Modified natural cycle IVF : feasibility and results

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Modified Natural Cycle IVF

feasibility and results

Marie-José Pelinck

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Stellingen behorende bij het proefschrift

'Modified natural cycle IVF: feasibility and results'

Marie-José Pelinck

1. Modified natural cycle IVF is een geschikte behandelingsmodaliteit voor alle indicaties voor IVF *(dit proefschrift)*.

2. De kans dat een embryo implanteert is bij modified natural cycle IVF-behandeling minstens net zo groot als bij IVF-behandeling met ovariële hyperstimulatie *(dit proefschrift)*.

3. De kans op zwangerschap per gestarte modified natural cycle-behandelingscyclus daalt niet in hogere cyclusnummers. Dit wordt mede veroorzaakt door selectieve drop-out van patiënten met een relatief lage kans op zwangerschap *(dit proefschrift)*.

4. IVF-behandeling bestaande uit modified natural cycle IVF, als nodig gevolgd door IVF met ovariële hyperstimulatie is even effectief als IVF behandeling bestaande uit IVF met ovariële hyperstimulatie alleen en gaat gepaard met minder complicaties *(dit proefschrift)*.

5. Het nut van het gebruik van een GnRH-antagonist bij modified natural cycle IVF staat niet vast *(dit proefschrift)*.

6. Zolang vergoeding van IVF-behandeling gebonden is aan een maximum aantal uit te voeren cycli, zullen patiënten kiezen voor meer eieren voor hun geld.

7. De snelle invoering van vaccinatie tegen HPV is een voorbeeld van marktwerking in de gezondheidsraad.

8. De kunst van het dokter zijn is de patiënt te amuseren terwijl de natuurlijke genezing doorzet *(Voltaire)*.

9. Een succesvolle IVF-behandeling betaalt zichzelf terug *(Hum Reprod 2009, 24: 626-632)*.

10. Een afweging van de voor- en nadelen kan objectief gezien niet tot de keuze voor kinderen voeren. Een kinderwens is dan ook biologisch ingegeven, wat met zich brengt dat de discussie over de maatschappelijke wenselijkheid van IVF niet op basis van rationele argumenten gevoerd kan worden.

11. Aaisykje is niet alleen op het Friese platteland maar ook in een IVF-kliniek aan strenge regels gebonden.

12. Een cowboy met zijn handen omhoog juicht waarschijnlijk niet.

13. If you come to a fork in the road, take it *(Yogi Berra)*.

Modified natural cycle IVF
Marie-Jose Pelinck
PhD thesis, University of Groningen - with summary in Dutch

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Rijksuniversiteit Groningen

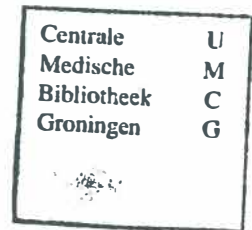# Modified natural cycle IVF:
feasibility and results

Proefschrift

ter verkrijging van het doctoraat in de
Medische Wetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
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door

Maria Josephina Pelinck

geboren op 12 maart 1969
te Apeldoorn

Contents

Chapter 1

Introduction

Introduction

The first successful in vitro fertilization (IVF) treatment was performed in a natural, unstimulated menstrual cycle (Steptoe and Edwards, 1978). In those days, oocyte harvesting was done by laparoscopy, necessitating general anaesthesia and was often unsuccessful. Timing of oocyte retrieval was based on the LH surge, requiring intensive cycle monitoring, and untimely ovulations often occurred. In the laboratory, in-vitro fertilization was not very efficient, especially in cases with suboptimal semen quality.

Thus, IVF in the natural cycle was largely replaced by IVF with controlled ovarian hyperstimulation (COH) in order to obtain a larger number of growing follicles, making oocyte retrieval more efficient and increasing the oocyte yield and number of embryos available for transfer. Injectable medication was used for ovulation triggering (hCG), enabling planning of oocyte retrieval, as well as GnRH-agonists for pituitary downregulation and prevention of LH-surges. For ovarian stimulation, oral anti-estrogens (clomiphene citrate) were used initially. The introduction of injectable human menopausal gonadotrophins (HMG) for ovarian hyperstimulation further increased the oocyte yield.

The use of COH and the practice of multiple embryo transfer led to an increase in pregnancy rates, but along with it to an increase in complications and costs. Complications occurring after COH are the ovarian hyperstimulation syndrome (OHSS), bleeding and infection of the ovaries after oocyte retrieval and multiple pregnancies. OHSS is characterized by increased vascular permeability and overexpression of vascular endothelial growth factor, leading to a shift of fluid from the intravascular compartment and haemoconcentration, which can result in thromboembolism and even death (Elchalal and Schenker, 1997). The most important complication, related to the practice of transferring multiple embryos, is the occurrence of multiple pregnancies, which are associated with increased adverse maternal outcome, poor neonatal outcome and high costs (Fauser et al., 2005). Maternal complications include hypertensive disorders, preeclampsia, postpartum haemorrhage, gestational diabetes, venous thromboembolism and increased risk of caesarean section (Walker et al., 2004). The main neonatal complications associated with multiple pregnancies are caused by preterm birth: neonatal mortality, low birth weight, respiratory distress syndrome, cerebral haemorrhage and necrotizing enterocolitis, in turn leading to long-term complications such as cerebral palsy, bronchopulmonary dysplasia and neurological disorders (Bergh et al., 1999). Evidence is increasing however, that also singleton pregnancies derived from COH-IVF are associated with poor neonatal outcome as compared to spontaneous pregnancies (Helmerhorst et al., 2004).

Reporting of success rates of IVF treatment was mainly focussed on the number of pregnancies per cycle or embryo transfer, with little attention for multiple pregnancy rates or other complications.

In recent years, efficiency of IVF treatment has increased due to several developments. Monitoring became easier due to the possibility of transvaginal ultrasound. The performance of oocyte retrieval by laparoscopy was replaced by the transvaginal route, increasing the oocyte yield per retrieval and abolishing the necessity of general anesthesia. Intracytoplasmic sperm injection (ICSI) was developed, making fertilization far more efficient in cases of severe semen deficiency (Palermo et al., 1992). An increased knowledge

of implantation potential of embryos, based on morphological criteria, enabled a better selection for transfer (Van Royen *et al.*, 1999).

These new developments, along with growing awareness of the risks and long-term consequences of multiple pregnancies, gave room for a change in approach in IVF treatments, with more focus on patient (dis)comfort and prevention of multiple pregnancies.

Elective single embryo transfer was introduced as a means to prevent multiple pregnancies (Gerris *et al.*, 1999). Milder stimulation regimens, with less patient discomfort and shorter duration per treatment cycle were proposed as an alternative for COH (Heijnen *et al.*, 2007).

In the reporting of IVF outcome, a shift toward a focus on live birth rates (instead of pregnancy or ongoing pregnancy), success per started cycle or full treatment (instead of success per oocyte retrieval or embryo transfer), and singleton pregnancies is visible. In this regard, several new outcome definitions are proposed, among which the 'BESST' (Birth Emphasizing a Successful Singleton at Term) (Min *et al.*, 2004). This outcome definition emphasizes the term live birth rate of singletons, acknowledging the importance of singleton pregnancies as a desired outcome of IVF treatment. The general adoption of an outcome parameter defining success as the number of pregnancies leading to the birth of a healthy child, per started cycle or better still, per full treatment, will enable a realistic comparison of results of treatment among different IVF-centers. The reporting of success rates per full treatment, i.e. cumulative pregnancy rates obtained after all IVF-cycles performed by one patient, provides the possibility of a realistic comparison of results of different treatment strategies. For instance, in comparing mild stimulation to COH-IVF, the lower *per cycle* success rate of mild stimulation is compensated by a shorter duration of treatment and lower drop-out of patients, eventually leading to similar success rates per full treatment (Heijnen *et al.*, 2007).

The development of GnRH-antagonists has renewed interest in the use of the natural cycle for IVF (Rongières-Bertrand *et al.*, 1999). As competitive inhibitors of the GnRH-receptor on the pituitary, GnRH-antagonists are able to block LH-surges while allowing for the development of one single dominant follicle. Since the occurrence of untimely LH-rises were an important cause of the lack of efficacy in natural cycle IVF, GnRH-antagonists are expected to be able to raise its efficacy.

For the approach in which treatment is aimed at the use of the one dominant follicle in the natural cycle, using a GnRH-antagonist for prevention of LH-rises, together with gonadotrophins for substitution, several different terminologies have been used, such as minimal stimulation, semi-natural cycle and modified natural cycle IVF. In a recent consensus meeting, it was agreed that the correct terminology for this approach should be modified natural cycle (MNC)-IVF (Nargund *et al.*, 2007).

MNC-IVF offers several advantages. Since gonadotrophins are administered in a low dose and only one or few follicles develop, the risk of the ovarian hyperstimulation syndrome (OHSS) is negligible. Since usually no more than one single embryo is available for transfer, it is associated with a low chance of multiple pregnancies. MNC-IVF is also a patient-friendly treatment since medication is administered for a few days only, causing few side effects, and the duration of a treatment cycle is considerably shorter than standard IVF

with COH. Since usually only one follicle is aspirated, oocyte retrieval is easy and short-lasting and can be performed without analgesia (Ramsewak *et al.,* 1990). As opposed to COH-IVF, in MNC-IVF no resting cycle is necessary after a failed treatment cycle and treatments are easily repeated in consecutive cycles. Since usually no spare embryos are generated, MNC-IVF is an attractive treatment option for patients who, for ethical or religious reasons, oppose to the generation of spare embryos (Biggers and Summers, 2004).

The low-risk and patient-friendly profile of MNC-IVF make it worthwhile to investigate its efficacy.

Scope of the thesis

The studies reported in this thesis aim to explore the efficacy of MNC-IVF. For this purpose, a cohort of patients was formed, who were offered either three or nine cycles of MNC-IVF preceding regular COH-IVF. Cumulative pregnancy rates, drop-out behaviour of patients and results according to indication for IVF were studied, as well as cost-effectiveness, implantation rates of embryos according to their morphological appearance and perinatal outcome of singletons resulting from these treatments.

In some of the studies in this thesis (chapters 3 and 4) the term 'minimal stimulation IVF' is used instead of 'modified natural cycle IVF', due to the fact that these studies were published before consensus was reached on terminology for this approach (Nargund *et al.,* 2007).

Outline

Chapter 2 provides an overview of published reports on the efficacy of natural cycle IVF without the use of GnRH-antagonists.

Chapter 3 reports the results of a maximum of three cycles of modified natural cycle IVF in a cohort of 50 patients.

Chapter 4 reports a multicenter study involving a cohort of 350 patients. The results of three cycles of modified natural cycle IVF, according to indication for IVF and according to cycle number are reported.

Chapter 5 addresses the question what would be the optimal number of cycles of modified natural cycle IVF. For this purpose, results of treatment and drop-out behaviour were studied in a cohort of 268 patients who were offered a maximum of nine cycles of modified natural cycle IVF.

Chapter 6 provides follow-up of patients described in chapter 5. Results of full treatment, consisting of modified natural cycle IVF followed by COH-IVF are given.

Chapter 7 reports on cost-effectiveness of modified natural cycle IVF compared to COH-IVF.

Chapter 8 provides a description of morphological characteristics of embryos derived from modified natural cycle IVF and according implantation rates.

Chapter 9 reports on perinatal outcome of singleton pregnancies derived from modified natural cycle IVF and gives a comparison with COH-IVF singletons.

Chapter 10 provides a general discussion of the findings from our studies and discusses implications for clinical practice and future research.

Chapter 2

Efficacy of natural cycle IVF: a review of the literature

MJ Pelinck, A Hoek, AHM Simons, MJ Heineman

ABSTRACT

Since the introduction of in vitro fertilization (IVF) treatments, natural cycle IVF has been largely replaced by IVF with ovarian stimulation. However, natural cycle IVF has several advantages. It is associated with a close to zero multiple pregnancy rate and a zero risk of ovarian hyperstimulation syndrome. Per cycle, natural cycle IVF is less time consuming, physically and emotionally less demanding for patients and cheaper than stimulated IVF but also less effective. This systematic literature review adresses the issue of effectiveness of natural cycle IVF. Herein, 20 studies describing natural cycle IVF are presented, 12 case series and eight in which a comparison was made between natural cycle IVF and IVF with ovarian stimulation. Good-quality randomised controlled trials and formal cost-effectiveness analyses are lacking. The 20 selected studies comprised a total of 1800 cycles of natural cycle IVF, resulting in 819 embryo transfers (45.5% per cycle) and 129 ongoing pregnancies (7.2% per cycle and 15.8% per embryo transfer). Efficacy of natural cycle IVF is hampered by high cancellation rates because of premature LH rise and premature ovulations.

It is concluded that natural cycle IVF is a low-risk, low-cost and patient-friendly procedure. A randomized controlled trial comparing natural cycle IVF with current standard treatment strategies is warranted.

Introduction

The first successful in vitro fertilization (IVF) treatment was performed in an unstimulated menstrual cycle (Steptoe and Edwards, 1978), since when IVF in natural cycles has been largely replaced by IVF with ovarian stimulation. The use of exogenous gonadotrophins and gonadotrophin-releasing hormone (GnRH) agonists lowers cancellation rates and raises the number of oocytes obtained, thus leading to a higher number of embryos and consequently better results in terms of pregnancy rates.

As a consequence of IVF with ovarian stimulation, two important complications arise, namely multiple pregnancies and ovarian hyperstimulation syndrome (OHSS).

In order to maximize the chance of pregnancy, multiple embryos are usually transferred to the uterus, leading to a 20-30% multiple pregnancy rate in most IVF programmes (Nygren and Andersen, 2001). Compared with naturally conceived pregnancies, the risk for twins in IVF-pregnancies is increased about 20-fold, and the risk for higher order multiples about 400-fold (Kauma 1997). Twin and higher order multiple pregnancies are associated with a high risk of prematurity, causing considerable morbidity and mortality of the neonates (Bergh *et al.,* 1999; Elster *et al.,* 2000). Compared to singletons, twins have a 5-6 fold higher perinatal mortality rate (Lieberman, 1998).

Up to ten percent of IVF treatments with ovarian stimulation lead to the ovarian hyperstimulation syndrome which is a severe and sometimes life-threatening condition (Elchalal and Schenker, 1997; Beerendonk *et al.*, 1998).

Moreover, although available data are reassuring, the possibility of heightened risk of ovarian cancer after repeated ovarian stimulation remains a matter of concern. (Duckitt and Templeton, 1998). The generation of spare embryos also causes ethical and religious dilemmas.

Currently, attention is focussed on developing strategies to avoid twin and higher-order multiple pregnancies, as well as strategies to make IVF more 'patient-friendly' (Edwards *et al.,* 1996; Olivennes and Frydman, 1998; Templeton, 2000; Olivennes, 2000; Jones and Schnorr, 2001). Strategies proposed to restrict the incidence of multiple pregnancies are e.g. elective transfer of a single embryo (Vilska *et al.,* 1999; Gerris and Van Royen, 2000; ESHRE Campus Course Report, 2001; Martikainen *et al.,* 2001) or blastocyst (Gardner *et al.,* 2000a,b).

Various regimens of minimal ovarian stimulation for IVF have been proposed as patient-friendly strategies, with no risk of OHSS and pregnancy rates of 17-33% per oocyte retrieval, yet still leading to multiple pregnancy rates of 5 –14% (Fauser *et al.,* 1999; Branigan and Estes, 2000; De Jong *et al.,* 2000; DeVane *et al.,* 2000; Macklon and Fauser, 2000; Ingerslev *et al.,* 2001).

In natural cycle IVF, a low-risk profile is combined with a patient-friendly treatment.
Natural cycle IVF is associated with a close to zero multiple pregnancy rate and a zero risk of OHSS. Per cycle, natural cycle IVF is less time consuming, physically less demanding and requires far less hormonal medication than stimulated IVF. In light of the advantages of natural cycle IVF, it seems important to evaluate its effectiveness. The present report is a systematic literature review focussing on the pregnancy rates after natural cycle IVF and its cost-effectiveness.

Literature search
Objectives
The aim of this review is to determine ongoing pregnancy rates per started cycle of natural cycle IVF. As secundary aims, we wished to compare results of natural cycle IVF to stimulated IVF, and to determine the cost-effectiveness of natural cycle IVF.

Selection criteria
Only studies concerning natural cycle IVF or intracytoplasmic sperm injection (ICSI) with no other intervention than the administration of human chorionic gonadotrophin (HCG) for ovulation triggering were included in the analysis.

Studies concerning IVF or ICSI with the use of minimal stimulation by clomiphene citrate (CC) or otherwise, were thus not included. An accurate estimation of the effectiveness of treatments can only be made taking into account the number of started cycles. Therefore, only studies from which the actual ongoing pregnancy rate per started cycle could be calculated were included.

Case series with retrospectively or prospectively collected data were included in the search. Controlled trials with a historical control group, pseudorandomized and randomized controlled trials were included in the review provided that the natural cycle IVF treatment arm fitted the selection criteria. Case reports and expert opinions were not included.

Search strategy
Publications of potential interest were identified through comprehensive searches of the Embase and Medline databases. The following headings were used: IVF, ICSI, spontaneous cycle, natural cycle, and unstimulated cycle. Inclusive dates for the on-line search were 1989 to July 2001. In addition, bibliographies of retrieved studies were hand searched for other potentially relevant publications. Abstract books of The American Society for Reproductive Medicine (1989-2000) and European Society for Human Reproduction and Embryology (1989-2001) meetings were hand searched for potentially relevant publications.

Two independent reviewers (MJP and AH) selected the publications to be included in accordance with the above mentioned criteria.

The studies were analysed to obtain an estimate of the pregnancy rate per started cycle of natural cycle IVF and an estimate of the cancellation rate per started cycle. No reports were found which dealt primarily with the issue of cost-effectiveness of natural cycle IVF. Three of the studies included in the review provided an estimate of costs of natural cycle IVF (Aboulghar *et al.* 1995; Daya *et al.*, 1995; Nargund *et al.*, 2001).

Studies excluded from the review
Of 33 potentially relevant reports, 13 were not included in the review. Five studies were excluded because the number of started cycles was not mentioned (Monks *et al.*, 1993; Turner *et al.*, 1994; Kumar *et al.*, 1997; Reljic and Vlaisavljević, 1999; Tomazevie *et al.*, 1999). Four studies were excluded because the number of ongoing pregnancies was not specified (Ueno *et al.*, 1991; Fahy *et al.*, 1995; Cahill *et al.*, 1996; Reljic *et al.*, 2001). One

study was excluded because treatments were performed only in women of advanced age (>44 years) and this was judged not to be representative for the general IVF patient population (Bar-Hava *et al.*, 2000). Another three publications were not included since the presented figures overlapped with subsequent (Paulson *et al.*, 1990; Lindheim *et al.*, 1996) or former (Paulson *et al.*, 1994a) publications by the same authors.

Studies included in the review

Of the 20 studies included in the review, 15 were published in peer-reviewed journals (Foulot *et al.*, 1989; Paulson *et al.*, 1992; Claman *et al.*, 1993; MacDougall *et al.*, 1994; Aboulghar *et al.*, 1995; Daya *et al.*,1995; Seibel *et al.*, 1995; Kim *et al.*, 1996; Lindheim *et al.*, 1997; Zayed *et al.*, 1997; Bassil *et al.*, 1999; Janssens *et al.*, 2000; Ingerslev *et al.*, 2001; Nargund *et al.*, 2001; Ng *et al.*, 2001) and five were published in conference abstract books (Hillensjö *et al.*, 1990; Levy *et al.*, 1991; Svalander *et al.*, 1991; Tomazevic *et al.*, 1996; Feldman *et al.*, 1998).

Of the 20 selected studies, eight were case series of natural cycle IVF with prospectively collected data (Foulot *et al.*, 1989; Hillensjö *et al.*, 1990; Aboulghar *et al.*, 1995; Daya *et al.*,1995; Feldman *et al.*, 1998; Janssens *et al.*, 2000; Nargund *et al.*, 2001; Ng *et al.*, 2001). Six studies were retrospective case series (Svalander *et al.*, 1991; Paulson *et al.*, 1992; Claman *et al.*, 1993; Seibel *et al.*, 1995; Lindheim *et al.*, 1997; Zayed *et al.*, 1997). In five studies, a comparison was made with stimulated IVF cycles from the same time period (Svalander *et al.*, 1991; Paulson *et al.*, 1992; Claman *et al.*, 1993; Lindheim *et al.*, 1997; Ng *et al.*, 2001). One report was a comparative study, in which natural cycle IVF was compared to previous failed cycles of stimulated IVF in the same patients (Bassil *et al.*, 1999). In one study, results of natural cycle IVF were compared to results of simultaneous embryo transfer of cryopreserved embryos together with embryos obtained after natural cycle IVF (Kim *et al.*, 1996). One study was a randomized controlled trial comparing two embryo transfer policies (Tomazevic *et al.*, 1996). Two studies were randomized controlled trials comparing natural cycle IVF with IVF in CC-stimulated cycles (MacDougall *et al.*, 1994; Ingerslev *et al.*, 2001). One study was a randomized controlled trial with crossover design comparing natural cycle IVF with stimulated IVF (Levy *et al.*, 1991).

The 20 selected studies comprised 1800 natural cycle IVF treatment cycles. The total number of participating patients remained uncertain because two trials did not mention this number.

The results of natural cycle IVF of the above-mentioned studies are presented in tables I and II.

Case series

All case series, as well as the natural cycle IVF treatment arms of the controlled trials included in the review were analysed together.

Patient selection and inclusion criteria
Indications for IVF

In ten studies, the indications for IVF were tubal infertility (Hillensjö *et al.*, 1990; Svalander *et al.*, 1991; Claman *et al.*, 1993; Aboulghar *et al.* 1995; Tomazevic *et al.*, 1996;

Table I. Case series of natural cycle IVF

| Study | N° of patients | Age (years)[a] | N° of cycles | N°of cancelled cycles/ reason for cancellation[b] | | | timing OR | |
				LH surge/ovulation	other	total	LH	HCG
Foulot 1989	71	32; na; 24-40	80	7 (8.8)	5 (6.3)	12 (15.0)	yes	3000
Hillensjö 1990	18	30.8; na; na	48	12 (25.0)	0	12 (25.0)	no	5000
Levy 1991	22	na	22	na	na	6 (27.3)	no	4000
Svalander 1991	44	34; na; na	51	7 (13.7)	12 (23.5)	19 (37.3)	no	5000
Paulson 1992	46	33.9 ± 3.2; na; 28-39	101	na	na	23 (22.8)	no	10000
Claman 1993	na	<38	75	na	na	35 (46.7)	no	2500 / 5000
MacDougall 1994	14	32.1 ± 0.9; na; na	14	8 (57.1)	2 (14.3)	10 (71.4)	no	5000
Aboulghar 1995	58	32 ± 4.1; na; 23-39	229	na	na	117 (51.1)	no	5000
Daya 1995	na	34.5 ± 3.0; na; na	240	56 (23.3)	28 (11.7)	84 (35.0)	no	10000
Seibel 1995	48	32.8; na; 26-38	64	na	na	16 (25.0)	no	2500
Kim 1996	45	32.2 ± 2.8; na; na	80	13 (16.3)	0	13 (16.3)	yes	10000
Tomazevic 1996	73	33.9 ± 3.6; na; na	110	na	na	na	no	5000
Lindheim 1997	30	< 40	35	5 (14.3)	0	5 (14.3)	no	10000
Zayed 1997	117	na; na; 24-44	162	10 (6.2)	7 (4.3)	17 (10.5)	yes	no
Feldman 1998	22	na; na; na	44	8 (18.2)	8 (18.2)	16 (36.4)	no	5000
Bassil 1999	11	36.6 ± 6; na; na	16	2 (12.5)	1 (6.3)	3 (18.8)	yes	10000
Janssens 2000	50	na; na; 22-38	81	13 (16.0)	1 (1.2)	14 (17.3)	no	10000
Nargund 2001	52	na ; 34; 24-40	202	7 (3.5)	21 (10.4)	28 (13.9)	no	5000
Ingerslev 2001	64	30.7 ± 2.5; na; na	114	34 (29.8)	6 (5.3)	40 (35.1)	yes	5000
Ng 2001	19	na; 32; 25-40	32	17 (53.1)	3 (9.4)	20 (62.5)	no	10000
Total			1800	199/1199 (16.6)	94/1199 (7.8)	490 (28.9)[c]		

[a]values are mean ± SD; median; range
[b]values in parentheses are percentages per cycle
[c]Tomazevic 1996 not included
na: not available

Table II. Case series of natural cycle IVF

Study	OR (%/cycle)	successful OR (%/attempt)	fertilization rate (%/oocyte) IVF	ICSI	embryo transfer (%/cycle; %/oocyte)	implantation (%/embryo)	clinical PR (%/cycle)	ongoing PR (%/cycle; %/ET)
Foulot 1989	68 (85.0)	63 (92.6)	na		53 (66.3; 77.9)	17 (32.1)	17 (21.3)[a]	13 (16.3; 24.5)
Hillensjö 1990	36 (75.0)	30 (83.3)	na		20 (41.7; 55.6)	na	2 (4.2)	2 (4.2; 10.0)
Levy 1991	16 (72.7)	13 (81.3)	84.6		11 (50.0; 68.8)	0	0	0
Svalander 1991	32 (62.7)	31 (96.9)	64.5		20 (39.2; 62.5)	6 (30.0)	6 (11.8)	6 (11.8; 30.0)
Paulson 1992	78 (77.2)	na	71.7		63 (62.4; 80.8)	11 (13.4)	11 (10.9)	9 (8.9; 14.3)
Claman 1993	40 (53.3)	24 (60.0)	na		18 (24.0; 45.0)	2 (11.1)	2 (2.7)	2 (2.7; 11.1)
MacDougall 1994	4 (28.6)	4 (100.0)	100.0		4 (28.6; 100.0)	0	0[a]	0
Aboulghar 1995	112 (48.9)	98 (87.5)	na		86 (37.6; 76.8)	11 (12.1)	11 (4.8)	11 (4.8; 12.8)
Daya 1995	156 (65.0)	130 (83.3)	80.0		92 (38.3; 58.9)	na	11 (4.6)	9 (3.8; 9.8)
Seibel 1995	48 (75.0)	36 (75.0)	69.4		25 (39.1; 52.1)	na	8 (12.5)	7 (10.9; 28.0)
Kim 1996	67 (83.8)	64 (95.5)	97.0		61 (76.3; 91.0)	10 (16.4)	10 (12.5)	8 (10.0)
Tomazevic 1996	na	na	77.0		59 (53.6; na)	13 (22.0)	13 (11.8)	11 (10.0; 18.6)
Lindheim 1997	30 (85.7)	30 (100.0)	89.1		28 (80.0; 9.33)	na (33)	na	5 (14.3; 17.9)
Zayed 1997	145 (89.5)	138 (95.2)	73.4		89 (54.9; 61.4)	12 (13.5)	12 (7.4)	9 (5.6; 10.1)
Feldman 1998	28 (63.6)	16 (57.1)	-	62.5	10 (22.7; 35.7)	2 (20.0)	2 (4.5)	2 (4.5; 20.0)
Bassil 1999	13 (81.3)	11 (84.6)	83.3	60.0	6 (37.5; 46.2)	3 (50.0)	3 (18.8)	3 (18.8; 50.0)
Janssens 2000	67 (82.7)	56 (83.6)	89.3		41 (50.6; 61.2)	10 (24.4)	10 (12.3)	8 (11.1;19.5)
Nargund 2001	174 (86.1)	142 (81.6)	65.8		96 (47.5; 55.2)	na	na	16 (7.9; 16.7)
Ingerslev 2001	74 (64.9)	68 (91.9)	44.2	56.3	29 (25.4; 39.2)	4 (13.8)	4 (3.5)	4 (3.5; 13.8)
Ng 2001	12 (37.5)	10 (31.3)	80.0		8 (25.0; 66.7)	na	4 (12.5)	4 (12.5; 50.0)
Total					819 (45.5; na)			129 (7.2; 15.8)

[a]Pregnancy after intraperitoneal or intrauterine insemination after canceled or unsuccesful oocyte retrieval: not included in calculation of clinical PR
na: not available
OR: oocyte retrieval; PR: pregnancy rate; ET: embryo transfer

Janssens *et al.*, 2000; Nargund *et al.*, 2001), unexplained infertility (Zayed *et al.*, 1997) or both (Paulson *et al.*, 1992; Ng *et al.*, 2001). In one study, indications for IVF were tubal infertility, unexplained infertility, endometriosis or cervical factor infertility (Foulot *et al.*, 1989). In one study, indications for IVF were tubal infertility, endometriosis or male factor infertility (Bassil *et al.*, 1999). In one study, indications for IVF were tubal infertility, unexplained infertility or failed donor insemination (MacDougall *et al.*, 1994). In one study, indications for IVF were tubal infertility, unexplained infertility or male factor infertility (Ingerslev *et al.*, 2001). In one study, indication for IVF was not specified, but fertilization in a previous IVF treatment cycle was an inclusion criterium (Daya *et al.*,1995). In one study, the indication for IVF was not specified, but male factor infertility was an exclusion criterion (Kim *et al.*, 1996). In four studies, indication for IVF was not specified at all (Levy *et al.*, 1991; Seibel *et al.*, 1995; Lindheim *et al.*, 1997; Feldman *et al.*, 1998).

In eleven of the 20 studies, normal semen quality was an inclusion criterion (Foulot *et al.*, 1989; Hillensjö *et al.*, 1990; Levy *et al.*, 1991; Paulson *et al.*, 1992; Claman *et al.*, 1993; MacDougall *et al.*, 1994; Aboulghar *et al.*, 1995; Kim *et al.*, 1996; Lindheim *et al.*, 1997; Nargund *et al.*, 2001; Ng *et al.*, 2001). In one study, a total motile sperm count of > 5×10^6 was required (Daya *et al.*,1995).

In two studies, ICSI was performed where applicable (Bassil *et al.*,1999; Ingerslev *et al.*, 2001). In one study, ICSI was performed in all cases (Feldman *et al.*, 1998).

Previous IVF treatments

In several studies, included patients had undergone previous stimulated IVF treatments. In five studies, all included patients had undergone IVF treatment before enrollment (Daya *et al.*,1995; Tomazevic *et al.*, 1996; Lindheim *et al.*, 1997; Feldman *et al.*, 1998; Bassil *et al.*, 1999) and in three of these, poor response in previous cycles was an inclusion criterion (Lindheim *et al.*, 1997; Feldman *et al.*, 1998; Bassil *et al.*, 1999).

In three studies, some of the included patients had undergone IVF treatment before enrollment (Foulot *et al.*, 1989; Paulson *et al.*, 1992; Nargund *et al.*, 2001). In two of these, some of the patients had been poor responders in previous IVF cycles (Paulson *et al.*, 1992; Nargund *et al.*, 2001).

In 11 studies, it was not specified whether patients had undergone IVF treatment before inclusion (Hillensjö *et al.*, 1990; Levy *et al.*, 1991; Svalander *et al.*, 1991; Claman *et al.*, 1993; MacDougall *et al.*, 1994; Aboulghar *et al.*, 1995; Seibel *et al.*, 1995; Kim *et al.*, 1996; Zayed *et al.*, 1997; Janssens *et al.*, 2000; Ng *et al.*, 2001).

In one study, none of the patients had undergone IVF treatment before enrollment (Ingerslev *et al.*, 2001).

Assessment of ovulatory function

Clearly, an ovulatory cycle is required to be able to perform natural cycle IVF. All patients included in the studies were judged to have ovulatory menstrual cycles, based on regularity of the cycle (Foulot *et al.*, 1989; Hillensjö *et al.*, 1990; Levy *et al.*, 1991; Svalander *et al.*, 1991; Aboulghar *et al.*, 1995; Daya *et al.*,1995; Zayed *et al.*, 1997; Bassil *et al.*, 1999; Ingerslev *et al.*, 2001; Nargund *et al.*, 2001), biphasic basal body temperature charts (Claman *et al.*, 1993; Seibel *et al.*, 1995; Janssens *et al.*, 2000), elevated midluteal

progesterone values (Paulson *et al.*, 1992; MacDougall *et al.*, 1994; Kim *et al.*, 1996; Ng *et al.*, 2001) or in-phase endometrial biopsies (Claman *et al.*, 1993; Janssens *et al.*, 2000). In three studies, this was not specified (Tomazevic *et al.*, 1996; Lindheim *et al.*, 1997; Feldman *et al.*, 1998).

Patient age

The mean, median and range of age of the study patients are presented in table I.

Cycle monitoring and timing of oocyte retrieval

In almost all studies, cycle monitoring was performed with ultrasound, either starting on cycle day 6-11 or 2-4 days before anticipated ovulation (Foulot *et al.*, 1989; Hillensjö *et al.*, 1990; Levy *et al.*, 1991; Svalander *et al.*, 1991; Paulson *et al.*, 1992; Claman *et al.*, 1993; MacDougall *et al.*, 1994; Aboulghar *et al.*, 1995; Daya *et al.*,1995; Seibel *et al.*, 1995; Kim *et al.*, 1996; Tomazevic *et al.*, 1996; Lindheim *et al.*, 1997; Feldman *et al.*, 1998; Bassil *et al.*, 1999; Janssens *et al.*, 2000; Ingerslev *et al.*, 2001; Nargund *et al.*, 2001; Ng *et al.*, 2001). In one study, monitoring was done by serial measurements of serum estradiol (E2) and luteinizing hormone (LH) without ultrasound (Zayed *et al.*, 1997).

In most studies, the timing of oocyte retrieval was done by ovulation triggering with HCG, with cancellation or advancement of oocyte retrieval in case of an LH surge (table I). HCG was administered when the follicle size was 15-20 mm. In two studies, the follicle size at which ovulation was triggered was not mentioned (Tomazevic *et al.*, 1996; Lindheim *et al.*, 1997). The interval between HCG injection and oocyte retrieval ranged from 31 to 36 hours. In one study, oocyte retrieval was planned on the basis of spontaneous LH surges only (Zayed *et al.*, 1997). Patients were requested to draw blood samples twice to five times daily. The interval between the onset of the spontaneous LH surge and oocyte retrieval was 34-36 hours.

In eight studies, E2 levels were used together with follicle size to determine the optimal time for ovulation triggering (Foulot *et al.*, 1989; Levy *et al.*, 1991; Svalander *et al.*, 1991; Paulson *et al.*, 1992; Claman *et al.*, 1993; Seibel *et al.*, 1995; Kim *et al.*, 1996; Ng *et al.*, 2001).

Cancellation of oocyte retrieval

One of the disadvantages of natural cycle IVF is a high cancellation rate because of premature LH surges or premature ovulation.

There are two approaches in planning oocyte retrieval. The first is to plan oocyte retrieval by ovulation triggering with HCG and to cancel the cycle in case a spontaneous LH surge occurs. In the studies where this approach was applied, oocyte retrieval was performed in 28.6% to 86.1% per started cycle (Hillensjö *et al.*, 1990; Levy *et al.*, 1991; Svalander *et al.*, 1991; Paulson *et al.*, 1992; Claman *et al.*, 1993; MacDougall *et al.*, 1994; Aboulghar *et al.*, 1995; Daya *et al.*, 1995; Seibel *et al.*, 1995; Lindheim *et al.*, 1997; Feldman *et al.*, 1998; Janssens *et al.*, 2000; Nargund *et al.*, 2001; Ng *et al.*, 2001). The total number of oocyte retrievals in these studies was 833 out of 1238 started cycles (67.3%).

The second approach is to plan oocyte retrieval according to the timing of sponta-neous LH surges, either in all cases (Zayed *et al.*, 1997) or only in those cases where an unexpected LH surge occurs before ovulation triggering (Foulot *et al.*, 1989; Kim *et al.*, 1996; Bassil *et al.*, 1999; Ingerslev *et al.*, 2001). In the studies where this approach was applied, oocyte retrieval was performed in 64.9% - 89.5% per started cycle (Foulot *et al.*, 1989; Kim *et al.*, 1996; Zayed *et al.*, 1997; Bassil *et al.*, 1999; Ingerslev *et al.*, 2001). The total number of oocyte retrievals in these studies was 367 out of 452 started cycles (80.6%).

One study reports on the successful use of the cyclooxygenase inhibitor indomethacin to postpone follicle rupture, with 3.5% cancellations because of ovulation and 10.4% for other reasons (Nargund *et al.*, 2001).

Reasons for cancellation of oocyte retrieval were specified in 14 studies, in which a total of 1199 cycles were described (table I). Oocyte retrieval was cancelled because of premature LH surge or ovulation in 199 out of 1199 cycles (16.6%). Ninety-four out of 1199 cycles (7.8%) were abandoned because of poor follicular growth, ovarian cyst forma-tion or for other reasons.

Follicle aspiration and oocyte recovery rate

In seven studies, the aspiration needle which was used for transvaginal oocyte retrieval was specified. In two studies single-lumen needles were applied without flushing of the follicle, and with a successful oocyte recovery rates of 83% and 83.6% respectively (Tomazevic *et al.*, 1996; Janssens *et al.*, 2000). In four studies, either single- or double-lumen needles were applied with flushing of the follicle. The successful oocyte recovery rate was 60.0% to 95.2% in these studies (Claman *et al.*, 1993; Zayed *et al.*, 1997; Ingerslev *et al.*, 2001; Ng *et al.*, 2001). The total number of successful oocyte retrievals in these stud-ies was 240 out of 271 procedures (88.6%). In one study, both single- and double-lumen needles were used (Daya *et al.*, 1995). In this study, the succesful oocyte recovery rate rose from 68.5% with single-lumen needles to 91.2% after the introduction of double-lumen needles with flushing of the follicle. Oocyte recovery was successful in 57.1% to 100% of retrievals in studies where the choice of needle was not specified (Foulot *et al.*, 1989; Hillensjö *et al.*, 1990; Levy *et al.*, 1991; Svalander *et al.*, 1991; Paulson *et al.*, 1992; MacDougall *et al.*, 1994; Aboulghar *et al.*, 1995; Seibel *et al.*, 1995; Kim *et al.*, 1996; Lind-heim *et al.*, 1997; Feldman *et al.*, 1998; Bassil *et al.*, 1999; Nargund *et al.*, 2001).

In eight studies, more than one oocyte was obtained on some occasions (Paulson *et al.*, 1992; Aboulghar *et al.*, 1995; Daya *et al.*, 1995; Kim *et al.*, 1996; Lindheim *et al.*, 1997; Ingerslev *et al.*, 2001; Nargund *et al.*, 2001; Ng *et al.*, 2001).

Almost all oocyte retrievals were transvaginal and ultrasound-guided. In one case, a laparoscopy was performed for oocyte retrieval because of anatomical reasons (Foulot *et al.*, 1989).

Analgesia during oocyte retrieval

In three studies, analgesia was standard for oocyte retrieval (Paulson *et al.*, 1992; Claman *et al.*, 1993; Ingerslev *et al.*, 2001), whilst in two studies analgesia was only given on patient request (Daya *et al.*, 1995; Janssens *et al.*, 2000). In three studies, all oocyte retrievals were performed without analgesia (Aboulghar *et al.*, 1995; Tomazevic *et al.*,

1996; Feldman *et al.,* 1998), whilst in 12 studies it was not mentioned whether analgesia was used (Foulot *et al.,* 1989; Hillensjö *et al.,* 1990; Levy *et al.,* 1991; Svalander *et al.,* 1991; MacDougall *et al.,* 1994; Seibel *et al.,* 1995; Kim *et al.,* 1996; Lindheim *et al.,* 1997; Zayed *et al.,* 1997; Bassil *et al.,* 1999; Nargund *et al.,* 2001; Ng *et al.,* 2001).

Luteal phase support

It is not clear whether luteal phase support is necessary in natural cycle IVF. In 10 studies, luteal phase support was given after embryo transfer (Foulot *et al.,* 1989; Paulson *et al.,* 1992; Claman *et al.,* 1993; Aboulghar *et al.,* 1995; Daya *et al.,* 1995; Kim *et al.,* 1996; Tomazevic *et al.,* 1996; Zayed *et al.,* 1997; Bassil *et al.,* 1999; Ng *et al.,* 2001) and from a total of 535 embryo transfers, 79 ongoing pregnancies resulted (14.8%). In two studies, no luteal phase support was given (Janssens *et al.,* 2000; Ingerslev *et al.,* 2001) and 12 ongoing pregnancies resulted from a total of 70 embryo transfers (17.1%). In eight studies, it was not mentioned whether luteal phase support was given (Hillensjö *et al.,* 1990; Levy *et al.,* 1991; Svalander *et al.,* 1991; MacDougall *et al.,* 1994; Seibel *et al.,* 1995; Lindheim *et al.,* 1997; Feldman *et al.,* 1998; Nargund *et al.,* 2001) and among a total of 214 embryo transfers, 38 ongoing pregnancies resulted (17.8%).

Fertilization rate and embryo transfer rate

In treatment cycles where IVF was applied, fertilization rate per inseminated oocyte was 44.2%-100%. In treatment cycles where ICSI was applied, fertilization rate per injected oocyte was 56.3-62.5% (table II). Triploidy was observed in from 0 to 18.2% of the fertilized oocytes (Paulson *et al.,* 1992; MacDougall *et al.,* 1994; Daya *et al.,* 1995; Kim *et al.,* 1996; Tomazevic *et al.,* 1996; Bassil *et al.,* 1999; Janssens *et al.,* 2000).

The percentage of embryo transfers per started cycle was 22.7 to 80.0%, whilst the percentage of embryo transfers per attempted oocyte retrieval was 35.7 to 100.0% (table II). Although in eight studies on some occasions more than one oocyte was obtained, in most cases only one embryo was available for transfer (Paulson *et al.,* 1992; Aboulghar *et al.,* 1995; Daya *et al.,* 1995; Kim *et al.,* 1996; Lindheim *et al.,* 1997; Ingerslev *et al.,* 2001; Nargund *et al.,* 2001; Ng *et al.,* 2001). In 12 studies, all transfers were of a single embryo (Foulot *et al.,* 1989; Levy *et al.,* 1991; Svalander *et al.,* 1991; Claman *et al.,* 1993; MacDougall *et al.,* 1994; Kim *et al.,* 1996; Zayed *et al.,* 1997; Feldman *et al.,* 1998; Bassil *et al.,* 1999; Janssens *et al.,* 2000; Ingerslev *et al.,* 2001; Nargund *et al.,* 2001). In four studies, in some patients two embryos were transferred (Paulson *et al.,* 1992; Aboulghar *et al.,* 1995; Daya *et al.,* 1995; Lindheim *et al.,* 1997), whilst in another four studies the number of embryos per transfer was not specified (Hillensjö *et al.,* 1990; Seibel *et al.,* 1995; Tomazevic *et al.,* 1996; Ng *et al.,* 2001).

Implantation rate and pregnancy rate

Fourteen studies provided sufficient data to calculate the clinical implantation rate per transferred embryo (table II). The clinical implantation rate per transferred embryo in these studies ranged from 0 to 50% (table II).

Eighteen studies provided sufficient data to calculate the clinical pregnancy rate per started cycle and per embryo transfer (table II). The clinical pregnancy rate per started

Table III. Comparison of natural cycle IVF with stimulated IVF

Study	N° of patients	Age (years)	N° of cycles	ET (%/cycle)	implantation (%/embryo)	PR (%/cycle)	ongoing PR (%/cycle ;%/ET)	multiple PR (%/pregnancy)	study design
Levy 1991									
natural cycle	22	na	22	11 (50.0)	0	0	0	0	randomised
GnRHa/HMG	26	na	26	23 (88.5)	na	6 (23.1)	na	na	cross-over
Svalander 1991									
natural cycle	44	34; na; na	51	20 (39.2)	6 (30.0)	6 (11.8)	6 (11.8; 30.0)	0	retrospective
CC/HMG	121	35; na; na	122	87 (71.3)	na	27 (22.1)	19 (15.6;21.8)	na	
Paulson 1992									
natural cycle	46	33.9 ±3.2; -; 28-39	101	63 (62.3)	11 (13.4)	11 (10.9)	9 (8.9; 14.3)	0	retrospective
(GnRHa)/HMG	na	<40	na	na	66 (9.0)	49 (na)[a]	35 (na)[a]	na	
Claman 1993									
natural cycle	na	<38	75	18 (24.0)	2 (11.1)	2 (2.7)	2 (2.7; 11.1)	0	retrospective
GnRHa/HMG	na	na	450	298 (66.2)	na	65 (14.4)	48 (10.7; 16.1)	13 (20.0)	
MacDougall 1994									
natural cycle	14	32.1 ± 0.9; na; na	14	4 (28.6)	0	0	0	0	randomised
CC	16	32.8 ± 1.1; na; na	16	11 (68.8)	na	2 (12.5)	2 (12.5; 18.2)	0	controlled
Lindheim 1997									
natural cycle	30	<40	35	28 (80.0)	na (33.0)[b]	na	5 (14.3; 17.9)	0	retrospective
GnRHa/HMG	27	<40	27	21 (77.8)	na (7.0)[b]	2 (7.4)	2 (7.4; 9.5)	na	
Ingerslev 2001									
natural cycle	64	30.7 ± 2.5; na; na	114	29 (25.4)	4 (13.8)	4 (3.5)	4 (3.5; 13.8)	0	randomised
CC	68	30.2 ± 2.9; na; na	111	59 (53.2)	21 (24.7)	19 (17.1)	18 (16.2; 30.5)	2 (10.5	controlled
Ng 2001									
natural cycle	19	32 ; na; 25-40	32	8 (25.0)	na	4 (12.5)	4 (12.5; 50.0)	0	retrospective
'stimulated'	268	na	301	278 (92.4)	na	63 (20.9)	na	na	

[a]pregnancies ensuing from 178 oocyte retrievals; number of started cycles and number of embryo transfers not available
[b]implantation rate mentioned in the text; number of implantations not available
na: not available;CC: clomiphene citrate; ET: embryo transfer; PR: pregnancy rate

cycle was 0 to 21.3% (table II); in one study, it was not specified how pregnancy was defined (Nargund *et al.*, 2001).

