET. The mild stimulation consisted of cycle day five start with a fixed daily dose (150 IU) of gonadotrophins, and GnRH antagonist co-treatment from the late follicular phase. In women with mono or bifollicular growth (17%), the cycle was cancelled and treatment adjusted in a second treatment cycle by starting recFSH on cycle day two. A longer duration of infertility, short menstrual cycle length,

secondary infertility and higher BMI were associated with an insufficient ovarian response. These variables were included in a prediction model. The area under the receiver-operating characteristics curve of the model was 0.69. A probability cut-off for cancellation of 0.3 yielded an expected sensitivity of 33%, specificity of 92%. Analysis of ovarian response in the subsequent treatment cycle showed an improved ovarian response and a significant reduction in cancellation rate. With the presented model it is possible to identify patients at risk for cycle cancellation during mild ovarian stimulation due to insufficient response. The contributing factors of the model suggest a relationship between ovarian aging, BMI and insufficient response to mild stimulation.

CHAPTER 6

In this chapter factors that influence the decision of couples to discontinue treatment were analysed, including the treatment strategy applied.

The incidence of drop-out from IVF and factors associated with withdrawal from treatment were analysed in 384 patients aged < 38 years undergoing IVF/ ICSI for standard indications. Patients were treatment either with a mild (cycle day five start with a low fixed dose (150 IU/day) gonadotrophins and late follicular phase GnRH antagonist co-treatment and SET) or a standard treatment strategy (conventional GnRH agonist co-treatment stimulation protocol and DET) for a planned maximum of four (mild) or three (conventional) treatment cycles. 17% of the patients dropped out of IVF treatment before a pregnancy was achieved or the planned number of cycles had been carried out. The physical or psychological burden of treatment was the most frequent cause of drop-out (28%). The application of a mild treatment strategy significantly reduced the chance of drop-out (Hazard Ratio 0.55 (95% CI 0.31-0.96)). When a mild IVF strategy was applied, the association between the baseline anxiety score and drop-out was reduced by more than 50%. Couples were also more likely to discontinue treatment in the presence of severe male subfertility (Hazard Ratio 4.80 (95 % CI 1.63-14.13)) and the failure to achieve embryo transfer (Odds Ratio 0.41 (95% CI 0.24-0.72)). Couples at increased risk of dropping out of IVF can be identified. An important factor determining this risk is the burden of the treatment strategy itself. Reducing drop out from IVF is necessary to improve the efficacy and cost-effectiveness of treatment.

CHAPTER 7

This chapter discusses the conclusions which could be drawn from the work presented in the current thesis. In this thesis it was shown that fear of using mild stimulation with regard to a reduction in ovarian response or regarding drop-outs when the number of treatment cycles needed is increased is unjustified.

Furthermore, prediction models were developed to decrease the number of cancellations following mild stimulation to a normal level and to provide an evidence based method for when to apply SET. These interventions should increase the efficacy and therefore the uptake of milder treatment strategies for IVF.

SAMENVATTING

HOOFDSTUK 1

In de introductie van dit proefschrift worden, naast de voor- en nadelen van de huidige strategieën voor IVF behandeling, de doelen en opzet van dit proefschrift besproken.

De voornaamste nadelen van het huidige behandelprotocol voor IVF zijn de hoge kosten, het hoge percentage meerlingen, de psychische en fysieke belasting voor patiënten en de potentieel slechtere neonatale uitkomst van IVF baby's. Het gebruik van milde ovariële stimulatie en het toepassen van single embryo transfer (SET) kan deze complicaties verminderen. Echter, door de mogelijke vermindering van de kans op zwangerschap per cyclus ten gevolge van deze aanpassingen bestaat er weerstand tegen het gebruik van deze methodes. Het doel van dit proefschrift is het weerleggen van de argumenten tegen het gebruik van milde ovariële stimulatie en het zoeken naar factoren die de effectiviteit en het acceptatie van mildere behandelstrategieën zullen verhogen.

HOOFDSTUK 2.1

In dit hoofdstuk wordt de literatuur betreffende het aandeel van fertiliteitsbevorderende behandelingen op het aantal meerling-zwangerschappen besproken.