In two studies, intraperitoneal or intrauterine insemination was performed in cycles where oocyte retrieval was either cancelled or unsuccessful, and two pregnancies were obtained (Foulot *et al.*, 1989; MacDougall *et al.*, 1994). These pregnancies were not included in the calculation of pregnancy rate per cycle .

All studies provided sufficient data to calculate the ongoing pregnancy rate per started cycle and per embryo transfer (table II). The ongoing pregnancy rate per started cycle and per embryo transfer was 0 to 18.8% and 0 to 50.0%, respectively (table II).

Of 129 ongoing clinical pregnancies, one was a monozygotic twin (Seibel *et al.*, 1995) and 128 were singleton. Of these, spontaneous abortion of the twin pregnancy occurred at 20 weeks gestation, 56 were reported as ongoing, and 72 as live births.

Cumulative pregnancy rates

In three studies, life table analysis was performed (Paulson *et al.*, 1992; Aboulghar *et al.*, 1995; Nargund *et al.*, 2001). In two of these studies (Paulson *et al.*, 1992; Aboulghar *et al.*, 1995), the analysis was performed on those cycles where follicle aspiration was performed; in one study (Nargund *et al.*, 2001), the analysis was performed on the total number of started cycles. Cumulative pregnancy rates were 43.0% and 41.7% after three (Paulson *et al.*, 1992) and five (Aboulghar *et al.*, 1995) follicle aspirations respectively. A 46.0% cumulative pregnancy rate and a 32% probability of live birth after four cycles was calculated in one study (Nargund *et al.*, 2001).

Controlled trials

The studies in which a comparison was made between natural cycle IVF and stimulated IVF were analysed separately and are presented in table III.

Description of the studies

One study (Levy *et al.*, 1999) was a series of 22 cycles of natural cycle IVF compared with 26 cycles of stimulated IVF. This study was randomized with a cross-over design, though the method of randomization was not mentioned. There was no mention of either the sample size estimation by power calculation or the drop-out rate (Levy *et al.*, 1991). Four studies reported on series of natural cycle IVF that were retrospectively compared with stimulated IVF cycles of the same time period (Svalander *et al.*, 1991; Paulson *et al.*, 1992; Claman *et al.*, 1993; Ng *et al.*, 2001). In three of these studies, drop-out rates were not mentioned (Svalander *et al.*, 1991; Claman *et al.*, 1993; Ng *et al.*, 2001). In one study, it was possible to extrapolate drop-out rates after one, two and three cycles. The reasons for drop-out were not specified in this study (Paulson *et al.*, 1992). Others (Lindheim *et al.*, 1997) reported on 35 natural cycle IVF cycles in 30 patients and retrospectively compared these with 27 cycles of stimulated IVF. The 30 patients in the natural cycle IVF group were patients whose oocyte retrieval was cancelled in a former treatment cycle because of poor response to ovarian stimulation. The 27 patients in the stimulated IVF group were patients with a poor response to ovarian stimulation who proceeded with oocyte aspiration (Lindheim *et al.*, 1997). Two studies were randomized controlled trials comparing natural

cycle IVF with IVF in clomiphene citrate (CC)-stimulated cycles (MacDougall *et al.*, 1994; Ingerslev *et al.*, 2001). In the former study (MacDougall *et al.*, 1994), randomization was performed using computer-selected random numbers. There was no mention of sample size estimation by power calculation, and drop-out rates were not mentioned (MacDougall *et al.*, 1994). In the latter study (Ingerslev *et al.*, 2001), block randomization was performed by sealed envelope method. Power calculation was not done in this study, and although drop-out rates were mentioned for the total group of patients, they were not specified for the natural cycle arm (Ingerslev *et al.*, 2001).

Ovarian stimulation

For stimulated IVF, luteal phase-initiated down-regulation with GnRH agonists and ovarian stimulation with human menopausal gonadotrophins (HMG) was used in three studies (Levy *et al.*, 1991; Claman *et al.*, 1993; Lindheim *et al.*, 1997). In one study, either a down-regulation protocol with a GnRH agonist and HMG or HMG alone was used for ovarian stimulation (Paulson *et al.*, 1992). In one study, ovarian stimulation was performed with a CC/HMG protocol (Svalander *et al.*, 1991), while in another study the stimulation protocol was not specified (Ng *et al.*, 2001). In two studies, ovarian stimulation was performed with CC (MacDougall *et al.*, 1994; Ingerslev *et al.*, 2001).

Embryo transfer rate

For natural cycle IVF, the proportion of embryo transfers per started cycle was 24 to 80%. For gonadotrophin-stimulated IVF cycles, the proportion of embryo transfers per started cycle was 66.2 to 92.4%. For CC-stimulated cycles, the proportions of embryo transfers per started cycle were 53.2% and 68.8%, respectively (MacDougall *et al.*, 1994; Ingerslev *et al.*, 2001).

For natural cycle IVF, in five studies all transfers were of a single embryo (Levy *et al.*, 1991; Svalander *et al.*, 1991; Claman *et al.*, 1993; MacDougall *et al.*, 1994; Ingerslev *et al.*, 2001). In two studies, some transfers were of two or three embryos (Paulson *et al.*, 1992; Lindheim *et al.*, 1997). In one study, the number of embryos per transfer was not specified (Ng *et al.*, 2001). For gonadotrophin-stimulated IVF cycles, a mean number of 1.14-3.35 embryos were transferred in three studies where this was specified (Svalander *et al.*, 1991; Claman *et al.*, 1993; Lindheim *et al.*, 1997). In three studies, the number of embryos per transfer was not specified (Levy *et al.*, 1991; Paulson *et al.*, 1992; Ng *et al.*, 2001). For CC-stimulated cycles, the number of embryos per transfer was not mentioned in one study (MacDougall *et al.*, 1994) and 1.44 in another (Ingerslev *et al.*, 2001).

Implantation rate and pregnancy rates

For natural cycle IVF, seven studies provided sufficient data to calculate clinical implantation rate per transferred embryo (table III) and this ranged from 0 to 33.0%. For gonadotrophin-stimulated IVF cycles, two studies provided sufficient data to calculate clinical implantation rates per transferred embryo of 7.0-9.0% (table III). For CC-stimulated IVF cycles, clinical implantation rate per transferred embryo was 24.7% in the only study where this was specified (Ingerslev *et al.*, 2001).

For natural cycle IVF, seven studies provided sufficient data to calculate clinical

pregnancy rate per started cycle (table III), and this ranged from 0 to12.5%. All studies provided sufficient data to calculate ongoing pregnancy rate per started cycle and per embryo transfer (table III). Ongoing pregnancy rates per started cycle and per embryo transfer was 0 to14.3% and 0 to 50.0%, respectively. For gonadotrophin-stimulated IVF cycles, clinical pregnancy rate per started cycle could be calculated from five studies (table III), and ranged from 7.4 to 23.1%. Three studies provided data which enabled the calculation of ongoing pregnancy rate per started cycle and per embryo transfer (table III). Ongoing pregnancy rate per started cycle and per embryo transfer was 7.4 to 15.6% and 9.5 to 21.8%, respectively. For CC-stimulated IVF cycles, the clinical pregnancy rate per started cycle was 12.5 to 17.1%. Ongoing pregnancy rate per started cycle and per embryo transfer was 12.5 to 16.2% and 18.2% to 30.5%, respectively (table III).

Multiple pregnancy rate

Of 32 clinical pregnancies reported from natural cycle IVF, 30 were ongoing. All pregnancies were singleton. Of 139 clinical pregnancies reported from gonadotrophin-stimulated IVF, 104 were ongoing. In two studies, the number of ongoing pregnancies was not mentioned for the stimulated IVF group (Levy *et al.*, 1991; Ng *et al.*, 2001). In only one study the multiple pregnancy rate was specified; in this study, of 48 ongoing pregnancies, eight were twins, four were triplets and one was a quadruplet (Claman *et al.*, 1993). In the other studies, multiple pregnancy rates were not specified (Svalander *et al.*, 1991; Paulson *et al.*, 1992; Lindheim *et al.*, 1997). Of 21 clinical pregnancies reported from CC-stimulated cycles, 20 were ongoing; of these, two were twin pregnancies (MacDougall *et al.*, 1994; Ingerslev *et al.*, 2001).

Cost-effectiveness

Although in almost all studies, low costs of natural cycle IVF are mentioned as an advantage, formal cost-effectiveness analyses are lacking.

One group (Aboulghar *et al.*, 1995) stated that in their centre the total costs of one complete trial of unstimulated IVF treatment including average costs of cancelled cycles, was 20% of that of a stimulated cycle. Others (Nargund *et al.*, 2001) reported that in their experience the per treatment cost of natural cycle IVF was 23% of that of stimulated IVF, costs related to OHSS included. These authors also stated that natural cycles offer savings of between £4796 and £9857 per pregnancy as compared with stimulated cycles (Nargund *et al.*, 2001). A cost per cycle for natural cycle IVF $1200 was also mentioned (Daya *et al.*, 1995); considering a live birth rate of 6.8%, these authors concluded that costs per live birth are $17 650 for natural cycle IVF. They also assume costs of IVF with ovarian stimulation of $5680 per cycle (one transfer of cryopreserved embryos included). Assuming a live birth rate of 14.3% from fresh embryo transfers plus 1.8% from cryopreserved embryo transfers, it was concluded that costs per live birth were $35 000 after stimulated IVF (Daya *et al.*, 1995).

Discussion

In the studies included in this review, 129 ongoing pregnancies out of 1800 started cycles are described (7.2%).

In these studies, natural cycle IVF and ICSI is described for all causes of infertility. Some authors have proposed natural cycle IVF as a valuable alternative to stimulated IVF in poor responders (Lindheim *et al.,* 1997; Feldman *et al.,* 1998; Bassil *et al.,* 1999). Moreover, patient characteristics and protocols for monitoring of IVF treatment cycles also vary considerably, and as a consequence the outcome of the IVF treatments is variable.

In natural cycle IVF, results are hampered by high (28.9%) cancellation rates per started cycle.

The timing of oocyte retrieval is usually performed after ovulation triggering with HCG. In case an LH rise is found before ovulation triggering, oocyte retrieval is either cancelled or advanced. Although in studies where oocyte retrieval was performed in case of an LH rise, the number of oocyte retrievals per started cycle was higher, there seemed to be little impact on overall results. The ongoing pregnancy rate per started cycle was 6.8% and 8.2% in those studies where oocyte retrieval was and was not cancelled in case of an LH rise. Two groups (Foulot *et al.,* 1989; Kim *et al.,* 1996) found similar fertilization and cleavage rates in patients with or without an LH surge. The pregnancy rate, however, was higher in patients without an LH surge (32.0% and 15.9% per cycle) than in those with an LH surge (11.5% and 8.3% per cycle). The planning of oocyte retrieval based on an LH rise requires intensive monitoring and an almost round-the-clock service, which most laboratories cannot provide.

One study reported on the succesful use of indomethacin to postpone follicular rupture (Nargund *et al.,* 2001). An interesting strategy to lower cancellation rates because of LH rise or ovulation is the use of GnRH antagonists, which are given after follicular dominance has developed and for a few days only. There are three reports on this approach (Meldrum *et al.,* 1994; Paulson *et al.,* 1994b; Rongières-Bertrand *et al.,* 1999). In these studies cancellation rates because of ovulation ranged from 0.0 to 9.0%.

Another factor contributing to low success rates of natural cycle IVF is a rather low percentage of successful oocyte retrievals (57.1 to 100.0%). Flushing of the follicle during oocyte retrieval might raise the efficacy of the procedure, but makes the procedure less comfortable for patients.

Implantation rates per embryo after natural cycle IVF are quite acceptable up to 50.0%. The studies with lower implantation rates included poor responders and patients with a history of several unsuccessful IVF treatments. Implantation rates of embryos obtained after IVF with ovarian stimulation are rather low in the controlled trials included in this review (7.0 - 9.0%), though considering that these studies are not very recent, this may partly reflect poor laboratory skills (Paulson *et al.,* 1992; Lindheim *et al.,* 1997).

It is possible that, compared with stimulated IVF cycles, the endometrium in natural cycle IVF is more receptive. Some reports claim a diminished endometrial receptivity caused by supraphysiologic levels of steroid hormones after ovarian stimulation for IVF (Fossum *et al.,* 1989; Simón *et al.,* 1998; Basir *et al.,* 2001). Physiologic levels of steroid hormones as present in unstimulated IVF cycles may theoretically lead to better endometrial receptivity. On the other hand, implantation rates per embryo may be lowered by the fact that usually no selection of embryos is possible as only one is obtained.

In natural cycle IVF, multiple embryos are available for transfer in some cases, after aspiration of secondary follicles. In one study (Paulson *et al.,* 1992), a lower fertiliza-

tion rate was found in oocytes derived from secondary follicles compared with oocytes derived from dominant follicles (41.0 versus 100.0%), but a higher pregnancy rate after multiple embryo transfer than after transfer of a single embryo (31.2 versus 13.0% per embryo transfer). This suggests that embryos ensuing from secondary oocytes are able to produce a pregnancy, or alternatively, that the production of multiple embryos is a marker of pregnancy success of the dominant follicle (Paulson *et al.*, 1992).

In theory, the depletion of granulosa cells by follicle fluid aspiration might cause corpus luteum dysfunction, making luteal support necessary (Garcia *et al.*, 1981). This could not be concluded from the included studies however, as ongoing pregnancy rates were comparable in studies where luteal support was and was not given (14.8 and 17.1% per embryo transfer, respectively).

For accurate assessment of efficacy of natural cycle IVF, cumulative pregnancy rates are more useful than pregnancy rates per started cycle only. Life-table analysis in three studies included in this review showed cumulative pregnancy rates of 43.0 and 41.7% after three and five oocyte aspirations (Paulson *et al.*, 1992; Aboulghar *et al.*, 1995) and 46.0% after four started cycles (Nargund *et al.*, 2001).

Natural cycle IVF has many potential advantages. In the studies included in this review, in the majority of cases a single embryo was transferred and the multiple pregnancy rate was close to zero. In controlled trials included herein, for the stimulated IVF groups, the multiple pregnancy rate was not mentioned in most studies, but it was very high (13 out of 48 pregnancies were multiple) where this was mentioned (Claman *et al.*, 1993). In studies where minimal ovarian stimulation with CC was applied, the multiple pregnancy rate was 9.5% (MacDougall *et al.*, 1994; Ingerslev *et al.*, 2001). Clearly, multiple pregnancies are a consequence of embryo transfer policy and not of ovarian stimulation in itself and can be avoided by replacing a single embryo. This strategy still allows for selection of good-quality embryos, which raises pregnancy rates while leading to singleton pregnancies. Reports on this subject show excellent pregnancy rates of 27.0 to 48.3% per single embryo transfer (Vilska *et al.*, 1999; Gerris *et al.*, 1999; ESHRE Campus Course Report, 2001; Martikainen *et al.*, 2001). The addition of pregnancies after transfer of frozen-thawed spare embryos raises the delivery rate per oocyte retrieval even further (Tiitinen *et al.*, 2001). However, so far, elective single embryo transfer is only proposed for selected, good-prognosis patients and is far from standard in most IVF centres (Nygren and Andersen, 2001).

When natural cycle IVF is applied, there is no risk of OHSS.

Although the cryopreservation of spare embryos and their subsequent transfer contribute to pregnancy rates of stimulated IVF, legal, ethical and religious dilemmas caused by the generation of spare embryos after stimulated IVF are avoided by applying natural cycle IVF.

Per cycle, natural cycle IVF is physically less demanding than stimulated IVF and probably less emotionally so (Højgaard *et al.*, 2001).

Per cycle, natural cycle IVF is cheaper than stimulated IVF, as it is less time consuming for laboratory and medical personnel as well as patients, and requires less hormonal medication. The per-cycle costs of natural cycle IVF are 20-23% of those of stimulated IVF (Aboulghar *et al.*, 1995; Nargund *et al.*, 2001).

Controlled trials comparing natural cycle IVF with stimulated IVF are lacking. For a correct comparison, pregnancy rates after transfer of cryopreserved embryos should be included in the pregnancy rates of stimulated IVF. Considering the lower costs and shorter duration of a natural cycle IVF treatment, it seems logical to compare a number of natural cycle IVF treatments with one cycle of stimulated IVF, either with or without elective single embryo transfer. In such a study, the cumulative pregnancy rates of natural cycle IVF should be compared with the pregnancy rate of one cycle of stimulated IVF, frozen embryo transfers included. The complication rate of both strategies should also be taken into account.

From a cost-effectiveness point of view, the costs per live birth should be reported, and the costs related to complications should be included in such an analysis. Since the total birth costs of a twin pregnancy are 4- to 10-fold higher than those of a singleton pregnancy (Callahan *et al.*, 1994; Luke *et al.*, 1996), the prevention of twin pregnancies by applying natural cycle IVF will lead to huge savings. The prevention of OHSS will also lead to a reduction in costs. On the other hand, more cycles of natural cycle IVF are needed to obtain pregnancy rates comparable to those of stimulated IVF, and therefore direct treatment-related costs per pregnancy may be higher with natural cycle IVF than with stimulated IVF. The cost-effectiveness of either strategy will thus depend on their efficacy.

Conclusions

Natural cycle IVF is a low-risk and patient-friendly procedure with an ongoing pregnancy rate of about 7% per started cycle and about 16% per embryo transfer. The success rates of natural cycle IVF are hampered by high cancellation rates because of premature LH rise and premature ovulations. Recent reports using GnRH antagonists to prevent such a LH rise, or indomethacin to prevent rupture of the follicle before planned oocyte retrieval, appear promising in this respect.

Improvements in laboratory conditions and fertilization techniques such as ICSI may increase the success rate of natural cycle IVF. In light of the many potential advantages of natural cycle IVF, it seems advisable to re-evaluate its place in the wide range of possible fertility treatments. A randomized controlled trial, comparing natural cycle IVF with current standard treatments, is warranted. Such a study should include a cost-effectiveness analysis and focus on cumulative pregnancy rates.

Acknowledgement

Chapter 3

Minimal stimulation IVF with late follicular phase administration of the GnRH-antagonist cetrorelix and concomitant substitution with recombinant FSH: a pilot study.

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ABSTRACT

BACKGROUND: The use of the natural cycle for IVF offers the advantage of a patient-friendly and low-risk protocol. Its effectiveness is limited, but may be improved by using a GnRH antagonist to prevent untimely LH surges.

METHODS: In this pilot study, minimal stimulation IVF with late follicular phase administration of the GnRH antagonist cetrorelix and simultaneous substitution with recombinant FSH was applied for a maximum of three cycles per patient. Main outcome measures were pregnancy rates per started cycle and cumulative pregnancy rates after three cycles.

RESULTS: A total of 50 patients completed 119 cycles (2.4 per patient). Fifty-two embryo transfers resulted in 17 ongoing pregnancies (14.3% per started cycle; 32.7% per embryo transfer; 95% confidence interval (CI) 7.9-20.7% and 19.7-45.7%, respectively). One dizygotic twin pregnancy occurred after transfer of two embryos, the other pregnancies were singletons. The cumulative ongoing pregnancy rate after three cycles was 34.0% (95% CI 20.6-47.4%). Live birth rate was 32.0 % per patient (95% CI 18.8-45.2%).

CONCLUSIONS: Pregnancy rates after IVF with minimal, late follicular phase stimulation are encouraging. Considering the low-risk and patient-friendly nature of this protocol, it may be a feasible alternative to IVF with ovarian hyperstimulation.

Introduction

Natural cycle IVF has received much attention in recent literature (Bassil *et al.*, 1999; Reljic *et al.*, 1999; Janssens *et al.*, 2000; Feldman *et al.*, 2001; Ingerslev *et al.*, 2001; Nargund *et al.*, 2001; Ng *et al.*, 2001; Omland *et al.*, 2001; Reljic *et al.*, 2001; Bauman *et al.*, 2002; Lukassen *et al.*, 2003; Morgia *et al.*, 2004). The use of natural cycle IVF offers several advantages. Since no ovarian stimulation is performed, ovarian hyperstimulation syndrome (OHSS) does not occur, and compared with standard IVF treatment with controlled ovarian hyperstimulation (COH), natural cycle IVF is a cheap and patient-friendly procedure (Aboulghar *et al.*, 1995; Daya *et al.*,1995; Olivennes and Frydman, 1998; Højgaard *et al.*, 2001; Nargund *et al.*, 2001).

The efficacy of natural cycle IVF, however, is limited. Reported ongoing pregnancy rates per started cycle are 7.2% on average, with a range of 0 - 18.8%, as reviewed in Pelinck *et al.*, 2002. The lack of efficacy is related to high cancellation rates (28.9%) mainly caused by the occurrence of spontaneous LH surges (Pelinck *et al.*, 2002).

The efficacy of natural cycle IVF can be improved by using a GnRH antagonist to prevent untimely LH surges and premature ovulations. In minimal stimulation IVF, a GnRH antagonist is started in the late follicular phase, after follicular dominance has developed. To substitute for the fall in gonadotrophins, HMG or FSH are administered together with the GnRH antagonist. Various protocols are described with the GnRH antagonists Nal-Glu and cetrorelix at different doses, and substitution with HMG or urinary purified FSH (Meldrum *et al.*, 1994; Paulson *et al.*, 1994b; Rongières-Bertrand *et al.*, 1999). In these studies, a total of 54 cycles are described resulting in seven ongoing pregnancies (13% per started cycle). In the largest of these three studies, only patients with severe male factor infertility were included and ICSI was applied. A total of 44 cycles in 33 patients led to seven clinical pregnancies, of which five were ongoing (Rongières-Bertrand *et al.*, 1999).

So far, the use of recombinant FSH (rFSH) has not been described for the minimal stimulation protocol.

Minimal stimulation IVF offers the same advantages as natural cycle IVF, being associated with a zero risk of OHSS since no ovarian hyperstimulation is performed and being a cheap and patient-friendly protocol compared to COH IVF. Per cycle, minimal stimulation IVF is cheaper than COH IVF as considerably less hormonal medication is used, and laboratory procedures are less time-consuming as, in general, only one oocyte is obtained. The patient-friendliness of minimal stimulation IVF lies in the fact that hormonal medication is used for a few days only, and therefore few side-effects are experienced by patients, and that the oocyte retrieval is less painful than in COH IVF since, in general, only one follicle is aspirated. With minimal stimulation, usually no spare embryos are created, and therefore ethical dilemmas concerning them are avoided.

In this pilot study we performed IVF with minimal stimulation. The GnRH antagonist cetrorelix was administered in the late follicular phase and rFSH was used for substitution. The goal of this study was to make an estimation of pregnancy rate per started cycle and of cumulative pregnancy rates after three cycles. This is the first study in which a minimal stimulation protocol is described using rFSH for substitution.

Materials and methods

Study protocol

This study protocol was reviewed and approved by the ethical committee of the Academic Hospital of Groningen, the Netherlands. Inclusion criteria for this study were: female patient age 18-36 years, first IVF treatment ever or first IVF treatment after a pregnancy, the presence of a regular and proven ovulatory menstrual cycle with a length of 26-35 days and body mass index (BMI) of 18-28 kg/m^2. Indications for IVF were tubal pathology, unexplained subfertility, male factor, endometriosis, cervical factor or failed donor inseminations.

Patients with male factor or unexplained subfertility had undergone treatment with intra-uterine insemination for three to six cycles before starting IVF treatment, as is standard protocol in our centre. Patients were excluded from the study if an endometriosis cyst was seen on ultrasound. Patients requiring ICSI were not included in this study.

Patients were offered a maximum of three free treatment cycles. Treatments were performed in three consecutive menstrual cycles, unless patients otherwise requested. Patients who decided not to participate in this study underwent COH IVF treatment according to our standard protocol.

Ultrasound monitoring was started on cycle day 3 or 8, and repeated daily or every other day, according to the size of the lead follicle. Follicle diameter was measured in three perpendicular planes, and the mean value was taken. When a lead follicle with a mean diameter of at least 14 mm was observed, daily injections of cetrorelix 0.25mg (Cetrotide®, Serono, the Hague, the Netherlands) together with 150 IU rFSH (75 IU FSH per ampoule: Gonal-F®, Serono Benelux BV, the Netherlands) were started. Cetrorelix was continued up to and including the day of ovulation triggering, r-FSH was continued up to the day of ovulation triggering. Patients were instructed to have their injections in the evening and at the same time daily, to ensure a 24-h interval between injections.

Serum concentrations of LH and estradiol (E$_2$) were assessed on the days that ultrasound was performed. Blood samples were taken in the morning, so serum concentrations reflected levels 12 - 16 h after administration of the medication.

Ovulation triggering was achieved by subcutaneous injection of 10 000 IU of human chorionic gonadotrophin (HCG; Pregnyl®, Organon, Oss, the Netherlands) when a follicle with a diameter of at least 18 mm was observed and plasma E$_2$ levels were $\geq$ 0.8 nmol/L (equivalent to 218 pg/ml; Immulite 2000) or $\geq$ 1.06 nmol/L (equivalent to 288 pg/ml; Architect i-2000). In case a follicle with a diameter of 18 mm was observed together with an E$_2$ level of < 0.8 nmol/L (218 pg/ml; Immulite 2000) or < 1.06 nmol/L (288 pg/ml; Architect i-2000), ovulation triggering was postponed for one day. If an LH rise was noticed, cycles were not cancelled since we hypothesized that cetrorelix administered on the day of ovulation triggering should be capable of blunting the LH surge, thus allowing for planned oocyte retrieval. Transvaginal ultrasound-guided follicle aspiration was performed 34 h after ovulation triggering. A single lumen aspiration needle (MDT®, Hilvarenbeek, the Netherlands) was used. No flushing of the follicle was performed. The aspiration pressure was - 50 to - 100 mm Hg according to standard procedure. Analgesia (fentanyl 2 mg intravenously) was only given on patient request.

Oocytes were inseminated 2-5 h after oocyte retrieval with 10.000 motile spermatozoa prepared by centrifugation for 15 min at 300 g over a 45/90% gradient of Suprasperm® (Medicult a/s, Jylling, Denmark), followed by washing and a swim-up procedure in culture medium. Oocytes were cultured in human tubal fluid medium (Cambrex Bio Science, Verviers, Belgium), supplemented with 10% plasma solution (CLB, Amsterdam, the Netherlands). Fertilization was assessed 17-20 hours after insemination. Embryo transfer was performed 72-76 h after oocyte retrieval, using a TDT® (Prodimed, Neuilly-en-Thielle, France) or Wallace® (SIMS Portex Ltd., Hythe, UK) catheter. For luteal support, HCG 1500 IU was given 5, 8 and 11 days after oocyte retrieval. Since no conclusive data are available on the need for luteal phase support in minimal stimulation IVF, luteal support was administered in all cycles where embryo transfer was performed.

Clinical pregnancy was defined as the ultrasound visualisation of a intrauterine gestational sac. Ongoing pregnancy was defined as the presence of a intrauterine gestational sac with fetal heart beat at 12 weeks amenorrhoea.

Serum assays

Serum LH was measured using immunoassay station AutoDELFIA® (EEG Wallac, Turku, Finland), with inter-assay coefficients of variation (lower, middle and upper range of concentrations) of 5.4%, 5.7% and 6.0%.

E_2 concentrations were measured on the Immulite 2000 station® (Diagnostic Products Corporation, Los Angeles, CA, USA) for the first 94 cycles of the study. In the other 25 cycles in this study, the Architect-i2000® (Abbott Diagnostics, Chicago, IL, USA) was used for E_2 measurements. The inter-assay coefficients of variation (lower, middle and upper range of concentrations) were 17.7%, 8% and 9.3% for Immulite 2000 and 7.9%, 3.6% and 2.8% for Architect i-2000. E_2 values measured with Architect-i2000 = values measured with Immulite 2000 x 1,20 + 0.1 nmol/l.

Data analysis

The percentage and 95% confidence interval (CI) of successful oocyte retrievals was calculated per attempt, the 2PN fertilization rate was calculated per inseminated oocyte, and the number of embryo transfers and clinical and ongoing pregnancy rates were calculated per started cycle. The percentage and 95% CIs of cumulative ongoing pregnancy and live birth rate after three cycles was calculated per patient. The group of cycles where premature ovulation occurred (n=5) was compared to the group where oocyte retrieval was performed as planned (n=104), in order to investigate whether patient or cycle characteristics were different between these groups. Patient and cycle characteristics were compared using Student's t-test and the Mann-Whitney U-test where appropriate. A P-value of < 0.05 was considered significant.

Results

Of 51 consecutive patients asked, 50 agreed to participate in this study. Patient characteristics are shown in table I. The median age of the patients was 32 years (range 26-36). Indications for IVF are shown in table I. Cycle characteristics are shown in table II.

Table I. Patient characteristics

N° of patients	50
Female patient age (years)	32.0 (26-36)
BMI (kg/m^2)	22.6 (17.9-31.9)
Subfertility	
primary	38
secundary	12
Duration of subfertility (months)	40 (3 – 92)
Indication	
tubal	18
unexplained	15
male factor	11
endometriosis	3
cervical factor	1
failed donor insemination	2

Values are median (range)
BMI = body mass index

Table II. Cycle characteristics

	first cycle	second cycle	third cycle	total
Cycles	50	41	28	119
Cetrorelix administration (days)	3.0 (1-7)	4.0 (2-11)	4.0 (2-9)	4.0 (1-11)
Follicle size at HCG administration (mm)	19.0 (16-25)	19.0 (16-24)	19.5 (18-22)	19.0 (16-25)
Oocyte retrievals	43	37	24	104
Succesful oocyte retrievals	33	27	20	80
% / attempt (95% CI)	76.7	73.0	83.3	76.9 (68.7-85.2)
2 PN fertilization (% / oocyte)	71.4	71.0	59.1	68.2
Embryo transfer	23	17	12	52
% / cycle (95% CI)	46.0	41.5	42.9	43.7 (34.6-52.8)
Clinical PR	7	7	5	19
% / cycle (95% CI)	14.0	17.1	17.9	16.0 (9.3-22.7)
% / ET (95% CI)	30.4	41.2	41.7	36.5 (23.2-49.9)
abortion	1	1	-	2
ongoing	6	6	5	17
% / cycle (95% CI)	12.0	14.6	17.9	14.3 (7.9-20.7)
% / ET (95% CI)	26.1	35.3	41.7	32.7 (19.7-45.7)
live birth	6	6	4	16
% / cycle (95% CI)	12.0	14.6	14.3	13.4 (7.2-19.7)
% / ET (95% CI)	26.1	35.3	33.3	30.8 (18.0-43.6)
Cumulative ongoing PR				17
% / patient (95% CI)				34.0 (20.6-47.4)
Live birth				16
% / patient (95% CI)				32.0 (18.8-45.2)

Values are median (range) unless stated otherwise
ET = embryo transfer; PR = pregnancy rate

Nine patients completed one cycle, 13 patients completed two cycles and 28 patients completed three cycles, for a total of 119 treatment cycles (2.4 cycles per patient).

The median number of days of cetrorelix administration was 4 (range 1-11). Follicle size at which ovulation was triggered was 19 mm (range 16-25)(table II).

Ten out of 119 started cycles were cancelled before the start of medication, because of lack of follicular development, premature luteinization or personal reasons of the patient. Five planned oocyte retrievals were cancelled (4.2 % of started cycles). In these cases, premature ovulation had occurred at the time of planned oocyte retrieval and no follicle was present. In two of these, two co-dominant follicles were present at the time of ovulation triggering.

Patient and cycle characteristics of the group where premature ovulation occurred (n=5) and of the group where oocyte retrieval was performed as planned (n=104) are shown in table III. Female patient age, BMI and number of days of cetrorelix administration were not different between groups. Mean LH levels at ovulation triggering were higher in cancelled oocyte retrievals compared with cycles where oocyte retrieval was performed (13.7 $\pm$ 3.3 versus 4.8 $\pm$ 4.1 IU/l; P = 0.001; table III). Fourteen LH surges occurred (LH value on the day of ovulation triggering > 10.0 IU/L). In all these cycles, ovulation was triggered and oocyte retrieval was planned according to protocol. In four of them (LH value at ovulation triggering 13 - 16 IU/l), premature ovulation had occurred at the time of oocyte retrieval. In the other 10 cases (LH value 11 - 28 IU/L), ovulation had not yet taken place and oocyte retrieval was successful in seven cases. In one cycle, premature ovulation had occurred at the time of oocyte retrieval, despite the absence of a detected LH surge (LH value at ovulation triggering 8.3 IU/L).

Table III. Characteristics of cycles with and without planned oocyte retrieval performed.

	OR performed	OR planned and cancelled	P
N° of cycles	104	5	
Female patient age (years)	31.6 (2.7); 32.0 (26-37)	30.8 (2.7); 32.0 (28-34)	0.50[a]
BMI (kg/m^2)	23.2 (3.2); 22.5 (18-32)	23.0 (4.2); 22.6 (18-29)	0.79[b]
Cetrorelix administration (days)	3.9 (1.6); 4.0 (1-11)	3.2 (2.0); 4.0 (1-5)	0.31[a]
LH at ovulation triggering (IU/L)	4.8 (4.1); 3.5 (0.7-28.0)	13.7 (3.3); 15.0 (8.3-16.0)	0.001[b]
E2 at ovulation triggering (nmol/L)			
Immulite 2000	0.91 (0.50); 0.90 (0.2-4.5)	0.93 (0.17); 0.84 (0.8-1.2)	0.95[a]
Architect-i 2000	1.09 (0.38); 1.0 (0.7-2.2)	-	
Follicle size at ovulation triggering	19.6 (1.5); 19.0 (17-25)	18.0 (2.1); 18.0 (16-21)	0.02[a]

OR: oocyte retrieval
Values are mean ($\pm$ SD); median (range)
[a]Student t-test; [b]Mann-Whitney U

Mean E_2 levels at ovulation triggering were not different in cancelled oocyte retrievals compared with cycles where oocyte retrieval was performed (0.93 ± 0.17 nmol/l, 253 ± 46 pg/ml versus 0.91 ± 0.50 nmol/l, 248 ± 136 pg/ml; $P = 0.95$). The follicle size at ovulation triggering was smaller in cycles where oocyte retrieval was cancelled than in cycles where oocyte retrieval was performed (18 ± 2.1 mm versus 19.6 ± 1.5 mm; $P = 0.02$)(table III).

Planned oocyte retrieval was cancelled because of premature ovulation in five different patients, who completed a further seven cycles. In these cycles, oocyte retrieval was planned at a 1-2 mm smaller follicle size than in the preceding cycle, in order to avoid another cancellation. None of these cycles were cancelled, and six out of seven oocyte retrievals were successful.

Eighty out of 104 oocyte retrievals were successful (76.9 %; table II). In eight of these, two co-dominant follicles were aspirated. In one case, five co-dominant follicles were present and in one case six. One, two and three oocytes were obtained in 73, six and one case respectively. In 60 out of a total of 88 oocytes, 2PN fertilization occurred (normal fertilization rate 68.2 %).

Fifty-two embryo transfers were performed (43.7 % per started cycle), leading to 19 clinical pregnancies (16 % per started cycle; 36.5 % per embryo transfer). In 49 and three cases respectively, one and two embryos were transferred. Eighteen pregnancies were singleton, and one pregnancy was a dichorionic twin pregnancy after transfer of two embryos. Two miscarriages occurred. The ongoing pregnancy rate was thus 14.3 % per started cycle and 32.7 % per embryo transfer. The cumulative ongoing pregnancy rate after three cycles was 34% per patient. One singleton pregnancy ended with immature birth at gestational age of 18 weeks. The other pregnancies ended with live births, giving a live birth rate of 32% per patient (table II).

Results according to indication are shown in Table IV. For male factor subfertility, the fertilization rate per inseminated oocyte was 46.7%. For the other indications, fertilization rate ranged from 75% to 100%. Owing to the low fertilization rate in male factor subfertility, the number of embryos per started cycle was only 25%, whereas for the other indications, he number of embryo transfers per started cycle ranged from 45.7% to 100%.

Discussion

In this study, minimal stimulation IVF with late follicular phase administration of the GnRH antagonist cetrorelix and concomitant substitution with rFSH is described. This is the first study in which the use of rFSH is described for this purpose.

We hypothesized that during daily administration of cetrorelix 0.25 mg, substitution with gonadotrophins is necessary since follicular developmental arrest is expected in some cases if cetrorelix is administered without any substitution (Duijkers *et al.*, 1998).

E_2 production in the follicle is both LH- and FSH-dependent. Androgens, which are needed as a substrate for FSH-dependent production of E_2 in the granulosa cells, are produced in the theca cells in response to LH. In two studies where rFSH was administered after downregulation with GnRH agonists, normal follicular development was demonstrated in the presence of very low or even undetectable LH levels (Ben-Chetrit *et al.*, 1996;

Table IV. Results according to indication

Indication	Tubal factor	Unexplained	Male factor	Endometriosis	Cervical factor	Failed donor	Total
No of patients	18	15	11	3	1	2	50
Age (years)	32 (27-36)	32 (26-35)	32 (27-36)	29 (28-30)	29	33 (32-34)	32 (26-36)
No. of cycles	43	35	28	6	1	6	119
Cetrorelix administration (days)	4.0 (2-11)	4.0 (2-8)	3.0 (1-9)	3.0 (1-6)	4	3.0 (1-4)	4.0 (1-11)
Follicle size at HCG administration (mm)	19.8 (18-25)	19.0 (16-22)	19.0 (16-21)	19.0 (18-24)	21	19.0 (18-21)	19.0 (16-25)
Oocyte retrievals	39	27	26	6	1	5	104
Succesful oocyte retrievals	31	25	15	4	1	4	80
% per attempt (95% CI)	79.5	92.6	57.7	66.7	100	80.0	76.9 (68.7-85.2)
2PN fertilization (% per oocyte)	77.4	76.0	46.7	75.0	100	75.0	68.2
Embryo transfer	22	16	7	3	1	3	52
% per cycle (95% CI)	51.2	45.7	25.0	50.0	100	50.0	43.7 (34.6-52.8)
Clinical PR	7	6	3	2	1	-	19
% per cycle (95% CI)	16.3	17.1	10.7	33.3	100	-	16.0 (9.3-22.7)
% per ET (95% CI)	31.8	37.5	42.9	66.7	100	-	36.5 (23.2-49.9)
Abortion	1	1	-	-	-	-	2
Ongoing	6	5	3	2	1	-	17
% per cycle (95% CI)	14.0	14.3	10.7	33.3	100	-	14.3 (7.9-20.7)
% per ET (95% CI)	27.3	31.3	42.9	66.7	100	-	32.7 (19.7-45.7)
Live birth	6	4	3	2	1	-	16
% per cycle (95% CI)	14.0	11.4	10.7	33.3	100	-	13.4 (7.2-19.7)
% per ET (95% CI)	27.3	25.0	42.9	66.7	100	-	30.8 (18.0-43.6)

Values are median (range) unless stated otherwise
ET= embryo transfer; PR = pregnancy rate

Sullivan *et al.*, 1999). We therefore hypothesized that residual LH levels after administration of cetrorelix 0.25 mg daily would suffice for androgen production and maintenance of a physiological E_2 level, provided that exogenous FSH was administered for substitution.

In this study, no developmental arrest of the dominant follicle was seen after start of medication. It seems therefore that substitution with 150 IU of rFSH after administration of 0.25 mg of cetrorelix is effective in this protocol.

The overall premature ovulation rate (4.2 % per started cycle) in our study is in accordance with the ovulation rate in an earlier study, where HMG was used for substitution after administration of a single dose of 0.5 or 1 mg of cetrorelix in the late follicular phase. In this study, four out of 44 planned oocyte retrievals were cancelled because of premature ovulation (Rongières-Bertrand *et al.*, 1999). In one of these cycles, an early LH surge was detected after inadvert omission of cetrorelix injection. In the other three, no LH surge was detected but at the time of oocyte retrieval no follicle was seen.

The suppressive effect of cetrorelix is dose-dependent, 0.25 mg daily being the lowest effective dose (Albano *et al.*, 1997; Duijkers *et al.*, 1998). However, in individual cases, LH surges and subsequent ovulations have been demonstrated during daily administration of 0.25 mg of cetrorelix (Duijkers *et al.*, 1998; Ragni *et al.*, 2001; Al-Inany and Aboulghar, 2002). In our study, serum LH levels on the day of ovulation triggering in the cycles that were cancelled because of premature ovulation were rather high, and in four out of five cancelled oocyte retrievals a LH surge (LH > 10 IU/L) was detected. Medication was administered in the evening, while blood was drawn 12 – 16 hours before. It is therefore possible that a LH surge occurred without detection. However, in ten cycles, LH value was > 10 IU/L on the day of ovulation triggering, but ovulation had not occurred at the time of oocyte retrieval. Apparently, in some cases, cetrorelix 0.25 mg is indeed capable of blunting the LH surge enough to allow for planned oocyte retrieval.

The overall premature ovulation rate (4.2 %) we found in this study is low, and probably lower than the ovulation rate with natural cycle IVF without use of GnRH antagonists, which is about 16.6 % (Pelinck *et al.*, 2002). Further research should be performed comparing natural cycle IVF with minimal stimulation IVF to clarify whether the benefit of a lowered cancellation rate with a minimal stimulation protocol outweighs the inconvenience of treatment with GnRH antagonist and gonadotrophins. On the other hand, an increase in GnRH antagonist dose might further reduce ovulation rates and thus lead to better results in minimal stimulation IVF. In this study, we chose to use 150 IU r-FSH as substitution, and this seems to be effective in the minimal stimulation protocol. However, we do not know whether a lower dose of gonadotrophins or the use of LH-containing preparations will be equally effective. This also should be a subject of further research.

In the present study, medication was started at a follicle size of 14 mm, assuming that follicular dominance had developed, in order to avoid multiple follicular recruitment. In 97 cycles, a single dominant follicle developed and subsequently, no more than one oocyte and one embryo for transfer was obtained. In 10 cycles, two co-dominant follicles were present when medication was started and both follicles continued to grow during administration of cetrorelix and rFSH. In two cycles, five and six co-dominant follicles developed, whereas only one was present when medication was started. It seems therefore that in most but not all cases, multiple follicle development is prevented by starting med-

ication no earlier than at a follicular size of 14 mm. Out of 52 embryo transfers, 49 were of one single embryo.

In the present study, the overall ongoing pregnancy rate was 32.7 % per embryo transfer. The ongoing implantation rate was 30.9 % per transferred embryo. This implantation rate seems to be comparable to implantation rates of embryos obtained after COH IVF. This is surprising since, other than in COH IVF, in our protocol no selection of the best-quality embryo was possible since in most cases only one was obtained. It may be that the oocyte from the dominant follicle represents the best-quality oocyte in a cohort of oocytes, leading to an embryo with good implantation potential. An alternative explanation for the surprisingly good implantation rates found in our study is that the endometrium is more receptive after minimal stimulation than in stimulated IVF. Some authors claim a disturbed endometrial receptivity after ovarian stimulation for IVF, related to supraphysiologic levels of E_2 (Fossum *et al.*, 1989; Simón *et al.*, 1998; Basir *et al.*, 2001; Bourgain and Devroey, 2003). Physiologic levels of steroid hormones as present in minimal stimulation cycles may thus be associated with better endometrial receptivity compared to endometrial recepticity after COH IVF.

In this study, the patient population is probably somewhat biased towards a good chance for pregnancy, the maximum patient age at inclusion being 36 years. The overall ongoing pregnancy rate was 14.3 % per started cycle, which seems to be higher than pregnancy rates obtained after natural cycle IVF without the use of a GnRH-antagonist (Pelinck *et al.*, 2002).

The pregnancy rates in the present study seem comparable to those found in an earlier study, where a single injection of cetrorelix 0.5 or 1 mg was given together with HMG (Rongières-Bertrand *et al.*, 1999). In that study, where in all cases ICSI was applied, seven clinical pregnancies, of which five ongoing, were obtained in 44 cycles (15.9% clinical and 11.4% ongoing pregnancy rate per started cycle)(Rongières-Bertrand *et al.*, 1999).

Cancellation rates and number of unsuccessful oocyte retrievals were rather high, leading to a disappointingly low number of embryo transfers per cycle (43.7%). Implantation rates were high, leading to a 14.3% ongoing pregnancy rate per started cycle. Compared with pregnancy rates after COH IVF, this ongoing pregnancy rate per cycle is low. However, minimal stimulation IVF is easily repeatable in consecutive cycles, and the duration of one cycle of minimal stimulation IVF is about one-third of a COH IVF treatment cycle, downregulation preceding the actual treatment cycle and a resting cycle afterwards included. Therefore, in terms of effectiveness, it seems logical to compare cumulative pregnancy rates after three cycles of minimal stimulation IVF to those after one cycle of COH IVF.

Future research should clarify which patients will benefit most from minimal stimulation IVF. From the present study, it seems that this protocol is effective for all indications studied, albeit possibly less so for male factor subfertility, where, owing to a low fertilization rate, the number of embryos per started cycle seems lower than for the other indications. Of course, no firm conclusions can be drawn owing to the small numbers involved. In analogy with natural cycle IVF, this protocol could be a valuable alternative for poor responders (Lindheim *et al.*, 1997; Bassil *et al.*, 1999; Feldman *et al.*, 2001; Morgia *et al.*, 2004). Since with ovarian stimulation, these patients will have only few

oocytes, it seems feasible to apply a minimal stimulation IVF protocol, where with less costs and less time per cycle, comparable results could be obtained. In addition, patients at risk for OHSS or with a history of OHSS could also benefit from this protocol.

Minimal stimulation IVF has the advantage of being a patient-friendly protocol, since medication is administered for a few days only and therefore few side-effects are experienced. Oocyte retrieval is less painful than in COH IVF, since usually only one follicle is aspirated. Also, minimal stimulation IVF is associated with zero risk of OHSS and therefore is a low-risk alternative to COH IVF.

As yet there are no cost-effectiveness analyses available on minimal stimulation IVF, but per cycle, minimal stimulation IVF will be far cheaper than COH IVF, since considerably less hormonal medication is used.

We consider the very low multiple pregnancy rate in minimal stimulation IVF an advantage. Clearly, multiple pregnancies are a consequence of embryo transfer policy and not of ovarian stimulation, and so can be prevented after COH IVF by performing elective single embryo transfer (SET) in cases where at least one embryo of good quality is available (Gerris and Van Royen, 2000; Ozturk *et al.*, 2001). The addition of pregnancies after transfer of frozen-thawed spare embryos raises the delivery rate per oocyte retrieval even further (Tiitinen *et al.*, 2001). With judicious application of elective SET, the multiple pregnancy rate is reduced to 7.5 – 21 % without a drop in overall pregnancy rates (Gerris *et al.*, 2002; De Sutter *et al.*, 2003; Tiitinen *et al.*, 2003). However, this strategy still requires ovarian hyperstimulation, leading to high costs, patient discomfort and risk for OHSS, and is only possible if patients are willing to undergo elective SET, which is often not the case (Gerris *et al.*, 2004; Murray *et al.*, 2004).

Of course, it is not known how willing patients will be to undergo minimal stimulation IVF, since the high inclusion rate in the present study is probably, at least in part, caused by the fact that treatment cycles were offered for free.

A randomized controlled study comparing minimal stimulation IVF with COH IVF seems warranted. In such a study, focus should not be on success rates per cycle, but rather on pregnancy rates per time spent by the patient; so for instance, three or four cycles of minimal stimulation IVF can be compared to one cycle of COH IVF, frozen embryo transfers included. In such a study, elective SET could be applied in the COH IVF cycles as well as in those cycles of minimal stimulation IVF where two or more embryos are obtained. Concerning cost-effectiveness, costs per live birth should be calculated, including costs of pregnancy and delivery and costs related to OHSS. Also, quality of life of patients should be taken into account in such a study.

In conclusion, pregnancy rates after minimal stimulation IVF with use of the GnRH antagonist cetrorelix in the late follicular phase are encouraging. For substitution, rFSH alone seems to be sufficient. A randomized controlled trial comparing minimal stimulation IVF with current standard treatment protocols is warranted, to clarify whether minimal stimulation IVF is a feasible alternative to COH IVF.

Chapter 4

Cumulative pregnancy rates after three cycles of minimal stimulation IVF and results according to subfertility diagnosis: a multicentre cohort study

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ABSTRACT

BACKGROUND: In minimal stimulation IVF, treatment is aimed at using the single oocyte that spontaneously develops to dominance. To prevent untimely ovulation, a GnRH antagonist is administered in the late follicular phase of the natural cycle together with recombinant FSH for substitution. Owing to the lack of ovarian stimulation, minimal stimulation IVF is a low-risk and patient-friendly treatment. In this study, effectiveness of minimal stimulation IVF was studied.

METHODS: In this prospective multicentre cohort study, minimal stimulation IVF was offered to 350 patients. All indications for conventional IVF were included. Main outcome measures were pregnancy rates per cycle and cumulative pregnancy rates after three cycles.

RESULTS: A total of 336 patients completed 844 cycles (2.5 per patient). The overall ongoing pregnancy rate per started cycle was 8.3% [95% confidence interval (CI) 6.4-10.2%]. The cumulative ongoing pregnancy rate after up to three cycles was 20.8% (95% CI 16.4-25.3%) per patient. No differences were found according to indication for IVF.

CONCLUSIONS: Minimal stimulation IVF seems suitable for all indications studied. Pregnancy rates are encouraging. Due to the low-risk and patient-friendly nature of this protocol, it seems a feasible treatment option for patients requiring IVF.

Introduction

In minimal stimulation IVF, treatment is aimed at using the one dominant follicle that spontaneously develops in a natural cycle. Because of the minimum use of medication, minimal stimulation IVF offers several advantages. A GnRH antagonist is only used in the late follicular phase, to prevent untimely LH surges and the consequent cancellation of oocyte retrieval, as is the administration of gonadotrophins to substitute for the expected fall in estradiol (E_2) (Paulson et al., 1994b; Rongières-Bertrand et al., 1999).

Because gonadotrophins are administered in a low dose and only one or few follicles develop, the risk of the ovarian hyperstimulation syndrome (OHSS) is negligible. Minimal stimulation IVF is also a patient-friendly treatment as medication is administered for a few days only, causing few side effects, and the duration of a treatment cycle is considerably shorter than standard IVF with controlled ovarian stimulation (COS). As usually only one follicle is aspirated, oocyte retrieval is easy and short lasting and can be performed without analgesia (Ramsewak et al., 1990). As opposed to COS-IVF, in minimal stimulation IVF, no resting cycle is necessary after a failed treatment cycle, and treatments are easily repeated in consecutive cycles. Because usually no spare embryos are generated, minimal stimulation IVF is an attractive treatment option for patients who, for ethical or religious reasons, are opposed to the generation of spare embryos (Biggers and Summers, 2004).

Data on efficacy of minimal stimulation IVF according to cause of subfertility are scarce.

So far, only small studies describing minimal stimulation IVF have been published with pregnancy rates per started cycle varying between 0.0 and 18.3% (Rongières-Bertrand et al., 1999; Ubaldi et al., 2003; Kadoch et al., 2003; Vogel et al., 2003; Kolibianakis et al., 2004; Weghofer et al., 2004; Zhioua et al., 2004; Elizur et al., 2005; Pelinck et al., 2005). In most of these studies, ICSI was performed in all cycles, either electively because of the expected small number of oocytes, or because of severe male factor infertility (Rongières-Bertrand et al., 1999; Ubaldi et al., 2003; Vogel et al., 2003; Weghofer et al., 2004; Zhioua et al., 2004). In a pilot study performed in the University Medical Center Groningen, where cumulative pregnancy rates after a maximum of three cycles of minimal stimulation IVF were assessed, only conventional IVF was performed. In this study, ongoing pregnancy rates per started cycle were 14.0, 14.3 and 10.7% for tubal factor, unexplained and male factor subfertility, respectively, indicating an advantage for tubal factor and unexplained subfertility (Pelinck et al., 2005).

It is unclear how efficacy of minimal stimulation IVF compares to COS-IVF. In two recent studies, similar pregnancy rates per cycle were found in minimal stimulation IVF and COS-IVF (Weghofer et al., 2004; Elizur et al., 2005). However, these studies included only women of $\geq$ 40 years of age or poor-responder patients, so these results are not applicable to the general IVF population (Weghofer et al., 2004; Elizur et al., 2005).

To evaluate effectiveness of minimal stimulation IVF however, cumulative pregnancy rates are more relevant than pregnancy rates per started cycle. Owing to the patient-friendly nature of the minimal stimulation protocol, it is possible that dropout rates will be relatively low. Moreover, as treatments are easily repeated in consecutive cycles, pregnancy rates per time spent by the patient may be favourable. In the pilot study performed in our

centre (University Medical Center Groningen), we found a cumulative ongoing pregnancy rate of 34.0% after three cycles of minimal stimulation IVF (Pelinck *et al.*, 2005).

The purpose of the present multicentre cohort study was 2-fold. First, we wished to evaluate effectiveness of minimal stimulation IVF according to cause of subfertility and secondly, cumulative pregnancy rates after three cycles were calculated. So far, this is the largest series of minimal stimulation IVF.

Materials and methods
Study protocol

In this multicentre cohort study, the University Medical Center Groningen (centre A), the Academic Medical Center Amsterdam (centre B), the Vrije Universiteit Medical Center Amsterdam (centre C) and the Isala Clinics Zwolle (centre D) participated.

The study protocol was reviewed and approved by the local ethical committees of the participating centres. Inclusion criteria for this study were female patient age 18-36 years, first IVF treatment ever or first IVF treatment after a pregnancy, the presence of a regular and proven ovulatory menstrual cycle with a length of 26-35 days and BMI (kg/m^2) of 18-28. Indications for IVF were tubal pathology, unexplained subfertility, male factor, endometriosis, cervical factor or failed artificial inseminations with donor semen (AID). Patients were not included in the study in case an endometriosis cyst was seen on ultrasound. Patients requiring ICSI were not included in this study. Patients with male factor or unexplained subfertility had undergone treatment with intra-uterine insemination (IUI) for three to six cycles before starting IVF treatment, as is standard protocol in the Netherlands.

Patients were offered a maximum of three free treatment cycles. In a subgroup of patients in centre A, nine free cycles were offered. For this study, the first three cycles were analysed. Treatments were performed in three consecutive menstrual cycles, unless patients requested otherwise. Patients who decided not to participate in this study, underwent COS-IVF treatment according to standard protocol.

Inclusion of the patients took place from January 2001 to June 2004. Treatments were performed between January 2001 and January 2005. This study is an extension of a pilot study performed in centre A, in which 50 patients were studied (Pelinck *et al.*, 2005).

Ultrasound monitoring was started on cycle day 3 or 8 and repeated daily or every other day, according to the size of the lead follicle. Follicle diameter was measured in three perpendicular planes, and the mean value was taken. When a lead follicle with a mean diameter of at least 14 mm was observed, daily injections of 0.25 mg of the GnRH antagonist cetrorelix (Cetrotide®, Serono, the Hague, the Netherlands) together with 150 IU recombinant FSH (r-FSH, Gonal-F®, Serono Benelux BV, the Netherlands) were started. Cetrorelix was continued up to and including the day of ovulation triggering, and r-FSH was continued up to the day of ovulation triggering. Patients were instructed to have their injections in the evening and at the same time daily, to ensure a 24 h interval between injections.

Blood was taken for assessment of serum concentrations of LH and E_2 on the days ultrasound was performed. In centres A and B, LH levels were determined the same day and were taken into account for planning of oocyte retrieval and ultrasound examinations. In centres C and D, LH levels were not taken into account for planning of oocyte retrieval

and ultrasound examinations, because results usually were not yet available at the time of planning. In centres A, B and C, E_2 levels were available at the time of planning, whereas in centre D they were not.

Blood samples were taken in the morning, so serum concentrations reflected levels 12 - 16 hours after administration of the medication.

Ovulation triggering was achieved by subcutaneous injection of 10 000 IU of HCG (Pregnyl®, Organon, Oss, the Netherlands) when a follicle with a diameter of at least 18 mm was observed and/or plasma E2 levels were $\geq$ 0.8 nmol/l.

Cycles were cancelled when an LH level of $\geq$ 20.0 IU/L was noticed at a follicle size of < 15 mm (before medication was started). In cases where an LH level of 10.0-30.0 IU/L was noticed at a follicle size of $\geq$ 15 mm (after medication start), the cycle was not cancelled since we hypothesized that cetrorelix should be capable of blunting the LH-surge enough to allow for planned oocyte retrieval. In cases where an LH level of $\geq$ 30.0 IU/L was noticed, planning of oocyte retrieval was cancelled.

Transvaginal ultrasound-guided follicle aspiration was performed 34 h after ovulation triggering. A single lumen aspiration needle was used. No flushing of the follicle was performed. Analgesia was only given on patient request. Only large (dominant) follicles were aspirated. In cases where at the time of planned oocyte retrieval unexpected ovulation had occurred and tubes were patent, intra-uterine insemination (IUI) was performed.

Conventional IVF was performed according to local standard procedures. Embryo transfer was performed on the third day after oocyte retrieval. For luteal support, HCG 1500 IU (Pregnyl®, Organon, Oss, the Netherlands) was given 5, 8 and 11 days after oocyte retrieval.

Pregnancy was defined as the ultrasound visualisation of an intrauterine gestational sac or a proven ectopic pregnancy. Ongoing pregnancy was defined as the presence of an intrauterine gestational sac with fetal heart beat at 12 weeks amenorrhoea.

Data analysis

Patient characteristics according to participating centre and indication for IVF were compared using Kruskal-Wallis and Chi-square test where applicable. Results (per started cycle, per oocyte retrieval, per embryo transfer and per patient, according to indication for IVF and cycle number) are given as percentages with 95% confidence intervals (CI). A separate analysis was performed of results in second and third cycles of patients who experienced a cancellation of oocyte retrieval, an unsuccessful oocyte retrieval or fertilization failure in their first cycle and compared with the results of second and third cycles of patients where these events did not occur in the first cycle.

Results

Patient characteristics

In centers A, B, C and D, 303, 21, 16, and 10 patients were included, respectively. Patient characteristics according to participating center were not significantly different (table I).

Patients characteristics according to indication for IVF are summarized in table II. The median age of the patients and the median duration of subfertility were significantly different between indications (p=0.02 and p<0.001, respectively). The median BMI was not

Table I. Patient characteristics according to participating center

	Center A	center B	center C	center D	Total	P
N⁰ of patients	303	21	16	10	350	
Female patient age (years)	33.0 (22-37)	34.0 (25-36)	32.0 (29-36)	32.0 (23-36)	33.0 (22-37)	0.94[a]
BMI (kg/m²)	23.0 (16-34)	24.0 (18-29)	21.5 (20-26)	23.5 (21-33)	23.0 (16-34)	0.35[a]
Subfertility (% of total)						
Primary	172 (56.8)	11 (52.4)	11 (68.8)	2 (20.0)	196 (56.0)	0.09[b]
Secondary	131 (43.2)	10 (47.6)	5 (31.3)	8 (80.0)	154 (44.0)	
Duration of subfertility (months)	46.0 (0-121)	47.0 (8-111)	52.0 (17-90)	42.0 (8-77)	46.0 (0-121)	0.31[a]
Indication (% of total)						
Tubal	95 (31.4)	7 (33.3)	5 (31.3)	2 (20.0)	109 (31.1)	0.22[b]
Unexplained	117 (38.6)	4 (19.0)	8 (50.0)	3 (30.0)	132 (37.7)	
Male factor	47 (15.5)	7 (33.3)	1 (6.3)	4 (40.0)	59 (16.9)	
Endometriosis	25 (8.3)	1 (4.8)	2 (12.5)	-	28 (8.0)	
Cervical factor	10 (3.3)	-	-	1 (10.0)	11 (3.1)	
Failed AID	9 (3.0)	2 (9.5)	-	-	11 (3.1)	

Values are median (range) unless stated otherwise
[a]Kruskal-Wallis
[b]Chi square

Table II. Patient characteristics according to indication for IVF

	tubal	unexplained	male factor	endometriosis	cervical factor	failed AID	total		P
No of patients	109	132	59	28	11	11	350		
Female patient age (years)	33.0 (22-36)	32.0 (23-36)	33.0 (23-37)	31.5 (26-36)	35.0 (30-36)	35.0 (30-36)	33.0 (22-37)		0.02[a]
BMI (kg/m^2)	22.0 (17-34)	23.0 (16-30)	23.0 (18-33)	22.0 (19-30)	23.0 (18-26)	21.0 (18-34)	23.0 (16-34)		0.92[a]
Subfertility (% of total)									
Primary	45 (41.3)	82 (62.1)	33 (55.9)	22 (78.6)	5 (45.5)	9 (81.8)	196 (56.0)		0.001[b]
Secondary	64 (58.7)	50 (37.9)	26 (44.1)	6 (21.4)	6 (54.5)	2 (18.2)	154 (44.0)		
Duration of subfertility									
(months)	34.0 (0-98)	51.0 (0-121)	47.0 (3-111)	47.5 (8-98)	50.5 (3-105)	64.5 (31-107)	46.0 (0-121)		0.000[a]

Values are median (range) unless stated otherwise
[a]Kruskal Wallis
[b]Chi square

different between indications (p=0.92). Subfertility was primary in 56.0% of patients and secondary in 44.0% and significantly different between indications, secondary subfertility being most frequent in patients with tubal factor (p=0.001).

Overall results

Results according to indication for IVF and cycle number are summarized in tables III and IV. Results according to participating center showed no significant differences (data not shown).

Figures 1 and 2 are summaries of both tables III and IV. Overall, 57 patients dropped out of the study, in 14 cases before start of any treatment (figure 1). In five of these, the reason for drop-out was the occurrence of a spontaneous pregnancy. Forty-three patients

Figure 1. Number of drop-outs and pregnancies according to cycle number
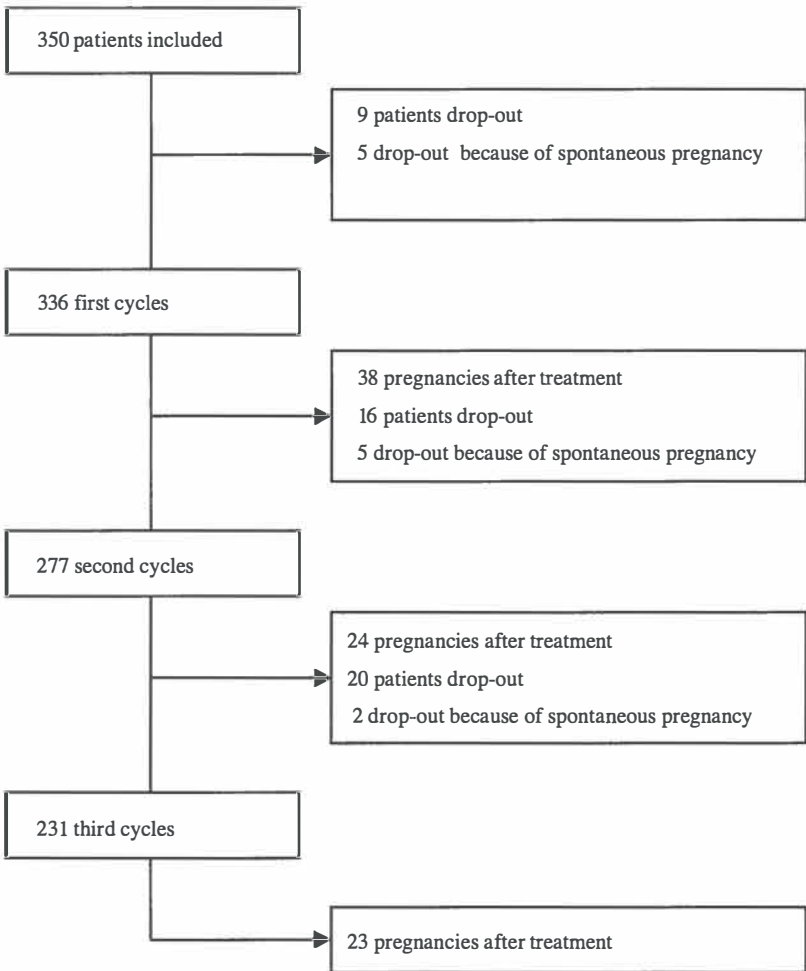
dropped out of the study after one or two unsuccesful treatment cycles, in seven of these because of the occurrence of a spontaneous pregnancy.

A summary of all started cycles is shown in figure 2. A total of 844 cycles were started in 336 patients. Out of 844 started cycles, 55 (6.5%) were cancelled before medication was started, because of lack of follicular development (16 cases), premature LH rise or ovulation (25 cases) or other reasons (14 cases). A further 25 cycles where cancelled after cetrorelix and r-FSH administration was started, because of an LH surge or ovulation (15 cases), stop in follicular growth (5 cases) or other reasons (5 cases).

Out of 844 started cycles, 764 oocyte retrievals were planned (90.5%). Of these, 69 (9.0%) were cancelled, in one case because of inaccessibility of the ovary during oocyte retrieval, and in 68 cases because of premature ovulation, where despite correct administration of medication, no follicle was present at the time of planned oocyte retrieval. Of the cycles where oocyte retrieval was planned, LH levels on the day medication was started and on the day ovulation was triggered were known in 730 and 735 cases, respectively. In 158 cases (21.6%), LH level was $\geq$ 10.0 IU/L at the time medication was started. Of these, 30 (19.0%) were cancelled at the time of oocyte retrieval. On the day ovulation was triggered, LH level was $\geq$ 10.0 IU/L in 111 cases (15.1%). Of these, 38 (34.2%) were cancelled due to ovulation at the time of planned oocyte retrieval.

Figure 2. Summary of overall results
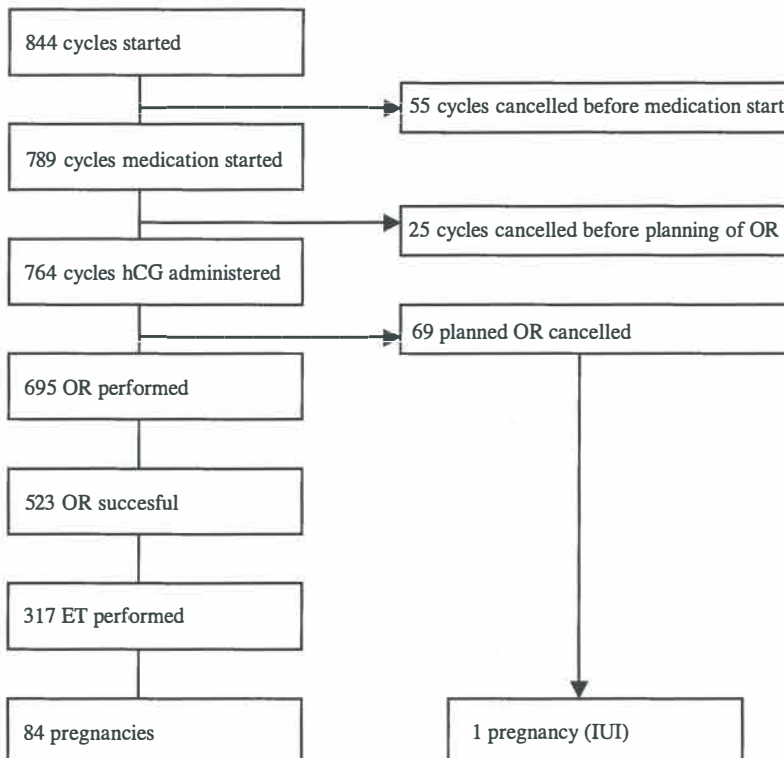
Out of 695 oocyte retrievals, 523 were succesful (75.3%). In 40 of these, two or more oocytes were obtained (two oocytes in 35 cases, three oocytes in two cases and four, nine and 20 oocytes in the remaining three cases). In most of the cases where two or more oocytes were obtained, one single follicle or two co-dominant follicles were aspirated. In two and four cases respectively, three and four follicles were aspirated. In the remaining three cases, five, six and eleven follicles were aspirated.

In 376 out of a total of 523 successful oocyte retrievals, fertilization occurred (71.9%). No transfer was carried out in 59 of these due to aberrant fertilization or defective embryo development (three pronuclei (PN), absence of cleavage or excessive fragmentation).

Overall, 317 embryo transfers were performed (37.6% per started cycle). In 299 of these, one single embryo was available for transfer. In 16 cases, two embryos were available. Three and four embryos were available in one case each. In all these cases, two embryos were transferred.

A total of 299 single-embryo transfers led to 78 pregnancies. In all centers exept centre C, embryos were screened for the presence of multinucleated blastomeres (MNB). Of 280 single embryo transfers where presence of MNB, amount of fragmentation (0%, ≤10%, 10-40% or >40%) and number of blastomeres on day 2 and day 3 was noted, 84 (30.0%) were of excellent quality, that is, no MNB, four or five blastomeres on day 2 and at least 7 on day 3 and ≤ 10% fragmentation (Van Royen et al., 1999). Of these, 34 implanted (40.5%). Out of 37 embryos showing MNB at any stage, five ongoing implantations occurred (13.5%).

Eighty-five pregnancies followed. One pregnancy occurred after cancelled oocyte retrieval and IUI, 78 occurred after transfer of one embryo and six after transfer of two embryos. Thirteen pregnancies ended in miscarriages, one was ectopic and one was a cervical pregnancy. Seventy pregnancies were ongoing. Four twin pregnancies occurred, two after transfer of one embryo and two after transfer of two embryos. Of the twin pregnancies, one miscarried and three were ongoing. Pregnancy rate per started cycle was 10.1%, of which 4.7% were twins. The ongoing pregnancy rate per started cycle was 8.3%, of which 4.3% were twins. The pregnancy rate and ongoing pregnancy rate per embryo transfer were 26.5% and 22.1%. The cumulative ongoing pregnancy rate after three cycles was 20.8% per patient.

One pregnancy was interrupted because of severe congenital abnormalities (limb-body wall complex). One pregnancy ended in fetal death at 17 weeks gestation. Outcome was unknown for one ongoing pregnancy. Live birth rate, not including the pregnancy lost to follow-up, was thus 19.9% per patient.

Of 208 patients completing three unsuccessful cycles, 127 continued with minimal stimulation IVF, 46 started COS-IVF and 35 refrained from further treatment.

Results according to indication for IVF

Results according to indication for IVF are summarized in table III. For the indications tubal factor, unexplained, male factor, endometriosis, cervical factor and failed AID respectively, 263, 323, 136, 62, 29 and 31 cycles were started. There were no significant differences in the number of cancelled cycles.

In failed AID, cryopreserved semen was used for IVF. Median total motile sperm count (TMSC) of the used semen in this group was 3.7×10^6. For the other indications, median TMSC ranged from 18.0×10^6 (male factor) to 130.0×10^6 (endometriosis). Fertilization per succesful oocyte retrieval was significantly lower in male factor and unexplained subfertility as compared with tubal factor and endometriosis (table III). The number of embryo transfers per started cycle was significantly lower for male factor as compared with tubal subfertility (table III).

Pregnancy rates and live birth rates were not significantly different between indications (table III).

Results according to cycle number

Results according to cycle number are shown in table IV and figure 1. Fifty-nine patients completed one cycle, 46 patients completed two cycles and 231 patients completed three cycles, for a total of 844 cycles.

Between cycle numbers, the differences in cancellation rates, oocyte retrieval rate, fertilization rate, embryo transfer rate and pregnancy rates were not significant (table IV and figure 1).

The results of second and third cycles of patients who experienced a cancellation of oocyte retrieval, an unsuccessful oocyte retrieval or fertilization failure in their first cycle and the results of second and third cycles of patients where these events did not occur in the first cycle are shown in table V.

In 56 patients, oocyte retrieval was not performed in the first cycle. In 36 of these, this was due to LH rise or ovulation during cetrorelix administration or because of unexpected ovulation at the time of planned oocyte retrieval. These 36 patients completed a further 62 cycles (34 second and 28 third cycles), of which 13 were cancelled again due to LH rise or ovulation (21.0% [95% CI 10.6-31.3]), nine were cancelled for other reasons (14.5%) and 40 oocyte retrievals (64.5% [95% CI 52.4-76.7]) were performed. For comparison, in 300 patients, oocyte retrieval was not cancelled in the first cycle, or was cancelled for reasons other than LH rise or ovulation. These patients completed a further 446 cycles (243 second and 203 third cycles), of which 35 were cancelled due to LH rise or ovulation (7.8% [95% CI 5.3-10.4]), significantly less than in the group where the first cycle was cancelled. Thirty-six second and third cycles were cancelled for reasons other than LH rise or ovulation (8.1%). The number of oocyte retrievals performed in second and third cycles was 375 (84.1% [95% CI 80.6-87.5]), significantly higher than in the group where the first cycle was cancelled.

For 70 patients, oocyte retrieval was not successful in the first cycle. These 70 patients completed a further 118 cycles (64 second and 54 third cycles), of which 14 were cancelled and 104 oocyte retrievals were performed (88.1%), of which 65 were successful (62.5% per attempt [95% CI 53.0-72.0]). For comparison, oocyte retrieval was successful in the first cycle in 210 patients. These patients completed a further 298 cycles (162 second and 136 third cycles), of which 50 were cancelled and 248 oocyte retrievals were performed (83.2%).

Table III. Results according to indication for IVF

Indication	tubal	unexplained
No of patients included	109	132
No of patients started	106	128
Cycles started (n° per patient)	263 (2.5)	323 (2.5)
Cycles cancelled before medication start	16	21
% per cycle (95% CI)	6.1 (3.1-9.0)	6.5 (3.8-9.2)
Cetrorelix administration (days: median, range)	3.0 (1-12)	3.0 (1-11)
Cyles cancelled before HCG	5	9
% per cyle (95% CI)	1.9 (0.2-3.6)	2.8 (1.0-4.6)
OR planned	242	293
% per cycle (95% CI)	92.0 (88.7-95.4)	90.7 (87.5-93.9)
Planned OR cancelled	22	26
% per planned OR (95% CI)	9.1 (5.4-12.8)	8.9 (5.6-12.2)
OR performed	220	267
% per cycle (95% CI)	83.7 (79.1-88.2)	82.7 (78.4-86.9)
OR succesful	170	201
% per attempt (95% CI)	77.3 (71.6-82.9)	75.3 (70.0-80.6)
TMSC (million: median, range)	89.0 (1.0-560.0)	58.0 (1.0-760.0)
Cycles with fertilization	140	136
% per succesful OR (95% CI)	82.4 (76.5-88.2)	67.7 (61.1-74.3)
Embryo transfer	120	112
% per cycle (95% CI)	45.6 (39.5-51.8)	34.7 (29.4-40.0)
single ET	114	106
double ET	6	6
Pregnancy	28	30[a]
% per cycle (95% CI)	10.6 (6.8-14.5)	9.3 (6.1-12.5)
% per ET (95% CI)	23.3 (15.6-31.1)	25.9 (17.6-34.2)[a]
abortion	3	6
ectopic	-	1
cervical	-	-
ongoing	25	23
% per cycle (95% CI)	9.5 (5.9-13.1)	7.1 (4.3-10.0)
% per ET (95% CI)	20.8 (13.4-28.2)	20.5 (12.9-28.2)
cumulative ongoing pregnancy rate	25	23
% per patient (95% CI)	23.6 (15.3-31.8)	18.0 (11.2-24.8)
live birth rate	24	21[b]
% per patient (95% CI)	22.6 (14.5-30.8)	16.5 (9.9-23.1)[b]

[a] one ectopic pregnancy after cancelled oocyte retrieval and intra-uterine insemination.
[b] one pregnancy: outcome unknown; not included in calculation

The number of successful oocyte retrievals per attempt was significantly higher compared with the group where oocyte retrieval was not successful in the first cycle (200 oocyte retrievals successful: 80.6% per attempt [95% CI 75.6-85.7]).

For 52 patients, no fertilization occurred after successful oocyte retrieval in the first cycle. Indications for IVF were tubal factor, unexplained subfertility, male factor, endometriosis, failed AID and cervical factor in 14, 23, 10, 1, 2 and 2 cases respectively.

nale factor	endometriosis	cervical factor	failed AID	Total
;9	28	11	11	350
;5	26	10	11	336
36 (2.5)	62 (2.4)	29 (2.9)	31 (2.8)	844 (2.5)
'	9	2	-	55
;.1 (1.4-8.9)	14.5 (5.6-23.5)	6.9 (0.0-16.3)	0.0	6.5 (4.8-8.2)
;.0 (1-9)	3.0 (1-6)	3.0 (1-5)	3.0 (1-9)	3.0 (1-12)
'	4	-	-	25
;.1 (1.4-8.9)	6.5 (0.2-12.7)	0.0	0.0	3.0 (1.8-4.1)
22	49	27	31	764
;9.7 (84.5-94.9)	79.0 (68.7-89.4)	93.1 (83.7-100.0)	100.0	90.5 (88.5-92.5)
'	6	3	5	69
;.7 (1.5-9.9)	12.2 (2.9-21.6)	11.1 (0.0-23.2)	16.1 (2.9-29.3)	9.0 (7.0-11.1)
15	43	24	26	695
;4.6 (78.4-90.8)	69.4 (57.6-81.1)	82.8 (68.7-96.8)	83.9 (70.7-97.1)	82.3 (79.7-85.0)
;6	35	14	17	523
'4.8 (66.7-82.9)	81.4 (69.5-93.3)	58.3 (38.2-78.5)	65.4 (46.7-84.0)	75.3 (72.0-78.5)
8.0 (0.807-120.0)	130.0 (30.8-750.0)	30.0 (4.3-260.0)	3.65 (0.300-8.3)	54.0 (0.300-760.0)
·6	32	10	12	376
3.5 (42.7-64.2)	91.4 (82.0-100.0)	71.4 (47.3-95.6)	70.6 (48.5-92.7)	71.9 (68.0-75.8)
·1	26	10	8	317
0.1 (22.3-38.0)	41.9 (29.4-54.5)	34.5 (16.8-52.1)	25.8 (10.1-41.5)	37.6 (34.2-40.9)
9	24	8	8	299
	2	2	-	18
5	8	2	2	85[a]
1.0 (5.7-16.4)	12.9 (4.4-21.4)	6.9 (0.0-16.3)	6.5 (0.0-15.3)	10.1 (8.0-12.1)
6.6 (21.5-51.6)	30.8 (12.7-48.9)	20.0 (5.3-45.3)	25.0 (0.0-55.6)	26.5 (21.5-31.5)[a]
	-	1	-	13
	-	-	-	1
	1	-	-	1
2	7	1	2	70
.8 (4.0-13.7)	11.3 (3.3-19.3)	3.4 (0.0-10.2)	6.5 (0.0-15.3)	8.3 (6.4-10.2)
9.3 (15.1-43.5)	26.9 (9.5-44.3)	10.0 (0.0-29.0)	25.0 (0.0-55.6)	22.1 (17.4-26.7)
2	7	1	2	70
1.8 (10.7-32.9)	26.9 (9.5-44.3)	10.0 (0.0-29.0)	18.2 (0.0-41.4)	20.8 (16.4-25.3)
2	7	1	2	67[b]
1.8 (10.7-32.9)	26.9 (9.5-44.3)	10.0 (0.0-29.0)	18.2 (0.0-41.4)	19.9 (15.6-24.3)[b]

These 52 patients completed a further 92 cycles (48 second and 44 third cycles), of which 15 were cancelled and 77 oocyte retrievals were performed (83.7%), of which 63 were successful (81.8% per attempt). Median TMSC in these cycles was 30.0 x 10^6 (range 1.30-410.0). Fertilization occurred in 30 cases (47.6% [95% CI 35.0-60.2]). Twenty-seven embryo transfers were performed (29.3% [95% CI 19.9-38.8]), leading to seven pregnancies (7.6%). For comparison, in 158 patients, fertilization did occur after successful oocyte

Table IV. Results according to cycle number

Cycle number	cycle 1	cycle 2	cycle 3	Total
Cycles started	336	277	231	844
Cycles cancelled before medication start	17	20	18	55
% per cycle (95% CI)	5.1 (2.7-7.5)	7.2 (4.1-10.3)	7.8 (4.3-11.3)	6.5 (4.8-8.2)
Cetrorelix administration (days: median, range)	3.0 (1-12)	3.0 (1-11)	3.0 (1-8)	3.0 (1-12)
Cyles cancelled before HCG	9	9	7	25
% per cycle (95% CI)	2.7 (0.9-4.4)	3.2 (1.1-5.4)	3.0 (0.8-2.3)	3.0 (1.8-4.1)
OR planned	310	248	206	764
% per cycle (95% CI)	92.3 (89.3-95.2)	89.5 (85.9-93.2)	89.2 (85.1-93.3)	90.5 (88.5-92.5)
Planned OR cancelled	30	22	17	69
% per planned OR (95% CI)	9.7 (6.3-13.0)	8.9 (5.3-12.5)	8.3 (4.4-12.1)	9.0 (7.0-11.1)
OR performed	280	226	189	695
% per cycle (95% CI)	83.3 (79.3-87.4)	81.6 (76.9-86.2)	81.8 (76.7-86.9)	82.3 (79.7-85.0)
OR succesful	210	167	146	523
% per attempt (95% CI)	75.0 (69.8-80.2)	73.9 (68.1-79.7)	77.2 (71.1-83.3)	75.3 (72.0-78.5)
TMSC (million: median,range)	68.0 (1.0-760.0)	54.0 (0.807-710.0)	51.0 (0.911-510.0)	56.0 (0.807-760.0)[a]
Cycles with fertilization	158	118	100	376
% per succesful OR (95% CI)	75.2 (69.3-81.2)	70.7 (63.6-77.7)	68.5 (60.8-76.2)	71.9 (68.0-75.8)
Embryo transfer	136	99	82	317
% per cycle (95% CI)	40.5 (35.1-45.8)	35.7 (30.0-41.5)	35.5 (29.2-41.8)	37.6 (34.2-40.9)
single ET	127	95	77	299
double ET	9	4	5	18
Pregnancy	38	24[b]	23	85[b]
% per cycle (95% CI)	11.3 (7.9-14.8)	8.7 (5.3-12.0)	10.0 (6.0-13.9)	10.1 (8.0-12.1)
% per ET (95% CI)	27.9 (20.2-35.6)	23.2 (14.7-31.7)[b]	28.0 (18.1-38.0)	26.5 (21.5-31.5)[b]
abortion	2	8	3	13
ectopic	-	1	-	1
cervical	-	-	1	1
ongoing	36	15	19	70
% per cycle (95% CI)	10.7 (7.3-14.1)	5.4 (2.7-8.1)	8.2 (4.6-11.8)	8.3 (6.4-10.2)
% per ET (95% CI)	26.5 (18.9-34.0)	15.2 (7.9-22.4)	23.2 (13.9-32.5)	22.1 (17.4-26.7)

[a]cryopreserved semen not included in calculation
[b]one ectopic pregnancy after cancelled oocyte retrieval and intra-uterine insemination

retrieval in the first cycle. Indications for IVF were tubal factor, unexplained subfertility, male factor, endometriosis, failed AID and cervical factor in 52, 61, 23, 16, 3 and 3 cases, respectively. These patients completed a further 206 cycles (114 second and 92 third cycles), of which 35 were cancelled and 171 oocyte retrievals were performed (83.0%), of which 137 were successful (80.1% per attempt). Median TMSC in these cycles was 59.0 x 10^6 (range 0.81-710.0). Fertilization rate and number of embryo transfers were significantly higher as compared with the group where fertilization failure occurred in the first cycle (fertilization in 115 cases: 83.9% [95% CI 77.7-90.2]; embryo transfer in 96 cases: 46.6% [95% CI 39.7-53.6]). Twenty-two pregnancies occurred (10.7%).

Discussion
This study describes the largest series of minimal stimulation IVF available so far. The overall ongoing pregnancy rate per started cycle was 8.3%, with a cumulative ongoing pregnancy rate after up to three cycles of 20.8%.

Pregnancy rates according to indication for IVF showed no significant differences. The results of this study suggest therefore that minimal stimulation IVF is applicable for all indications for conventional IVF. Although for male factor infertility compared with tubal factor, both fertilization and embryo transfer rates were significantly lower, these differences were not reflected in a lower pregnancy rate. Fertilization rate in male factor was lower than in endometriosis, but the embryo transfer rate was not. This lower fertilization rate in male factor infertility is not surprising since these patients have diminished semen quality. However, when fertilization did occur, implantation rates found in our study were very good. For unexplained infertility, the fertilization rate was significantly lower than for tubal factor and endometriosis, but the embryo transfer rate and pregnancy rates were not. This lower fertilization rate in unexplained infertility is not surprising either, since fertilization failure is a common finding in these patients (Takeuchi *et al.*, 2000; Hershlag *et al.*, 2002; Jaroudi *et al.*, 2003; Bungum *et al.*, 2004). For this category of patients also, once fertilization did occur, we found good implantation rates. For cervical factor infertility, results seemed rather poor, but since the number of patients was small, no firm conclusions can be drawn.

The number of cancelled oocyte retrievals was rather high (17.7%). Out of 844 started cycles, 80 (9.5%) were cancelled before planning of oocyte retrieval. An additional 69 cycles were cancelled at the time of planned oocyte retrieval. This cancellation rate seems to be in accordance with cancellation rates reported in literature. In earlier studies on minimal stimulation IVF, in 101 out of a total of 531 described cycles, oocyte retrieval was cancelled (19.0%) (Meldrum *et al.*, 1994; Paulson *et al.*, 1994b; Rongières-Bertrand *et al.*, 1999; Kadoch *et al.*, 2003; Vogel *et al.*, 2003; Kolibianakis *et al.*, 2004; Weghofer *et al.*, 2004; Zhioua *et al.*, 2004; Elizur *et al.*, 2005).

Of 149 cancellations, 108 (12.8% per started cycle) were related to a rise in LH or ovulation. In natural cycle IVF without the use of a GnRH antagonist, out of a total of 1572 described cycles, 314 were cancelled because of an LH rise or ovulation (20.0%) (Omland *et al.*, 2001; Ballesteros *et al.*, 2002; Bauman *et al.*, 2002; Pelinck *et al.*, 2002; Lukassen *et al.*, 2003). This raises the question whether the apparently small decrease in cancellation rate in the minimal stimulation protocol justifies the inconvenience and costs of treatment

Table V. Results in second and third cycle according to performance in first cycle

Results of first cycle	1st cycle cancelled (LH rise/ovulation)	1st cycle not cancelled (or cancel other reason)
Cycle 1 (n° of cycles)	36	300
Cycles 2 and 3 of same patients (n° of cycles)	62	446
cancel (LH rise / ovulation)	13	35
% per cycle (95% CI)	21.0 (10.6-31.3)[a]	7.8 (5.3-10.4)[a]
cancel (other reason)	9	36
% per cycle (95% CI)	14.5 (5.6-23.5)	8.1 (5.5-10.7)
cancel total	22	71
% per cycle (95% CI)	35.5 (23.3-47.6)[b]	15.9 (12.5-19.4)[t]
OR performed	40	375
% per cycle (95% CI)	64.5 (52.4-76.7)[c]	84.1 (80.6-87.5)[c]
OR successful		
% per attempt (95% CI)		
TMSC (million: median, range)		
cycles with fertilization		
% per successful OR (95% CI)		
embryo transfer	19	162
% per cycle (95% CI)	30.6 (18.9-42.4)	36.3 (31.8-40.9)
pregnancy	4	43[g]
% per cycle (95% CI)	6.5 (0.21-12.7)	9.6 (6.8-12.4)

a,b,c,d,e,f same letters: significant differences
g one pregnancy after cancelled oocyte retrieval and intra-uterine insemination

with GnRH antagonist and gonadotrophins. Since no studies comparing natural cycle IVF to minimal stimulation IVF are available, no conclusions can be drawn on this issue. A study comparing minimal stimulation IVF with natural cycle IVF, including a cost-effectiveness analysis, seems warranted.

On the other hand, changes in the minimal stimulation protocol may reduce the number of LH rises and premature ovulations, thus raising effectiveness. A higher dose or more frequent administration of cetrorelix, ovulation triggering at a smaller follicle size or a smaller interval between HCG administration and oocyte retrieval could all be helpful in this respect. Another approach to the reduction of the number of premature ovulations is the use of indomethacin to prevent follicular rupture (Nargund *et al.,* 2001).

In this study, the number of successful oocyte retrievals seems rather low (75.1% per attempt). In all oocyte retrievals, a single lumen aspiration needle was used, and no flushing of the follicle was done. Flushing of the follicle may raise effectiveness of the oocyte retrieval but also will make the procedure more painful and time-consuming (Tan *et al.,* 1992; Daya *et al.,* 1995).

1st cycle OR unsuccessful	1st cycle OR successful	1st cycle no fertilization	1st cycle fertilization
70	210	52	158
118	298	92	206
14	50	15	35
1.9 (5.9-17.8)	16.8 (12.4-21.1)	16.3 (8.6-24.0)	17.0 (11.8-22.2)
.04	248	77	171
8.1 (82.2-94.1)	83.2 (78.9-87.6)	83.7 (76.0-91.4)	83.0 (77.8-88.2)
5	200	63	137
2.5 (53.0-72.0)[d]	80.6 (75.6-85.7)[d]	81.8 (73.0-90.6)	80.1 (74.0-86.2)
		30.0 (1.30-410.0)	59.0 (0.81-710.0)
		30	115
		47.6 (35.0-60.2)[e]	83.9 (77.7-90.2)[e]
5	123	27	96
9.7 (21.3-38.1)	41.3 (35.6-47.0)	29.3 (19.9-38.8)[f]	46.6 (39.7-53.6)[f]
1[g]	29	7	22
.3 (4.0-14.7)	9.7 (6.3-13.2)	7.6 (2.1-13.1)	10.7 (6.4-15.0)

In this study, the high number of cancelled and unsuccessful oocyte retrievals led to a low number of embryo transfers per started cycle (37.3%), but due to a good implantation rate (25.7% per transferred embryo), the pregnancy rate was acceptable. Thirty percent of single embryos transferred were of excellent quality and showed an implantation rate of 40.5% per embryo. The overall implantation rate found in this study seems similar to implantation rates of embryos obtained after COS-IVF (Andersen *et al.*, 2005), which is surprising since in most cases, only one embryo was available for transfer and unlike the case for COS-IVF, selection of the best-quality embryo was not possible. An explanation for this could be that from a cohort of oocytes, the one that naturally develops to dominance represents the best-quality oocyte. An alternative explanation could be that the implantation environment in minimal stimulation IVF is better than in COS-IVF. The supraphysiological E_2 levels after ovarian stimulation are suggested to be correlated with disturbed endometrial receptivity (Devroey *et al.*, 2004). Therefore, the physiological hormone levels present in minimal stimulation IVF may be associated with a better endometrial receptivity as compared with COS.

In 40 oocyte retrievals, two or more oocytes were obtained (4.7% per cycle; 5.8% per oocyte retrieval). In most of these cases, two co-dominant follicles were present when medication was started and both continued to grow and were aspirated, or two oocytes were obtained from one dominant follicle. However, in nine of these cases (1.1% per cycle; 1.3% per oocyte retrieval), three or more large follicles were aspirated, although only one dominant follicle ($\geq$ 14 mm) had been present when medication was started. Apparently, in rare cases, the administration of r-FSH leads to ovarian stimulation, even when it is started after presumed follicular dominance at a follicle size of 14 mm. An alternative explanation is that an ovarian cyst was mistaken for a dominant follicle and follicular dominance had not yet developed in these cases.

In this study, the multiple pregnancy rate was very low with 4.7%, which is advantageous considering the many problems associated with multiple pregnancies (Fauser *et al.*, 2005). Although two twin pregnancies occurred after transfer of one single embryo, the application of elective single-embryo transfer in those cases where more than one is available, could lead to a further reduction in multiple pregnancies after minimal stimulation IVF (Gerris, 2005).