Meerling-zwangerschappen worden als nadelig beschouwd ten gevolge van de slechtere neonatale uitkomst, lange termijn ontwikkelingsproblemen, maternale complicaties en de hoge kosten die hiermee gepaard gaan. Meerling-zwangerschappen na fertiliteitsbehandelingen zijn het gevolg van de ovariële stimulatie, gebruikt om meer dan één follikel te laten uitrijpen, en het terug plaatsen van meer dan één embryo na de IVF of ICSI behandeling. Methoden om meerling-zwangerschappen te voorkomen zijn: het beperken van het gebruik van fertiliteitsbehandelingen bij patiënten met een goede kans op een spontane zwangerschap, beperkt gebruik van ovariële stimulatie, IUI in de natuurlijke cyclus en het

terugplaatsen van één embryo na IVF en ICSI behandeling. De uitdaging ligt in het implementeren van deze methodes ondanks een mogelijke vermindering van de kans op zwangerschap per behandel cyclus. Een van de mogelijkheden om de acceptatie van deze methoden te vergroten is het aanpassen van de methode waarop behandelresultaten gerapporteerd worden. Door de behandelresultaten te rapporteren per complete behandeling in plaats van per cyclus inclusief de complicaties en de kosten van de behandeling worden de voordelen van deze behandelmethodes benadrukt.

HOOFDSTUK 2.2

Dit hoofdstuk geeft een overzicht van de literatuur over milde ovariële stimulatie methodes voor IVF.

Milde ovariële stimulatie voor IVF heeft als doel een kosteffectieve, patiëntvriendelijk behandelprotocol te zijn met een optimale balans tussen resultaten, complicaties en risico's van de behandeling. De introductie van GnRH antagonisten in de kliniek en voortschrijdend inzicht in het proces van follikelrecrutering en dominante follikelselectie hebben geleid tot de ontwikkeling van mildere, meer fysiologische behandelmethodes. Huidige milde stimulatieprotocollen bevatten ondermeer het gebruik van alternatieve medicatie voor ovariële stimulatie, zoals anti-oestrogenen, aromatase inhibitors in de vroege folliculaire fase en hCG of LH toediening in de late folliculaire fase.

Naast een vermindering van kosten en complicaties zijn er aanwijzingen dat milde stimulatie tot een verbeterde embryo- en endometriumkwaliteit leidt. Over het algemeen betreffen de studies over de effectiviteit van milde stimulatieprotocollen relatief kleine onderzoekspopulaties en zijn van matige methodologische kwaliteit.

Het consequent rapporteren van de incidentie en ernst van de complicaties, het aantal behandeldagen, de hoeveelheid gebruikte medicatie, kosten, belasting voor de patiënt en het aantal uitvallers in studies naar verschillende behandelprotocollen is nodig om de bewustwording van de behoefte aan milde stimulatie te vergroten.

HOOFDSTUK 3

Het doel van dit hoofdstuk was het ontwikkelen van een model voor het voorspellen van de kans op een doorgaande zwangerschap met SET na IVF behandeling met een milde stimulatieprotocol.

Als onderdeel van een prospectieve gerandomiseerde studie werden 195 paren onder de 38 jaar met een normo-ovulatoire cyclus en een standaard indicatie voor IVF/ ICSI behandeld met een mild stimulatie protocol met ovariële stimulatie vanaf cyclus dag 5 en GnRH antagonist vanaf de mid-folliculaire fase en SET. Patiënten met een electieve SET (n=152) werden geïncludeerd in de analyse, de kans op een doorgaande zwangerschap in deze patiënten groep was 28% (42/152). Multivariate

logistische regressie-analyse werd toegepast op data van deze patiënten om een predictiemodel te ontwikkelen voor de kans op een doorgaande zwangerschap. BMI, het aantal eicellen, ovariële gevoeligheid voor stimulatie en de beschikbaarheid van een top kwaliteit embryo bleken gerelateerd aan de kans op een doorgaande zwangerschap en werden geïncludeerd in het model.

De voorspellende waarde van het model was 0,68 (area under the ROC curve). Een afkapwaarde van 0,2 van het model was leidde tot een sensitiviteit van 36% en een specificiteit van 90%. Het model biedt een evidence based methode om te bepalen onder welke condities SET toegepast moet worden. Na externe validatie kan toepassing van het model leiden tot een verhoging van de kans op een eenling zwangerschap.

HOOFDSTUK 4

In dit hoofdstuk worden de klinische implicaties van een lage oocyte opbrengst na milde ovariële stimulatie geanalyseerd.