It is unclear what the optimal number of cycles per patient would be. Overall, results were not significantly different according to cycle number. However, the occurence of a cancellation of oocyte retrieval, unsuccesful oocyte retrieval and fertilization failure all seem to be repeating phenomena in further cycles. Patient counselling on the number of cycles to be performed should therefore be individualized, taking into account the performance in the first cycle.

So far, no cost-effectiveness analyses concerning minimal stimulation IVF are available. Per cycle, minimal stimulation will be far cheaper than COS-IVF due to less medication use. On the other hand, more cycles of minimal stimulation compared with COS-IVF will be needed to obtain a comparable number of pregnancies per patient. Future research should clarify how costs per obtained pregnancy or live birth after minimal stimulation IVF compare to those after COS-IVF.

Minimal stimulation IVF offers a low-risk and patient-friendly protocol, being associated with a very low risk of OHSS and little hormonal medication use, short duration of a treatment cycle and easy oocyte retrieval. No resting cycle is necessary after a failed cycle, and treatments can be performed in consecutive cycles. Although effectiveness per started cycle is rather low, cumulative pregnancy rates after up to three cycles are reasonable and probably comparable with those after one treatment cycle of COS-IVF, which takes a comparable time span to be performed (Andersen *et al.*, 2005).

Based on the advantages of minimal stimulation IVF, it is our opinion that it is a feasible treatment for all patient categories studied. There are some groups of patients for whom minimal stimulation IVF forms a particularly valuable alternative to COS-IVF. These include patients with a history of OHSS, who will benefit from the lack of ovarian stimulation and patients who oppose to the generation of supernumery embryos, who will appreciate the fact that in most cases only one oocyte is obtained. Also in poor responders to COS-IVF, it seems logical to apply minimal stimulation IVF, as with COS, these patients will have only few oocytes and a low embryo transfer rate. With less time and costs, com-

parable results could be obtained with minimal stimulation. In one study comparing minimal stimulation IVF with COS-IVF in poor responders, similar pregnancy rates were found (Elizur *et al.*, 2005).

In conclusion, minimal stimulation seems suitable for all indications for conventional IVF. Due to considerable loss in every step of the procedure, the embryo transfer rate is low, but this is compensated by a favourable implantation rate. Pregnancy rates found in this study are encouraging. Because of the low-risk and patient-friendly nature of this protocol, it is our opinion that minimal stimulation is a feasible treatment option for patients requiring IVF.

Acknowledgement

Chapter 5

Cumulative pregnancy rates after a maximum of nine cycles of modified natural cycle IVF and analysis of patient drop-out: a cohort study

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ABSTRACT

BACKGROUND : In modified natural cycle IVF (MNC-IVF), treatment is aimed at using the one follicle that spontaneously develops to dominance, using a GnRH antagonist together with gonadotrophins in the late follicular phase only.

METHODS : In this single-centre cohort study, nine cycles of MNC-IVF were offered to 268 patients. Cumulative pregnancy rates (CPRs) were calculated and drop-out was analysed. The present study is an extension of earlier studies in which three cycles of MNC-IVF were offered to the same patients.

RESULTS : A total of 256 patients completed 1048 cycles (4.1 per patient). Embryo transfer rate was 36.5% per started cycle. Ongoing pregnancy rate was 7.9% per started cycle and 20.7% per embryo transfer. Including treatment-independent pregnancies, the observed CPR after up to nine cycles was 44.4% (95% CI 38.3-50.5) per patient. Pregnancy rates per started cycle did not decline in higher cycle numbers (overall 9.9%). Drop-out rates were high (overall 47.8%). We found that cancellation of oocyte retrieval, fertilization failure and failure to reach embryo transfer are repeating phenomena in subsequent cycles and furthermore that these events predispose for drop-out.

CONCLUSIONS: CPR after nine cycles of MNC-IVF in this study was 44.4%. Pregnancy rate per cycle did not decline in higher cycle numbers, possibly due to selective drop-out of poor prognosis patients. Due to the low-risk and patient-friendly nature of the MNC protocol, it seems a feasible treatment option for patients requiring IVF.

Introduction

In modified natural cycle IVF (MNC-IVF), the one follicle that spontaneously develops to dominance is used for IVF. A GnRH antagonist is administered in the late follicular phase of the natural cycle to prevent unwanted LH-surges and ovulations. Together with the GnRH antagonist, gonadotrophins are used to substitute for an expected fall in oestradiol (E_2) levels (Rongières-Bertrand et al., 1999).

Rather low pregnancy rates of 0.0-18.3% per started cycle have been found in several studies on MNC-IVF and ICSI (Rongières-Bertrand et al., 1999; Ubaldi et al., 2003; Vogel et al., 2003; Kolibianakis et al., 2004; Weghofer et al., 2004; Zhioua et al., 2004; Castelo Branco et al., 2005; Elizur et al., 2005; Pelinck et al., 2006).

However, MNC-IVF offers several advantages. It is a patient-friendly approach due to minimal use of medication, easy oocyte retrieval and negligible risk of ovarian hyperstimulation syndrome (OHSS). Treatment is easily repeated in consecutive cycles and no resting cycle is necessary in between. Since in most cases only one embryo is available for transfer, the multiple pregnancy rate is low, which is advantageous considering the many problems associated with multiple pregnancies (Fauser et al., 2005). For patients who, for ethical or religious reasons, are opposed to the generation of spare embryos, MNC-IVF forms an attractive alternative to IVF with controlled ovarian stimulation (COS), and legal problems associated with cryopreserved embryos are avoided.

Considering the low-risk and patient-friendly nature of the MNC protocol, it is our opinion that it forms a valuable treatment modality prior to, or as an alternative to, standard IVF with COS. Obviously, a higher number of MNCs will be required to obtain pregnancy rates comparable to those obtained with COS-IVF. In an earlier study, in which patients were interviewed who had undergone either natural cycle IVF or low stimulation IVF with clomiphene citrate, it was found that 66% of patients would opt to undergo six or more cycles before refraining from further treatment (Højgaard et al., 2001). In this study, patients who had undergone COS-IVF were also interviewed. Side-effects of hormone treatment as well as stress associated with cancellation of the cycle were perceived as severe or unacceptable by significantly more patients who had undergone COS-IVF as compared with those who had undergone natural cycle or low stimulation IVF (Højgaard et al., 2001).

Thus, it seems likely that patients are willing to accept the necessity of a higher number of MNC-IVF cycles in order to obtain acceptable pregnancy rates. Since one treatment cycle of MNC-IVF has a duration of just one menstrual cycle and treatment is easily repeated in consecutive cycles, pregnancy rates per time spent by the patient may be favourable.

So far, little is known about cumulative pregnancy rates (CPRs) after MNC-IVF. We previously found a cumulative ongoing pregnancy rate of 20.8% after three cycles of MNC-IVF (Pelinck et al., 2006).

The additional value of an increase in the number of MNC-IVF cycles to be offered to patients will depend mainly on the willingness of patients to undergo these cycles. In order to determine the optimal number of cycles per patient, it is also important to evaluate whether or not the pregnancy rate per cycle decreases in higher cycle numbers.

In the present cohort study, a maximum of nine cycles of MNC-IVF was offered to patients. We selected nine cycles in order to be able to detect a decline in pregnancy rate, if present, at higher cycle numbers. Available funding restricted the number of cycles and we arbitrarily chose nine as the upper limit. Drop-out rates after unsuccessful treatment cycles and pregnancy rates according to cycle number were calculated.

Materials and methods
Study protocol

The protocol for this study was reviewed and approved by the ethics committee of the University Medical Center Groningen, The Netherlands.

Inclusion criteria for this study were female patient age 18-36 years, first IVF treatment or first IVF treatment after a pregnancy (spontaneously conceived or obtained with COS-IVF), the presence of a regular and proven ovulatory menstrual cycle with a length of 26-35 days and body mass index (BMI: kg/m^2) of 18-28. Indications for IVF were tubal pathology, unexplained subfertility, male factor, endometriosis, cervical factor or failed donor inseminations. Patients were not included in the study when an endometriosis cyst was seen on ultrasound. Patients requiring ICSI were not included in this study. Patients with male factor or unexplained subfertility had undergone treatment with intrauterine insemination (IUI) for three to six cycles before starting IVF treatment, as is standard protocol in The Netherlands.

Patients were offered a maximum of nine treatment cycles. Treatments were performed in consecutive menstrual cycles, unless patients requested otherwise. Patients who decided not to participate in this study, underwent COS-IVF treatment according to standard protocol.

Enrolment of patients to the study took place from March 2001 to August 2004. Treatments were performed between March 2001 and September 2005. All treatments were offered free of charge and prior to starting standard COS-IVF. Until January 2004, medication was fully refunded by insurance companies. In January 2004, refunding policy changed and for some of the patients, medication was no longer refunded.

During the study period, patients who had conceived with MNC-IVF in the past and who returned for treatment after the pregnancy ended, were again offered MNC-IVF but treatments performed upon returning were not included in the cohort studied. Results of the treatments performed in patients after a previous MNC-IVF pregnancy were analysed separately.

End point in the study was pregnancy, defined as either the visualization of at least one intrauterine gestational sac or a proven ectopic pregnancy. Ongoing pregnancy was defined as the presence of an intrauterine gestational sac with fetal heart beat at 12 weeks gestational age. Live birth was defined as the birth of a living infant after pregnancy duration of at least 24 weeks.

The present study is an extension of earlier studies in which results of the first three cycles of the patients included in this study were described (Pelinck et al., 2005; 2006).

Cycle monitoring

Cycle monitoring was performed as described in detail previously (Pelinck *et al.*, 2005). In short, cycles were monitored with ultrasound and serum E_2 and LH measurements, and cetrorelix (GnRH antagonist, 0.25 mg / day: Cetrotide®, Serono, the Hague, the Netherlands) together with recombinant FSH (150 IU / day: Gonal-F®, Serono Benelux BV, the Netherlands) was started after follicular dominance had developed. Oocyte retrieval was carried out 34 h after ovulation triggering by 10 000 IU of HCG (Pregnyl®, Organon, Oss, the Netherlands) at a follicle size of at least 18 mm and/or plasma E_2 levels of ≥ 1.06 nmol/L.

In cycles where an LH-rise was noticed, planning of oocyte retrieval was cancelled according to previously described criteria (Pelinck *et al.*, 2006).

For oocyte retrieval, a single lumen aspiration needle was used and no flushing of the follicle was performed. In cases where at the time of planned oocyte retrieval, unexpected ovulation had occurred and tubes were patent, IUI was performed.

Conventional IVF was performed according to standard procedures. No ICSI was done, since patients included in the study had semen quality sufficient for conventional IVF and we expected no higher fertilization rate with ICSI.

Embryo transfer was performed on the third day after oocyte retrieval. For luteal support, HCG 1500 IU was given 5, 8 and 11 days after oocyte retrieval.

Data analysis

Results according to cycle number were calculated per started cycle, per planned, performed and successful oocyte retrieval and given as percentages. CPRs were calculated as observed CPR per patient, as well as using life table analysis. For life table analysis, two methods were used: the first being traditional life table analysis and the second a corrected estimation, taking into account the number of treatment-independent pregnancies (Kaplan EL, Meier P, 1958). Drop-outs, non-drop-outs and pregnant patients were analysed separately for patient and cycle characteristics. For comparisons, analysis of variance, chi square and 95% confidence intervals (CI) were used where applicable. A separate analysis was performed of results in subsequent cycles of patients who experienced a cancellation of oocyte retrieval, fertilization failure or failure to reach embryo transfer in their first cycle and compared with the results of subsequent cycles of patients where these events did not occur in the first cycle, using 95% CI. A $P < 0.05$ was considered statistically significant.

Results

Patient characteristics and results of treatment cycles

Patient characteristics are shown in table I. Out of 268 included patients, 27 had undergone COS-IVF leading to a pregnancy before entering the study.

Of 268 included patients, 12 withdrew from the study before starting treatment, 5 of these because of the occurrence of a spontaneous pregnancy (1 ectopic, 2 abortions and 2 ongoing). Of the remaining seven, three patients proceeded with COS-IVF and four refrained from all further treatment.

Results according to cycle number are shown in table II. Overall, 256 patients started 1048

Cumulative pregnancy rates after a maximum of nine cycles of modified natural cycle IVF

Table I. Patient characteristics

N° of patients	268
Female patient age (years)[a]	33.3 (23-36)
BMI (kg/m^2)[a]	23.0 (16-34)
Duration of subfertility (months)[a]	46.0 (0-121)
Subfertility (%)	
Primary	164 (61.2)
Secondary	104 (38.8)
Indication (%)	
Tubal	82 (30.6)
Unexplained	106 (39.6)
Male factor	41 (15.3)
Endometriosis	22 (8.2)
Cervical factor	8 (3.0)
Failed AID	9 (3.4)

[a]Values are median (range)

treatment cycles (4.1 per patient). Median duration of treatment was 19.8 weeks (range 3.7-107.7).

Ninety-four cycles (9.0%) were cancelled before planning of oocyte retrieval. Reasons for cancellation were LH rise or ovulation before or during cetrorelix administration (46 cycles), lack of follicular development or problems with monitoring due to difficult visual-

Table II. Results according to cycle number of modified natural cycle IVF

Cycle number	1	2	3	4
Cycles started	256	217	181	127
OR not planned (%/cycle)	23 (9.0)	21 (9.7)	21 (11.6)	12 (9.4)
Planned OR cancelled (%/planned OR)	23 (9.9)	18 (9.2)	13 (8.1)	17 (14.8)
OR performed (%/cycle)	210 (82.0)	178 (82.0)	147 (81.2)	98 (77.2)
OR successful (%/attempt)	152 (72.4)	134 (75.3)	111 (75.5)	70 (71.4)
Cycles with fertilization (%/ successful OR)	116 (76.3)	93 (69.4)	73 (65.8)	52 (74.3)
Embryo transfer (%/cycle)	99 (38.7)	76 (35.0)	60 (33.1)	43 (33.9)
Single ET	94	73	57	43
Double ET	5	3	3	-
Pregnancy rate (%/cycle)	27 (10.5)	20 (9.2)a	19 (10.5)	12 (9.4)ab
abortion	2	7	2	1
ectopic	-	-	1a	-
cervical	-	-	1	-
ongoing (%/cycle)	25 (9.8)	12 (5.5)	16 (8.8)	11 (8.7)ab
live birth (%/cycle)	24 (9.4)	12 (5.5)	15 (8.3)	11 (8.7)

OR: oocyte retrieval; ET: embryo transfer
[a]pregnancy after cancelled oocyte retrieval and IUI
[b]spontaneous conception during cycle that was cancelled because of LH surge

ization of the ovary (28 cycles), ovarian cysts not spontaneously disappearing (6 cycles), vaginal blood loss during the follicular phase (1 cycle), insufficient semen quality after a febrile episode (1 cycle) and illness or personal reasons (12 cycles).

A further 98 cycles (10.3% per planned oocyte retrieval) were cancelled at the time of planned oocyte retrieval, in one case because of inaccesibility of the ovary and in 97 cases because unexpected ovulation had occurred. Out of 856 oocyte retrievals, 625 were successful (73.0% per attempt). In most cases 1 or 2 oocytes were obtained (576 and 44 cycles, respectively). In five cycles, three or more oocytes were obtained (3, 3, 6, 9 and 20 oocytes, respectively).

In 453 cycles, fertilization occurred (72.5% per successful oocyte retrieval). Due to aberrant fertilization or defective embryo development no embryo transfer was done in 71 of these. In 382 cycles, embryo transfer was done (36.5% per started cycle; 61.1% per successful oocyte retrieval). In 20 cycles, 2 or more embryos were available for transfer and in all of these, double embryo transfer (DET) was done. In all other cycles, one single embryo was transferred (SET).

In 104 cycles, a pregnancy was obtained. One of these occurred spontaneously during a treatment cycle that was cancelled because of an LH surge, 6 occurred after IUI in cases where oocyte retrieval was cancelled because of unexpected ovulation and 97 pregnancies occurred after embryo transfer (91 after SET and 6 after DET). The pregnancy rate was 9.9% (95% CI: 8.1-11.8) per started cycle. Three out of 104 pregnancies were twins (2.9%), of which one occurred after transfer of one single embryo and two occurred after DET.

	6	7	8	9	Total
2	69	51	32	23	1048
(5.4)	8 (11.6)	4 (7.8)	-	-	94 (9.0)
)(11.5)	5 (8.2)	6 (12.8)	4 (12.5)	2 (8.7)	98 (10.3)
7 (83.7)	56 (81.2)	41 (80.4)	28 (87.5)	21 (91.3)	856 (81.7)
5 (72.7)	36 (64.3)	32 (78.0)	18 (64.3)	16 (76.2)	625 (73.0)
2 (75.0)	29 (80.6)	21 (65.6)	13 (72.2)	14 (87.5)	453 (72.5)
7 (40.2)	25 (36.2)	19 (37.7)	11 (34.4)	12 (52.2)	382 (36.5)
5	23	16	9	12	362
	2	3	2	-	20
l (12.0)[a]	5 (7.2)[a]	5 (9.8)[a]	3 (9.4)[a]	2 (8.7)	104 (9.9)
	-	2[a]	2	1	18
		-	1[a]	-	2
	-	-	-	-	1
)(10.9)[a]	5 (7.2)[a]	3 (5.9)	-	1 (4.3)	83 (7.9)
)(10.9)	5 (7.2)	3 (5.9)	-	1 (4.3)	81 (7.7)

Eighteen pregnancies, including one twin pregnancy, ended in spontaneous abortions, two were ectopic, one was a cervical pregnancy and 83 were ongoing at 12 weeks gestational age. Ongoing pregnancy rate was 7.9% (95% CI: 6.3-9.6) per started cycle. One pregnancy was interrupted because of severe congenital abnormalities (limb body wall complex). One pregnancy ended in fetal death at 17 weeks gestation. Live birth was thus 7.7% (95% CI: 6.1-9.4) per cycle.

OHSS did not occur after any of the cycles. Results according to cycle number were not significantly different (95% CI overlapping; not shown). Pregnancy rates per cycle according to subfertility diagnosis were not significantly different (data not shown). CPRs per patient were 43.0 (95% CI: 31.9-54.2), 41.2 (95% CI: 31.4-50.9), 38.5 (95% CI: 22.9-54.0), 35.0 (95% CI: 13.7-56.3), 42.9 (95% CI: 5.4-80.3) and 33.3 (95% CI: 1.9-64.8) for tubal factor, unexplained subfertility, male factor, endometriosis, cervical factor and failed donor inseminations, respectively.

During the study period, 27 patients returned for MNC-IVF after previous MNC-IVF had led to pregnancy (16 spontaneous abortions, 3 ectopic pregnancies and 8 ongoing pregnancies). These patients were again offered nine cycles of MNC-IVF and a further 105 cycles were performed (3.9 per patient). These 105 cycles led to 15 pregnancies, of which 1 miscarried, leading to a pregnancy rate per started cycle of 14.3% (95% CI: 7.5-21.1). All ongoing pregnancies ended in live births. Rate of ongoing pregnancy rate and live birth rate was 13.3% (95% CI: 6.7-20.0) per cycle.

Drop-out and CPRs
Drop-out rates and CPRs are specified in table III and figure 1.
Out of 268 included patients, 102 (38.1%) left the study before completing 9 cycles because a pregnancy was obtained. Fifteen (5.6%) left the study because of a treatment-independent pregnancy (2 abortions and 13 ongoing pregnancies). Of the remaining 151, 128 (84.8%) dropped out of the study after 0-8 unsuccessful cycles. Of these, 86 (67.2%) proceeded with COS-IVF treatment and 42 (32.8%) stopped treatment altogether. Reasons to stop treatment were related to marital or personal problems in seven cases, illness or operation needed in three cases, problem with sperm donor in one case, problem with semen quality in one case and a problem with the menstrual cycle in one case. One patient moved, and three patients planned to adopt a child. Five patients stated psychological stress or physical burden as the reason to stop further treatment. One patient stated financial problems as the reason to stop treatment. The remaining 19 patients did not state a specific reason for discontinuing treatment.
The drop-out rate (not including those who stopped treatment because of treatment-independent pregnancy) was low after the first and second cycle (3.5% and 6.5% respectively) and rose sharply thereafter to 13.0-25.5% in further cycles. CPR and ongoing pregnancy rate per patient starting treatment were 40.6% (95% CI: 34.5-46.8) and 32.4% (95% CI: 26.6-38.3). CPR and ongoing pregnancy rate per patient included in the study were 38.8% (95% CI: 32.9-44.8) and 31.0% (95% CI: 25.3-36.6) per patient (Figure 1). Including treatment-independent pregnancies, CPR and ongoing pregnancy rate per patient included in the study were 44.4% (95% CI: 35.2-53.6) and 35.8% (95% CI: 30.0-41.7) per patient (Figure 1).

Table III. Drop-out rates and cumulative pregnancy rates

Cycle number	patients	pregnancy	CPR[a]	TIP	CPR including TIP[b]	DO	CPR life table[c]	CPR life table[d]
0	268	-	-	5 (1.9)	5 (1.9)	7 (2.6)	-	1.9
1	256	27 (10.5)	27 (10.5)	3 (1.2)	35 (13.1)	9 (3.5)	10.5	13.4
2	217	20 (9.2)	47 (18.4)	2 (0.09)	57 (21.3)	14 (6.5)	18.8	22.1
3	181	19 (10.5)	66 (25.8)	1 (0.06)	77 (28.7)	34 (18.8)	27.3	30.8
4	127	12 (9.4)	78 (30.5)	2 (1.6)	91 (34.0)	21 (16.5)	34.2	38.4
5	92	11 (12.0)	89 (34.8)	-	102 (38.1)	12 (13.0)	42.1	45.8
6	69	5 (7.2)	94 (36.7)	1 (1.4)	108 (40.3)	12 (17.4)	46.3	50.5
7	51	5 (9.8)	99 (38.7)	1 (2.0)	114 (42.5)	13 (25.5)	51.5	56.3
8	32	3 (9.4)	102 (39.8)	-	117 (43.7)	6 (18.8)	56.1	60.4
9	23	2 (8.7)	104 (40.6)	-	119 (44.4)	na	59.9	63.8

Numbers in parentheses are percentages
CPR: cumulative pregnancy rate; TIP: treatment-independent pregnancy; DO: drop-out; na: not applicable
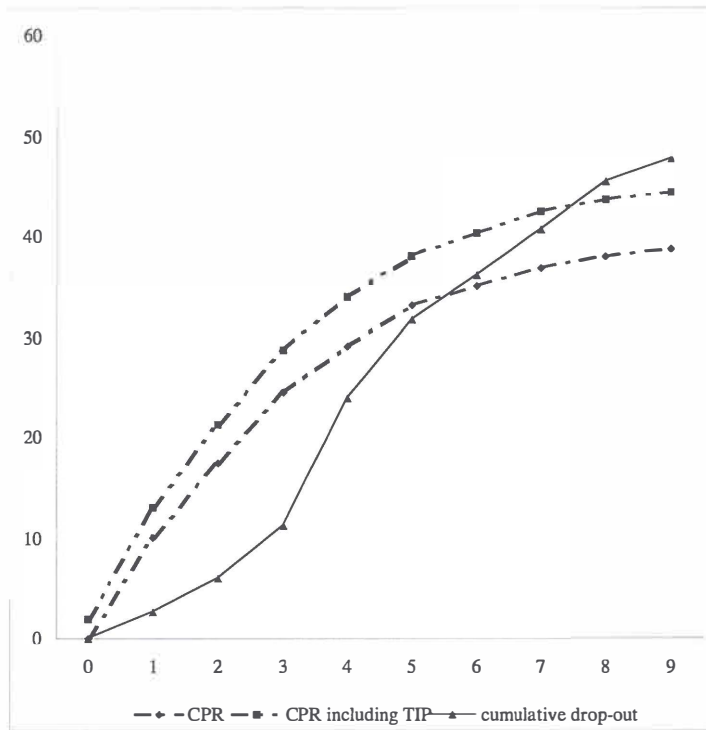[a]CPR calculated over patients starting treatment (n=256)
[b]CPR calculated over patients included in the study (n=268)
[c]life table analysis, treatment-independent pregnancies censored
[d]life table analysis, treatment-independent pregnancies not censored

Figure 1. Drop-out and cumulative pregnancy rates

CPR: cumulative pregnancy rate
TIP: treatment-independent pregnancy

CPRs were calculated with life table analysis according to two methods. In the first method, all patients who stopped treatment were censored, leading to a CPR of 59.9% (95% CI: 53.9-65.9). In the second method, patients who stopped treatment because of a spontaneous pregnancy were not censored and considered pregnant in the calculation. All other patients who stopped treatment were censored. CPR according to this method was 63.8% (95% CI: 57.9-69.7).

Analysis of drop-out
To analyse whether selective drop-out occurred, patients were divided into four groups (patients where a treatment-independent pregnancy occurred excluded): (i) patients dropping out after completing one to four unsuccessful cycles; (ii) patients dropping out after completing five to eight unsuccessful cycles; (iii) patients who completed nine unsuccessful cycles and (iv) patients whose treatment led to pregnancy (cycles in which the pregnancy occurred excluded).
Patient and cycle characteristics of these four groups are presented in table IV. Age, percentage of primary subfertility and duration of subfertility were not significantly different between groups. The number of oocyte retrievals performed per cycle, as well as fertilization rate and embryo transfer rate were significantly lower in group A compared to groups

Table IV. Patient and cycle characteristics of drop-outs, non-drop-outs and pregnant patients

Group[a]	A	B	C	D	P
N° of patients	78	43	21	77	
age (mean ± SD)	32.6 (3.2)	33.0 (2.6)	33.4 (2.3)	32.1 (3.0)	0.20[b]
subfertility primary (%)	50 (64.1)	27 (62.8)	15 (71.4)	47 (61.0)	0.85[c]
duration subfertility (mean ± SD)	51.6 (23.6)	46.8 (19.6)	45.8 (20.8)	43.7 (20.6)	0.16[b]
N° of cycles	223	271	189	230	
OR performed (%/cycle)	162 (72.6; 66.7-78.6)	211 (77.9; 72.8-82.9)	160 (84.7; 79.4-89.9)	199 (86.5; 82.0-91.0)	
OR successful (%/attempt)	123 (75.9; 69.2-82.6)	148 (70.1; 63.8-76.4)	112 (70.0; 62.8-77.2)	129 (64.8; 58.1-71.6)	
fertilization (%/successful OR)	64 (52.0; 43.0-61.0)	90 (60.8; 52.8-68.8)	90 (80.4; 72.8-87.9)	101 (78.3; 71.0-85.6)	
ET (%/cycle)	44 (19.7; 14.4-25.1)	72 (26.6; 21.2-31.9)	73 (38.6; 31.5-45.7)	89 (38.7; 32.3-45.1)	

[a]patients with treatment-independent pregnancies excluded from analysis
A: drop-out after 1-4 unsuccessful modified natural cycles
B: drop-out after 5-8 unsuccessful modified natural cycles
C: 9 unsuccessful modified natural cycles completed
D: pregnant (cycle in which pregnancy occurred not included)
[b]ANOVA
[c]Chi square
OR: oocyte retrieval; ET: embryo transfer

Table V. Results of subsequent cycles after cancellation of oocyte retrieval, fertilization failure or no embryo transfer in the first cycle

Results of first cycle	OR not performed	OR performed
N° of patients	46	210
Results of cycles 2-9		
N° of cycles	138	654
OR performed (%/cycle)	97 (70.3; 62.5-78.1)	549 (83.9; 81.1-86.8)
OR successful (%/attempt)	72 (74.2; 65.3-83.1)	401 (73.0; 69.3-76.8)
fertilization (%/successful OR)	45 (62.5; 51.1-73.9)	292 (72.8; 68.4-77.3)
ET (%/cycle)	35 (25.4; 18.0-32.8)	248 (37.9; 34.1-41.7)
pregnancy (%/cycle)	13 (9.4; 4.4-14.4)	64 (9.8; 7.5-12.1)

OR: oocyte retrieval ; ET: embryo transfer
Numbers in parentheses are percentages; 95% confidence interval

C and D. When comparing group B to groups C and D, the same trend was seen for number of oocyte retrievals and embryo transfer but differences were not significant. Fertilization rate was significantly lower in group B compared with groups C and D.

In order to analyse whether cancellation of oocyte retrieval, fertilization failure or failure to reach embryo transfer are repeating phenomena in further cycles, results of cycles 2-9 of patients where these events occurred were compared to those of patients where they did not. Results of this analysis are shown in table V. The number of performed oocyte retrievals as well as the embryo transfer rate was significantly lower in cycles 2-9 in the group where no oocyte retrieval was performed in the first cycle as compared with the group where oocyte retrieval was performed in the first cycle. Patients where fertilization failure occurred in the first cycle showed significantly lower fertilization rate and embryo transfer rate in cycles 2-9 as compared with those where fertilization did occur in the first cycle. In patients who failed to reach embryo transfer in the first cycle, fertilization rate and embryo transfer rate were significantly lower in subsequent cycles compared with patients where embryo transfer was done in the first cycle.

Discussion

The present study describes CPRs after nine cycles of MNC-IVF in a cohort of patients. The cohort described was formed by including consecutive patients, and the inclusion rate was almost 100%. Therefore, results of this study can be considered to be obtained in a representative sample of patients < 37 years of age with ovulatory cycles and indication for IVF.

In this study, we calculated actual observed CPRs as well as an estimation by life table analysis. The difference between the two estimates is rather large and increases in higher cycle numbers. The life table analysis represents an overestimation since it inherently assumes that the chance of pregnancy is the same in drop-outs and those who continue treatment while in our study drop-out was probably selective.

In patients dropping out of the study, age, duration of subfertility and percentage of primary subfertility was not different from those not dropping out. However, the number of cancelled cycles was higher and fertilization rate and embryo transfer rate were lower in the

fertilization failure	fertilization	no ET performed	ET performed
36	116	157	99
109	333	520	272
92 (84.4; 77.5-91.4)	281 (84.4; 80.4-88.4)	418 (80.4; 76.9-83.9)	228 (83.8; 79.4-88.3)
57 (72.8; 63.6-82.1)	218 (77.6; 72.6-82.6)	301 (72.0; 67.6-76.4)	172 (75.4; 69.7-81.1)
31 (46.3; 34.1-58.5)	178 (81.7; 76.4-86.9)	198 (65.8; 60.3-71.3)	139 (80.8; 74.8-86.8)
29 (26.6; 18.1-35.1)	149 (44.7; 39.3-50.2)	161 (31.0; 26.9-35.0)	122 (44.9; 38.8-50.9)
7 (6.4; 1.7-11.1)	33 (9.9; 6.6-13.2)	49 (9.4; 6.9-12.0)	28 (10.3; 6.6-14.0)

group of drop-outs, showing that cycle cancellation, fertilization failure and failure to reach embryo transfer predispose for drop-out of patients in subsequent cycles. Our analysis also shows that cancellation of the cycle, fertilization failure and failure to reach embryo transfer are repeating phenomena. Surprisingly, in patients with cycle cancellation or failure to reach embryo transfer in the first cycle, the higher rate of cycle cancellation and lower number of embryo transfers in subsequent cycles was not reflected in a lower pregnancy rate. Compared with patients where fertilization did occur in the first cycle, in patients with fertilization failure in the first cycle both fertilization rate and the number of embryo transfers were lower in subsequent cycles, and in this group of patients a lower (not statistically significant) pregnancy rate was found. It is likely that patients dropping out of the study would have had a reduced chance of pregnancy if they had continued treatment. The extent of overestimation of the CPR by the life table approach is of course unknown.

In studies on standard IVF with COS, the life table approach is often used for the analysis of CPRs. In COS-IVF, estimated success rates are also overrated due to selective drop-out of poor prognosis patients. Patients where oocyte yield is low or few embryos are available for transfer, as well as older patients, are often found to be more likely to withdraw from further treatment (Stolwijk et al., 2000; de Jong et al, 2002; Sharma et al., 2002), although in other studies no differences in prognostic factors between drop-outs and those continuing treatment were found (Roest et al., 1998; de Vries et al., 1999). Furthermore, patients considered to have a low chance of pregnancy are often advised to stop treatment (Land et al., 1997; Stolwijk et al., 2000; Olivius et al., 2002). In a recent publication, an overview is given of several studies using life table analysis to calculate estimated success rates after COS-IVF. Estimated success rates from these studies were compared with observed success rates, showing that the extent of overestimation increases with longer follow-up periods and lower observed success rates (Witsenburg et al., 2005). The observed CPR in our study represents an underestimation of the rate that could be reached in a cohort of patients, since the chance of pregnancy in patients dropping out of the study would not have been zero if they had continued treatment. A realistic estimate of the CPR, corrected for drop-out, will be somewhere between the observed CPR and the life table estimation.

 It is rather artificial to correct for drop-outs, since in analysis of IVF results, drop-outs are in most cases not lost to follow-up but rather patients deciding to stop treatment for various reasons. Therefore, drop-out is an inherent part of IVF performance. Corrected estimations can however be used in counselling patients when deciding whether or not to continue treatment.

In the present study, the cumulative drop-out rate was rather high, with 47.8% of the included patients leaving the study before completing nine cycles and without conceiving. Most of these proceeded with COS-IVF (67.2%). Patients refraining from further MNC-IVF may have done so for various reasons. In our study, treatments were offered for free and medication was refunded by insurance companies in the majority of treatment cycles and therefore patients' motivation to stop MNC-IVF will in most cases not have been financial in nature. Patients in our study were of course aware of the research setting in which their treatment took place and, being unsure of the chance of success, may have preferred standard COS-IVF. The low multiple pregnancy rate in MNC-IVF is advantageous, but in several studies, it was shown that by many patients, a multiple pregnancy is not considered an adverse outcome (Grobman *et al.,* 2001; Kalra *et al.,* 2003; Child *et al.,* 2004; Murray *et al.,* 2004). The very low risk of OHSS is an advantage of MNC-IVF but since OHSS is also rare in standard COS-IVF, patients may not perceive this as very important. Also, although the MNC protocol appears to be patient-friendly due to minimal use of hormonal medication and thus few side-effects, this may be perceived differently by patients. Frequent visits to the clinic and a high number of oocyte retrievals needed to obtain a pregnancy may be burdensome for patients, and disappointments due to cancellation of oocyte retrieval, unsuccessful oocyte retrieval, fertilization failure and failure to reach embryo transfer often occur.

In our study, no active censoring was done, as no patient was denied further treatment after unsuccessful cycles. However, since during the course of this study it became clear that cancellation of oocyte retrieval, fertilization failure and failure to reach embryo transfer are repeating phenomena in further cycles, patients were given information on this, which may have motivated some patients to discontinue MNC-IVF treatment.

The pregnancy rate per started cycle found in this study is low due to considerable loss in every step of the procedure. Further research into adjustments to the protocol, such as changes in timing of ovulation triggering or dose of cetrorelix, is needed to improve its effectiveness, as discussed previously (Pelinck *et al.,* 2006). The application of ICSI may improve fertilization rates and increase the number of embryo transfers. In our study, a rather large number of cycles were cancelled due to lack of follicular development or problems with visualization of the ovary, which could have been prevented by a stricter selection of patients.

Pregnancy rate per cycle and CPR per patient were not different according to indication for IVF. In our study, the proportion of patients with unexplained subfertility is rather high and seems to be higher than in the general population. This may be explained by the fact that only patients with regular and proven ovulatory cycles were included and patients with severe male factor infertility requiring ICSI were not.

In patients returning for treatment after previous successful MNC-IVF, the pregnancy rate was higher than in our study group (14.3% vs. 9.9% per started cycle, respec-

tively), illustrating that by selecting good prognosis patients results can be considerably improved. Further research is needed to optimize selection of patients who are likely to do well with MNC-IVF. In choosing between MNC-IVF and COS-IVF, benefits and drawbacks of both treatment modalities should be considered, as well as the expected pregnancy rates with either treatment. For instance, poor responders to COS are likely to benefit from MNC-IVF since with COS these patients will have only few oocytes and a low embryo transfer rate. With less time and costs, comparable results could be obtained with MNC-IVF (Tarlatzis *et al.*, 2003; Castelo Branco *et al.*, 2005; Elizur *et al.*, 2005; Ubaldi *et al.*, 2005). In specific situations, such as patients with a history of severe OHSS or those opposing to the creation of supernumery embryos, MNC-IVF may be preferred over COS-IVF. For normal responders to COS, comparative studies are warranted to evaluate the effectiveness of MNC-IVF relative to COS-IVF. Obviously, for these patients, a higher number of MNC-IVF cycles will be needed to obtain pregnancy rates comparable to those obtained after stimulated IVF. In this respect, the higher number of oocyte retrievals needed with MNC-IVF and associated risks of infection and bleeding should be taken into account. Any comparative study should not only evaluate effectiveness but also focus on cost-effectiveness and quality of life. Although the drop-out rates in standard COS-IVF also are high, the high drop-out rate in our study suggests that the physical and emotional burden for patients undergoing MNC-IVF is considerable.

MNC-IVF offers several advantages, being associated with a negligible risk of OHSS and a very low multiple pregnancy rate. A treatment cycle has a short duration and treatments are easily repeated in consecutive cycles. Per cycle, MNC-IVF is cheaper than COS-IVF due to minimal use of hormonal medication and in terms of costs per live birth, MNC-IVF may be cost-effective compared with COS-IVF (Groen *et al.*, 2005). In a recent study done in our centre, birth weights of singletons conceived by MNC-IVF and COS-IVF were compared. A significantly higher mean birth weight was found in the MNC-IVF group, whereas patient characteristics in both groups were similar (Keizer *et al.*, 2005). Although this should be confirmed in further studies, it is an important finding since higher birth weights probably reflect better health of newborns. Considering the advantages of MNC-IVF, it is our opinion that it is a valuable treatment option preceding standard IVF with COS, even if pregnancy rates per cycle are lower than those obtained with COS-IVF.

In recent papers, it is often proposed that the best outcome definition of success after IVF is the live birth rate per started treatment consisting of consecutive cycles (Fauser *et al.*, 2002; Vail and Gardener, 2003; Heijnen *et al.*, 2004). When MNC-IVF is applied before COS-IVF, the outcome parameter would be the cumulative live birth rate after sequential treatment with MNC-IVF, if necessary followed by COS-IVF.

Since the CPR after MNC-IVF found in our study is 40.6% and many of those not conceiving with MNC proceeded with COS-IVF, the overall CPR per patient probably will be favourable. In applying sequential treatment with MNC-IVF followed by COS-IVF, those patients conceiving with MNC-IVF will not be exposed to the risks and burden of COS-IVF. For now, implementation of MNC-IVF is hampered by existing reimbursement systems, which are usually based on a maximum number of cycles to be performed, leading to a need to maximize pregnancy rates per cycle rather than per treatment period.

The optimal number of treatment cycles per patient remains unclear. The pregnancy rate per cycle appears to remain constant throughout higher cycle numbers and the decline in steepness of the cumulative pregnancy curve is mainly caused by drop-out of patients during the study, suggesting that patients should be advised to undergo at least nine cycles of MNC-IVF before starting COS-IVF. However, the apparent absence of a decline in pregnancy rates in higher cycle numbers may be caused by selective drop-out of patients with a possible poor prognosis, so nine cycles may not be not be the suitable number for all patients. Since the occurrence of a cancellation of oocyte retrieval, fertilization failure and failure to reach embryo transfer all seem to be repeating phenomena in further cycles, patient counselling on the number of cycles to be performed should be individualized, taking into account the results of previous cycles.

In conclusion, a CPR of 40.6% was found after nine cycles of MNC-IVF. Including treatment-independent pregnancies, the CPR was 44.4%. Drop-out rates were high, especially in higher cycle numbers. The pregnancy rate per started cycle does not appear to decline in higher cycle numbers, possibly caused by selective drop-out of poor prognosis patients. Considering the advantages of MNC-IVF, the very low multiple pregnancy rate and negligible risk of OHSS in particular, it is our opinion that MNC-IVF offers a valuable treatment modality for patients requiring IVF.

Chapter 6

Cumulative pregnancy rates after sequential treatment with modified natural cycle IVF followed by IVF with controlled ovarian stimulation.

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ABSTRACT

BACKGROUND : In modified natural cycle IVF (MNC-IVF), treatment is aimed at using the one follicle that spontaneously develops to dominance, using a GnRH antagonist together with gonadotrophins in the late follicular phase only. The MNC-IVF is of interest because of its low-risk and patient-friendly profile. The effect of application of MNC-IVF preceding standard IVF with ovarian stimulation on overall results is unknown.

METHODS : This single-centre cohort study provides follow-up of an earlier study in which nine cycles of MNC-IVF were offered to 268 patients. Ongoing pregnancy rates and live birth rates, as well as time-to-pregnancy after controlled ovarian stimulation-IVF (COS-IVF) following MNC-IVF, were evaluated.

RESULTS : Actual observed cumulative ongoing pregnancy rates and live birth rates after sequential treatment with MNC-IVF followed by COS-IVF were 51.5 (95% CI: 45.4-57.6) and 50.0% (95% CI: 43.9-56.1) per patient, of which 8.0 and 6.7% were twins. Median time to ongoing pregnancy was 28.8 weeks. Including treatment-independent pregnancies, cumulative ongoing pregnancy rate was 56.7% (95% CI: 50.7-62.8).

CONCLUSIONS: Sequential treatment with MNC-IVF followed by COS-IVF does not appear to compromise overall success rates, while twin pregnancy rate is low. Because of its patient-friendly and low-risk profile, it seems appropriate to perform MNC-IVF preceding COS-IVF.

Introduction

Modified natural cycle IVF (MNC-IVF), in which treatment is aimed at the use of the one follicle that spontaneously develops in the natural menstrual cycle, is proposed as an alternative to standard IVF with controlled ovarian stimulation (COS-IVF), either for all patients with ovulatory cycles requiring IVF or ICSI (Rongières-Bertrand *et al.*, 1999; Vogel *et al.*, 2003; Zhioua *et al.*, 2004; Pelinck *et al.*, 2006) or for specific indications such as poor response to ovarian stimulation or advanced female age (Ubaldi *et al.*, 2003; Kadoch *et al.*, 2003; Weghofer *et al.*, 2004; Elizur *et al.*, 2005). A GnRH-antagonist is used in the late follicular phase of the unstimulated cycle, to prevent untimely LH-surges and ovulations and subsequent cancellation of the cycle. Together with the GnRH-antagonist, gonadotrophins are administered to substitute for the expected fall in FSH levels and as a consequence, oestradiol (E_2) levels (Rongières-Bertrand *et al.*, 1999). A recent paper addressed the terminology for this approach (Nargund *et al.*, 2007).

Compared to COS-IVF, the efficacy of MNC-IVF seems rather low with pregnancy rates per started cycle of 0.0-18.3% (Rongières-Bertrand *et al.*, 1999; Ubaldi *et al.*, 2003; Kadoch *et al.*, 2003; Vogel *et al.*, 2003; Kolibianakis *et al.*, 2004; Weghofer *et al.*, 2004; Zhioua *et al.*, 2004; Elizur *et al.*, 2005; Pelinck *et al.*, 2006; Pelinck *et al.*, 2007).

However, MNC-IVF has several advantages. It is a patient-friendly treatment, since medication is administered in a low dose and for a few days only, causing few side effects. Oocyte retrieval is easy and short lasting and can be performed without analgesia since usually only one follicle is aspirated (Ramsewak *et al.*, 1990). Moreover, it is a treatment with a low-risk profile due to the negligible risk of ovarian hyperstimulation syndrome (OHSS) and inherent low multiple pregnancy rate. We found higher birth weights in singletons conceived by MNC-IVF compared to COS-IVF (Keizer *et al.*, 2005). Although this should be confirmed in further studies, it is an important finding since higher birth weights probably reflect better health of newborns. Compared to COS-IVF, MNC-IVF may be cost-effective (Groen *et al.*, 2005). The duration of a treatment cycle is short and treatments are easily repeated in consecutive cycles and therefore, pregnancy rates per time spent by the patient may be acceptable (Pelinck *et al.*, 2007).
Given the advantages of MNC-IVF, it seems a feasible treatment option for patients requiring IVF.

Recently, it has been suggested that the best outcome parameter of IVF treatment is the ongoing pregnancy rate or live birth per patient starting treatment, or live birth after a given time period (Fauser *et al.*, 2002; Vail and Gardener, 2003; Heijnen *et al.*, 2004; Heijnen *et al.*, 2007).

So far, little is known about cumulative pregnancy rates after MNC-IVF. We found cumulative ongoing pregnancy rates of 20.8% and 32.4% after a maximum of three and nine consecutive cycles, respectively, with ongoing twin pregnancy rates of 4.3% and 2.4% (Pelinck *et al.*, 2006; Pelinck *et al.*, 2007). In our center, MNC-IVF is performed preceding COS-IVF, so full treatment consists of MNC-IVF followed by COS-IVF. No studies describing sequential treatment consisting of MNC-IVF followed by COS-IVF are available.
In the present study, results of MNC-IVF followed by COS-IVF are described in a cohort

of patients. Cumulative pregnancy rates per patient were calculated, as well as time to pregnancy or end of treatment. Fertilization rates were compared between MNC-IVF and COS-IVF.

Materials and methods
Patients
For the present study, a cohort of 268 patients who were offered nine cycles of MNC-IVF was evaluated and followed up concerning results of full treatment, consisting of MNC-IVF, followed by COS-IVF if necessary.

Inclusion criteria for this study were: female patient age 18-36 years, first IVF treatment ever or first IVF treatment after a pregnancy (spontaneously conceived or obtained with COS-IVF), the presence of a regular and proven ovulatory menstrual cycle with a length of 26-35 days and body mass index (BMI: kg/m^2) of 18-28. Indications for IVF were tubal pathology, unexplained subfertility, male factor, endometriosis, cervical factor or failed donor inseminations. Patients were not included in the study in case an endometriosis cyst was seen on ultrasound. Patients requiring ICSI because of insufficient semen quality were not included in this study. Patients with male factor or unexplained subfertility had undergone treatment with intra-uterine insemination (IUI) for three to six cycles before starting IVF treatment, as is standard protocol in the Netherlands.

The results of MNC-IVF for these patients have been described in detail recently (Pelinck et al., 2007). All patients who conceived with MNC-IVF who miscarried or experienced an ectopic pregnancy were offered again nine cycles of MNC-IVF. For the present study, results of MNC-IVF treatment following abortion or ectopic pregnancy were added to the previously described data, resulting in some patients undergoing more than nine cycles (Pelinck et al., 2007).

Patients who did not conceive with MNC-IVF after nine cycles or who refrained from further MNC-IVF treatment after 0-8 cycles, were offered COS-IVF.

All patients were followed up completely concerning treatments performed in our centre.

End point in the study was ongoing pregnancy, defined as the presence of an intrauterine gestational sac with fetal heart beat at 12 weeks gestational age. Pregnancy was defined as either the visualization of at least one intrauterine gestational sac or a proven ectopic pregnancy. Live birth was defined as the birth of a living infant after pregnancy duration of at least 24 weeks.

MNC-IVF treatment was offered for free. MNC- and COS-IVF treatments were performed between February 2001 and October 2006. Until January 2004, COS-IVF was fully refunded by the insurance companies for a maximum of three cycles. Owing to a change in refunding policy, from 1st January 2004 onwards, the first COS-IVF treatment cycle was not refunded anymore for part of the patients. Also, medication needed for MNC-IVF and the first COS-IVF cycle was no longer refunded for part of the patients. Second and third COS-IVF cycles, including medication, were fully refunded during the entire study period. Fourth and further COS-IVF cycles were never refunded.

The protocol for the study concerning nine cycles of MNC-IVF was reviewed and approved by the ethical committee of the University Medical Center Groningen, the Netherlands. The part of the study concerning COS-IVF treatment did not require approval

of the ethical committee because it concerned standard treatment, data of which were recorded anonymously.

Modified natural cycle

MNC-IVF was performed as described in detail previously (Pelinck *et al.*, 2006). In short, unstimulated cycles were monitored by ultrasound. Medication was started after follicular dominance had spontaneously developed. For the prevention of LH rises, the GnRH-antagonist cetrorelix (Cetrotide®, Serono Benelux BV, the Netherlands) was used in a daily dose of 0.25mg, starting at a follicle size of 14mm and given up to and including the day of ovulation triggering. For substitution, recombinant FSH (r-FSH: Gonal-F®, Serono Benelux BV, the Netherlands) was given concomitantly in a daily dose of 150 IU, up to the day of ovulation triggering. Ovulation was triggered at a follicle size of 18 mm (10 000 IU of HCG: Pregnyl®, Organon, the Netherlands) and oocyte retrieval was performed 34 hours later. In cycles where a serum LH rise was noticed, planning of oocyte retrieval was cancelled according to the previously described criteria (Pelinck *et al.*, 2006). In cases where at the time of planned oocyte retrieval, unexpected ovulation had occurred and tubes were patent, IUI was performed. Conventional IVF was performed according to standard procedures. Embryo transfer (ET) was performed on the third day after oocyte retrieval. Luteal support consisted of HCG 1500 IU at 5, 8 and 11 days after oocyte retrieval.

COS-IVF

COS-IVF was performed using a flare-up or down-regulation protocol, using triptorelin (Decapeptyl®, Ferring Nederland BV, Hoofddorp, the Netherlands) or leuproreline (Lucrin®, Abbott BV, Hoofddorp, the Netherlands) and r-FSH (Puregon®, Organon, Oss, the Netherlands). Cycles were monitored with ultrasounds and serum E_2 levels. When at least half of the dominant follicles were >18mm, ovulation was triggered by 10 000 IU of HCG (Pregnyl®, Organon, Oss, the Netherlands) and oocyte retrieval performed 36 h later under conscious sedation with fentanyl intravenously. Oocyte retrieval was cancelled in the cases of poor response (< 3 large follicles) or of threatening OHSS (E_2 levels >15 nmol/l and/or >20 follicles). Conventional IVF was performed according to standard procedures. In one case where in MNC-IVF, fertilization failure had occurred repeatedly, ICSI was performed. In second and third cycles of COS, ICSI was performed if fertilization failure (< 10% of all oocytes fertilized) had occurred in a previous cycle of COS. ET was performed on the second, third or fourth day after oocyte retrieval. Luteal support consisted of progesterone vaginal suppositories 3x100mg/day starting on the day of oocyte retrieval until pregnancy test, or HCG 1500 IU at 5, 7 and 9 days after oocyte retrieval.

Data analysis

Cumulative ongoing pregnancy rate and live birth rate after full treatment, consisting of MNC-IVF followed by COS-IVF, were calculated per patient. Time-to-pregnancy or end of treatment was calculated per patient. End-point in the study was an ongoing pregnancy. For patients who conceived with MNC-IVF or COS-IVF and who miscarried or experienced an ectopic pregnancy, subsequent cycles were included in the analysis for the same patient.

Cumulative pregnancy rates after sequential treatment with modified natural cycle IVF

Table I. Patient characteristics

	at inclusion	at start COS-IVF
N° of patients	268	109
Female patient age (years)[a]	33.3 (23-36)	34.2 (23-38)
BMI (kg/m^2)[a]	23.0 (16-34)	23.0 (18-31)
Duration of subfertility (months)[a]	46.0 (0-121)	55.8 (7-113)
Subfertility (%)		
Primary	164 (61.2)	69 (63.3)
Secondary	104 (38.8)	40 (36.7)
Indication (%)		
Tubal	82 (30.6)	28 (25.7)
Unexplained	106 (39.6)	44 (40.4)
Male factor	41 (15.3)	19 (17.4)
Endometriosis	22 (8.2)	12 (11.0)
Cervical factor	8 (3.0)	3 (2.8)
Failed AID	9 (3.4)	3 (2.8)

[a]Values are median (range)

Table II. Results according to cycle number of modified natural cycle IVF

Cycle number	0	1	2	3	4	5
cycles started	-	256	219	191	139	102
OR (%/cycle)	-	211 (82.4)	181 (82.6)	157 (82.2)	109 (78.4)	83 (81.4
OR successful (%/attempt)	-	153 (72.5)	136 (75.1)	120 (76.4)	77 (70.6)	60 (72.3
ET (%/cycle)	-	99 (38.7)	76 (34.7)	64 (33.5)	50 (36.0)	41 (40.2
single ET	-	94	73	61	49	38
double ET	-	5	3	3	1	3
pregnancy rate (%/cycle)	-	27 (10.5)	20 (9.1)[a]	20 (10.5)	13 (9.4)[ab]	11 (10.8
abortion/ectopic	-	2	8a	3	1	1
ongoing	-	25 (9.7)	12 (5.5)	17 (8.9)	12 (8.6)[ab]	10 (9.8)
COPR (%/patient)	-	25 (9.3)	37 (13.8)	54 (20.1)	66 (24.6)	76 (28.4
tip (%/patient)	5	3	2	1	2	1
abortion/ectopic	3	1	-	-	-	1
ongoing (%/patient)	2	2	2	1	2	-
COPR tip included	2 (0.7)	29 (10.8)	43 (16.0)	61 (22.8)	75 (28.0)	85 (31.7
continued COS (%/patient)	3	4	9	24	18	7
stop treatment (%/patient)	7	6	5	10	5	7

[a] one pregnancy after cancelled oocyte retrieval and IUI
[b] one spontaneous conception during cycle that was cancelled because of LH surge
OR: oocyte retrieval; ET: embryo transfer; COPR: cumulative ongoing pregnancy rate; tip: treatment-independent pregnancy

Fertilization rates in MNC-IVF and COS-IVF were compared and given in percentages with 95% CI.

Results

Modified natural cycle

Patient characteristics are shown in table I. Out of 268 included patients, 27 had undergone COS-IVF leading to a pregnancy before entering the study. Twelve patients refrained from MNC-IVF after inclusion in the study, in two cases because a spontaneous (ongoing) pregnancy had occurred. Three patients started COS-IVF instead of MNC-IVF and seven patients refrained from treatment altogether.

Results of MNC-IVF are shown in table II. Overall, a total of 1123 cycles were performed, leading to 112 pregnancies, of which 90 were ongoing beyond twelve weeks gestational age. Four out of 112 pregnancies were twins (three after double ET (DET) and one after single ET (SET)), of which one miscarried. One pregnancy was interrupted because of severe congenital abnormalities (limb body wall complex) and one pregnancy ended with fetal death at 17 weeks gestation. Eighty-eight pregnancies ended with live birth, of which nine were preterm (< 37 weeks gestational age). Of three ongoing twin pregnancies, two ended preterm. Live birth rate and term live birth rate were thus 34.4% and 30.9%, respectively, per patient starting MNC-IVF.

	7	8	9	10	11	12	Total
8	57	38	31	5	5	2	1123
0 (76.9)	44 (77.2)	31 (81.6)	27 (87.1)	2 (40.0)	4 (80.0)	2 (100.0)	911 (81.1)
9 (65.0)	35 (79.5)	21 (67.7)	20 (74.1)	1 (50.0)	4 (100.0)	1 (50.0)	667 (73.2)
0 (38.5)	24 (42.1)	15 (39.5)	15 (48.4)	-	1 (20.0)	1 (50.0)	416 (37.0)
8	21	13	14	-	1	1	393
	3	2	1	-	-	-	23
(7.7)[a]	7 (12.3)[a]	4 (10.5)[a]	3 (9.7)	-	-	1 (50.0)	112 (10.0)
	2[a]	3[a]	1	-	-	-	22
(6.4)[a]	5 (8.8)	1 (2.6)	2 (6.5)	-	-	1 (50.0)	90 (8.0)
1 (30.2)	86 (32.1)	87 (32.5)	89 (33.2)	89 (33.2)	89 (33.2)	90 (33.6)	90 (33.6)
	1	-	-	-	-	1	17 (6.3)
	1	-	-	-	-	1	7
	-	-	-	-	-	-	10 (3.7)
1 (34.0)	96 (35.8)	97 (36.2)	99 (36.9)	99 (36.9)	99 (36.9)	100 (37.3)	100 (37.3)
)	10	3	19	-	2	-	109 (40.7)
	4	3	5	-	1	1	59 (22.0)

Eight patients conceived twice with MNC-IVF. Out of 21 patients whose first pregnancy was not ongoing, 19 patients continued with MNC-IVF (75 cycles, leading to eight pregnancies, of which seven ongoing). The remaining two patients refrained from further treatment. One of these subsequently conceived spontaneously and miscarried.

A total of 17 spontaneous conceptions occurred. One patient proceeded with COS-IVF after spontaneous abortion of spontaneous conception.

Pregnancy rates per patient were not different according to indication for IVF (Chi square: $P = 0.58$; data not shown).

In summary, for MNC-IVF, cumulative ongoing pregnancy rate, live birth rate and term live birth rate were 35.2 (95% CI: 29.2-41.1), 34.4 (95% CI: 28.4-40.3) and 30.9% (95% CI: 25.1-36.6), respectively, per patient starting treatment, of which 3.3, 3.4 and 1.3% twins. Including treatment-independent pregnancies, cumulative ongoing pregnancy rate was 38.3% (95% CI: 32.2-44.4) per patient starting treatment.

Per patient included in the study, cumulative ongoing pregnancy rate, live birth rate and term live birth rate after treatment were 33.6 (95% CI: 27.8-39.4), 32.8 (95% CI: 27.1-38.6) and 29.5% (95% CI: 23.9-35.0), respectively. Including treatment-independent pregnancies, cumulative ongoing pregnancy rate was 37.3% (95% CI: 31.4-43.2) per patient included in the study.

Table III. Results according to cycle number of COS-IVF

Cycle number	1	2	3
cycles started	109	81	53
oocyte retrieval (%/cycle)	91 (83.5)	70 (86.4)	52 (98.1)
ivf	90	57	32
icsi	1	13	20
fresh ET (%/cycle)	66 (60.6)	58 (71.6)	46 (86.8)
single ET	7	8	8
double ET	59	50	38
ET cryopreserved embryos (%/cycle)	3 (2.8)	2 (2.5)	3 (5.7)
pregnancy rate (%/cycle)	18 (16.5)[b]	17 (21.0)	10 (18.9)
abortion/ectopic	2[c]	3	1
ongoing (%/cycle)	16[d] (14.7)	14 (17.3)	9 (17.0)
COPR (%/patient)	16 (14.7)	30 (27.5)	39 (35.8)
tip (%/patient)	4	1	2
abortion/ectopic	1	-	2
ongoing (%/patient)	3	1	-
COPR tip included (%/patient)	19 (17.4)	34 (31.2)	43 (39.4)

[a]cancellation of oocyte retrieval: threatening OHSS (n=11), poor response (n=12), vaginal spotting (n=1), personal reasons (n=6)
[b]one pregnancy after transfer of cryopreserved embryos
[c]one pregnancy interrupted because of trisomy 21
[d]one twin pregnancy selectively reduced to singleton because of trisomy 21
ET: embryo transfer ; COPR : cumulative ongoing pregnancy rate ; tip : treatment-independent pregnancy

COS-IVF

Out of 268 included patients, 109 proceeded with COS-IVF, results of which are shown in table III.

Eleven patients developed OHSS, of which three were hospitalized for treatment.

Fifty-four pregnancies were obtained, of which 48 were ongoing beyond 12 weeks gestational age. Of these, five were vanishing twin pregnancies, one twin pregnancy was selectively reduced to singleton because of trisomy 21, seven were ongoing twin pregnancies and 35 were ongoing singleton pregnancies.

One twin pregnancy ended with immature birth at 23 weeks gestational age and one singleton pregnancy ended with fetal death at 16 weeks gestational age. Forty-six pregnancies ended with live birth, of which nine were preterm. Of six twin pregnancies ending with live birth, four were delivered preterm. Live birth rate and term live birth rate were thus 42.2% and 33.9%, respectively, per patient starting COS.

Two patients conceived twice with COS. Out of six patients, whose first COS-pregnancy was not ongoing, three continued COS-IVF treatment (four cycles, leading to one singleton ongoing pregnancy and one vanishing twin). One patient, whose COS-pregnancy miscarried, subsequently conceived naturally.

A total of seven spontaneous conceptions occurred after COS-IVF (one abortion, two

4	5	6	7	8	Total
18	7	3	1	1	273
18 (100)	7 (100)	3 (100)	1 (100)	1 (100)	243 (89.0)[a]
8	1	-	-	-	188
10	6	3	1	1	55
16 (88.9)	7 (100)	3 (100)	1 (100)	1 (100)	198 (72.5)
2	-	-	-	-	25
14	7	3	1	1	173
2 (11.1)	-	-	-	-	10 (3.7)
6 (33.3)	1 (14.3)	2 (66.7)	-	-	54 (19.8)
-	-	-	-	-	6
6 (33.3)	1 (14.3)	2 (66.7)	-	-	48 (17.6)
45 (41.3)	46 (42.2)	48 (44.0)	48 (44.0)	48 (44.0)	48 (44.0)
-	-	-	-	-	7 (6.4)
-	-	-	-	-	3
-	-	-	-	-	4 (3.7)
49 (45.0)	50 (45.9)	52 (47.7)	52 (47.7)	52 (47.7)	52 (47.7)

ectopic and four ongoing). One patient continued with COS-IVF after spontaneous conception of an ectopic pregnancy.

Pregnancy rates per patient were not different according to indication for IVF (Chi square: $P = 0.75$; data not shown).

In summary, for COS-IVF, cumulative ongoing pregnancy rate, live birth rate and term live birth rate were 44.0 (95% CI: 34.5-53.5), 42.2 (95% CI: 32.7-51.7) and 33.9% (95% CI: 24.9-43.0), respectively, per patient starting treatment, of which 14.6, 13.0 and 5.4% were twins. Including treatment-independent pregnancies, cumulative ongoing pregnancy rate was 47.7% (95% CI: 38.1-57.3) per patient.

Results of sequential treatment of MNC-IVF followed by COS-IVF

Of all patients included in the study, cumulative ongoing pregnancy rate, live birth rate and term live birth rate were 51.5 (95% CI: 45.4-57.6), 50.0 (95% CI: 43.9-56.1) and 43.3% (95% CI: 37.2-49.3), respectively, per patient after MNC-IVF followed by COS-IVF. Of these, 8.0, 6.7 and 2.6% were twins. Including treatment-independent pregnancies occurring before and after treatment, cumulative ongoing pregnancy rate was 56.7% (95% CI: 50.7-62.8), of which 6.6% were twins.
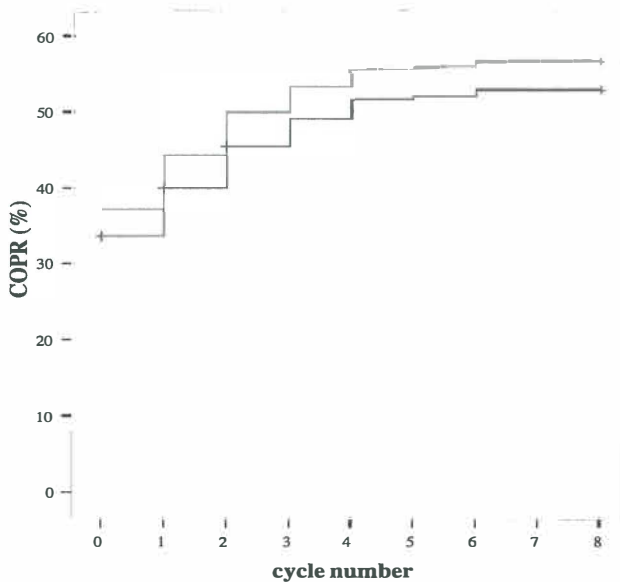
Actual observed cumulative ongoing pregnancy rates according to cycle number of MNC-IVF and COS-IVF are shown in Fig 1a and b.

Figure 1a. COPR according to cycle number of MNC-IVF
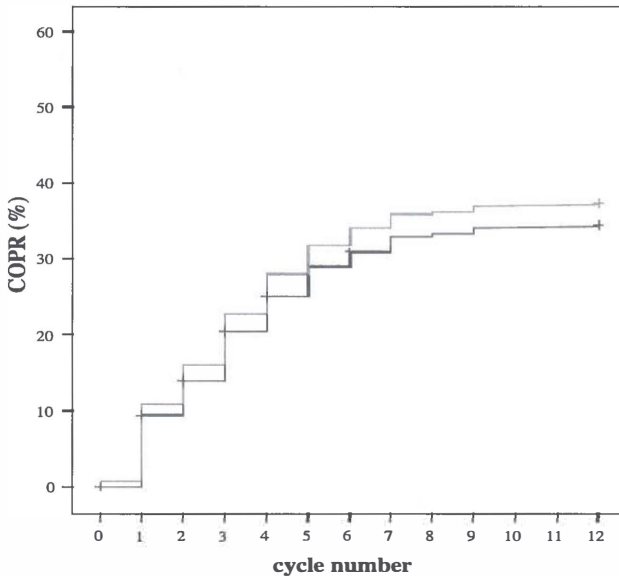
lower line: COPR after MNC treatment

upper line: COPR, treatment independent pregnancies included

Figure 1b. COPR according to cycle number of COS-IVF


lower line: COPR after COS treatment

upper line: COPR, treatment independent pregnancies included

Actual observed cumulative ongoing pregnancy rates according to treatment time period are shown in Fig 2. Cumulative ongoing pregnancy rate was 38.8% (95% CI: 32.9-44.8) and 51.5% (95% CI: 45.4-57.6) after one and two years of treatment, respectively. Fourteen ongoing pregnancies occurred after this period (one after MNC-IVF, ten after COS-IVF and three spontaneous conceptions). The median time of treatment was 34.1 (0-220) weeks for all patients included in the study. The median time to ongoing pregnancy was 28.8 (0-220) weeks.

Fertilization rates
Of patients who proceeded with COS-IVF after unsuccessful MNC-IVF, fertilization rates after MNC and after COS with conventional IVF were compared. Of 109 patients, who underwent COS-IVF, in 32 (29.4%) ICSI was performed, in one case in the first COS-cycle, and in 31 cases after fertilization failure (< 10% of all oocytes fertilized) in a COS-cycle. In the first cycle with successful oocyte retrieval in patients undergoing COS with conventional IVF, fertilization failure occurred in 25.3% (95% CI: 16.0-34.6). In those patients where none of the oocytes had fertilized in MNC, fertilization failure occurred in 48.0% (95% CI: 28.0-68.0) of first COS-cycles. In those patients where all oocytes had fertilized in MNC, fertilization failure occurred in 9.1% (95% CI: 0.92-19.1) of first COS-cycles.

Figure 2. Cumulative ongoing pregnancy rate (%) according to treatment time period (weeks)
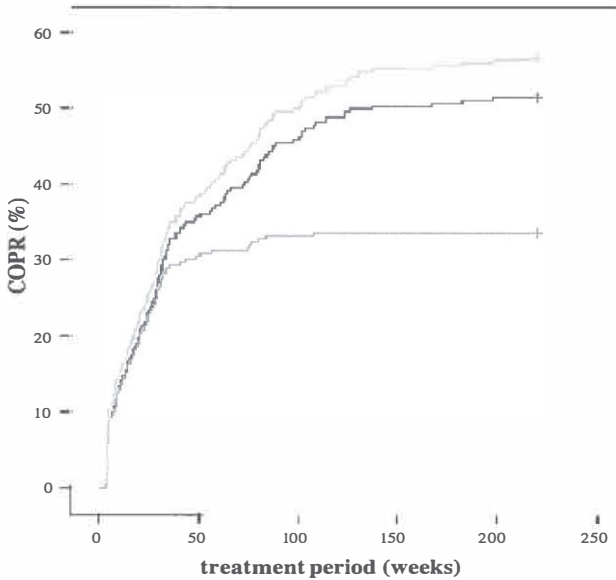
lower line: MNC-IVF only

middle line: MNC-IVF and COS-IVF

upper line: MNC-IVF and COS-IVF, treatment independent pregnancies included

Discussion

In the present study, results of sequential treatment with MNC-IVF followed by COS-IVF were studied and, including treatment-independent pregnancies, a cumulative ongoing pregnancy rate of 56.7%, of which 6.6% twins, was found.

Cumulative ongoing pregnancy rates were 38.8% and 51.5%, respectively, after one and two years of treatment. The number of ongoing pregnancies obtained within one year seems to be somewhat lower than those found in a study comparing minimal stimulation (gonadotrophins started on day 5 of the cycle and GnRH-antagonist used for LH-suppression) to standard stimulated IVF, in which 46.8% ongoing pregnancies were found after minimal stimulation IVF with SET and 51.8% after standard stimulated IVF with DET (Heijnen *et al.*, 2007). In our study, treatment time is somewhat lengthened because of the inclusion of patients restarting treatment after an abortion or ectopic pregnancy, and also due to some waiting time before starting COS-IVF, caused by limitations in laboratory capacity.

We found a live birth rate after sequential treatment with MNC-IVF and COS-IVF of 50.0%. This seems to be in accordance with reported cumulative live birth rates of 35.7-59.1% found after full treatment with COS-IVF only (Goverde *et al.*, 2000; Witsenburg *et al.*, 2005; Elizur *et al.*, 2006). However, the multiple pregnancy rate in our study (6.7%) is substantially lower than the reported multiple pregnancy rate of 21.2-25.7% in these studies (Goverde *et al.*, 2000; Witsenburg *et al.*, 2005). The live birth rate after full treatment may be slightly underestimated as we did not register if patients underwent treatment elsewhere after leaving our centre.

In our opinion therefore, the application of sequential treatment with MNC-IVF followed by COS-IVF if necessary is a realistic strategy. This way, not only a large proportion of patients is spared the risk of OHSS and the discomfort of COS, but also the overall multiple pregnancy rate is substantially reduced.

On the other hand, in COS-IVF, OHSS does not occur frequently and multiple pregnancy rates can be further reduced by applying SET more frequently (Tiitinen *et al.*, 2003; Gordts *et al.*, 2005; De Neubourg and Gerris, 2006). MNC-IVF is a patient-friendly treatment but also has several drawbacks. Compared to COS-IVF, a higher number of MNC-cycles are needed to obtain comparable pregnancy rates. Although the possibility of a quicker succession of treatment cycles is beneficial in MNC-IVF, repeated visits to the clinic and frequent disappointments due to cancellation of oocyte retrieval, unsuccessful oocyte retrieval, fertilization failure and failure to reach ET are burdensome for patients. Unfortunately, formal studies on quality of life in MNC-IVF are lacking, but studies on natural cycle IVF without the use of GnRH-antagonists show that patients seem to prefer natural cycle IVF over stimulated IVF, based on a preference for simplicity and short duration of treatment cycles (Højgaard *et al.*, 2001) or anxiety for hormone injections (Pistorius *et al.*, 2006).

In the present study, an unselected group of patients aged less than 37, reflecting a good prognosis population, was studied and results seem favourable. Further studies should investigate whether MNC-IVF is also feasible in patients of higher age and whether MNC-IVF is indeed appropriate for all patients, or should only be applied in a selection of patients with specific characteristics. In certain situations, like a history of OHSS, or patients opposed to the generation of spare embryos, MNC-IVF seems to be preferable over COS-IVF in all cases. Also poor responders to COS are likely to benefit from MNC-IVF, since these patients have poor oocyte yield and low ET rate with COS and considering cumulative pregnancy rates, may benefit from a quicker succession of treatment cycles in MNC-IVF. In studies comparing MNC-IVF to COS-IVF in poor responders, reported pregnancy rates per cycle were similar (Weghofer *et al.*, 2004; Elizur *et al.*, 2005).

It is unclear what should be the maximum number of cycles of MNC-IVF to be performed. In a previous study with the same cohort of patients as described in the present study, we found the occurrence of a cancellation of oocyte retrieval and failure to reach ET to be repeating phenomena in further cycles, although this was not reflected in a lower pregnancy rate. Fertilization failure also was found to be a repeating phenomenon, and an associated (although not statistically significant) lower pregnancy rate was found (Pelinck *et al.*, 2007). The number of cycles to be performed before COS-IVF should therefore be individualized, taking into account the results of previous cycles.

In the present study, we found fertilization failure in MNC to be predictive of fertilization failure in COS. Performing ICSI in patients showing fertilization failure in MNC-IVF probably would improve success rates.

In conclusion, sequential treatment with MNC-IVF followed by COS-IVF does not appear to compromise overall success rates, while twin pregnancy rate is very low. Because of the patient-friendly and low-risk profile of MNC-IVF, this seems an appropriate strategy.

Comparative studies on MNC-IVF and COS-IVF are warranted. Such a study should evaluate effectiveness, cost-effectiveness and risks of either treatment modality, but also focus on quality of life and patients' preferences and include time to pregnancy as an end-point.

Chapter 7

Cost-effectiveness of modified natural cycle versus controlled ovarian hyperstimulation in in-vitro fertilisation

ABSTRACT

OBJECTIVE: To compare the cost-effectiveness of modified natural cycle IVF (MNC-IVF) with controlled ovarian hyperstimulation (COH-IVF) and its short flare-up protocol and long down-regulation protocol.

DESIGN: prospective cohort study of MNC-IVF.

SETTING: Four Dutch fertility centres participated in prospective inclusion of patients for MNC-IVF. COH-IVF data from the Groningen University Medical Centre were used for comparison.

PATIENTS: female patients aged 18–36 years with a regular and proven ovulatory menstrual cycle with a length of 26–35 days and BMI (kg/m^2) of 18–28.

INTERVENTIONS: MNC-IVF consisted of daily injections of 0.25 mg of the GnRH antagonist cetrorelix together with 150 IU recombinant FSH until ovulation triggering. COH-IVF consisted of a down-regulation or flare-up protocol with triptorelin.

OUTCOME MEASURES: Pregnancy rates and costs of treatment.

RESULTS: Ongoing pregnancy rates after MNC-IVF were 8.3% versus 19.7% for COH-IVF. Average costs per treatment cycle were € 1407 for MNC-IVF versus € 3168 for COH-IVF. The incremental cost-effectiveness ratio was € 15447 per additional ongoing pregnancy. The down-regulation protocol was slightly less cost-effective than the flare-up protocol. CONCLUSIONS: MNC-IVF may be a cost-effective alternative for COH-IVF, especially when lower long-term costs of premature births are included. We recommend MNC-IVF as first-choice treatment, followed by COH-IVF if success is not obtained.

Introduction

The use of exogenous gonadotrophins and gonadotrophin-releasing hormone (GnRH) agonists in in vitro feritilisation (IVF) leads to high pregnancy rates, but they are accompanied by a 20 to 30% chance of multiple pregnancies and the risk of ovarian hyper-stimulation syndrome (OHSS). The former is associated with a substantial risk of neonatal morbidity and mortality caused by prematurity (Fauser *et al.*, 2005). The latter can be a potentially life-threatening condition in its severe form, which occurs in 0.5 to 5% of IVF treatments (Delvigne and Rozenberg, 2002).

These problems, alongside with ongoing ethical and religious dilemmas surround-ing the generation of spare embryos, have contributed to discussions about the allocation of resources to assisted reproduction techniques in general during the last decade. Several cost-effectiveness studies have been performed to offer support to decision makers in health care on this issue (Callahan *et al.*, 1994; Garceau *et al.*, 2002; Goldfarb *et al.*, 1996; Neumann *et al.*, 1994; Van Voorhis *et al.*, 1998). These studies showed that favourable clin-ical effects with respect to pregnancy rates were counteracted by high costs of neonatal care associated with premature twin or higher order multiple births. Clinicians have responded by focusing their attention on developing strategies to avoid multiple pregnancies and to make IVF more 'patient-friendly'. These strategies include elective single embryo transfer (ESET) instead of double embryo transfer (DET) and modifications to the hyperstimulation regimen, such as milder stimulation, use of GnRH antagonists instead of agonists, and nat-ural cycle IVF (Heijnen *et al.*, 2004; Heijnen *et al.*, 2007; Højgaard *et al.*, 2001; Rongières-Bertrand *et al.*, 1999). ESET and DET have in turn been the subject of cost-effectiveness studies (De Sutter *et al.*, 2002; Fiddelers *et al.*, 2006; Gerris *et al.*, 2004; Kjellberg *et al.*, 2006; Lukassen *et al.*, 2005; Wølner-Hanssen and Rydhstroem, 1998), but not much is known about the cost-effectiveness of natural cycle IVF (Daya *et al.*, 1995; Nargund *et al.*, 2001) or modified natural cycle IVF (Pelinck *et al.*, 2002; Sophonritsuk *et al.*, 2005).

At the Groningen University Medical Center, experience has been gained with IVF in the modified natural cycle (MNC-IVF) over the last few years (Pelinck *et al.*, 2002; Pelinck *et al.*, 2006). This method aims at the use of the one follicle that naturally develops to dominance. The GnRH antagonist cetrorelix is administered during the late follicular phase to prevent untimely LH surges and ovulations, in combination with simultaneous substitution with recombinant FSH. Usually, this results in retrieval of a single oocyte, and therefore practically obliterates the risk of multiple pregnancies. Although pregnancy rates are inferior to IVF with controlled ovarian hyperstimulation (COH-IVF), MNC-IVF is con-sidered as an alternative for COH-IVF because it is more patient-friendly. Benefits for the patient include zero risk of OHSS and quicker treatment succession because the milder stimulation regimen is better tolerated. This may improve pregnancy rates per time period so that the difference with COH-IVF becomes less pronounced. Moreover, MNC-IVF may potentially be cost-effective since costs of twin pregnancies are almost completely avoided and medication costs per cycle are lower. Similar effects may be achieved using ESET, but recent studies show that results of ESET may vary (Ryan *et al,* 2007; van Montfoort *et al.*, 2006). We investigated the cost-effectiveness of MNC-IVF using data from a prospective cohort study supported by a grant from the Dutch Health Care Efficiency Program (ZonMw). The results were compared to the costs per live birth following COH-IVF with

double embryo transfer based on data from our hospital database. Comparisons were made for a single treatment cycle as well as for multiple treatment cycles, with partial costs in case a treatment cycle was cancelled. The long COH protocol with down-regulation and the flare-up COH protocol as different medication schedules were analysed separately.

Patients and methods

Data regarding MNC-IVF were collected in the context of a multi-centre cohort study. Results of this study have been described in detail previously (Pelinck *et al.*, 2006). Patients with an indication for IVF (no ICSI) were offered three cycles of MNC-IVF for free. The study protocol was reviewed and approved by the local ethics committees of all participating centres.

Inclusion criteria for participation in this study were: 1) Age 18-36 years; 2) First IVF treatment ever or first IVF treatment after pregnancy; 3) Proven ovulatory cycle of 26-35 days. Data regarding COH-IVF were extracted from the database at the department of Obstetrics and Gynaecology. Selected patients were below 37 years of age, with an indication for IVF, treated between January 2001 en January 2004 but without previous MNC-IVF treatment. Extracted data concerned demographic characteristics, treatment data such as number of cycles started, number of oocyte retrievals, embryo transfers, pregnancy rates and outcomes, as well as medication use, separate for flare-up and down-regulation schedules. To allow a more detailed comparison of MNC-IVF and COH-IVF, data for the first treatment cycle of both methods are presented separately.

Modified natural cycle IVF was performed as previously described (Pelinck *et al.*, 2005). The GnRH antagonist cetrorelix (Cetrotide®, Serono, The Hague, the Netherlands) 0.25 mg daily, was started at follicle size of 14 mm together with 150 IU r-FSH daily (Gonal-F®, Serono, The Hague, the Netherlands). Cycles were monitored by transvaginal ultrasound examinations and measurements of estradiol (E2) and LH levels. Ovulation was triggered by 10,000 IU of hCG (Pregnyl®, Organon, Oss, the Netherlands) when the lead follicle reached a size of ≥ 18 mm. Oocyte retrieval was performed 34 hours after ovulation triggering. Fertilisation of the retrieved oocyte was performed according to standard procedures. Embryo transfer was done on the third day after oocyte retrieval. Luteal support was provided by administration of three times 1500 IU hCG (Pregnyl®, Organon, Oss, the Netherlands).

Controlled ovarian hyperstimulation IVF was performed using a down-regulation or flare-up protocol with triptorelin (Decapeptyl®, Ferring Nederland BV, Hoofddorp, the Netherlands) and r-FSH (Puregon®, Organon, Oss, the Netherlands). The down-regulation protocol was applied in patients with polycystic ovaries or endometriosis. All other patients were treated with the flare-up protocol. Ovulation was triggered by 10,000 IU of hCG (Pregnyl®, Organon, Oss, the Netherlands) when at least half of the follicles reached a size of >18 mm. Oocyte retrieval was performed 36 hours after ovulation triggering. Oocyte retrieval was not planned in cases of threatening OHSS (E2 levels >15 nmol/L and >20 follicles) or poor response (< 2-3 follicles). Fertilisation was performed according to standard procedures. Double embryo transfer was done on the second or third day after oocyte retrieval if two or more embryos were available. Luteal support was provided by adminis-

tering progesterone (Progestan®, Organon, Oss, The Netherlands) intravaginally or three times 1500 IU hCG (Pregnyl®, Organon, Oss, the Netherlands).

Follow-up of patients took place until one week after completion of the treatment cycle(s) for non-pregnant patients and until eight weeks after completion of the treatment cycle for pregnant patients (i.e. pregnancy duration of twelve weeks). Pregnancies of twelve weeks were considered to be ongoing and to result in a live birth.

In the economic evaluation the costs-effectiveness of MNC-IVF versus COH-IVF were compared in a cost-effectiveness analysis from the health care insurer's perspective with the live birth rate as the primary effect measure. All IVF-procedure-related costs, pregnancy-related costs and neonatal costs were included. Because no volume data were collected for the intake phase of the treatment (information session, intake by the gynaecologist, basic fertility work-up etc.) the costs of this phase were not included. Moreover, costs of OHSS in patients receiving COH-IVF and effects of cycles with cryopreserved embryos were not taken into account.

Unit costs of items included in the economic evaluation are specified in table I. Costs of medication were calculated based on actual data on use of medication and priced according to the 'Farmacotherapeutisch Kompas', 2003, a formulary published by the Dutch Health Insurance Board. A prescription charge of Euro 6.45 was added to each course of medication prescribed. Costs of IVF procedures were calculated using Dutch tariffs for the follicular stimulation phase, the oocyte aspiration, the actual in vitro fertilisation, and embryo transfer. In case of incomplete treatment cycles, which occurs relatively frequent in MNC-IVF, only costs of completed phases were calculated. Costs of ongoing pregnancy for twin and singleton pregnancies were taken from the publication by Lukassen *et al.* (2004), including costs of pregnancy and costs up to six weeks after delivery (Lukassen *et al.*, 2004).

Table I. Unit costs of items included in the economic evaluation

Cost item	Unit price (€)
Triptorelin/day	9.26
Cetrorelix/day	36.15
r-FSH/Unit	0.41
hCG 3 x 1500 IU (luteal support)	10.08
hCG 10,000 IU (ovulation triggering)	12.01
Progestan 50 capsules	10.75
Stimulation phase (tariff)	381
Oocyte retrieval (tariff)	249
Fertilisation (tariff)	328
Embryo transfer (tariff)	203
Cost of singleton pregnancy and delivery*	2,250
Cost of twin pregnancy and delivery*	13,469

*: Lukassen et al., 2004

Because it can be debated whether a twin pregnancy is a desirable outcome of IVF, we performed sensitivity analyses with different weights for twin births. The primary analyses were performed with the outcome defined as 'at least one live born child', so twin and single births were weighted as equal. For the sensitivity analysis the weight of twin births was varied between 2, i.e. with 'the number of live born children' as outcome measure, and 0.5. The latter value expresses the undesirable nature of this outcome with respect to the risk for mother and children and the associated immediate additional health care costs, and the possibility of high future health care costs related to premature birth.

Statistical analyses were performed using SPSS. Comparisons of pregnancy rates were performed using Chi-square test and differences in costs were evaluated using Student's T-test or the Mann-Whitney U-test depending on the distribution of the data. Analyses were aimed at the most relevant comparisons: overall MNC-IVF versus overall COH-IVF, 1st cycle MNC-IVF versus 1st cycle COH-IVF, and flare-up versus down-regulation. Non-parametric 95% confidence intervals around incremental cost-effectiveness ratios (ICER, i.e. cost-difference divided by difference in pregnancy rate) were estimated using bootstrap analysis (Briggs *et al.*, 1997) with 5000 replications (R-project statistical software).

Results

A total of 350 patients participated in the multi-centre study on MNC-IVF as described in detail previously (Pelinck *et al.*, 2006). Of the patients included, 14 did not start the study treatment. The remaining patients (n=336) underwent at least one treatment cycle: 277 (82.4%) started the second cycle and 68.7% at least partially completed three cycles. Overall a total of 844 cycles were completed. Treatment succession was quick: 79.1% of the patients who underwent a second treatment cycle did so immediately after the first. Of the third cycles performed, 74.6% followed immediately after the second. Median interval between the first and second cycle, calculated as the interval between last menstruation dates before the respective treatment cycles, was 31 days and between the second and third cycle 34 days. With respect to the indications for IVF in the two groups, the major differences concerned unexplained subfertility (COH: 19.3% vs. MNC: 38.1%), male factor (COH: 24.5% vs. MNC: 16.4%) and endometriosis (COH: 13.% vs. MNC: 7.7%). Tubal factor (COH: 25% vs. MNC: 31.5%) differed less and failed donor insemination and cervix factor were minor categories in both groups. The patients undergoing COH-IVF (n=212) completed a total number of 386 cycles during the study period, i.e. 1.8 cycles/patient. Patients in the MNC-IVF cohort completed an average of 2.5 cycles during the study period.

Age, number of cycles and treatment results of the patients are presented in table II. The mean age was comparable in both groups. The mean duration of subfertility was 46.9 months in the MNC group (median 46, SD 23.2) and 42.4 months in the COH group (median 41, SD 26.2). MNC-IVF resulted in considerably fewer oocyte retrievals and embryo transfers per cycle than COH-IVF. Overall, no oocytes were retrieved in 24.7% of retrievals in MNC-IVF, and one oocyte was retrieved in 69.5% of retrievals. Two, three or four oocytes were occasionally retrieved. For COH-IVF, failure of retrieval did not occur and at least five oocytes were retrieved in 83.8% of the cycles. With respect to embryo

Table II. Treatment characteristics of patients undergoing in vitro fertilisation

	MNC-IVF overall (n=336)	1st cycle (n=336)	COH-IVF overall (n=212)	1st cycle (n=212)	flare-up (n=132)#	down-reg (n=78)#
mean age	32.8	32.7	33.4	33.0	32.9	32.3
number of cycles	844	336	386	212	229	153
oocyte retrievals/cycle*	82.3%	83.3%	92.7%‡	91.5%†	92.6%	92.8%
oocytes retrieved	0.82	0.81	12.35	12.16	10.71	13.63
embryo transfers/cycle	37.6%	40.5%	80.6%‡	78.8%‡	80.3%	80.4%
1 embryo transferred	94.3%	93.4%	13.8%	14.4%	14.7%	13.0%
2 embryos transferred	5.7%	6.6%	83.7%	83.8%	82.1%	87.0%
3 embryos transferred	-	-	2.2%	1.8%	2.7%	-
pregnancies/transfer	22.1%	26.5%	24.4%	24.6%	23.4%	26.0%
ongoing pregnancies/cycle	8.3%	10.7%	19.7%‡	19.3%†	18.8%	20.9%
multiple pregnancies	4.3%	5.6%	18.4%†	19.5%	13.9%	25.0%

*: retrievals with and without success
#: in four patients the stimulation protocol was unknown
†: $p<0.01$ versus MNC-IVF (Chi-square test)
‡: $p<0.001$ versus MNC-IVF (Chi-square test)

Table III. Average treatment costs per cycle and per live birth*

	MNC-IVF		COH-IVF			
	overall	1st cycle	overall	1st cycle	flare-up[#]	down-reg[#]
Number of cycles	844	336	386	212	229	153
Triptorelin/cetrorelix						
days/cycle	3.18	3.18	17.04	16.61	10.35	26.71
costs/cycle	121.12	121.41	157.78	153.79	95.88	247.30
r-FSH						
days/cycle	2.28	2.26	10.40	10.22	9.47	11.65
costs/cycle	149.32	148.83	1100.82	995.31	1044.54	1166.47
hCG/luteal support	14.71	15.16	19.53	19.19	19.48	19.52
Total medication costs/cycle (95% CI)	285.14 (274.4-295.9)	284.26 (266.4-302.1)	1278.12‡ (1216-1340)	1168.30‡ (1087-1250)	1159.91 (1082-1237)	1433.28† (1335-1531)
Follicle stimulation	381	381	381	381	381	381
Oocyte retrieval	205	208	231	228	231	231
Fertilisation + transfer	278	287	428‡	418‡	426	427
Treatment costs excluding pregnancy	1180	1192	2318‡	2196‡	2198	2472†
Pregnancy and delivery	227	308	850‡	858♠	716	1057
Total costs/cycle (95% CI)	1407 (1327-1487)	1500 (1349-1651)	3168‡ (2903-3433)	3054‡ (2687-3421)	2914 (2612-3218)	3529† (3042-4017)
Costs/live birth	16952	14019	16081	15824	15500	16885
Incremental cost-effectiveness ratio (overall)		15447				
Incremental cost-effectiveness ratio (1st cycle)			18070			

*: partial costs in case of partially completed cycle
#: in four patients the stimulation protocol was unknown
†: p<0.001 versus flare-up (Mann-Whitney U-test)

♠: p<0.005 versus MNC-IVF (Mann-Whitney U-test)
‡: p<0.001 versus MNC-IVF (Mann-Whitney U-test)

transfers, the data reflect the standard procedures. One embryo was transferred in 94.3% of MNC cycles while the majority of COH cycles (83.7%) concerned double embryo transfer, with occasional transfer of 3 embryos. Despite these differences, the pregnancy rates per transfer for MNC-IVF and for COH-IVF did not differ significantly. However, the overall pregnancy rate per cycle for MNC-IVF was about half that for COH-IVF (p<0.001). As expected, the multiple pregnancy rate was considerably reduced with MNC-IVF in comparison with COH-IVF, i.e. 4.3% versus 17.1%, respectively (p<0.01). For both treatment methods, the first cycle compared favourably to the overall results with respect to pregnancy rates. Pregnancy rates for the long down-regulation protocol and the short flare-up protocol did not differ significantly, but there was a marked difference in the percentage of multiple pregnancies, which was considerably higher for the down-regulation protocol than for any of the other COH treatment types without reaching statistical significance (p=0.2 versus flare-up).