De relatie tussen het aantal eicellen en de kans op zwangerschap na milde en conventionele ovariële stimulatie vergeleken. Data van drie prospectieve, gerandomiseerde studies waarin de effectiviteit van een mild behandelprotocol met GnRH antagonist (313 patiënten) en een conventioneel behandelprotocol met GnRH agonist downregulatie (279 patiënten) bestudeerd wordt bij patiënten onder de 38 jaar werden geanalyseerd. Het milde stimulatieprotocol resulteerde in een lagere eicellenopbrengst in vergelijking met conventionele stimulatie (mediaan 6 vs. 9 respectievelijk, $p < 0.001$). De hoogste embryo-implantatiekans was geassocieerd met vijf eicellen na milde stimulatie (31%) ten opzichte van tien eicellen na conventionele stimulatie (29%) ($p = 0.045$). Deze studie laat zien dat het optimale aantal eicellen afhankelijk is van het gebruikte stimulatieschema. Daarnaast wordt bevestigd dat, in tegenstelling tot bij standaard stimulatie, na milde stimulatie een beperkte eicelopbrengst geassocieerd is met de optimale zwangerschapskans. Deze bevinding impliceert dat milde ovariële stimulatie zorgt voor de selectie van een homogene groep eicellen van goede kwaliteit.

De observatie van de lage kans op zwangerschap na milde stimulatie als er veel eicellen zijn gevonden is mogelijk gerelateerd aan suboptimale suppressie bij het gebruik van een flexibel GnRH antagonist downregulatieprotocol en vraagt om verdere analyse.

HOOFDSTUK 5

Het doel van dit hoofdstuk is het ontwikkelen van een prognostisch model voor het voorspellen van de kans op een afgebroken cyclus (*cancelled*) ten gevolge van een onvoldoende ovariële response na milde ovariële stimulatie.

De gegevens van 174 IVF patiënten onder 38 jaar met een BMI onder 28 die met milde ovariële stimulatie en SET werden behandeld werden met behulp van multivariate logistische regressie geanalyseerd. Patiënten werden gestimuleerd vanaf CD 5 met 150 IE rFSH waarna een GnRH antagonist werd toegediend in de late folliculaire fase. Bij patiënten met mono of bifolliculaire groei (17%), werd de cyclus afgebroken en het behandelprotocol aangepast in een tweede behandelcyclus waarbij de rFSH werd gestart op cyclus dag twee.

Een langere infertiliteitsduur, kortere menstruele cyclus, secundaire infertiliteit en een hogere BMI bleken geassocieerd met onvoldoende ovariële response. Deze variabelen werden geïncludeerd in een predictiemodel. De "area under the receiver operating characteristic curve" van het model was 0,69. Een afkapwaarde van 0,3 van het model leidde tot een sensitiviteit van 33% en een specificiteit van 92%. Analyse van de ovariële response in de volgende behandelcyclus liet een verbetering van de response zien en een significante vermindering van de kans op afbreken.

Met het gepresenteerde model is het mogelijk patiënten te identificeren met een verhoogd risico op afbreken tijdens milde ovariële stimulatie ten gevolge van een onvoldoende ovariële respons. De factoren in het model suggereren een relatie tussen ovariële veroudering, BMI en een onvoldoende ovariële respons op milde ovariële stimulatie.

HOOFDSTUK 6

In dit hoofdstuk worden factoren, inclusief behandel protocol, geanalyseerd die maken dat patiënten vroegtijdig de IVF behandeling staken.

De incidentie van drop-out van IVF behandeling en factoren die geassocieerd zijn met het staken van de behandeling werden geanalyseerd bij 384 patiënten onder de 38 jaar die IVF of ICSI behandeling ondergingen voor standaard indicaties. Patiënten werden met een mild (cyclusdag vijf start met 150 IU/ dag gonadotrophines, GnRH antagonist en SET) of een conventioneel behandelprotocol (met GnRH agonist downregulatie en DET) behandeld voor een vooraf besloten maximaal aantal van drie (conventioneel) of vier (mild) behandelingscycli. Zeventien procent van de patiënten staakten de behandeling voordat ze zwanger werden of het maximum aantal behandelcycli was behaald. De fysieke of psychische belasting van de behandeling werd het meest frequent genoemd als reden (28%). Het toepassen van een milde behandelstrategie was gerelateerd aan een significante vermindering van de kans op drop-out (Hazard Ratio 0.55 (95% CI 0.31-0.96)). Als een milde stimulatieprotocol werd toegepast, was de associatie tussen de baseline angstscore en drop-out verminderd met meer dan 50%. De kans op drop-out was ook groter als de indicatie voor behandeling een ernstige mannelijke factor betrof (Hazard Ratio 4.80 (95 % CI 1.63-14.13)) of de cyclus niet resulteerde in een embryotransfer (Odds Ratio 0.41 (95% CI 0.24-0.72)). Patiënten met een verhoogd risico op drop-out kunnen dus geïdentificeerd worden.