Average costs per treatment cycle are summarized in table III. For four treatment cycles there were no data regarding the type of treatment (long or short protocol). Costs of medication were about four times higher for COH-IVF than for MNC-IVF (p<0.001). This was mainly caused by longer administration of FSH. The long down-regulation protocol was associated with the highest medication costs (p<0.001 versus flare-up). Differences in average treatment costs were mainly due to the differences in costs of medication. Slightly higher average costs of oocyte retrieval and embryo transfer per cycle were a result of higher retrieval and transfer rates in COH-IVF. Costs of pregnancies and delivery differed significantly (p<0.001 and p<0.005 overall and for cycle 1, respectively) due to the higher percentage of twin pregnancies with COH-IVF. Total treatment costs, including pregnancy and delivery, were up to 2.5-fold higher for the various COH-IVF cycles than for MNC-IVF (p<0.001 overall and for cycle 1), with flare-up protocol as the most favourable and the down-regulation protocol as the least favourable alternative (p<0.001 versus flare-up). The

Figure 1. Average costs and pregnancy rates of IVF treatments
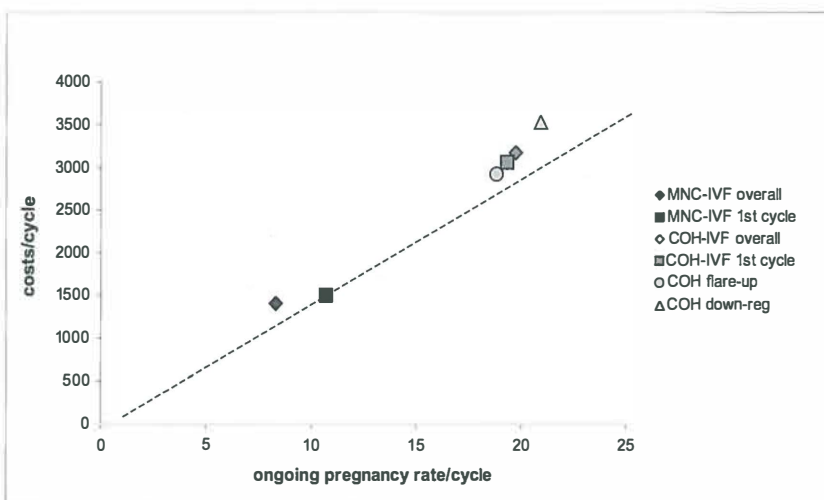
Figure 2. Effect of variable twin weight
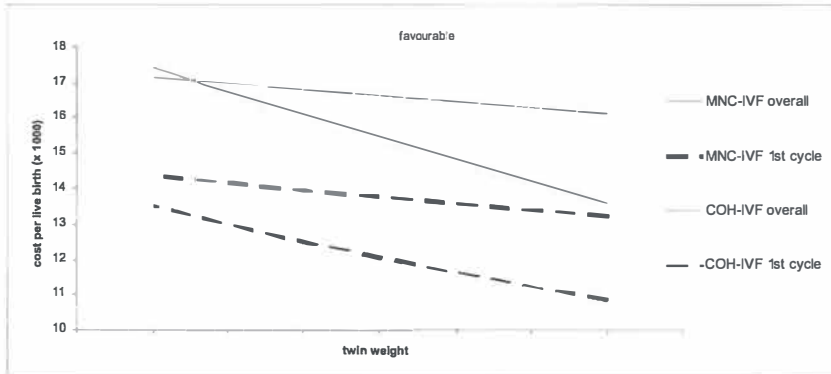
cost-effectiveness ratios for all treatment types, i.e. costs per cycle versus the pregnancy rates, are presented graphically in figure 1. The dotted reference line indicates a cost-effectiveness ratio of 15000 Euro per live birth. As shown in the graph, costs and pregnancy rate for MNC-IVF are both clearly below COH-IVF. However, the ratio of costs and birth rate are largely similar for MNC-IVF and COH-IVF, as indicated by their proximity to the reference line. Exact data are presented in table III. Among the COH-IVF variants, the first cycle has a slightly more favourable ratio of costs versus effects than the other three options. Additionally, the average MNC-IVF costs per live birth over three cycles were about 17.000 Euro at a cumulative pregnancy rate of 20.8% (average treatment costs 3536 Euro, data not shown), which is about as good as the average COH-IVF performance.

Incremental cost-effectiveness ratios were calculated for the comparisons COH-IVF overall versus MNC-IVF overall and for COH-IVF 1st cycle versus MNC-IVF 1st cycle and are presented in table III. Since the cost difference and the difference in pregnancy rate are both negative if MNC is regarded as the experimental treatment, the ICER yields a positive number, representing the difference in cost per additional live born child. The non-parametric 95% confidence interval for the ICER based on bootstrap analyses was 11896 - 22605 for the overall comparison and 11446 – 51405 for the 1st cycle. The high upper confidence limit in the latter analysis is caused by replications with near-zero differences in effects, which cause the ratio to inflate.

The sensitivity analyses (figure 2) show the influence of varying the weight of twin births on the costs per live birth. For the MNC-IVF categories, the influence is minor because of the small number of twins. For COH-IVF the impact of the sensitivity analysis was much larger, with a 16% decrease in the costs per live birth when a twin birth was weighted as a double birth for the overall COH-IVF group. If the weight of a twin birth was reduced to 0.5, the difference in cost-effectiveness of MNC-IVF (all cycles) and COH-IVF (all cycles) diminished to zero.

Discussion

In this study we compared the cost-effectiveness of modified natural cycle IVF and controlled ovarian hyperstimulation IVF based on the results of a prospective cohort study (Pelinck *et al.,* 2006). Moreover, an evaluation of the short flare-up protocol versus the long down-regulation protocol for COH-IVF was performed. Our data suggest that MNC-IVF may be a cost-effective alternative for COH-IVF, since the costs per live birth do not differ substantially. Moreover, the long down-regulation protocol for COH-IVF required for patients with PCOS or endometriosis provides poorer cost-effectiveness than the flare-up protocol.

One limitation of the study was that it was not performed as a randomized controlled trial. Instead, the effectiveness data for MNC-IVF were taken from a prospective cohort study and the data for COH-IVF were retrieved from our hospital database. However, since the selection criteria for the COH-IVF patients, e.g. treatment period and age, were largely the same as for the MNC-IVF patients, we believe that this did not compromise the validity of the results of the present study. A second limitation may be the use of tariffs instead of actual resource use for the costs of IVF procedures. Since we were mainly interested in incremental costs, i.e. differences in costs between the treatments, and not absolute costs of both treatments, we accepted tariffs as a sufficiently close approximation of actual costs. Moreover, separate tariffs could be applied for the different phases in the treatment, so incomplete treatment cycles could be valued according to the phases that were completed, e.g. in case oocyte retrieval was unsuccessful because of premature ovulation, which is more frequent in MNC-IVF than in COH-IVF. Detailed data for medication use were available to allow a precise calculation of the medication costs for both treatments, as well as for the flare-up and down-regulation protocols separately. As our data confirm, these costs are a major contribution to the total treatment costs. Finally, effects of cryopreservation of embryos for subsequent use were not included in this analysis. Although this is common practice in many centers, the added benefit is not undisputed. In an analysis by De Jong *et al.,* the use of cryopreserved embryos increased pregnancy rates after up to three cycles of COH-IVF by only 1.3% (De Jong *et al.,* 2002).

An evaluation of IVF involves many aspects, including pregnancy rates, neonatal outcomes, costs and the patients' perspective. All of these aspects are relevant to decision makers when a preference for a particular treatment policy has to be determined. In MNC-IVF, reduction of risk-bearing multiple pregnancies, annihilation of the risk of OHSS and a more patient-friendly treatment protocol are achieved at the expense of a lower pregnancy rate per cycle. Our data show that the cost-effectiveness of MNC-IVF is more or less comparable to COH-IVF because the lower effectiveness is associated with equally lower costs, both of medication and neonatal morbidity due to multiple pregnancies. A similar reduction of multiple pregnancies can be achieved with elective single embryo transfer (ESET) after controlled ovarian hyperstimulation. Studies performed so far indicate that while high pregnancy rates can be maintained if only good quality embryos are selected for ESET (Gerris *et al.,* 2004; Lukassen *et al.,* 2005; Thurin *et al.,* 2004), systematic application of ESET in unselected patients is likely to result in up to 50% lower pregnancy rates (Gerris *et al.,* 2002; van Montfoort *et al.,* 2006). Recently, Heijnen *et al.* reported similar

live birth rates and lower costs after up to four cycles of a 'mild' stimulation IVF strategy with ESET compared to up to three cycles of conventional COH-IVF with DET (Heijnen *et al.*, 2007). Because of the disparity of embryo replacement strategies, this study sheds no further light on the effect of systematic ESET. Moreover, OHSS is not completely avoided with this mild IVF regimen.

Future research involving a direct comparison of MNC-IVF and systematic ESET will have to be performed to determine which of these two treatments deserves preference. MNC-IVF may have some advantages to offer (Højgaard *et al.*, 2001), such as less medication, shorter treatment and the possibility of quicker succession of treatment cycles. Moreover, the risk of severe ovarian hyperstimulation syndrome is reduced to zero (none were observed in the prospective cohort). Our cost estimates did not include the cost reduction due to prevention of OHSS because we believe that the precision of incidence rates of relatively rare complications is likely to be higher in large patient series than in smaller studies such as our own. Kjellberg *et al.* have calculated the mean costs of OHSS per cycle between 80 (SD 338) and 124 Euro (SD 459), i.e. 900 to 1100 Euro per occurrence based on incidence rates of around 10% (all grades of severity). Another estimate, based on UK tariffs, amounted to 800 UK pounds (about 1100 Euro) per occurrence (Daya *et al.*, 2001). Although inclusion of costs of OHSS would be to the benefit of MNC-IVF, the impact on total mean treatments costs would be relatively small.

The patients' acceptance of MNC-IVF also has to be taken into account. Studies have shown that couples are reluctant to accept ESET as a less effective treatment option (Murray *et al.*, 2004; Pinborg *et al.*, 2003; Porter and Bhattacharya, 2005). Unawareness of the risks of multiple pregnancies and cost considerations if the couples have to pay for their treatment were identified as factors underlying this attitude towards ESET. However, in countries where ESET is now standard treatment, such as Belgium and the Scandinavian countries, excellent results are obtained (Gerris *et al.*, 2002; Thurin *et al.*, 2004; Wølner-Hanssen and Rydhstroem, 1998).

With respect to the choice for either the long down-regulation protocol for COH-IVF or the shorter flare-up protocol, our data seem to point in favour of the latter. Not only are the costs of medication for the flare-up protocol lower than for the down-regulation protocol, the overall cost-effectiveness of the down-regulation is also negatively influenced by the high percentage of twins in the group using the long protocol. Guidance provided by the UK National Centre for Health and Clinical Excellence (NICE, www.nice.org.uk) in 2004 favoured the long protocol because of the better pregnancy rates it achieved, but neither costs of medication nor costs of multiple pregnancies were explicitly included in this analysis. Our results suggest that use of the long protocol should be restricted to conditions warranting the extra medication costs and increased risk of twins.

A comprehensive evaluation of the impact of an IVF strategy avoiding twin pregnancies such as MNC-IVF should include lifetime costs of care for disabled children. Estimates of these costs are not readily available. Wølner-Hanssen and Rhydstroem (1998) used data from a French estimate dating back to 1982 (Papiernik, 1983) in an effort to include lifetime costs in their cost-effectiveness calculations (Wølner-Hanssen and Rhydstroem, 1998). They arrived at average costs for singletons of about 1,500 Euro and almost 14 times this number for twins (20,500 Euro). Another estimate can be derived from

the field of paediatrics, where lifetime costs of severely handicapped children as a result of metabolic disease have been estimated at $ 552,000 (Insinga *et al.*, 2002). A similar figure is mentioned in an evaluation of the US Centers for Disease Control (Centers for Disease Control, 1995). It is clear that inclusion of these costs in the cost-effectiveness calculations of IVF strategies with different percentages of twin pregnancies has a considerable impact. The results of our sensitivity analyses support this notion from a different perspective, following debate in the literature about the most relevant success measure in assisted reproduction, advocating singleton term live birth rate (BESST) as optimum outcome (Heijnen *et al.*, 2004; Min *et al.*, 2004). Clearly, valuing twin births as double births disregarding all costs and morbidity ensuing from premature birth is too simple. If singleton pregnancies are the outcome of interest, then multiple pregnancies should be regarded as unwanted. Our sensitivity analyses show that under these assumptions, differences in cost-effectiveness between MNC-IVF and COH-IVF may diminish to zero, depending on the degree of devaluation of twin births.

In conclusion, the results of this study show that MNC-IVF may be a cost-effective alternative for COH-IVF. Prevention of long-term costs associated with morbidity due to premature birth in multiple pregnancies, as observed more frequently after COH-IVF than after MNC-IVF, may tip the balance in favour of MNC-IVF. We recommend a policy of preferential MNC-IVF, followed by COH-IVF if success is not obtained.

Chapter 8

Embryo quality and impact of specific embryo characteristics on ongoing implantation in unselected embryos derived from modified natural cycle IVF

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ABSTRACT

OBJECTIVE: To study the implantation potential of unselected embryos derived from modified natural cycle IVF according to their morphological characteristics.

DESIGN: Cohort study.

SETTING: Academic department of reproductive medicine.

PATIENTS: A series of 449 single embryo transfers derived from modified natural cycle IVF.

INTERVENTIONS: None.

MAIN OUTCOME MEASURES: Ongoing implantation rate according to embryo characteristics.

RESULTS: The best implantation was found in embryos with 4 and 8 cells on day 2 and 3 respectively, $\leq 10\%$ fragmentation and absence of multinucleated blastomeres. In contrast to findings from other studies, we found embryos with less than four blastomeres on day 2 to do relatively well. Furthermore, we found the implantation potential of embryos containing multinucleated blastomeres to be less severely impaired than expected.

CONCLUSIONS: Findings from this study suggest that in currently used embryo scoring systems, the implantation potential of embryos with low numbers of blastomeres on day 2, as well as embryos containing multinucleated blastomeres is underestimated. However, it is unclear whether the results of our study apply to embryos derived from COH cycles.

Introduction

With multiple embryos available after IVF, it is important to select the embryos that are most likely to implant in order to be able to limit the number of embryos transferred while maintaining acceptable pregnancy rates. The increasing application of elective single embryo transfer (ESET) has made the selection of the most viable embryo from a cohort of embryos all the more important.

Most embryo scoring systems are non-invasive and based on morphological criteria. A variety of criteria has been described, including pronuclear zygote morphology, early cleavage, symmetry of blastomeres, fragmentation pattern, number of blastomeres, presence of multinucleation in blastomeres, and cleavage rate (Scott, 2003).

Embryo characteristics are often combined in graduated scoring systems with various degrees of complexity, providing a more precise estimation of implantation potential (Van Royen *et al.,* 1999; Fisch *et al.,* 2001; De Placido *et al.,* 2002; Sjöblom *et al.,* 2006; Holte *et al.,* 2007; Scott *et al.,* 2007).

Improvement of selection has been proposed by incorporating other embryo characteristics in the scoring systems, such as developmental potential and genetic constitution. For instance, prolonged culture to the blastocyst stage has been suggested as a means to select the best embryo for transfer, but is time-consuming. Concerning the efficacy of blastocyst transfers as compared to the transfer of cleavage-stage embryos, results are conflicting (Blake *et al.,* 2005; Papanikolaou *et al.,* 2008). Preimplantation genetic screening (PGS) is used to select against embryos carrying some form of aneuploidy, but is invasive and laborious and so far has not proven to improve success rates (Staessen *et al.,* 2004; Twisk *et al.,* 2006; Mastenbroek *et al.,* 2007).

Currently used embryo scoring systems have invariably been developed in cohorts of embryos derived from ovarian stimulation cycles. If validation of such scoring systems is done by confirmation of implantation, a certain degree of bias is unavoidable due to the selection of embryos for transfer. Alternatively, validation can be based on evaluation of developmental potential by blastocyst formation rate, but since the correlation between implantation potential and blastocyst formation in vitro is not 100%, this leaves some uncertainty too (Scott, 2003).

In contrast to standard IVF with ovarian stimulation, in modified natural cycle IVF (MNC-IVF), treatment is aimed at using the one follicle that spontaneously develops to dominance, in most cases leading to the availability of one single embryo for transfer (Rongières-Bertrand *et al.,* 1999; Pelinck *et al.,* 2006). This offers the unique possibility to relate embryo characteristics to their implantation potential, without any selection.

In the present study, a series of 449 single embryo transfers after modified natural cycle IVF is described. A detailed analysis of embryo characteristics and their impact on ongoing implantation is provided.

Materials and methods
Study protocol

For the present study, cycles of modified natural cycle IVF where one single embryo was available for transfer were identified. All cycles from which these embryos originated, were performed in a research setting, details of which have been described pre-

viously (Pelinck *et al.,* 2005; Pelinck *et al.,* 2006; Pelinck *et al.,* 2007). In short, IVF was performed in unstimulated cycles in which a GnRH-antagonist together with recombinant FSH was administered in the late follicular phase only. Treatment is aimed at the one follicle that spontaneously develops to dominance and in most cases, one oocyte is obtained and one single embryo is available for transfer. All treatments were performed between January 2001 and September 2005.

Laboratory procedures

Only conventional IVF was performed. For inclusion in the study, a minimum of 0.5 million motile sperm had to be present after a swim up procedure. Oocytes were inseminated 2-5 hours after oocyte retrieval with 10 000 motile spermatozoa prepared by centrifugation for 15 minutes at 300 *g* over a 45/90% gradient of Suprasperm® (Medicult a/s, Jylling, Denmark), followed by washing and a swim-up procedure in culture medium. Oocytes were cultured in human tubal fluid medium (Cambrex Bio Science, Verviers, Belgium), supplemented with 10% plasma solution (Sanquin, Amsterdam, the Netherlands). Fertilization was assessed 17-20 hours after insemination by determination of the number of pronuclei (PN). Embryos were scored 42-46 hours and 66-70 hours after insemination. Embryos were scored for number of blastomeres, percentage of fragmentation and the presence of (multi)nuclei on day 2 and day 3. Embryos with more than two pronuclei and with more than 50% fragmentation were discarded for transfer. All other embryos were transferred.

Embryo transfer was performed 72-76 hours after oocyte retrieval, using a TDT®

Table 1. Ongoing implantation according to number of blastomeres on day 2 and day 3, MNB excluded

N° of blastomeres day 3		2	3	4	5	6
	N° of blastomeres day 2					
Fragmentation ≤10%	1	3/0 (0.0)	2/0 (0.0)	0 (-)	0 (-)	0 (-)
	2	1/0 (0.0)	4/2 (50.0)	29/6 (20.7)	12/6 (50.0)	8/2 (25.0)
	3	0 (-)	1/0 (0.0)	5/0 (0.0)	2/0 (0.0)	7/0 (0.0)
	4	0 (-)	0 (-)	16/2 (12.5)	13/2 (15.4)	28/7 (25.0
	5	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
	6	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
	7	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
	Total	4/0 (0.0)	7/2 (28.6)	50/8 (16.0)	27/8 (29.6)	43/9 (20.9
Fragmentation >10%	1	1/0 (0.0)	1/0 (0.0)	1/0 (0.0)	0 (-)	0 (-)
	2	3/0 (0.0)	4/0 (0.0)	15/3 (20.0)	7/2 (28.6)	6/0 (0.0)
	3	1/0 (0.0)	5/0 (0.0)	3/1 (33.3)	3/1 (33.3)	10/2 (20.0
	4	0 (-)	0 (-)	4/0 (0.0)	7/2 (28.6)	8/1 (12.5)
	5	0 (-)	0 (-)	2/0 (0.0)	2/0 (0.0)	2/0 (0.0)
	6	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
	7	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
	Total	5/0 (0.0)	10/0 (0.0)	25/4 (16.0)	19/5 (26.3)	26/3 (11.5

Numbers are n° of embryos/n° implanted (percentage)

(Prodimed, Neuilly-en-Thielle, France) or Wallace® (SIMS Portex Ltd, Hythe, UK) catheter.

Pregnancy was defined as either the visualization of at least one intrauterine gestational sac or a proven ectopic pregnancy. Ongoing pregnancy was defined as the presence of an intrauterine gestational sac with fetal heart beat at 12 weeks gestational age.

Data analysis

Logistic regression analysis was applied to determine the influence of embryo and patient characteristics on ongoing implantation. Embryo characteristics tested were: PN score, blastomere number on day 2 and day 3, amount of fragmentation on day 3, multinucleation present and cleavage rate. Fragmentation on day 3 and day 2 were not entered both because of their strong correlation. Fragmentation on day 3 was chosen over day 2 since this offers the most relevant information just prior to transfer. Patient characteristics tested were female patient age, nature of subfertility (primary or secondary) and indication for IVF. After univariate analysis, factors with $P < 0.20$ were retained for multivariate analysis. Analyses were performed with Statistics package for Social Sciences (SPSS) 12.0.1.

Results

Patient characteristics

The median patient age at embryo transfer was 33.8 years (range 23-38). Indication for IVF was tubal pathology, unexplained subfertility, male factor, endometrio-

	8	9	10	12	total
(-)	0 (-)	0 (-)	0 (-)	0 (-)	5/0 (0.0)
0 (0.0)	5/1 (20.0)	0 (-)	0 (-)	0 (-)	62/17 (27.4)
1 (50.0)	3/2 (66.7)	0 (-)	0 (-)	0 (-)	20/3 (15.0)
)/7 (24.1)	76/33 (43.4)	3/0 (0.0)	0 (-)	0 (-)	165/51 (30.9)
0 ()	6/2 (33.3)	1/0 (0.0)	1/0 (0.0)	0 (-)	12/2 (16.7)
(-)	1/0 (0.0)	0 (-)	0 (-)	0 (-)	1/0 (0.0)
0 (0.0)	1/0 (0.0)	0 (-)	0 (-)	0 (-)	2/0 (0.0)
)/8 (20.5)	92/38 (41.3)	4/0 (0.0)	1/0 (0.0)	0 (-)	267/73 (27.3)
(-)	0 (-)	0 (-)	0 (-)	0 (-)	3/0 (0.0)
0 (0.0)	1/0 (0.0)	0 (-)	0 (-)	0 (-)	40/5 (12.5)
0 (0.0)	0 (-)	0 (-)	0 (-)	0 (-)	25/4 (16.0)
1 (12.5)	12/4 (33.3)	1/0 (0.0)	1/0 (0.0)	0 (-)	41/8 (19.5)
0 (0.0)	2/0 (0.0)	1/0 (0.0)	1/0 (0.0)	0 (-)	11/0 (0.0)
(-)	0 (-)	0 (-)	0 (-)	1/0 (0.0)	1/0 (0.0)
(-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
)/1 (6.3)	15/4 (26.7)	2/0 (0.0)	2/0 (0.0)	1/0 (0.0)	121/17 (14.0)

sis, failed donor inseminations and cervical hostility in 38.9, 37.5, 10.3, 7.9, 2.7 and 2.7% of cases, respectively. Embryo transfers were performed in 216 patients, with a mean number of cycles with embryo transfer of 2.08 ($\pm$ 1.3) per patient (median 2.0, range 1-6). Median total motile sperm count of unprepared semen, calculated as volume (ml) x concentration (number of spermatozoa/ml) x proportion of motile spermatozoa, was 60.6 million (range 0.72-787.5). After embryo transfer, 120 pregnancies, of which 99 ongoing beyond 12 weeks gestational age, were obtained, the ongoing implantation rate per transfer being 22.0% (95% CI 18.1-26.0).

Embryo characteristics
PN score on day 1 after oocyte retrieval

Out of 449 transferred embryos, 431 (96.0%) contained 2 PN on day 1, with 97 (22.5%) ongoing implantation. Eighteen (4.0%) embryos showed either none or one pronucleus on day one, with 2 (11.1%) ongoing implantations.

Embryo characteristics on day 2 and day 3 after oocyte retrieval

Table 1 shows ongoing implantations according to the number of blastomeres on day 3 versus day 2 and fragmentation ($\leq$10% or >10%), excluding embryos with MNBs (day 2, day 3 or both). Table 2 shows the same for embryos containing MNBs at any time.

Embryos scored as having 0%, 1-10% and >10% fragmentation on day 2, showed ongoing implantation rates of 24.4, 25.7% and 15.4%, respectively. The highest ongoing

Table 2. Ongoing implantation according to number of blastomeres on day 2 and day 3, MNB day 2 or day 3 or both

N° of blastomeres day 3		2	3	4	5	6
	N° of blastomeres day 2					
Fragmentation ≤10%	1	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
	2	0 (-)	3/0 (0.0)	12/4 (33.3)	3/0 (0.0)	0 (-)
	3	0 (-)	0 (-)	1/0 (0.0)	1/0 (0.0)	1/0 (0.0)
	4	1/0 (0.0)	0 (-)	1/0 (0.0)	1/1 (100.0)	1/0 (0.0)
	5	0 (-)	0 (-)	0 (-)	0 (-)	2/0 (0.0)
	6	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
	7	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
	Total	1/0 (0.0)	3/0 (0.0)	14/4 (28.6)	5/1 (20.0)	4/0 (0.0)
Fragmentation >10%	1	1/0 (0.0)	1/0 (0.0)	0/0 (0.0)	0 (-)	0 (-)
	2	1/0 (0.0)	2/0 (0.0)	7/2 (28.6)	3/0 (0.0)	1/0 (0.0)
	3	0 (-)	1/0 (0.0)	0 (-)	2/0 (0.0)	0 (-)
	4	0 (-)	2/0 (0.0)	1/0 (0.0)	2/1 (50.0)	1/0 (0.0)
	5	0 (-)	0 (-)	0 (-)	1/0 (0.0)	0 (-)
	6	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
	7	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
	Total	2/0 (0.0)	6/0 (0.0)	8/2 (25.0)	8/1 (12.5)	2/0 (0.0)

Numbers are n° of embryos/n° implanted (percentage)

implantation rate was found in embryos showing 4 blastomeres on day 2 (overall 27.8%). For embryos with 2, 3, 5 or > 5 blastomeres on day 2, ongoing implantation rates were 20.7, 13.7, 7.7 and 0.0%, respectively.

For day 3 embryos, ongoing implantation rate was 26.8 and 27.5% for embryos with 0% and 1-10% fragmentation respectively, while for embryos with fragmentation of >10%, ongoing implantation rate was 13.5%. The highest ongoing implantation rate was found in embryos showing 8 blastomeres (overall 38.1%). Few embryos (2.2% of all embryos) showed > 8 blastomeres on day 3, and no pregnancies resulted.

Of 388 embryos not containing MNBs at any time, 90 implanted (23.2%). Combining the number of blastomeres identified as optimal for day 2 and 3 (i.e. four on day 2 and eight on day 3), 88 embryos showed these numbers, of which 37 implanted (42.0%). Of these, 76 showed <10% fragmentation, with 43.4% ongoing implantation.

A total of 61 embryos showed MNBs at any time (day 2 only: n=8, day 3 only: n=11 and both days: n= 42). These led to nine ongoing implantations (14.8%), all of which originated from embryos with four, five or eight blastomeres.

Cleavage rate

Table 3 shows ongoing implantation according to cleavage rate, calculated as the number of blastomeres on day 3 divided by the number of blastomeres on day 2. Overall, the highest implantation rate was found in embryos showing cleavage rates 2 and >2<3 (30.1% and 28.9%, respectively).

	8	9	10	12	total
(-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
'0 (0.0)	0 (-)	0 (-)	0 (-)	0 (-)	19/4 (21.1)
(-)	0 (-)	0 (-)	0 (-)	0 (-)	3/0 (0.0)
(-)	6/1 (16.7)	0 (-)	0 (-)	0 (-)	10/2 (20.0)
(-)	0 (-)	0 (-)	0 (-)	0 (-)	2/0 (0.0)
(-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
(-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
'0 (0.0)	6/1 (16.7)	0 (-)	0 (-)	0 (-)	34/6 (17.6)
(-)	0 (-)	0 (-)	0 (-)	0 (-)	2/0 (0.0)
(-)	0 (-)	0 (-)	0 (-)	0 (-)	14/2 (14.3)
(-)	0 (-)	0 (-)	0 (-)	0 (-)	3/0 (0.0)
'0 (0.0)	0 (-)	0 (-)	0 (-)	0 (-)	7/1 (14.3)
(-)	0 (-)	0 (-)	0 (-)	0 (-)	1/0 (0.0)
(-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
(-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
'0 (0.0)	0 (-)	0 (-)	0 (-)	0 (-)	27/3 (11.1)

Table 3. Ongoing implantation rate according to cleavage rate

Cleavage rate[a]		≤1	>1<2	2	>2<3	≥3	total
	fragmentation						
MNB not present	≤10%	19/2 (10.5)	94/20 (21.3)	116/39 (33.6)	20/9 (45.0)	18/3 (16.7)	267/73 (27.3)
	>10%	17/0 (0.0)	39/6 (15.4)	40/9 (22.5)	12/2 (16.7)	13/0 (0.0)	121/17 (14.0)
	total	36/2 (5.6)	133/26 (19.5)	156/48 (30.8)	32/11 (34.4)	31/3 (9.7)	388/90 (23.2)
MNB ever	≤10%	2/0 (0.0)	9/1 (11.1)	19/5 (26.3)	3/0 (0.0)	1/0 (0.0)	34/6 (17.6)
	>10%	6/0 (0.0)	8/1 (12.5)	8/2 (25.0)	3/0 (0.0)	2/0 (0.0)	27/3 (11.1)
	total	8/0 (0.0)	17/2 (11.8)	27/7 (25.9)	6/0 (0.0)	3/0 (0.0)	61/9 (14.8)
	total	44/2 (4.5)	150/28 (18.7)	183/55 (30.1)	38/11 (28.9)	34/3 (8.8)	449/99 (22.0)

[a] cleavage rate: number of blastomeres on day 3 divided by number of blastomeres on day 2
Numbers are n° of embryos/n° implanted (percentage)

Impact of patient and embryo characteristics on implantation potential

Table 4 shows results of logistic regression analysis. For this analysis, embryos with one, six or seven blastomeres on day 2, as well as those with either two or more than eight blastomeres on day three were excluded since no pregnancies resulted from transfer of any of these embryos. Arrested embryos (cleavage rate $\leq$1) were excluded since these are clearly aberrant, leaving 383 embryos for analysis.

With univariate analysis, PN score, female patient age, nature of subfertility and indication for IVF proved to have no significant effect on ongoing pregnancy rate. For each embryo characteristic (except PN score) the adjusted OR for ongoing pregnancy was calculated with multiple regression analysis, using an unconditional forward procedure (table 4). No significant interactions were found.

Discussion

The embryo characteristics identified as most optimal (4 cells day 2, 8 cells day 3, $\leq$10% fragmentation, no MNB) in this study correspond exactly with what is theoretically expected and generally accepted to be the best embryo. Embryos with deviations from this optimal cleavage pattern are associated with a lower implantation. Among these, a cleavage rate of > 2 but <3, as well as the presence of two blastomeres on day 2, is advantageous.

Considering blastomere number on day 2, we found that 2-cell embryos showed only slightly worse implantation than 4-cell embryos, especially with fragmentation $\leq$10%, while embryos with 3 or 5 blastomeres on day 2 did significantly worse. Few embryos (0.89% of all embryos) showed 6 or more blastomeres on day 2 and no pregnancies resulted. In contrast to our findings, in other studies, embryos with 5 or 6 cells on day 2 are often found to have better developmental potential than those with 2 or 3 (Ziebe *et al.*, 1997; Saldeen and Sundström, 2005; Sjöblom *et al.*, 2006; Fauque *et al.*, 2007; Holte *et al.*, 2007). In only one study, 2-cell embryos were found to contribute more to the total number of pregnancies than 3-cell and >4-cell embryos (Scott *et al.*, 2007).

Roughly summarized, we found that embryos deviating from the optimal cleavage pattern by lower numbers of blastomeres on day 2, show less impaired implantation than those with higher numbers, while in other studies, these findings are reversed.

There are several possible explanations for these contrasting findings. First, no ICSI embryos, who are known to show higher numbers of blastomeres on day 2 compared to embryos derived from conventional IVF (Nagy *et al.*, 1998; Dumoulin *et al.*, 2000), were included in this study, while in most studies part of the embryos studied were derived from ICSI (Saldeen and Sundström, 2005; Sjöblom *et al.*, 2006; Fauque *et al.*, 2007; Holte *et al.*, 2007). In one study on embryos originating from conventional IVF only, >4-cell embryos showed better implantation than those with <4 cells on day 2 (Ziebe *et al.*, 1997). Second, in our study, the interval between hCG and oocyte retrieval was 34 hours, which is two hours shorter than in general. Therefore, the time interval between hCG administration and embryo evaluation is shortened by two hours. Supposing a set time between hCG administration and cleavage divisions (Scott *et al.*, 2003), and considering that the exact time of fertilization is not known since only conventional IVF was applied, a shift towards the observation of lower numbers of blastomeres is possible in our study. Third, in other studies, embryos studied are invariably derived from ovarian stimulation cycles (Ziebe *et al.*, 1997;

Table 4. Regression analysis

	Univariate			Multivariate		
	OR	95% CI	P	ORadj	95% CI	P
Embryo characteristic						
PN score day 1 (2PN vs other)	1.90	0.41-8.73	0.41			
Blastomere number day 2 (2 or 4 vs 3 or 5)	2.12	1.00-4.49	0.05	2.06	0.93-4.55	0.08
Blastomere number day 3 (8 vs 3-7)	2.55	1.57-4.14	<0.001	2.10	1.13-3.92	0.02
Fragmentation day 3 (≤10% vs >10%)	1.92	1.11-3.32	0.02	1.48	0.83-2.65	0.19
Multinucleation present (never vs ever)	1.68	0.79-3.60	0.18	1.56	0.70-3.46	0.28
Cleavage rate			0.02			0.07
>2 <3 vs 2	1.09	0.50-2.41	0.83	1.89	0.79-4.57	0.16
>1 <2 vs 2	0.52	0.31-0.87	0.01	0.85	0.44-1.62	0.61
≥ 3 vs 2	0.25	0.07-0.87	0.03	0.31	0.09-1.09	0.07
Patient characteristics						
female patient age (years)			0.38			
subfertility (primary or secondary)	0.96	0.60-1.52	0.85			
indication for IVF			0.65			
tubal vs unexplained	1.35	0.79-2.33	0.28			
tubal vs male factor	1.89	0.87-4.09	0.11			
tubal vs endometriosis	1.62	0.67-3.88	0.28			
tubal vs failed donor inseminations	1.26	0.32-4.93	0.74			
tubal versus cervical hostility	1.62	0.40-6.62	0.50			

Saldeen and Sundström, 2005; Holte *et al.*, 2007). It is possible that the endometrium after COH-IVF, being more advanced than after modified natural cycle, favours embryos with high number of blastomeres and vice versa, the endometrium after modified natural cycle IVF favours those with lower numbers. Finally, the number of blastomeres may vary with culture conditions (like the type of culture media used) and with timing of evaluation on day 2 and day 3. Therefore, the findings of our study cannot be generalized and comparisons with other centers are speculative.

Notwithstanding these explanations, it is also possible that in studies on embryos derived from COH cycles, a bias is introduced by selection of embryos for transfer leading to an underestimation of the implantation potential of embryos with low numbers of blastomeres on day 2. In most studies, implantation rates are evaluated in retrospect (Ziebe *et al.*, 1997; Saldeen and Sundström, 2005; Fauque *et al.*, 2007; Holte *et al.*, 2007) and in general, embryos with higher numbers of blastomeres are preferred for transfer. This can induce an underestimation of the implantation potential of embryos with lower numbers of blastomeres, since they are only chosen for transfer when no others are available. It is remarkable that in one study, where numbers of blastomeres on day 2 were noted but not used as a selection criterion for transfer, 2- and 4-cell embryos showed better implantation than others, which is in accordance with our findings (Scott *et al.*, 2007).

No influence on ongoing implantation was found for fragmentation up to 10%. This observation is in accordance with findings from others, suggesting that slight fragmentation is a normal phenomenon in human embryos (Van Royen *et al.*, 1999; Alikani *et al.*, 2000; Hardarson *et al.*, 2001; Van Royen *et al.*, 2001; Holte *et al.*, 2007).

The frequency of occurrence of multinucleation found in this study (13.6%) is in accordance with the frequency of 11.9-33.6% reported by others (Balakier and Cadesky, 1997; Jackson *et al.*, 1998; Alikani *et al.*, 2000; Van Royen *et al.*, 2003). It is well known that embryos containing multinucleated blastomeres often are chromosomally abnormal and show low blastocyst formation rate (Pickering *et al.*, 1995; Kligman *et al.*, 1996; Balakier and Cadesky, 1997; Staessen and Van Steirteghem, 1998; Alikani *et al.*, 2000; Meriano *et al.*, 2004; Yakin *et al.*, 2005). On the other hand, live births of healthy babies from embryos with MNBs are also reported (Balakier and Cadesky 1997; Jackson *et al.*, 1998; Pelinck *et al.*, 1998).

In our study, we found a compromised ongoing implantation rate in embryos with MNBs (14.8%). In other studies, reported ongoing implantation rates after transfer of embryos with multinucleated blastomeres are 3.3-13.0% (Balakier and Cadesky 1997; Jackson *et al.*, 1998; Pelinck *et al.*, 1998; Alikani *et al.*, 2000; Van Royen *et al.*, 2003) and in one study, an OR of 0.066 was found for implantation of embryos with MNB versus without (Van Royen *et al.*, 2001). It seems therefore that the implantation potential of embryos with MNBs found in our study is somewhat less severely impaired than expected. Obviously, no real comparison is possible due to differences in patient population and small numbers, but if a difference really exists, the higher implantation rate we found may be explained by the fact that in our study no selection of embryos for transfer was done, while in other studies embryos with MNBs were only transferred if no others were available.

An interesting finding from our study is that ongoing implantation rates seem to be less strongly influenced by the presence of MNBs and fragmentation than by number of

blastomeres. This suggests that in some specific cases, embryos with MNBs or fragmented embryos may have a larger implantation potential than sibling embryos without these characteristics and should be preferred for transfer.

In conclusion, in this series of unselected single embryos derived from modified natural cycle IVF, we described the exact fate of each distinct type of embryo and calculated the impact of individual embryo characteristics on implantation. Two or four blastomeres on day 2, eight on day 3, cleavage rate of 2-3, fragmentation $\leq$10% and absence of MNBs prove to be favourable characteristics. More than eight blastomeres on day 3 and accelerated cleavage rate ($\geq$3) appear unfavourable, whereas embryos with less than four blastomeres on day 2 do relatively well. This suggests that in currently used embryo scoring systems, the implantation potential of embryos with lower numbers of blastomeres on day 2 is underestimated. Furthermore, we found the implantation potential of embryos containing MNBs to be less severely impaired than expected. It is unclear however if the results of our study are applicable to embryos derived from COH cycles. Further research into this matter is warranted, but if the results of our study are confirmed, according changes in embryo transfer policy could be helpful in improvement of selection of the best embryo for transfer.

Chapter 9

Perinatal outcome in singletons born after modified natural cycle IVF and standard IVF with ovarian stimulation

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ABSTRACT

OBJECTIVE: Singletons born after IVF treatment are at risk for adverse pregnancy outcome, the cause of which is unknown. The aim of the present study was to investigate the influence of ovarian stimulation on perinatal outcome.

STUDY DESIGN: In this single-centre retrospective study, perinatal outcome of singleton pregnancies resulting from IVF treatment with (n=106) and without ovarian stimulation (n=84) were compared. For IVF without ovarian stimulation, a modified natural cycle protocol was used.

RESULTS: No differences were found in pregnancy duration, proportion of prematurity and proportion of low birth weight. Mean birth weight of modified natural cycle versus standard IVF singletons was 3485 ($\pm$ 527) grams vs 3218 ($\pm$ 670) grams; P=0.003. After adjustment for prognostic factors by linear regression analysis, the difference in birth weight remaining was 134 grams; P=0.045.

CONCLUSIONS: Birth weights of modified natural cycle IVF singletons found in this study are higher than standard IVF singletons, suggesting that ovarian stimulation may be a causative factor in the occurrence of low birth weight in standard IVF.

Introduction

Compared to spontaneous conceptions, singleton IVF pregnancies are associated with worse perinatal outcome (Helmerhorst *et al.*, 2004; Jackson *et al.*, 2004).

There are four possible explanations for the differences found in perinatal outcome of singletons born after IVF as compared to singletons born after spontaneous conception.

Firstly, the IVF laboratory procedure may have a negative influence on perinatal outcome (Jackson *et al.*, 2004).

Secondly, differences in maternal characteristics like age and parity or subfertility-related factors such as smoking or obesity may partly explain differences in neonatal outcome (Helmerhorst *et al.*, 2004). Subfertility itself may be associated with poor neonatal outcome, depending on the cause of subfertility (Pandian *et al.*, 2001; Thomson *et al.*, 2005; Romundstad *et al.*, 2008).

A third explanation for worse perinatal outcome in IVF pregnancies could be that ovarian stimulation may affect oocyte quality, or alternatively, cause diminished endometrial receptivity and poor implantation environment.

Finally, singleton pregnancies after stimulated IVF often occur after transfer of two embryos and in a number of cases are twin pregnancies spontaneously reduced to singletons. These so-called vanishing twins are reported to be associated with preterm delivery and low birth weight (Dickey *et al.*, 2002; Pinborg *et al.*, 2005; Pinborg *et al.*, 2007).

We investigated the influence of ovarian stimulation on perinatal outcome by comparing two groups of singleton IVF-pregnancies. The first group consisted of pregnancies resulting from modified natural cycle IVF, in which treatment is aimed at the use of the monofollicular cycle, without ovarian stimulation and with physiological estradiol levels during the follicular phase (Pelinck *et al.*, 2006; Pelinck *et al.*, 2007). The second group consisted of pregnancies resulting from standard IVF with controlled ovarian hyperstimulation (COH) and multifollicular growth.

Materials and Methods
Patient selection

The study group was formed by singleton pregnancies arising from modified natural cycle IVF (MNC-IVF), performed in a research setting, details of which have been described previously (Pelinck *et al.*, 2006; Pelinck *et al.*, 2007). Female patients aged 18-36 years, undergoing their first IVF treatment ever or first IVF treatment after a pregnancy (spontaneously conceived or obtained with COH-IVF), with regular and proven ovulatory menstrual cycles with a length of 26-35 days and body mass index (BMI: kg/m^2) of 18-28 were eligible for the MNC-IVF protocol. Indications for IVF were tubal pathology, unexplained subfertility, male factor, endometriosis, cervical factor or failed donor inseminations. The presence of an endometriosis cyst was an exclusion criterium. Only conventional IVF (no ICSI) was performed. All singleton pregnancies resulting from single or occasional double embryo transfers were included in the analysis. Pregnancies resulting from multifollicular cycles (> three dominant follicles at ovulation triggering) were excluded from the analysis since we considered these to be the result of ovarian stimulation.

Pregnancies in women in utero exposed to diethylstilbestrol (DES) were excluded, since these are at risk for adverse pregnancy outcome independent of IVF-treatment. Only pregnancies with duration of >24 weeks were included.

The control group was formed by COH-IVF singleton pregnancies from the same treatment period and with the same age limit (age <37 years at start of treatment). This group consisted of patients who either refrained from participation in the modified natural cycle study protocol or did not fit into the inclusion criteria (n=94) or who continued with COH-IVF after unsuccessful MNC-IVF (n=12). Only pregnancies resulting from fresh (single or double) embryo transfer after conventional IVF (no ICSI) were included. Again, pregnancies in women in utero exposed to DES were excluded and only pregnancies with duration >24 weeks were included.

Details on patient demographics, lifestyle habits and pregnancy outcome were collected by chart review and additionally by patient questionnaires. Gestational age was calculated as duration between oocyte retrieval and delivery plus fourteen days. Low birth weight and very low birth weight were defined as birth weight < 2500 gram and < 1500 gram, respectively. Preterm and very preterm delivery were defined as delivery before 37 and 32 completed gestational weeks, respectively. Small for gestational age and very small for gestational age were defined as <10th and <2.3^{d} percentile for gestational age.

IVF treatment protocols

Modified natural cycle IVF was performed as described previously (Pelinck *et al.,* 2006; Pelinck *et al.,* 2007). In short, cycles were monitored with ultrasound and serum oestradiol (E2) and LH measurements. Cetrorelix (0.25 mg / day: Cetrotide®, Serono, the Hague, the Netherlands) together with recombinant FSH (150 IU / day: Gonal-F®, Serono Benelux BV, the Netherlands) was started after follicular dominance had developed (size of the dominant follicle ≥ 14mm). Follicular aspiration was done 34 hours after ovulation triggering by 10 000 IU of HCG (Pregnyl®, Organon, Oss, the Netherlands) at a follicle size of at least 18 mm and/or plasma E2 levels of ≥ 1.06 nmol/L.

Conventional IVF was performed according to standard procedures. Embryo transfer was performed on the third day after oocyte retrieval. Luteal support consisted of HCG 1500 IU five, eight and eleven days after oocyte retrieval. In a small number of patients (n=3), menotrofine (Menopur®, Ferring Nederland BV, Hoofddorp, the Netherlands) was given for substitution instead of r-FSH, with a further identical protocol.

In COH-IVF, a flare-up or downregulation protocol was used with triptorelin (Decapeptyl®, Ferring Nederland BV, Hoofddorp, the Netherlands) or leuproreline (Lucrin®, Abbott BV, Hoofddorp, the Netherlands) and recombinant FSH (Puregon®, Organon, the Netherlands). Cycles were monitored with ultrasound. Ovulation was triggered by 10 000 units of HCG when at least half of the dominant follicles were > 18 mm. Follicular aspiration was performed 36 hours later under conscious sedation with fentanyl. Conventional IVF was performed according to standard procedures. Transfer of a maximum of two embryos was performed on the second or third day after oocyte retrieval. Luteal support consisted of progesterone vaginal suppositories (100 mg three times per day, started on the day of oocyte retrieval and continued until the day of pregnancy test) or HCG 1500 IU five, seven and nine days after oocyte retrieval.

Statistical analysis

We first performed a univariable analysis, using Chi square test for categorical outcome variables and Student-t test for continuous outcome variables. A *P*-value of < 0.05 was considered significant.

Linear regression was applied for the analysis of birth weight, with adjustment for prognostic factors. Factors entered in the model were pregnancy duration, pregnancy factors (hypertension and diabetes during pregnancy, infant sex), maternal lifestyle factors (smoking and alcohol consumption during pregnancy), maternal pre-pregnancy factors (age, height, BMI, ethnicity, level of education, parity (nulliparous versus multiparous), cause of subfertility, duration of subfertility) and paternal factors (height, ethnic background).

For the analyses, SPSS 12.0 (SPSS inc., Chicago, IL, USA) was used.

Results

From February 2001 to July 2004, 84 and 106 singleton pregnancies occurred after modified natural cycle IVF and COH-IVF, respectively. Of these, 12 occurred in patients who underwent COH-IVF after MNC-IVF had not been successful.

The mean number of oocytes obtained were 1.11 ± 0.31 and 10.6 ± 6.4 after MNC and COH-IVF, respectively. Peak E2 levels were 1.06 ± 0.47 and 8.25 ± 4.99 nmol/L after MNC and COH-IVF, respectively. In the COH group, a flare-up and downregulation protocol was used in 70 and 36 patients, respectively, with no difference in birth weight ($P = 0.25$) or other perinatal outcomes. In the COH-group, progesterone and HCG was given for luteal support in 42 and 64 patients, respectively, with no difference in birth weight ($P = 0.13$) or other perinatal outcomes. In the MNC-group, HCG was given for luteal support in all patients.

Of the MNC pregnancies, 81 occurred after single embryo transfer (SET) and three occurred after double embryo transfer (DET). Of the COH-IVF pregnancies, 14 occurred after SET, 91 after DET and one after the transfer of three embryos. Of these 106 pregnancies, fourteen were vanishing twins.

Baseline characteristics of all patients are shown in table 1. Except for duration of subfertility which was longer in the COH-IVF group ($P = 0.04$), we found no differences in patient characteristics.

Pregnancy characteristics according to treatment modality are shown in table 2. In the COH-IVF group, significantly more women smoked during pregnancy ($P = 0.02$). No other differences were found.

Birth weights in the MNC-IVF group were significantly higher than in the COH-IVF group (3485 vs 3218 grams: $P = 0.003$), whereas the proportions of low and very low birth weight and small for gestational age were not. Malformation rate, neonatal admission rate and perinatal mortality were also not different between groups (table 3).

In the COH-IVF group, fourteen pregnancies were vanishing twins. Mean birth weight and proportion of SGA and VSGA in these pregnancies were not significantly different from the 92 remaining singletons (3326 ± 472.2 vs 3202 ± 695.7 grams; $P = 0.52$; 0.0% and 0.0% vs 9.8% and 2.2%; $P = 0.39$). Mean duration of pregnancy and proportion

Table 1. Patient characteristics according to treatment modality

	MNC-IVF	n[a]	COH-IVF	n[a]	P[b]
maternal age (years)	33.0 (3.0)	84	33.5 (2.8)	106	0.22
maternal height (cm)	171.3 (5.7)	80	169.5 (6.9)	98	0.07
BMI (kg/m^2)	23.1 (3.3)	77	23.7 (3.6)	96	0.23
maternal ethnicity					
european/white	76 (95.0)	80	86 (87.8)	98	0.11
mediterranean	1 (1.3)		1 (1.0)		
asian	3 (3.8)		4 (4.1)		
other	0 (0.0)		7 (7.1)		
maternal level of education					
low	5 (6.3)	79	11 (11.2)	98	0.15
moderate	44 (55.7)		62 (63.3)		
high	30 (38.0)		25 (25.5)		
parity					
nulliparous	57 (67.9)	84	71 (67.0)	106	0.89
parous	27 (32.1)		35 (33.0)		
cause of subfertility					
tubal	23 (27.4)	84	30 (28.3)	106	0.12
male factor	12 (14.3)		20 (18.9)		
unexplained	34 (40.5)		26 (24.5)		
endometriosis	7 (8.3)		10 (9.4)		
cervix	4 (4.8)		5 (4.7)		
failed donor inseminations	4 (4.8)		9 (8.5)		
hormonal	0 (0.0)		6 (5.7)		
duration subfertility (months)	43.8 (23.3)	84	50.8 (23.5)	106	0.04
paternal height	184.6 (6.8)	75	183.3 (7.1)	89	0.22
paternal ethnicity					
european/white	76 (96.2)	79	85 (91.4)	93	0.41
mediterranean	0 (0.0)		1 (1.1)		
asian	3 (3.8)		5 (5.4)		
other	0 (0.0)		2 (2.2)		

numbers in parentheses are SD or percentages where applicable
[a]number of cases of which information was available
[b]Student-t test or Chi square where applicable

of preterm or very preterm birth were also not different between these groups (275 ± 11.1 vs 275 ± 14.8 days; $P = 0.93$; 7.1% and 0.0% vs 7.6% and 2.2%; $P = 0.85$).

Results of regression analysis

The crude difference in birth weight was 267 grams in favour of MNC-IVF ($P = 0.003$). After correction for pregnancy duration, pregnancy factors, lifestyle factors, and pre-pregnancy patient characteristics, the difference remaining was 134 grams in favour of MNC-IVF ($P = 0.045$; table 4). No significant interactions were found.

Table 2. Pregnancy characteristics according to treatment modality

	MNC-IVF	n[a]	COH-IVF	n[a]	p[b]
smoking during pregnancy					
yes	6 (7.5)	80	20 (20.4)	98	0.02
no	74 (92.5)		78 (79.6)		
alcohol use during pregnancy					
yes	3 (3.8)	80	5 (5.1)	98	0.67
no	77 (96.2)		93 (94.9)		
diabetes					
yes	2 (2.5)	79	0 (0.0)	99	0.11
no	77 (97.5)		99 (100.0)		
hypertension					
yes	6 (7.6)	79	7 (7.1)	99	0.89
no	73 (92.4)		92 (92.9)		
pregnancy duration (days)	276 (12.5)	84	275 (14.3)	106	0.48
pregnancy duration (weeks)					
> 37	78 (92.9)	84	96 (90.6)	106	0.44
32-37	6 (7.1)		8 (7.5)		
24-32	0 (0.0)		2 (1.9)		
induction of labour					
yes	15 (18.3)	82	17 (16.2)	105	0.71
no	67 (81.7)		88 (83.8)		
mode of delivery					
spontaneous vaginal	46 (54.8)	84	67 (65.0)	103	0.56
instrumental vaginal	18 (21.4)		17 (16.5)		
Caesarean section	20 (23.8)		19 (18.4)		

numbers in parentheses are SD or percentages where applicable
[a]number of cases of which information was available
[b]Student-t test or Chi square where applicable

Discussion

In this retrospective comparative cohort study, we found a difference in birth weight of 134 grams between singletons born after MNC-IVF and COH-IVF. Birth weights found in the MNC-IVF group were comparable to those after spontaneous conceptions in the Netherlands (3459 ± 16 grams; www.cbs.nl).

The difference in birth weight found in our study cannot be accounted for by IVF laboratory procedures, since they were the same in both groups.

Since laboratory procedures were the same in both groups and patient and pregnancy characteristics were adjusted for, the results of our study suggest that the lower birth weight in IVF children is related to the ovarian stimulation protocol.

By what mechanism ovarian stimulation would exert an influence on birth weight remains to be elucidated, but high estradiol levels associated with ovarian stimulation may play a causative role. This is suggested by findings from a study in which a negative corre-

Table 3. Neonatal outcome according to treatment modality

	MNC-IVF	n[a]	COH-IVF	n[a]	p[b]
infant sex					
male	39 (46.4)	84	51 (48.1)	106	0.82
female	45 (53.6)		55 (51.9)		
birth weight (grams)	3485 (526.6)	84	3218 (670.0)	106	0.003
birth weight (grams)					
> 2500	80 (95.2)	84	96 (90.6)	106	0.25
1500-2500	4 (4.8)		7 (6.6)		
< 1500	0 (0.0)		3 (2.8)		
birth weight (percentile)					
> P10	81 (96.4)	84	95 (89.6)	106	0.16
P2.3-P10 (SGA)	3 (3.6)		9 (8.5)		
< P2.3 (VSGA)	0 (0.0)		2 (1.9)		
congenital malformation					
yes	3 (3.8)[c]	80	6 (5.8)[d]	103	0.52
no	77 (96.3)		97 (94.2)		
neonatal admission					
yes	13 (16.0)	81	21 (20.2)	104	0.47
no	68 (84.0)		83 (79.8)		
perinatal mortality					
yes	0 (0.0)	84	2 (1.9)[e]	106	0.21
no	84 (100.0)		104 (98.1)		

numbers in parentheses are SD or percentages where applicable
[a]number of cases of which information was available
[b]Student-t test or Chi square where applicable
[c]Down syndrome, hip dysplasia, hypospadias
[d]Down syndrome, hip dysplasia, hypospadias (two cases), cleft lip, duodenal atresia
[e]one fetal death at 32 weeks gestational age and one neonatal death due to asphyxia

lation between estradiol levels and birth weight was found in stimulated IVF (Mitwally *et al.*, 2004). Also, in a recent study, ovarian hyperstimulation syndrome, which is associated with high estradiol levels, was shown to be associated with adverse outcome in pregnancies achieved through IVF (Chung *et al.*, 2006).

Whether the effect of ovarian stimulation is through an effect on endometrium, leading to diminished quality of implantation environment or through an effect on oocyte quality, or both, is unknown and difficult to distinguish. In children born after transfer of cryopreserved embryos compared to fresh embryo transfer higher birth weight and less prematurity is found by some (Bergh *et al.*, 1999; Källén *et al.*, 2005; Wang *et al.*, 2005) but not others (Wada *et al.*, 1994; Wennerholm *et al.*, 1997; Aytoz *et al.*, 1999). Since cryopreserved embryos, while resulting from ovarian stimulation, are usually transferred in unstimulated cycles, higher birth weights, if present, would be attributable to better quality implantation environment.

Table 4. Linear regression analysis of the relation between treatment modality and birth weight

Factors in the model	Difference in birth weight (grams), MNC-IVF vs COS-IVF	P
none	267	0.003
pregnancy duration	223	0.001
pregnancy duration and pregnancy factors (hypertension during pregnancy, diabetes during pregnancy, infant sex)	200	0.003
pregnancy duration, pregnancy factors and lifestyle factors (smoking and alcohol consumption during pregnancy)	157	0.018
pregnancy duration, pregnancy factors, lifestyle factors and maternal and paternal pre-pregnancy characteristics (maternal age, height, BMI, ethnicity, level of education, parity, cause of subfertility, duration of subfertility, paternal height and ethnicity)	134	0.045

Unfortunately, in most studies, cycle preparation before transfer of cryopreserved embryos was not specified according to pregnancy outcome (Wada *et al.,* 1994; Wennerholm *et al.,* 1997; Aytoz *et al.,* 1999; Bergh *et al.,* 1999; Källén *et al.,* 2005; Wang *et al.,* 2005). Also, since only part of cryopreserved embryos survive the thawing procedure, those surviving may have inherent better quality and growth potential, ultimately leading to higher birth weight.

There are several limitations to the present study. First, this is a retrospective study in which two unrelated cohorts are compared. Although differences in patient and pregnancy characteristics were adjusted for by regression analysis, it is possible that unknown factors have influenced the results.

Second, 11.3% of the patients in the control group underwent COH-IVF after MNC-IVF had failed. Although MNC-failures in some cases may be coincidental, this suggests that these patients' fertility potential may have been more strongly diminished as compared to the patients who did conceive with MNC-IVF. This may have negatively influenced perinatal outcome in the COH-group since several studies have shown a relation between diminished fertility potential and adverse pregnancy outcome (Ghazi *et al.,* 1991; Williams *et al.,* 1991; Olivennes *et al.,* 1993; Draper *et al.,* 1999; Pandian *et al.,* 2001; Basso *et al.,* 2003; Basso and Olsen, 2005; Thomson *et al.,* 2005; Romundstad *et al.,* 2008).

Third, in most COH-IVF cycles in our study two embryos were transferred, while in MNC-IVF in the majority of cases one single embryo was transferred. Patients conceiving a singleton after double embryo transfer may have more strongly diminished fertility than those conceiving twins. A poor pregnancy outcome in singletons after DET is also sug-

gested in a recent study comparing outcome of singleton pregnancies after single or double embryo transfer in assisted reproduction with COH. In this study, birth weight was higher and pregnancy duration was longer in the group of pregnancies occurring after SET (De Sutter *et al.*, 2006). Also, double embryo transfer often leads to vanishing twin pregnancies, which are associated with worse perinatal outcome (Dickey *et al.*, 2002; Pinborg *et al.*, 2005; Pinborg *et al.*, 2007). Contrary to results of recent studies, in our study, we found neither lower birth weights nor higher rate of prematurity in survivors from vanishing twin pregnancies, possibly due to small numbers.

The difference in preterm birth we found in our study was small and not significant (7.1% in the MNC-IVF group vs 9.4% in the COH-IVF group; p=0.55). These numbers seem to differ slightly from results from the meta-analysis of Helmerhorst *et al.*, where the proportion of preterm births found in singletons after assisted conception was 11.4% (Helmerhorst *et al.*, 2004). In studies reporting on pregnancy outcome after SET in COH-IVF and ICSI, preterm birth was found in 10.0-11.1% of cases, compared to 3.9-6.2% after spontaneous conceptions in fertile controls (De Neubourg *et al.*, 2006a; Poikkeus *et al.*, 2007). In studies comparing pregnancy outcome after SET and DET in COH-IVF and ICSI, results are conflicting. In one study, higher birth weight and lower preterm birth rate was found after SET (De Sutter *et al.*, 2006), while in another study, no differences were found (Poikkeus *et al.*, 2007). The preterm birth rate in our study may be somewhat underestimated due to exclusion of patients in utero exposed to DES and furthermore, numbers are relatively small, so no firm conclusion can be drawn on this matter.

Notwithstanding the limitations to the present study, the finding that birth weights of children born after modified natural cycle IVF are higher than those after COH-IVF as well as comparable to those born from natural conceptions, is clinically relevant since in general, birth weight reflects the health of newborns. The relation between low birth weight and development of cardiovascular and metabolic disease later in life has been well established in many studies (Barker 2002; Eriksson *et al.*, 2002; Gluckman *et al.*, 2004; De Boo and Harding 2006).

In conclusion, we found higher birth weights in singletons born after modified natural cycle IVF compared to COH-IVF, suggesting that ovarian stimulation may be a causative factor in the occurrence of low birth weight in COH-IVF children.

Chapter 10

General discussion

General discussion

The results of the studies in this thesis suggest that modified natural cycle (MNC) is a cost-effective alternative for COH-IVF and suitable for all indications for conventional IVF. The application of MNC-IVF preceding COH-IVF does not compromise overall success rate and is associated with a low multiple pregnancy rate.

However, these conclusions are based on a comparison with COH-IVF with application of double embryo transfer. In COH-IVF, applying elective single embryo transfer (ESET) substantially reduces the multiple pregnancy rate without compromising overall effectiveness (De Neubourg *et al.,* 2006b). In comparing cost-effectiveness of MNC-IVF and COH-IVF, the multiple pregnancy rate has a rather strong influence on results and therefore, if MNC-IVF is compared with COH-IVF with ESET, the cost-effectiveness ratio might change in favour of COH-IVF.

Still, when results are considered as cumulative pregnancy rates per patient starting treatment, the pregnancy rates after MNC-IVF are similar to those after COH-IVF. The rather low pregnancy rate per cycle of MNC-IVF is compensated by a shorter treatment duration and quicker succession of cycles, leading to acceptable cumulative pregnancy rates. Furthermore, MNC-IVF offers several advantages, being a low-risk and patient-friendly treatment modality.

MNC-IVF is a low-risk treatment for several reasons. The risk of OHSS is negligible. Our finding that birth weights are higher after MNC-IVF than after COH-IVF is potentially interesting in this respect, since higher birth weights reflect better health of newborns. The most important contribution to the low-risk profile of MNC-IVF is its associated very low multiple pregnancy rate, which is beneficial due to the risks and longterm consequences of multiple pregnancies (Fauser *et al.,* 2005). Obviously, multiple pregnancies are a consequence of embryo transfer policy and in COH-IVF can be prevented by performing single embryo transfer. Contrary to COH-IVF however, in MNC-IVF, usually one embryo is available. Therefore, no selection of embryos for transfer is necessary and the performance of a single instead of double embryo transfer is not dependent on the willingness of patients to do so. Patients' motivation to undergo single embryo seems to be dependent on its effectiveness compared to double embryo transfer (Twisk *et al.,* 2007), their knowledge of risks associated with twin pregnancies (Newton *et al.,* 2007), and reimbursement of treatment (Gerris, 2005).

MNC-IVF is a patient-friendly treatment modality due to little use of hormonal medication and therefore few side-effects, short duration of a treatment cycle, easy oocyte retrieval and the possibility of quick succession of treatment cycles. Unfortunately, formal studies on quality of life in MNC-IVF are lacking, but studies on natural cycle IVF without the use of GnRH-antagonists show that patients seem to prefer natural cycle IVF over stimulated IVF, based on a preference for simplicity and short duration of treatment cycles (Højgaard *et al.,* 2001) or anxiety for hormone injections (Pistorius *et al.,* 2006). On the other hand, the high drop-out rates found in our studies suggest that the burden for patients undergoing MNC-IVF is considerable.

The per cycle pregnancy rate in MNC-IVF is rather low due to considerable loss in every step of the procedure. Despite the use of a GnRH-antagonist, the number of can-

celled cycles due to untimely LH-rises and unexpected ovulations is disappointingly high. Compared to natural cycle IVF without the use of a GnRH-antagonist (chapter 2), the addition of a GnRH-antagonist to the natural cycle IVF protocol seems to decrease cancellation rates, but not to a great extent. This raises the question whether the apparently small decrease in cancellation rate in the MNC protocol justifies the inconvenience and costs of treatment with GnRH-antagonist and gonadotrophins. No studies comparing natural cycle IVF to MNC-IVF are available, and therefore, a study comparing MNC-IVF with natural cycle IVF, including a cost-effectiveness analysis, seems warranted. On the other hand, changes in the MNC protocol may reduce the number of LH rises and premature ovulations, thus raising effectiveness. A higher dose or more frequent administration of cetrorelix (or another GnRH-antagonist), ovulation triggering at a smaller follicle size or a smaller interval between HCG administration and oocyte retrieval could all be helpful in this respect. Another approach to the reduction of the number of premature ovulations is the use of indomethacin to prevent follicular rupture (Nargund et al., 2001). Furthermore, it is unclear why the dosage of cetrorelix as used in our studies is not as effective in preventing LH-rises and ovulations as expected (Duijkers et al., 1998), and elucidation of the mechanism by which unexpected LH rises and ovulations occur may contribute to appropriate adjustments to the MNC-protocol.

The success rate of oocyte retrieval in our studies was rather low. In all oocyte retrievals, a single lumen aspiration needle was used, and no flushing of the follicle was done. Flushing of the follicle may raise effectiveness of the oocyte retrieval but also will make the procedure more painful and time-consuming (Tan et al., 1992; Daya et al., 1995).

In-vitro fertilization rates are never 100%, and since usually one single oocyte is available in MNC-IVF, fertilization failure of one oocyte implies cancellation of the whole cycle. In our studies, only conventional IVF was done, and application of ICSI in cases of semen deficiency or fertilization failure in a preceding cycle may raise effectiveness. It should be kept in mind however, that fertilization failure or aberrant fertilization also occurs in about 20% of oocytes when ICSI is applied and compared to conventional IVF, the ICSI procedure is more labour-intensive and costly. Therefore, it does not seem feasible to apply ICSI in all cycles of MNC-IVF.

The considerable loss in every step of the procedure of MNC-IVF leads to a low embryo transfer rate. However, implantation rates found in our studies are good, and comparable to implantation rates found in COH-IVF. This is surprising since, other than in COH-IVF, no selection of the best-quality embryo was possible since in most cases only one was obtained. It may be that the oocyte from the dominant follicle represents the best-quality oocyte in a cohort of oocytes, leading to an embryo with good implantation potential, or alternatively, that ovarian stimulation is detrimental to oocyte quality. Another explanation for the good implantation rates found in our studies could be that the endometrium in a modified natural cycle is more receptive than in stimulated IVF (Devroey et al., 2004). Further research should clarify whether this is indeed the case and if so, how this is caused. Results of such studies in turn may lead to appropriate adjustments of stimulation protocols in COH-IVF. The results of our study on embryo characteristics suggest that the implantation potential of embryos showing certain characteristics may be under-

estimated in commonly used embryo scoring systems used in COH-IVF. Further research into this matter may contribute to improvement of selection of the best embryo for transfer in COH-IVF.

In our studies, we found cancellation of oocyte retrieval, fertilization failure and failure to reach embryo transfer to be repeating phenomena in subsequent cycles. This finding suggests the possibility of the construction of a prediction model estimating the chance of success in further cycles of MNC-IVF, based on performance in the first cycle. Such a model, in which patient characteristics should be included also, would make an individualized counselling on the number of modified natural cycles to be performed possible.

The results of the studies in this thesis suggest that MNC-IVF is a feasible treatment, to be performed prior to or instead of standard IVF. Before MNC-IVF can be introduced as a standard treatment modality however, its effectiveness relative to standard COH-IVF should be confirmed in a randomized trial. Such a trial should focus on the identification of patient categories for which MNC is especially beneficial as compared to standard IVF, and include not only a cost-effectiveness analysis but also study patients' preferences and quality of life. Furthermore, such a trial would make it possible to investigate whether the natural selection of one single oocyte is indeed associated with higher implantation rates compared to embryos obtained after ovarian stimulation. In this respect, a comparison with mild stimulation regimens would be relevant too. With mild stimulation, treatment is aimed at the development of a limited number of oocytes (Heijnen *et al.,* 2007; Nargund *et al.,* 2007). This approach has the same advantages as MNC-IVF, such as little medication use and short cycle duration, although to a lesser extent, but will be associated with a lower cancellation rate and higher embryo transfer rate compared to MNC-IVF. In such a comparison it should be investigated if mild stimulation has a negative effect on oocyte or endometrium quality, and if so, whether this effect is outweighed by a lower cancellation and higher embryo transfer rate.

The possible future acceptance of MNC-IVF as a standard treatment modality is dependent on adjustments to excisting reimbursement systems, focussing on full treatment instead of number of cycles to be reinbursed. A reimbursement system based on a maximum number of cycles to be performed inevitably leads to a need to maximize the per cycle success. A reimbursement system based on full treatment, consisting of MNC-IVF for a (individually determined) suitable number of cycles, followed by mild stimulation or COH-IVF if necessary, could be helpful in stimulating both patients and doctors to use MNC-IVF, and the associated risk reduction may in the end lead to a reduction in costs to society.

References

Aboulghar MA, Mansour RT, Serour GA, Amin YM, Sattar MA and Ramzy AM (1995) In vitro fertilization in a spontane-ous cycle: a successful simple protocol. J Obstet Gynaecol 21, 337-340.

Albano C, Smitz J, Camus M, Riethmuller-Winzen H, Van Steirteghem A and Devroey P (1997) Comparison of different doses of gonadotropin-releasing hormone antagonist Cetrorelix during controlled ovarian hyperstimulation. Fertil Steril 67, 917-922.

Alikani M, Calderon G, Tomkin G, Garrisi J, Kokot M, Cohen J (2000) Cleavage anoma-lies in early human embryos and survival after prolonged culture in-vitro. Hum Reprod 15, 2634-2643.

Al-Inany H and Aboulghar M (2002) GnRH antagonist in assisted reproduction: a Cochrane review. Hum Reprod 17, 874-885.

Andersen AN, Gianaroli L, Felberbaum R, de Mouzon J, Nygren KG; The European IVF-monitoring programme, European Society of Human Reproduction and Embryology (ESHRE) (2005) Assisted reproductive technology in Europe, 2001. Results genera-ted from European registers by ESHRE. Hum Reprod 20, 1158-1176.

Aytoz A, Van den Abbeel E, Bonduelle M, Camus M, Joris H, Van Steirteghem A, Devroey P (1999) Obstetric outcome of pregnancies after the transfer of cryopreserved and fresh embryos obtained by conventional in-vitro fertilization and intracytoplasmic sperm injection. Hum Reprod 14, 2619-2624.

Balakier H and Cadesky K (1997) The frequency and developmental capability of human embryos containing multinucleated blastomeres. Hum Reprod 12, 800-804.

Ballesteros A, Landeras J, Sagastegui CG, Molla M, Pérez-Cano I, Remohi J (2002) Natural cycle in poor responders. Fertil Steril 78 Suppl 1, P-108.

Bar-Hava I, Ferber A, Ashkenazi J, Dicker D, Ben-Rafael Z, Orvieto R (2000) Natural cycle in vitro fertilization in women aged over 44 years. Gynecol Endocrinol 14, 248-252.

Barker DJ (2002) Fetal programming of coronary heart disease. Trends Endocrinol Metab 13, 364-368.

Basir GS, O W, Ng EHY, Ho PC (2001) Morphometric analysis of peri-implantation endo-metrium in patients having excessively high oestradiol concentrations after ovarian stimulation. Hum Reprod 16, 435-440.

Bassil S, Godin PA, Donnez J (1999) Outcome of in-vitro fertilization through natural cycles in poor responders. Hum Reprod 14, 1262-1265.

Basso O, Baird DD (2003) Infertility and preterm delivery, birthweight and Caesarean sec-tion: a study within the Danish National Birth Cohort. Hum Reprod 18, 2478-2484.

References

Basso O, Olsen J (2005) Subfecundity and neonatal mortality: longitudinal study within the Danish national birth cohort. BMJ 330, 393-394.

Bauman R, Mihaljevic D, Kupesic S, Kurjak A (2002) In vitro fertilization in spontaneous cycles – our experience. Eur J Obstet Gynecol Reprod Biol 102, 184-187.

Beerendonk CCM, van Dop PA, Braat DDM, Merkus JMWM (1998) Ovarian hyperstimulation syndrome: facts and fallacies. Obstet Gynecol Surv 53, 439-449.

Ben-Chetrit A, Gotlieb L, Wong PY, Casper RF (1996) Ovarian response to recombinant human follicle-stimulating hormone in luteinizing hormone-depleted women: examination of the two cell, two gonadotropin theory. Fertil Steril 65, 711-717.

Bergh T, Ericson A, Hillensjö T, Nygren KG, Wennerholm UB (1999) Deliveries and children born after in-vitro fertilisation in Sweden 1982-95: a retrospective cohort study. Lancet 354, 1579-1585.

Biggers JD, Summers MC (2004) When to avoid creating surplus human embryos. Hum Reprod 19, 2457-2459.

Blake D, Proctor M, Johnson N, Olive D (2005) Cleavage stage versus blastocyst stage embryo transfer in assisted conception. Cochrane Database Syst Rev 4, CD002118.

Bourgain C, Devroey P (2003) The endometrium in stimulated cycles for IVF. Hum Reprod Update 9, 515-522.

Branigan EF, Estes MA (2000) Minimal stimulation IVF using clomiphene citrate and oral contraceptive pill pretreatment for LH suppression. Fertil Steril 73, 587-590.

Briggs AH, Wonderling DE, Mooney CZ (1997) Pulling cost-effectiveness analysis up by its bootstraps: a non-parametric approach to confidence interval estimation. Health Econ 6, 327-340.

Bungum L, Bungum M, Humaidan P, Andersen CY (2004) A strategy for treatment of couples with unexplained infertility who failed to conceive after intrauterine insemination. Reprod Biomed Online 8(5): 584-589.

Cahill DJ, Wardle PG, Harlow CR, Hull MGR (1996) Effect of progestogen therapy on follicular development, related hormone concentrations and fertilization in vitro in unstimulated cycles and unexplained and endometriosis-associated infertility. Hum Reprod 11, 647-650.

Callahan TL, Hall JE, Ettner SL, Christiansen CL, Greene MF, Crowley WF Jr (1994) The economic impact of multiple-gestation pregnancies and the contribution of assisted-reproduction techniques to their incidence. N Engl J Med 331, 244-249.

Castelo Branco A, Achour-Frydman N, Kadoch J, Fanchin R, Tachdjian G, Frydman R (2005) In vitro fertilization and embryo transfer in seminatural cycles for patients with ovarian aging. Fertil Steril 84, 875-880.

Centers for Disease Control (1995) Economic costs of birth defects and cerebral palsy – United States, 1992. Morb Mortal Wkly Rep 44, 694-699.

Child TJ, Henderson AM, Tan SL (2004) The desire for multiple pregnancy in male and female infertility patients. Hum Reprod 19, 558-561.

Chung K, Coutifaris C, Chalian R, Lin K, Ratcliffe SJ, Castelbaum AJ, Freedman MF, Barnhart KT (2006) Factors influencing adverse perinatal outcomes in pregnancies achieved through use of in vitro fertilization. Fertil Steril 86, 1634-1641.

Claman P, Domingo M, Garner P, Leader A, Spence JE (1993) Natural cycle in vitro fertilization-embryo transfer at the University of Ottawa: an inefficient therapy for tubal infertility. Fertil Steril 60, 298-302.

Daya S, Gunby J, Hughes EG, Collins JA, Sagle MA, YoungLai EV (1995) Natural cyles for in-vitro fertilization: cost-effectiveness analysis and factors influencing outcome. Hum Reprod 10, 1719-1724.

Daya S, Ledger W, Auray JP, Duru G, Silverberg K, Wikland M, Bouzayen R, Howles CM, Beresniak A (2001) Cost-effectiveness modelling of recombinant FSH versus urinary FSH in assisted reproduction techniques in the UK. Hum Reprod 16, 2563-2569.

De Boo HA, Harding JE (2006) The developmental origins of adult disease (Barker) hypothesis. Aust N Z J Obstet Gynaecol 46, 4-14.

De Jong D, Macklon NS, Fauser BCJM (2000) A pilot study involving minimal ovarian stimulation for in vitro fertilization: extending the 'follicle –stimulating hormone window' combined with the gonadotropin-releasing hormone antagonist cetrorelix. Fertil Steril 73, 1051-1054.

De Jong D, Eijkemans MJ, Beckers NG, Pruijsten RV, Fauser BC, Macklon NS (2002) The added value of embryo cryopreservation to cumulative ongoing pregnancy rates per IVF treatment: is cryopreservation worth the effort? J Assist Reprod Genet 19, 561-568.

Delvigne A, Rozenberg S (2002) Epidemiology and prevention of ovarian hyperstimulation syndrome (OHSS): a review. Hum Reprod Update 8, 559-577.

De Neubourg D, Gerris J (2006) What about the remaining twins since single-embryo transfer? How far can (should) we go? Hum Reprod 21, 843-846.

De Neuborg D, Gerris J, Mangelschots K, Van Royen E, Vercruyssen M, Steylemans A, Elseviers M (2006a) The obstetrical and neonatal outcome of babies born after single-embryo transfer in IVF/ICSI compares favourably to spontaneously conceived babies. Hum Reprod 21, 1041-1046.

De Neubourg D, Gerris J, van Royen E, Mangelschots K, Vercruyssen M (2006b) Impact of a restriction in the number of embryos transferred on the multiple pregnancy rate. Eur J Obstet Gynecol Reprod Biol 124, 212-215.

De Placido G, Wilding M, Strina I, Alviggi E, Alviggi C, Mollo A, Varicchio MT, Tolino A, Schiattarella C, Dale B (2002) High outcome predictability after IVF using a combined score for zygote and embryo morphology and growth rate. Hum Reprod 17, 2402-2409.

De Sutter P, Gerris J, Dhont M (2002) A health-economic decision-analytic model comparing double with single embryo transfer in IVF/ICSI. Hum Reprod 17, 2891-2896.

De Sutter P, Van der Elst J, Coetsier T and Dhont M (2003) Single embryo transfer and multiple pregnancy rate reduction in IVF/ICSI: a 5-year appraisal. Reprod Biomed Online 6, 464-469.

De Sutter P, Delbaere I, Gerris J, Verstraelen H, Goetgeluk S, Van der Elst J, Temmerman M, Dhont M (2006) Birthweight of singletons after assisted reproduction is higher after single- than after double-embryo transfer. Hum Reprod 21, 2633-2637.

References

DeVane GW, Gangrade BK, Wilson R, Loy RA (2000) Optimal pregnancy outcome in a minimal-stimulation in vitro fertilization program. Am J Obstet Gynecol 183, 309-315.

De Vries MJ, De Sutter P, Dhont M (1999) Prognostic factors in patients continuing in vitro fertilization or intracytoplasmic sperm injection treatment and dropouts. Fertil Steril 72, 674-678.

Devroey P, Bourgain C, Macklon NS, Fauser BCJM (2004) Reproductive biology and IVF: ovarian stimulation and endometrial recepticity. Trends Endocrinol Metab 15, 84-90.

Dickey RP, Taylor SN, Lu PY, Sartor BM, Storment JM, Rye PH, Pelletier WD, Zender JL, Matulich JM (2002) Spontaneous reduction of multiple pregnancy: incidence and effect on outcome. Am J Obstet Gynecol 186, 77-83.

Draper ES, Kurinczuk JJ, Abrams KR, Clarke M (1999) Assessment of seperate contributions to perinatal mortality of infertility history and treatment: a case-control analysis. Lancet 353, 1746-1749.

Duckitt K, Templeton AA (1998) Cancer in women with infertility. Curr Opin Obstet Gynecol 10, 199-203.

Duijkers IJM, Klipping C, Willemsen WNP, Krone D, Schneider E, Niebch G, Hermann R (1998) Single and multiple dose pharmacokinetics and pharmacodynamics of the gonadotrophin-releasing hormone antagonist Cetrorelix in healthy female volunteers. Hum Reprod 13, 2392-2398.

Dumoulin JC, Coonen E, Bras M, van Wissen LC, Ignoul-Vanvuchelen R, Bergers-Jansen JM, Derhaag JG, Geraedts JP, Evers JL (2000) Comparison of in-vitro development of embryos originating from either conventional in-vitro fertilization or intracytoplasmic sperm injection. Hum Reprod 15, 402-409.

Edwards RG, Lobo R, Bouchard P (1996) Time to revolutionize ovarian stimulation. Hum Reprod 11, 917-919.

Elchalal U, Schenker JG (1997) The pathophysiology of ovarian stimulation syndrome-views and ideas. Hum Reprod 12, 1129-1137.

Elizur SE, Aslan D, Shulman A, Weisz B, Bider D, Dor J (2005) Modified natural cycle using GnRH antagonist can be an optional treatment in poor responders undergoing IVF. J Assist Reprod Genet 22, 75-79.

Elizur SE, Lerner-Geva L, Levron J, Shulman A, Bider D, Dor J (2006) Cumulative birth rate following in vitro fertilization: study of 5310 cycles. Gynecol Endocrinol 22, 25-30.

Elster N and the Institute for Science, Law, and Technology Working Group on Reproductive Technology (2000) Less is more: the risks of multiple births. Fertil Steril 74, 617-623.

Eriksson JG, Forsén T, Tuomilehto J, Jaddoe VW, Osmond C, Barker DJ (2002) Effects of size at birth and childhood growth on the insulin resistance syndrome in elderly individuals. Diabetologia 45, 342-348.

ESHRE Campus Course Report (2001) Prevention of twin pregnancies after IVF/ICSI by single embryo transfer. Hum Reprod 16, 790-800.

Fahy UM, Cahill DJ, Wardle PG, Hull MGR (1995) In-vitro fertilization in completely natural cycles. Hum Reprod 10, 572-575.

Fauque P, Léandri R, Merlet F, Juillard JC, Epelboin S, Guibert J, Jouannet P, Patrat C (2007) Pregnancy outcome and live birth after IVF and ICSI according to embryo quality. J Assist Reprod Genet 24, 159-165.

Fauser BCJM, Devroey P, Yen SSC, Gosden R, Crowley WF Jr, Baird DT, Bouchard P (1999) Minimal ovarian stimulation for IVF: appraisal of potential benefits and drawbacks. Hum Reprod 14, 2681-2686.

Fauser BCJM, Bouchard P, Coelingh Bennink HJT, Collins JA, Devroey P, Evers JLH, van Steirteghem A (2002) Alternative approaches in IVF. Hum Reprod Update 8, 1-9.

Fauser BCJM, Devroey P, Macklon NS (2005) Multiple birth resulting from ovarian stimulation for subfertility treatment. Lancet 365, 1807-1816.

Feldman B, Levron J, Bider D, Shulman S, Shine S, Dor J (1998) Unstimulated cycles in IVF treatment of patients with low ovarian response. Fertil Steril 70 Suppl 1, S327.

Feldman B, Seidman DS, Levron J, Bider D, Shulman A, Shine S and Dor J (2001) In vitro fertilization following natural cycles in poor responders. Gynecol Endocrinol 15, 328-334.

Fiddelers AA, van Montfoort AP, Dirksen CD, Dumoulin JC, Land JA, Dunselman GA, Janssen JM, Severens JL, Evers JL (2006) Single versus double embryo transfer: cost-effectiveness analysis alongside a randomized clinical trial. Hum Reprod 21, 2090-2097.

Fisch JD, Rodriguez H, Ross R, Overby G, Sher G (2001) The Graduated Embryo Score (GES) predicts blastocyst formation and pregnancy rate from cleavage-stage embryos. Hum Reprod 16, 1970-1975.

Fossum GT, Davidson A, Paulson RJ (1989) Ovarian hyperstimulation inhibits embryo implantation in the mouse. J In Vitro Fertil Embryo Transfer 6, 7-10.

Foulot H, Ranoux C, Dubuisson JB, Rambaud D, Aubriot FX, Poirot C (1989) In vitro fertilization without ovarian stimulation: a simplified protocol applied in 80 cycles. Fertil Steril 52, 617-621.

Garceau L, Henderson J, Davis LJ, Petrou S, Henderson LR, McVeigh E, Barlow DH, Davidson LL (2002) Economic implications of assisted reproductive techniques: a systematic review. Hum Reprod 17, 3090-3109.

Garcia J, Jones GS, Acosta AA, Wright GL (1981) Corpus luteum function after follicle aspiration for oocyte retrieval. Fertil Steril 36, 565-572.

Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB (2000a) Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil Steril 73, 1155-1158.

Gardner DK, Lane M, Schoolcraft WB (2000b) Culture and transfer of viable blastocysts: a feasible proposition for human IVF. Hum Reprod 15 Suppl 6, 9-23.

Gerris J, De Neubourg D, Mangelschots K, Van Royen E, Van de Meerssche M, Valkenburg M (1999) Prevention of twin pregnancy after in-vitro fertilization or intracytoplasmic sperm injection based on strict embryo criteria: a prospective randomized clinical trial. Hum Reprod 14, 2581-2587.

Gerris J, Van Royen E (2000) Avoiding multiple pregnancies in ART: a plea for single embryo transfer. Hum Reprod 15, 1884-1888.

References

Gerris J, De Neubourg D, Mangelschots K, Van Royen E, Vercruyssen M, Barudy-Vasquez J, Valkenburg M, Ryckaert G (2002) Elective single day 3 embryo transfer halves the twinning rate without decrease in the ongoing pregnancy rate of an IVF/ICSI programme. Hum Reprod 17, 2626-2631.

Gerris J, De Sutter P, De Neubourg D, Van Royen E, Van der Elst J, Mangelschots K, Vercruyssen M, Kok P, Elseviers M, Annemans L, Pauwels P, Dhont M (2004) A real-life prospective health economic study of elective single embryo transfer versus two-embryo transfer in first IVF/ICSI cycles. Hum Reprod 19, 917-923.

Gerris JM (2005) Single embryo transfer and IVF/ICSI outcome: a balanced appraisal. Hum Reprod Update 11, 105-121.

Ghazi HA, Spielberger C, Kallen B (1991) Delivery outcome after infertility- a registry study. Fertil Steril 55, 726-732.

Gluckman PD, Hanson MA (2004) The developmental origins of the metabolic syndrome. Trends Endocrinol Metab 15, 183-187.

Goldfarb JM, Austin C, Lisbona H, Peskin B, Clapp M (1996) Cost-effectiveness of in vitro fertilization. Obstet Gynecol 87, 18-21.

Gordts S, Campo R, Puttemans P, Brosens I, Valkenburg M, Norre J, Renier M, Coeman D, Gordts S (2005) Belgian legislation and the effect of elective single embryo transfer on IVF outcome. Reprod Biomed Online 10, 436-441.

Goverde AJ, McDonnell J, Vermeiden JPW, Schats R, Rutten FFH, Schoemaker J (2000) Intrauterine insemination or in vitro fertilisation in idiopathic subfertility and male subfertility: a randomised trial and cost-effectiveness analysis. Lancet 355, 13-18.

Grobman WA, Milad MP, Stout J, Klock SC (2001) Patient perceptions of multiple gestations: an assessment of knowledge and risk aversion. Am J Obstet Gynecol 185, 920-924.

Groen H, Pelinck MJ, Hoek A, ten Vergert EM (2005) Cost-effectiveness of in vitro fertilisation (IVF) in the manipulated natural cycle versus conventional IVF. Ital J Public Health, 3 S1, 181.

Hardarson T, Hanson C, Sjögren A, Lundin K (2001) Human embryos with unevenly sized blastomeres have lower pregnancy and implantation rates: indications for aneuploidy and multinucleation. Hum Reprod 16, 313-318.

Heijnen EM, Macklon NS, Fauser BC (2004) What is the most relevant standard of success in assisted reproduction? The next step to improving outcomes of IVF: consider the whole treatment. Hum Reprod 19, 1936-1938.

Heijnen EM, Eijkemans MJ, De Klerk C, Polinder S, Beckers NG, Klinkert ER, Broekmans FJ, Passchier J, Te Velde ER, Macklon NS, Fauser BC (2007) A mild treatment strategy for in-vitro fertilisation: a randomised non-inferiority trial. Lancet 369 (9563), 743-749.

Helmerhorst FM, Perquin DAM, Donker D, Keirse MJNC (2004) Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. BMJ 328, 261-264.

Hershlag A, Paine T, Kvapil G, Feng H, Napolitano B (2002) In vitro fertilization-intracy toplasmic sperm injection split: an insemination method to prevent fertilization failure. Fertil Steril 77, 229-232.

Hillensjö T, Hammer M, Nilsson L, Olsson JH, Sjöblom P (1990) Natural cycle IVF: preliminary results and characteristics of 48 cycles with minimal monitoring. Hum Reprod 5 Suppl 1, 36.

Højgaard A, Ingerslev HJ, Dinesen J (2001) Friendly IVF: patient opinions. Hum Reprod 16, 1391-1396.

Holte J, Berglund L, Milton K, Garello C, Gennarelli G, Revelli A, Bergh T (2007) Construction of an evidence-based integrated morphology cleavage embryo score for implantation potential of embryos scored and transferred on day 2 after oocyte retrieval. Hum Reprod 22, 548-547.

Ingerslev HJ, Højgaard A, Hindkjær J, Kesmodel U (2001) A randomized study comparing IVF in the unstimulated cycle with IVF following clomiphene citrate. Hum Reprod 16, 696-702.

Insinga RP, Laessig RH, Hoffman GL (2002) Newborn screening with tandem mass spectrometry: examining its cost-effectiveness in the Wisconsin Newborn Screening Panel. J Pediatr 141, 524-531.

Jackson KV, Ginsburg ES, Hornstein MD, Rein MS, Clarke RN (1998) Multinucleation in normally fertilized embryos is associated with an accelerated ovulation induction response and lower implantation and pregnancy rates in in vitro fertilization-embryo transfer cycles. Fertil Steril 70, 60-66.

Jackson RA, Gibson KA, Wu YW, Croughan MS (2004) Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. Obstet Gynecol 103, 551-563.

Janssens RMJ, Lambalk CB, Vermeiden JPW, Schats R, Schoemaker J (2000) In-vitro fertilization in a spontaneous cycle: easy, cheap and realistic. Hum Reprod 15, 314-318.

Jaroudi K, Al-Hassan S, Al-Sufayan H, Al-Mayman H, Qeba M, Coskun S (2003) Intracytoplasmic sperm injection and conventional in vitro fertilization are complementary techniques in management of unexplained infertility. J Assist Reprod Genet 20, 377-381.

Jones HW, Schnorr JA (2001) Multiple pregnancies: a call for action. Fertil Steril 75, 11-17.

Kadoch J, Frydman N, Castelo Branco A, Lédéé Bataille N, Franchin R, Frydman R (2003) IVF-ET using naturally-selected dominant follicles for poor prognosis patients: a hypothesis. Fertil Steril 80 Suppl 3, P-199.

Källén B, Finnström O, Nygren KG, Olausson PO (2005). In vitro fertilization (IVF) in Sweden: infant outcome after different IVF fertilization methods. Fertil Steril 84, 611-617.

Kalra SK, Milad MP, Klock SC, Grobman WA (2003) Infertility patients and their partners: differences in the desire for twin gestations. Obstet Gynecol 102, 152-155.

Kaplan EL, Meier P (1958) Non-parametric estimation from incomplete observations. J Am Stat Assoc 53, 457-481.

Kauma S (1997) Discussant for 'Multiple pregnancy rate and embryo number transferred during in vitro fertilization.' Am J Obstet Gynecol 177, 355-356.

Keizer M, Schelling K, Pelinck M, Hoek A, Simons A, Heineman M (2005) Larger birth weight in singletons born after minimal stimulation IVF compared to singletons born after COH-IVF. Fertil Steril 84 Suppl 1, O-198.

References

Kjellberg A, Carlsson P, Bergh C (2006) Randomized single versus double embryo trans-
    fer: obstetric and paediatric outcome and cost-effectiveness analysis. Hum Reprod 21,
    210-216.
Kligman I, Benadiva C, Alikani M, Munné S (1996) The presence of multinucleated
    blastomeres in human embryos is correlated with chromosomal abnormalities. Hum
    Reprod 11, 1492-1498.
Kim SH, Kim CH, Suh CS, Moon SY, Lee JY, Chang YS (1996) Simultaneous program of
    natural-cycle in vitro fertilization and crypopreserved- thawed embryo transfer. J Ass
    Reprod Genet 13, 716-721.
Kolibianakis E, Zikopoulos K, Camus M, Tournaye H, Van Steirteghem A, Devroey P
    (2004) Modified natural cycle for IVF does not offer a realistic chance of parenthood
    in poor responders with high day 3 FSH levels, as a last resort prior to oocyte dona-
    tion. Hum Reprod 19, 2545-2549.
Kumar A, Benny P, Lenton EA, Cooke ID (1997) Retrograde tubal embryo transfer in natu-
    ral cycle in-vitro fertilization. Hum Reprod 12, 484-486.
Land JA, Courtar DA, Evers JL (1997) Patient dropout in an assisted reproductive techno-
    logy program: implications for pregnancy rates. Fertil Steril 68, 278-281.
Levy MJ, Gindoff P, Hall J, Stillman RJ (1991) The efficacy of natural versus stimulated
    cycle IVF-ET. Fertil Steril 56, Suppl, S15-16.
Lieberman B (1998) An embryo too many? Hum Reprod 13, 2664-2666.
Lindheim SR, Sauer MV, Francis MM, Macaso TM, Lobo RA, Paulson RJ (1996) The sig-
    nificance of elevated early follicular-phase follicle stimulating hormone (FSH) levels:
    observations in unstimulated in vitro fertilization cycles. J Ass Reprod Genet 13, 49-
    52.
Lindheim SR, Vidali A, Ditkoff E, Sauer MV (1997) Poor responders to ovarian hypersti-
    mulation may benefit from an attempt at natural-cycle oocyte retrieval. J Assist
    Reprod Genet 14, 174-176.
Lukassen HG, Kremer JA, Lindeman EJ, Braat DD, Wetzels AM (2003) A pilot study of
    the efficacy of intracytoplasmic sperm injection in a natural cycle. Fertil Steril 79,
    231-232.
Lukassen HG, Schönbeck Y, Adang EM, Braat DD, Zielhuis GA, Kremer JA (2004)
    Cost analysis of singleton versus twin pregnancies after in vitro fertilization. Fertil
    Steril 81, 1240-1246.
Lukassen HG, Braat DD, Wetzels AM, Zielhuis GA, Adang EM, Scheenjes E, Kremer JA
    (2005) Two cycles with single embryo transfer versus one cycle with double embryo
    transfer: a randomized controlled trial. Hum Reprod 20, 702-708.
Luke B, Bigger H, Leurgans S, Sietsema D (1996) The cost of prematurity: a case-control
    study of twins vs singletons. Am J Public Health 86, 809-814.
MacDougall MJ, Tan SL, Hall V, Balen A, Mason BA, Jacobs HS (1994) Comparison of
    natural with clomiphene citrate-stimulated cycles in in vitro fertilization: a prospecti-
    ve, randomized trial. Fertil Steril 61, 1052-1057.
Macklon NS, Fauser BCJM (2000) Regulation of follicle development and novel ap-
    proaches to ovarian stimulation for IVF. Hum Reprod Update 6, 307-312.