Een belangrijke factor die de kans op drop-out bepaalt is de belasting van de behandeling zelf. Het verminderen van het aantal patiënten dat voortijdig de behandeling staakt is noodzakelijk om de effectiviteit van de behandeling te verhogen.

HOOFDSTUK 7

In dit hoofdstuk worden de conclusies die uit de studies in dit proefstuk getrokken konden worden bediscussieerd. Dit proefschrift laat zien dat de angst voor het gebruik van milde stimulatie ten aanzien van het verminderen van de ovariële response of het vermeerderen van het aantal behandelcycli per patiënt om tot een gelijk behandelresultaat te komen ongegrond zijn. Daarnaast bleek het mogelijk met behulp van predictiemodellen te voorspellen welke patiënten een verhoogd risico hadden op een afgebroken cyclus of juist een lage kans op zwangerschap na SET. Door de behandeling aan te passen voor deze patiënten wordt de effectiviteit van de gehele behandeling hoger. Door deze bevindingen kan de effectiviteit en daarmee ook het gebruik van milde ovariële stimulatie vergroot worden.

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CURRICULUM VITAE

Marieke Verberg werd op zondag 4 september 1977 geboren in Leiden. Na het behalen van haar middelbare school diploma aan het Bonaventura college in Leiden ging zij in Groningen geneeskunde studeren. Na haar co-schappen in het UMC Groningen volgde zij een wetenschappelijke stage aan de McMaster University in Hamilton, Canada onder begeleiding van prof. dr. Salim Daya. Na het behalen van haar artsenbul in 2003 verhuisde zij naar Amsterdam en werkte ze een jaar als AGNIO op de afdeling gynaecologie en verloskunde van het Spaarne Ziekenhuis in Haarlem. Vervolgens deed zij acht maanden onderzoek naar hyperemesis gravidarum aan het St. Bartholomew's Hospital in Londen onder begeleiding van prof. J.G. Grudzinskas. In 2005 keerde ze weer terug naar Nederland en kreeg zij een aanstelling als IVF-arts/ onderzoeker aan het UMC Utrecht waar zij onder begeleiding van prof. N.S. Macklon en prof. B.C.J.M. Fauser startte met haar promotie-onderzoek. Sinds mei 2007 is ze in opleiding tot gynaecoloog in het cluster Utrecht. Momenteel werkt zij in het Diakonessenhuis te Utrecht (opleider dr. P.C. Scholten) en woont zij samen met Rutger in Amsterdam.

Nicole Horrée (paranimf)

LIST OF ABBREVIATIONS

AI	aromatase inhibitors
ART	assisted reproductive techniques
AUC	area under the receiver-operating characteristics curve
BMI	body mass index
CC	Clomiphene Citrate
CD	cycle day
CI	confidence interval
DET	double embryo transfer
E_2	17ß-oestradiol
ET	embryo transfer
FISH	fluorescence in situ hybridization
FSH	follicle-stimulating hormone
FSH-CTP	follicle-stimulating hormone -C-terminal peptide
GEE approach	generalized estimating equation approach
GIFT	gamete intrafallopian transfer
GnRH	gonadotropin-releasing hormone
HADS	hospital anxiety and depression scale
hCG	human chorionic gonadotrophin
hMG	human menopausal gonadotrophin
HR	hazard ratio
ICSI	intracytoplasmic sperm injection
IU	international unit
IUI	intra uterine insemination
IVF	in vitro fertilisation
IVM	in-vitro maturation
LH	luteinizing hormone
LOD	laparoscopic ovarian drilling
MFPR	multifetal pregnancy reduction

n.a.	not available.
NPV	negative predictive value
NS	non significant
OAC	oral anticonceptive
OHSS	ovarian hyperstimulation syndrome
OI	ovulation induction
OR	odds ratio
PCOS	polycystic ovary syndrome
PGS	preimplantation genetic screening
PPV	positive predictive value
r	recombinant
RCT	randomized controlled trial
ROC curve	receiver-operating characteristics curve
RR	relative risk
s.c.	subcutaneously
SD	standard deviation
SET	single embryo transfer