Martikainen H, Tiitinen A, Tomás C, Tapanainen J, Orava M, Tuomivaara L, Vilska S, Hyden-Granskog C, Hovatta O (2001) One versus two embryo transfer after IVF and ICSI: a randomized study. Hum Reprod 16, 1900-1903.

Mastenbroek S, Twisk M, van Echten-Arends J, Sikkema-Raddatz B, Korevaar JC, Verhoeve HR, Vogel NEA, Arts EGJM, de Vries JWA, Bossuyt PM, Buys CH, Heineman MJ, Repping S, van der Veen F (2007) In vitro fertilization with preimplantation genetic screening. NEJM 357, 9-17.

Meldrum DR, Rivier J, Garzo G, Wisot A, Stubbs C, Hamilton F (1994) Successful pregnancies with unstimulated cycle oocyte donation using an antagonist of gonadotropin-releasing hormone. Fertil Steril 61, 556-557.

Meriano J, Clark C, Cadesky K, Laskin CA (2004) Binucleated and micronucleated blastomeres in embryos derived from human assisted reproduction cycles. Reprod Biomed Online 9, 511-520.

Min JK, Breheny SA, MacLachlan V, Healy DL (2004) What is the most relevant standard of success in assisted reproduction? The singleton, term gestation, live birth rate per cycle initiated: the BESST endpoint for assisted reproduction. Hum Reprod 19, 3-7.

Mitwally MF, Abdel-Razeq SS, Suilivan M, Crickard K (2004). Estradiol production during controlled ovarian hyperstimulation correlates with the birth weight in pregnancies achieved after in vitro fertilization. Fertil Steril 82 Suppl 2, 50-51.

Monks NJ, Turner K, Hooper MAK, Kumar A, Verma S, Lenton EA (1993) Development of embryos from natural cycle in-vitro fertilization: impact of medium type and female infertility factors. Hum Reprod 8, 266-271.

Morgia F, Sbracia M, Schimberni M, Giallonardo A, Piscitelli C, Giannini P, Aragona C (2004) A controlled trial of natural cycle versus microdose gonadotropin-releasing hormone analog flare cycles in poor responders undergoing in vitro fertilization. Fertil Steril 81, 1542-1547.

Murray S, Shetty A, Rattray A, Taylor V, Bhattacharya S (2004) A randomized comparison of alternative methods of information provision on the acceptability of elective single embryo transfer. Hum Reprod 19, 911-916.

Nagy ZP, Janssenswillen C, Janssens R, De Vos A, Staessen C, Van de Velde H, Van Steirteghem AC (1998) Timing of oocyte activation, pronucleus formation and cleavage in humans after intracytoplasmic sperm injection (ICSI) with testicular spermatozoa and after ICSI or in-vitro fertilization on sibling oocytes with ejaculated spermatozoa. Hum Reprod 13, 1606-1612.

Nargund G, Waterstone J, Bland JM, Philips Z, Parsons J, Campbell S (2001) Cumulative conception and live birth rates in natural (unstimulated) IVF cycles. Hum Reprod 16, 259-262.

Nargund G, Fauser BC, Macklon NS, Ombelet W, Nygren K, Frydman R; for the Rotterdam ISMAAR Consensus Group on Terminology for Ovarian Stimulation for IVF (2007) The ISMAAR proposal on terminology for ovarian stimulation for IVF. Hum Reprod 22, 2801-2804.

Neumann PJ, Gharib SD, Weinstein MD (1994) The cost of a successful delivery with in-vitro fertilization. NEJM 331, 239-243.

References

Newton CR, McBride J, Feyles V, Tekpetey F, Power S (2007) Factors affecting patients'attitudes toward single- and multiple-embryo transfer. Fertil Steril 87, 269-278.

Ng EHY, Chui DKC, Tang OS, Lau EY, Yeung WS, Chung HP (2001) In vitro fertilizati on and embryo transfer during natural cycles. J Reprod Med 46, 95-99.

Nygren KG, Andersen AN (2001) Assisted reproductive technology in Europe, 1998. Results generated from European registers by ESHRE. Hum Reprod 16, 2459-2471.

Olivennes F, Rufat P, André B, Pourade A, Quiros MC, Frydman R (1993) The increased risk of complication observed in singleton pregnancies resulting from in-vitro fertilization (IVF) does not seem to be related to the IVF method itself. Hum Reprod 8, 1297-1300.

Olivennes F, Frydman R (1998) Friendly IVF: the way of the future? Hum Reprod 13, 1121-1124.

Olivennes F (2000) Avoiding multiple pregnancies in ART. Double trouble: yes a twin pregnancy is an adverse outcome. Hum Reprod 15, 1663-1665.

Olivius K, Friden B, Lundin K, Bergh C (2002) Cumulative probability of live birth after three in vitro fertilization/intracytoplasmic sperm injection cycles. Fertil Steril 77, 505-510.

Omland AK, Fedorsák P, Storeng R, Dale PO, Åbyholm T, Tanbo T (2001) Natural cycle IVF in unexplained, endometriosis-associated and tubal factor infertility. Hum Reprod 16, 2587-2592.

Ozturk O, Bhattacharya S, Templeton A (2001) Avoiding multiple pregnancies in ART: evaluation and implementation of new strategies. Hum Reprod 16, 1319-1321.

Palermo G, Joris H, Devroey P, Van Steirteghem AC (1992) Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 340 (8810): 17-18.

Pandian Z, Bhattacharya S, Templeton A (2001). Review of unexplained infertility and obstetric outcome: a 10 year review. Hum Reprod 16, 2593-2597.

Papanikolaou EG, Kolibianakis EM, Tournaye H, Venetis CA, Fatemi H, Tarlatzis B, Devroey P (2008) Live birth rates after transfer of equal number of blastocysts or cleavage-stage embryos in IVF. A systematic review and meta-analysis. Hum Reprod 23, 91-99.

Papiernik E (1983) Social cost of twin births. Acta Genet Med Gemellol 32, 105-111.

Paulson RJ, Sauer MV, Francis MM, Macaso TM, Lobo RA (1990) In vitro fertilization in unstimulated cycles: a clinical trial using hCG for timing of follicle aspiration. Obstet Gynecol 76, 788-791.

Paulson RJ, Sauer MV, Francis MM, Macaso TM, Lobo RA (1992) In vitro fertilization in unstimulated cycles: the University of Southern California experience. Fertil Steril 57, 290-293.

Paulson RJ, Sauer MV, Francis MM, Macaso T, Lobo RA (1994a) Factors affecting pregnancy success of human in-vitro fertilization in unstimulated cycles. Hum Reprod 9, 1571-1575.

Paulson RJ, Sauer MV, Lobo RA (1994b) Addition of a Gonadotropin Releasing Hormone (GnRH) antagonist and exogenous gonadotropins to unstimulated in vitro fertilization (IVF) cycles: physiologic observations and preliminary experience. J Ass Reprod Genet 11, 28-32.

Pelinck MJ, De Vos M, Dekens M, Van der Elst J, De Sutter P, Dhont M (1998) Embryos cultured in vitro with multinucleated blastomeres have poor implantation potential in human in-vitro fertilization and intracytoplasmic sperm injection. Hum Reprod 13, 960-963.

Pelinck MJ, Hoek A, Simons AHM, Heineman MJ (2002) Efficacy of natural cycle IVF: a review of the literature. Hum Reprod Update 8, 129-139.

Pelinck MJ, Vogel NEA, Hoek A, Arts EGJM, Simons AHM, Heineman MJ (2005) Minimal stimulation IVF with late follicular phase administration of the GnRH antagonist cetrorelix and concomitant substitution with recombinant FSH: a pilot study. Hum Reprod 20, 642-648.

Pelinck MJ, Vogel NEA, Hoek A, Simons AHM, Arts EGJM, Mochtar MH, Beemsterboer S, Hondelink MN, Heineman MJ (2006) Cumulative pregnancy rates after three cycles of minimal stimulation IVF and results according to subfertility diagnosis: a multicentre cohort study. Hum Reprod 21, 2375-2383.

Pelinck MJ, Vogel NE, Arts EG, Simons AH, Heineman MJ, Hoek A (2007) Cumulative pregnancy rates after a maximum of nine cycles of modified natural cycle IVF and analysis of patient drop-out: a cohort study. Hum Reprod 22, 2463-2470.

Pickering SJ, Taylor A, Johnson MH, Braude PR (1995) An analysis of multinucleated blastomere formation in human embryos. Hum Reprod 10, 1912-1922.

Pinborg A, Loft A, Schmidt L, Andersen AN (2003) Attitudes of IVF/ICSI-twin mothers towards twins and single embryo transfer. Hum Reprod 18, 621-627.

Pinborg A, Lidegaard O, la Cour Freiesleben N, Andersen AN (2005) Consequences of vanishing twins in IVF/ICSI pregnancies. Hum Reprod 20, 2821-2829.

Pinborg A, Lidegaard O, Freiesleben NC, Andersen AN (2007) Vanishing twins: a predictor of small-for-gestational age in IVF singletons. Hum Reprod 22, 2702-2714.

Pistorius EN, Adang EM, Stalmeier PF, Braat DD, Kremer JA (2006) Prospective patient and physician preferences for stimulation or no stimulation in IVF. Hum Fertil (Camb) 9, 209-216.

Poikkeus P, Gissler M, Unkila-Kallio L, Hyden-Granskrog C, Tiitinen A (2007) Obstetric and neonatal outcome after single embryo transfer. Hum Reprod 22, 1073-1079.

Porter M, Battacccharya S (2005) Investigation of staff and patients' opinions of a proposed trial of elective single embryo transfer. Hum Reprod 20, 2523-2530.

Ragni G, Vegetti W, Baroni E, Colomba M, Arnoldi M, Lombroso G, Crosignani G (2001) Comparison of luteal phase profile in gonadotrophin stimulated cycles with or without a gonadotrophin-releasing hormone antagonist. Hum Reprod 16, 2258-2262.

Ramsewak SS, Kumar A, Welsby R, Mowforth A, Lenton EA, Cooke ID (1990) Is analgesia required for transvaginal single-follicle aspiration in in vitro fertilization? A double-blind study. J In Vitro Fert Embryo Transf 7, 103-106.

References

Reljic M, Vlaisavljevic V (1999) The preovulatory serum estradiol pattern in natural IVF/ICSI cycles. J Ass Reprod Genet 16, 535-539.

Reljic M, Vlaisavljevic V, Gavric V, Kovacic B, Cizek-Sajko M (2001) Value of the serum estradiol level on the day of human chorionic gonadotropin injection and on the day after in predicting the outcome in natural in vitro fertilization/intracytoplasmic sperm injection cycles. Fertil Steril 75, 539-543.

Roest J, van Heusden AM, Zeilmaker GH, Verhoeff A (1998) Cumulative pregnancy rates and selective drop-out of patients in in-vitro fertilization treatment. Hum Reprod 13, 339-341.

Romundstad LB, Romundstad PR, Sunde A, von Düring V, Skjaerven R, Gunnell D, Vatten LJ (2008) Effects of technology or maternal factors on perinatal outcome after assisted fertilisation: a population-based cohort study. Lancet 372 (9640): 694-695.

Rongières-Bertrand C, Olivennes F, Righini C, Fanchin R, Taïeb J, Hamamah S, Bouchard P, Frydman R (1999) Revival of the natural cycles in in-vitro fertilization with the use of a new gonadotrophin-releasing hormone antagonist (Cetrorelix): a pilot study with minimal stimulation. Hum Reprod 14, 683-688.

Ryan GL, Sparks AE, Sipe CS, Syrop CH, Dokras A, Van Voorhis BJ (2007) A mandatory single blastocyst transfer policy with educational campaign in a United States IVF program reduces multiple gestation rates without sacrificing pregnancy rates. Fertil Steril 88, 354-360.

Saldeen, Sundström (2005) Nuclear status of four-cell preembryos predicts implantation potential in in vitro fertilization treatment cycles. Fertil Steril 84, 584-589.

Scott L (2003) The biological basis of non-invasive strategies for selection of human oocytes and embryos. Hum Reprod Update 9, 237-249.

Scott L, Finn A, O'Leary T, McLellan S, Hill J (2007) Morphologic parameters of early cleavage-stage embryos that correlate with fetal development and delivery: prospective and applied data for increased pregnancy rates. Hum Reprod 22, 230-240.

Seibel MM, Kearnan M, Kiessling A (1995) Parameters that predict success for natural cycle in vitro fertilization-embryo transfer. Fertil Steril 63, 1251-1254.

Sharma V, Allgar V, Rajkhowa M (2002) Factors influencing the cumulative conception rate and discontinuation of in vitro fertilization treatment for infertility. Fertil Steril 78, 40-46.

Simón C, Garcia Velasco JJ, Valbueña D, Peinado JA, Moreno C, Remohí J, Pellicer A (1998) Increasing uterine receptivity by decreasing estradiol levels during the pre-implantation period in high responders with the use of a follicle-stimulating hormone step-down regimen. Fertil Steril 70, 234-239.

Sjöblom P, Menezes J, Cummins L, Mathiyalagan B, Costello MF (2006) Prediction of embryo developmental potential and pregnancy based on early stage morphological characteristics. Fertil Steril 86, 848-861.

Sophonsritsuk A, Choktanasiri W, Weerakiet S, Rojanasakul A (2005) Comparison of outcomes and direct cost between minimal stimulation and conventional protocols on ovarian stimulation in in vitro fertilization. J Obstet Gynaecol Res 31, 459-463.

Staessen C, Van Steirteghem (1998) The genetic constitution of multinuclear blastomeres and their derivative daughter blastomeres. Hum Reprod 13, 1625-1631.

Staessen C, Platteau P, Van Assche E, Michiels A, Tournaye H, Camus M, Devroey P, Liebaers I, Van Steirteghem A (2004) Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. Hum Reprod 19, 2849-2858.

Steptoe, Edwards (1978) Birth after the preimplantation of a human embryo. Lancet 2, 366.

Stolwijk AM, Wetzels AM, Braat DD (2000) Cumulative probability of achieving an ongoing pregnancy after in-vitro fertilization and intracytoplasmic sperm injection according to a woman's age, subfertility diagnosis and primary or secondary subfertility. Hum Reprod 15, 203-209.

Svalander P, Green B, Haglund K, Hagström J, Lindstedt J, Marsk L, Nygren KG, Stefenson M, Töcksberg K, Törnblad AM (1991) Natural versus stimulated cycles in IVF-ET treatment for tubal factor infertility. Hum Reprod 6 Suppl 1, 101-102.

Sullivan MW, Stewart-Akers A, Krasnow JS, Berga SL, Zeleznik AJ (1999) Ovarian responses in women to recombinant follicle-stimulating hormone and luteinizing hormone (LH): a role for LH in the final stages of follicular maturation. J Clin Endocrinol Metab 84, 228-232.

Takeuchi S, Minoura H, Shibahara T, Shen X, Futamura N, Toyoda N (2000) In vitro fertilization and intracytoplasmic sperm injection for couples with unexplained infertility after failed direct intraperitoneal insemination. J Assist Reprod Genet 17, 515-520.

Tan SL, Waterstone J, Wren M, Parsons J (1992) A prospective randomized study comparing aspiration only with aspiration and flushing for transvaginal ultrasound-directed oocyte recovery. Fertil Steril 58, 356-360.

Tartlatzis BC, Zepiridis L, Grimbizis G, Bontis J (2003) Clinical management of low ovarian response to stimulation for IVF: a systematic review. Hum Reprod Update 9, 61-76.

Templeton, A (2000) Avoiding multiple pregnancies in ART: Replace as many embryos as you like - one at a time. Hum Reprod 15, 1662.

Thomson F, Shanbhag S, Templeton A, Bhattacharya S (2005) Obstetric outcome in women with subfertility. BJOG 112, 632-637.

Thurin A, Hausken J, Hillensjö T, Jablonowska B, Pinborg A, Strandell A, Bergh C (2004) Elective single-embryo transfer versus double-embryo transfer in in vitro fertilization. NEJM 351, 2392-2402.

Tiitinen A, Halttunen M, Härkki P, Vuoristo P, Hyden-Granskog C (2001) Elective single embryo transfer: the value of cryopreservation. Hum Reprod 16, 1140-1144.

Tiitinen A, Unkila-Kallio L, Halttunen M, Hyden-Granskog C (2003) Impact of elective single embryo transfer on the twin pregnancy rate. Hum Reprod 18, 1449-1453.

Tomazevic T, Gersak K, Meden-Vrtovec H, Pompe Tansek M, Kermavner L, Veble A, Valentincic B, Virant I, Zemva Z, Mivsek J (1996) Second versus fourth day embryo transfer in the natural IVF cycle. Hum Reprod 12 Suppl 1, 87-88.

Tomazevic T, Gersak K, Meden-Vrtovec H, Drobnic S, Veble A, Valentincic B, Kermavner-Bacer L, Virant-Klun I, Zemva Z (1999) Clinical parameters to predict the success of in vitro fertilization-embryo transfer in the natural cycle. Assist Reprod 9, 149-156.

Turner K, Martin KL, Woodward BJ, Lenton EA, Leese HJ (1994) Comparison of pyruvate uptake by embryos derived from conception and non-conception natural cycles. Hum Reprod 9, 2362-2366.

Twisk M, Mastenbroek S, van Wely M, Heineman MJ, Van der Veen F, Repping S (2006) Preimplantation genetic screening for abnormal number of chromosomes (aneuploidies) in in vitro fertilisation or intracytoplasmic sperm injection. Cochrane Database Syst Rev 25, CD005291.

Twisk M, van der Veen F, Repping S, Heineman MJ, Korevaar JC, Bossuyt PM (2007) Preferences of subfertile women regarding elective single embryo transfer: additional in vitro fertilization cycles are acceptable, lower pregnancy rates are not. Fertil Steril 88, 1006-1009.

Ubaldi F, Rienzi L, Baroni E, Ferrero S, Iacobelli M, Minasi M, Martinez F, Greco E (2003) GnRH antagonist and natural ICSI cycle in poor responder patients. Hum Reprod 18 Suppl 1, P-327.

Ubaldi FM, Rienzi L, Ferrero S, Baroni E, Sapienza F, Cobellis L, Greco E (2005) Management of poor responders in IVF. Reprod Biomed Online 10, 235-246.

Ueno J, Oehninger S, Brzyski RG, Acosta AA, Philput CB, Muasher SJ (1991) Ultrasonographic appearance of the endometrium in natural and stimulated in-vitro fertilization cycles and its correlation with outcome. Hum Reprod 6, 901-904.

Vail A, Gardener E (2003) Common statistical errors in the design and analysis of subfertility trials. Hum Reprod 18, 1000-1004.

Van Montfoort AP, Fiddelers AA, Janssen JM, Derhaag JG, Dirksen CD, Dunselman GA, Land JA, Geraedts JP, Evers JL, Dumoulin JC (2006) In unselected patients, elective single embryo transfer prevents all multiples, but results in significantly lower pregnancy rates compared with double embryo transfer: a randomized controlled trial. Hum Reprod 21, 338-343.

Van Royen E, Mangelschots K, De Neubourg D, Valkenburg M, Van de Meerssche M, Ryckaert G, Eestermans W, Gerris J (1999) Characterization of a top quality embryo, a step towards single-embryo transfer. Hum Reprod 14, 2345-2349.

Van Royen E, Mangelschots K, De Neubourg D, Laureys I, Ryckaert G, Gerris J (2001) Calculating the implantation potential of day 3 embryos in women younger than 38 years of age: a new model. Hum Reprod 16, 326-332.

Van Royen E, Mangelschots K, Vercruyssen M, De Neubourg D, Valkenburg M, Ryckaert G, Gerris J (2003) Multinucleation in cleavage stage embryos. Hum Reprod 18, 1062-1069.

Van Voorhis BJ, Stovall DW, Allen BD, Syrop CH (1998) Cost-effective treatment of the infertile couple. Fertil Steril 70, 995-1005.

Vilska S, Tiitinen A, Hydén-Granskog C, Hovatta A (1999) Elective transfer of one embryo results in an acceptable pregnancy rate and eliminates the risk of multiple birth. Hum Reprod 14, 2392-2395.

Vogel NEA, Pelinck MJ, Arts EGJM, Hoek A, Simons AHM, Heineman MJ (2003) Effectiveness of the modified natural cycle ICSI: results of a pilot study. Fertil Steril 80 Suppl 3, P-7.

Wada I, Macnamee MC, Wick K, Bradfield JM, Brinsden PR (1994) Birth characteristics and perinatal outcome of babies conceived from cryopreserved embryos. Hum Reprod 9, 543-546.

Walker MC, Murphy KE, Pan S, Yang Q, Wen SW (2004) Adverse maternal outcomes in multifetal pregnancies. BJOG 111, 1294-1296.

Wang YA, Sullivan EA, Black D, Dean J, Bryant J, Chapman M (2005) Preterm birth and low birth weight after assisted reproductive technology-related pregnancy in Australia between 1996 and 2000. Fertil Steril 83, 1650-1658.

Weghofer A, Margreiter M, Bassim S, Sevelda U, Beilhack E, Feichtinger W (2004) Minimal stimulation using recombinant follicle-stimulating hormone and a gonado-tropin-releasing hormone antagonist in women of advanced age. Fertil Steril 81, 1002-1006.

Wennerholm UB, Hamberger L, Nilsson L, Wennergren M, Wikland M, Bergh C (1997) Obstetric and perinatal outcome of children conceived from cryopreserved embryos. Hum Reprod 12, 1819-1825.

Williams MA, Goldman MB, Mittendorf R, Monson RR (1991) Subfertility and the risk of low birth weight. Fertil Steril 56, 668-671.

Witsenburg C, Dieben S, Van der Westerlaken L, Verburg H, Naaktgeboren N (2005) Cumulative live birth rates in cohorts of patients treated with in vitro fertilization or intracytoplasmic sperm injection. Fertil Steril 84, 99-107.

Wølner-Hanssen P, Rydhstroem H (1998) Cost-effectiveness analysis of in-vitro fertiliza-tion: estimated costs per successful pregnancy after transfer of one or two embryos. Hum Reprod 13, 88-94.

Yakin K, Balaban B, Urman B (2005) Impact of the presence of one or more multi-nucleated blastomeres on the developmental potential of the embryo to the blastocyst stage. Fertil Steril 83, 243-245.

Zayed F, Lenton EA, Cooke ID (1997) Natural cycle in-vitro fertilization in couples with unexplained infertility: impact of various factors on outcome. Hum Reprod 12, 2402-2407.

Zhioua F, Zhioua A, Chaker A, M'Solly S, Meriah S (2004) Efficacy of intracytoplasmic sperm injection in a natural cycle with GnRH antagonists. Hum Reprod 19 Suppl 1, P-299.

Ziebe S, Petersen K, Lindenberg S, Andersen AG, Gabrielsen A, Nyboe Andersen A (1997) Embryo morphology or cleavage stage: how to select the best embryo for transfer after in-vitro fertilization. Hum Reprod 12, 1545-1549.

Summary

Chapter 1 provides a historical overview of developments in IVF treatment and discusses the advantages of modified natural cycle IVF.

Chapter 2 provides a systematic literature review on the efficacy of natural cycle IVF without the use of GnRH-antagonists. Twenty studies were selected. Results per study varied greatly, due to differences in patient selection and intensity of monitoring. The 20 selected studies comprised a total of 1800 cycles of natural cycle IVF, resulting in 28.9% cancellations of oocyte retrieval and 45.5% embryo transfers per started cycle. The ongoing pregnancy rate was 7.2% per started cycle and 15.8% per embryo transfer.

In ***chapter 3***, a cohort of 50 patients is described who were offered a maximum of three consecutive cycles of MNC-IVF. Included in this study were patients aged 18-36 years, with a regular and proven ovulatory cycle and indication for conventional IVF (no ICSI). The GnRH antagonist cetrorelix was given in the late follicular phase of the natural, unstimulated cycle, together with r-FSH for substitution. In cases where at the time of planned oocyte retrieval, ovulation had occurred, IUI was performed.

In this pilot study, a 14.3% ongoing pregnancy rate per started cycle was found, with a twin pregnancy rate of 5.9%. The cumulative ongoing pregnancy rate after three cycles was 34.0% per patient. The number of embryo transfers was 43.7% per started cycle. Despite the correct use of cetrorelix, an LH surge (> 10 IU/l) was found in 11.8% of started cycles. In two-thirds of these cycles however, oocyte retrieval was performed as planned. It was concluded that in some but not all cases, cetrorelix is capable of blunting the LH surge enough to allow for planned oocyte retrieval. Overall, 4.2% of started cycles were cancelled because ovulation had occurred at the time of planned oocyte retrieval. In these cycles, LH-levels at ovulation triggering were significantly higher compared to cycles where oocyte retrieval was performed.

A remarkable finding in this study was that in two cycles, multifollicular growth (> two co-dominant follicles) occurred, although only one dominant follicle (≥ 14mm) was present when medication was started. Therefore, apparently, in rare cases, the administration of r-FSH can lead to ovarian stimulation, even when it is started after presumed follicular dominance at a follicle size of 14 mm. An alternative explanation is that an ovarian cyst was mistaken for a dominant follicle and follicular dominance had not yet developed in these cases.

The ongoing implantation rate found in this study was rather high (30.9% per transferred embryo), which is surprising since no selection of embryos for transfer was done. This may be caused by the inclusion of good-prognosis patients in this study but may also be explained by a better endometrial receptivity due to the lack of ovarian stimulation. It is also possible that the oocyte from the (naturally selected) dominant follicle represents

the best-quality oocyte in a cohort of oocytes, leading to an embryo with good implantation potential, or that ovarian stimulation is detrimental to oocyte quality, so that MNC-embryos have better quality than COH-embryos.

In *chapter 4*, an extension of the cohort described in chapter 3, with identical inclusion criteria and treatment protocol, is presented. In this multicentre study, in which four clinics participated, a total of 350 patients were included and pregnancy rate per started cycle, cumulative pregnancy rates after three cycles and results according to indication for IVF were evaluated.

In this study, a total of 844 MNC-cycles were started (2.4 per included patient), leading to 317 embryo transfers (37.6% per started cycle). Again, in rare cases, multifollicular growth developed. Overall, in 149 cycles (17.7%), no oocyte retrieval was performed, in 108 of these because of LH rise or ovulation. The ongoing pregnancy rate found in this study was 8.3% per started cycle, with 4.3% twins. Cumulative ongoing pregnancy rate after three cycles was 20.8% per patient.

Pregnancy rates and live birth rates were not significantly different between indications for IVF. No significant differences were found in results according to cycle number. However, events leading to an unsuccessful cycle, i.e. cancellation of oocyte retrieval due to untimely LH rise or ovulation, unsuccessful oocyte retrieval and fertilization failure, all proved to have a tendency to occur again in further cycles of the same patients. Based on this finding we concluded that patient counselling on the number of MNC cycles to be performed should be individualized, taking into account the performance in previous cycles.

A study in which nine cycles of MNC-IVF were offered to 268 patients, the first three cycles of which have been described in the study mentioned in chapter 4, is presented in *chapter 5*. The aim of this study was to evaluate what would be the optimal number of cycles to be offered to patients, and to identify a maximum number above which MNC-IVF becomes less effective. For this purpose, the pregnancy rate according to cycle number was calculated, and drop-out behaviour of patients was studied.

A total of 256 patients completed 1048 cycles (4.1 per patient). Embryo transfer rate was 36.5% per started cycle. Pregnancy rate was 9.9% per started cycle, of which 7.9% ongoing. Including treatment-independent pregnancies, the observed CPR after up to nine cycles was 44.4% per patient, with 2.9% twins.

Drop-out rates were high (overall 47.8%). We found cancellation of oocyte retrieval, fertilization failure and failure to reach embryo transfer to be repeating phenomena in subsequent cycles and furthermore, that these events predispose for drop-out.

Pregnancy rate per started cycle did not decline in higher cycle numbers, and we concluded that this is possibly due to selective drop-out of poor prognosis patients. No maximum number of cycles above which MNC-IVF becomes less effective was identified. Therefore, again, we concluded that patient counselling on the number of cycles to be performed should be individualized, taking into account the results of previous cycles.

Chapter 6 provides follow-up concerning further treatment of patients described in chapter 5. Results of full treatment, consisting of MNC-IVF followed by COH-IVF are given.

We found fertilization failure in MNC to be predictive of fertilization failure in COH and concluded that performing ICSI in patients showing fertilization failure in MNC-IVF probably would improve success rates.

Of 268 patients included in the study, 9 did not undergo any treatment, 147 underwent MNC-IVF only, three underwent COH-IVF only and 109 underwent both MNC and COH-IVF.

Including treatment-independent pregnancies, observed COPR after sequential treatment with MNC-IVF followed by COH-IVF was 56.7% per patient, of which 6.6% were twins. The median time of treatment was 34.1 (0-220) weeks for all patients included in the study. The median time to ongoing pregnancy was 28.8 (0-220) weeks.

From these data we concluded that sequential treatment with MNC-IVF followed by COH-IVF does not appear to compromise overall success rates, while twin pregnancy rate is very low and that the application of sequential treatment with MNC-IVF followed by COH-IVF if necessary, is a realistic strategy. This way, not only a large proportion of patients is spared the risk of OHSS and the discomfort of COH, but also the overall multiple pregnancy rate is substantially reduced.

In *chapter 7,* a comparison is made between cost-effectiveness of MNC-IVF and COH-IVF. For this analysis, all IVF-procedure-related costs, pregnancy-related costs and neonatal costs (up to six weeks after delivery) were included. For COH-IVF patients, costs of OHSS and effects of cycles with cryopreserved embryos were not taken into account.

Average costs per treatment cycle were 2.25 times higher for COH-IVF compared to MNC-IVF (largely caused by higher medication costs), while ongoing pregnancy rates were 2.37 times higher. When including costs of pregnancy and delivery, which were higher for COH-IVF due to a larger proportion of twin pregnancies, the costs per cycle were up to 2.5-fold higher for COH-IVF compared to MNC-IVF. The ratios of costs and birth rate were largely similar for MNC-IVF and COH-IVF.

Our data suggest that MNC-IVF may be a cost-effective alternative for COH-IVF, since its lower pregnancy rate is compensated by lower costs.

In *chapter 8,* a series of 449 unselected single embryos derived from MNC-IVF is studied. Morphological characteristics of these embryos and their according implantation rates are described.

The best implantation was found in embryos with ≤ 10% fragmentation, absence of multinucleated blastomeres, four cells on day 2 after oocyte retrieval and eight cells on day 3.
These characteristics correspond exactly with what is theoretically expected and generally accepted to be the best embryo.

In contrast with studies on embryos derived from COH-IVF we found embryos with less than four blastomeres on day 2 to do relatively well and furthermore, we found the implantation potential of embryos containing multinucleated blastomeres to be less severely impaired than expected.

It is possible that implantation behaviour according to morphological characteristics is actually different in embryos derived from MNC-IVF and COH-IVF, but it is also possible that in studies on embryos derived from COH cycles, the implantation potential of

embryos with low numbers of blastomeres on day 2, as well as embryos containing multi-nucleated blastomeres is underestimated. This underestimation could be caused by the selection of embryos for transfer since in general, these embryos are only chosen for transfer if no others are available.

We concluded that if our findings are confirmed in further studies, according changes in embryo scoring systems could be helpful in improvement of selection of the best embryo for transfer.

In *chapter 9,* perinatal outcome of 84 singletons resulting from MNC-IVF treatment is described and compared to 106 singletons resulting from COH-IVF treatments. Baseline and pregnancy characteristics of patients were not different between both groups, except for duration of subfertility which was longer in the COH-IVF group and proportion of women smoking during pregnancy which was higher in the COH-IVF group.

No differences were found in pregnancy duration, proportion of prematurity and proportion of low birth weight. Malformation rate, neonatal admission rate and perinatal mortality were also not different between groups.

Birth weights found in the MNC-IVF group were comparable to those after spontaneous conceptions in the Netherlands and significantly higher than of COH-IVF singletons (difference 267 grams). After adjustment for prognostic factors (pregnancy duration, pregnancy factors, lifestyle factors, and pre-pregnancy patient characteristics), the difference remaining was 134 grams in favour of MNC-IVF ($P = 0.045$).

From this study we concluded that since laboratory procedures were the same in both groups and patient and pregnancy characteristics were adjusted for, ovarian stimulation may be a causative factor in the occurrence of low birth weight in COH-IVF children.

In *chapter 10,* a general discussion of the findings from our studies is presented and possible implementation of MNC-IVF as a standard treatment, as well as recommendations for future research are being discussed.

MNC-IVF seems to be a feasible alternative to standard IVF treatment, and implementation into clinical practice may be a realistic option, under the condition of a suitable reinbursement system and after confirmation of its cost-effectiveness in further studies.

Samenvatting

In *hoofdstuk 1* wordt een historisch overzicht gegeven van ontwikkelingen in IVF-behandelingen. De voordelen van MNC-IVF worden besproken.

Hoofdstuk 2 bevat een systematisch literatuuroverzicht over de effectiviteit van IVF in de spontane cyclus zonder gebruik van GnRH-antagonisten. Er werden 20 publicaties geselecteerd. De resultaten per studie waren erg variabel door verschillen in patiëntenselectie en cyclusmonitoring. In de 20 geselecteerde publicaties worden 1800 cycli beschreven, resulterend in 28.9% afbreken van de cyclus en 45.5% embryo transfers per gestarte cyclus. Het aantal doorgaande zwangerschappen was 7.2% per gestarte cyclus en 15.8% per embryo transfer.

In *hoofdstuk 3* wordt een cohort van 50 patiënten beschreven die een maximum aantal van drie opeenvolgende cycli MNC-IVF aangeboden kregen. In deze studie werden patiënten van 18-36 jaar met een regelmatige en bewezen ovulatoire cyclus en een indicatie voor reguliere IVF (geen ICSI) geïncludeerd. De GnRH-antagonist cetrorelix werd in de laat-folliculaire fase van de spontane, ongestimuleerde cyclus toegediend, tezamen met recombinant FSH ter substitutie. Indien op het moment van geplande eicelpunctie een ovulatie bleek te zijn opgetreden werd IUI gedaan.

Het doorgaand zwangerschapscijfer was 14.3% per gestarte cyclus, met 5.9% tweelingzwangerschappen. Het cumulatief doorgaand zwangerschapscijfer na drie cycli was 34.0% per patiënt. Het aantal embryo transfers was 43.7% per gestarte cyclus.

In 11.8% van de gestarte cycli trad, ondanks correct gebruik van cetrorelix, een LH-stijging op. In tweederde van deze cycli werd desondanks de geplande eicelpunctie uitgevoerd. Wij concludeerden dat in sommige maar niet alle cycli, cetrorelix in staat is om de LH piek afdoende af te zwakken om de geplande eicelpunctie door te kunnen laten gaan. In totaal werden 4.2% van de gestarte cycli afgebroken omdat er op het moment van de geplande eicelpunctie een ovulatie bleek te zijn opgetreden. De LH-waarden op het moment van ovulatietriggering waren in deze cycli significant hoger dan in de cycli waar de eicelpunctie wel kon worden uitgevoerd.

Een opmerkelijke bevinding in deze studie was dat in twee cycli multifolliculaire groei (>2 co-dominante follikels) optrad, hoewel er slechts één dominante follikel ($\geq$ 14mm) aanwezig was op het moment dat medicatie gestart werd. Blijkbaar kan de toediening van recombinant FSH in zeldzame gevallen leiden tot ovariële stimulatie. Een andere verklaring zou kunnen zijn dat een ovariële cyste is aangezien voor een dominante follikel en dat in deze gevallen nog geen dominante follikel aanwezig was.

De kans op implantatie was tamelijk hoog (30.9% doorgaande zwangerschap per teruggeplaatst embryo), hetgeen verrassend is aangezien geen selectie van embryos voor terugplaatsing werd uitgevoerd. De hoge implantatiekans kan zijn veroorzaakt door de

inclusie van patiënten met een goede kans op zwangerschap maar zou ook kunnen worden verklaard door een betere kwaliteit van het endometrium door het ontbreken van een effect van ovariële stimulatie. Het is ook mogelijk dat de eicel afkomstig van de (natuurlijk geselecteerde) dominante follikel de beste kwaliteit heeft binnen een cohort van eicellen, zodoende leidend tot een embryo met goede implantatiekans, of dat ovariële stimulatie schadelijk is voor eicelkwaliteit, zodat MNC-embryos van betere kwaliteit zijn dan COH-embryos.

In *hoofdstuk 4* wordt een uitbreiding van het in hoofdstuk 3 beschreven cohort, met identieke inclusiecriteria en behandelingsprotocol gepresenteerd. In deze multicentrische studie, waaraan vier klinieken deelnamen, werden 350 patiënten geïncludeerd en wij bepaalden de kans op zwangerschap per gestarte cyclus, cumulatieve zwangerschapscijfers na drie cycli en resultaten volgens indicatie voor IVF.

In totaal werden 844 MNC-cycli gestart (2.4 per geïncludeerde patient), leidend tot 317 embryo transfers (37.6% per gestarte cyclus). Wederom trad enkele keren multifolliculaire groei op. In 149 cycli (17.7%) werd geen eicelpunctie uitgevoerd, in 108 hiervan vanwege een LH-stijging of ovulatie. Het doorgaand zwangerschapscijfer was 8.3% per gestarte cyclus, met 4.3% tweelingzwangerschappen. Het cumulatief doorgaand zwangerschapscijfer na drie cycli was 20.8% per patiënt.

Er werden geen significante verschillen gevonden tussen indicaties voor IVF wat betreft zwangerschapscijfers en cyclusnummer. Wij vonden dat het afbreken van de cyclus in verband met LH-stijging of ovulatie, onsuccesvolle eicelpunctie en het niet bevrucht raken van een eicel een tendens vertonen om opnieuw op te treden in volgende cycli van dezelfde patiënten. Op basis hiervan concludeerden wij dat het advies aan patiënten betreffende het aantal uit te voeren cycli zou moeten worden geïndividualiseerd, rekening houdend met de uitkomst van eerdere cycli.

In *hoofdstuk 5* wordt een onderzoek beschreven waarin 268 patiënten negen cycli MNC-IVF kregen aangeboden. De eerste drie cycli van deze patiënten werden in hoofdstuk 4 beschreven. Het doel van dit onderzoek was om het optimale aantal uit te voeren cycli te bepalen, en om te bepalen of er een maximum aantal is waarboven MNC-IVF minder effectief wordt. Derhalve werd het zwangerschapscijfer volgens cyclusnummer bepaald, en het drop-out gedrag van patiënten bestudeerd.

Er werden 1048 cycli uitgevoerd bij 256 patiënten (4.1 per patiënt). Het aantal embryo transfers was 36.5% per gestarte cyclus. De kans op zwangerschap was 9.9% per gestarte cyclus, waarvan 7.9% doorgaand. Inclusief spontaan opgetreden zwangerschappen was het cumulatieve zwangerschapscijfer na een maximum van negen cycli 44.4% per patiënt, met 2.9% tweelingzwangerschappen.

Het aantal drop-outs was hoog (47.8%). Wij stelden vast dat het niet doorgaan van de eicelpunctie, het niet bevrucht raken van de eicel en het niet doorgaan van een embryo transfer een tendens vertonen om opnieuw op te treden in volgende cycli van dezelfde patiënten en dat deze predisponeren voor drop-out van patiënten.

De kans op zwangerschap daalde niet in hogere cyclusnummers, en wij concludeerden dat dit mogelijk komt door selectieve drop-out van patiënten met een (relatief) lage kans op zwangerschap. Wij konden geen maximum aantal cycli vaststellen waarboven

MNC-IVF minder effectief werd en concludeerden wederom dat het advies aan patiënten betreffende het aantal uit te voeren cycli zou moeten worden geïndividualiseerd, rekening houdend met de uitkomst van eerdere cycli.

In *hoofdstuk 6* wordt de follow-up van patiënten beschreven in hoofdstuk 5 gepresenteerd. Resultaten van volledige behandeling, bestaande uit MNC-IVF gevolgd door COH-IVF worden beschreven.

Wij vonden dat het falen van bevruchting in MNC voorspellend is voor falen van bevruchting in COH-IVF en concludeerden dat ICSI de kans op succes waarschijnlijk vergroot.

Van 268 geïncludeerde patiënten ondergingen er 9 geen enkele behandeling, 147 alleen MNC-IVF, 3 alleen COH-IVF en 109 beide.

Inclusief spontaan opgetreden zwangerschappen was het cumulatief doorgaand zwangerschapscijfer na behandeling met MNC-IVF gevolgd door COH-IVF 56.7%, met 6.6% tweelingzwangerschappen. De mediane tijd dat behandeling duurde was 34.1 (0-220) weken voor alle geïncludeerde patiënten. De mediane tijd tot doorgaande zwangerschap was 28.8 (0-220) weken.

Op basis van deze gegevens concludeerden wij dat door sequentiële behandeling met MNC-IVF gevolgd door COH-IVF de totale resultaten niet negatief beïnvloed lijken te worden, terwijl het aantal tweelingzwangerschappen heel laag is en dat het toepassen van MNC-IVF gevolgd door COH-IVF (als nodig), een zinvolle behandelingsstrategie is. Hiermee wordt niet alleen een groot deel van de patiënten het risico op OHSS en het ongemak van COH bespaard, maar ook het aantal tweelingzwangerschappen sterk gereduceerd.

In *hoofdstuk 7* wordt een vergelijking gemaakt van kosteneffectiviteit van MNC-IVF en COH-IVF. In deze analyse werden alle IVF-procedure gerelateerde kosten, zwangerschapsgerelateerde en neonatale kosten (tot zes weken na de bevalling) opgenomen. OHSS-gerelateerde kosten en effecten van cryopreservatie van embryos (voor COH-IVF patiënten) werden niet in de analyse opgenomen.

Gemiddelde kosten per cyclus waren 2.25 maal hoger voor COH-IVF vergeleken met MNC-IVF (voornamelijk door hogere medicatiekosten), terwijl de kans op doorgaande zwangerschap 2.37 maal hoger was. Met opnemen van kosten van zwangerschap en bevalling, die voor COH-IVF hoger waren door een hoger aantal tweelingzwangerschappen, werden de kosten per cyclus 2.5 maal hoger voor COH-IVF vergeleken met MNC-IVF. De kosten / geboortecijfer ratio's waren vergelijkbaar tussen MNC-IVF en COH-IVF.

Uit deze data komt naar voren dat MNC-IVF een kosteneffectief alternatief lijkt voor COH-IVF, doordat de lagere kans op zwangerschap gecompenseerd wordt door lagere kosten.

In *hoofdstuk 8* wordt een serie van 449 transfers van één ongeselecteerd embryo verkregen met MNC-IVF gepresenteerd en morfologische karakteristieken van deze embryos en de bijbehorende kans op implantatie worden beschreven.

De beste kans op implantatie werd gevonden bij embryos met ≤ 10% fragmentatie, afwezigheid van blastomeren met multinucleatie, vier cellen op dag 2 na eicelpunctie en acht cellen op dag 3. Dit komt precies overeen met wat theoretisch verwacht wordt en algemeen geaccepteerd is als het beste embryo.

In tegenstelling tot studies over embryos verkregen met COH-IVF, vonden wij dat embryos met minder dan vier blastomeren op dag 2 relatief goed implanteerden en tevens dat embryos met multinucleaire blastomeren een minder slechte implantatie vertoonden dan verwacht.

Het is mogelijk dat implantatie van embryos volgens hun morfologische karakteristieken verschilt tussen MNC-IVF en COH-IVF, maar het is ook mogelijk dat in studies over embryos verkregen met COH-IVF de kans op implantatie van embryos met lage aantallen blastomeren op dag 2 en van embryos met multinucleaire blastomeren onderschat wordt. Deze onderschatting zou kunnen worden veroorzaakt door selectie van embryos voor transfer, aangezien deze in het algemeen alleen gekozen worden voor transfer als er geen andere beschikbaar zijn.

Wij concludeerden dat als onze bevindingen worden bevestigd in nader onderzoek, aanpassingen van bestaande embryo scoring systemen zouden kunnen bijdragen aan een verbetering van de selectie van het beste embryo voor transfer.

In *hoofdstuk 9* wordt de perinatale uitkomst van 84 eenlingen geboren na MNC-IVF beschreven en vergeleken met die van 106 eenlingen geboren na COH-IVF.

Patiënten- en zwangerschapskarakteristieken waren niet verschillend tussen de groepen, behalve de duur van subfertiliteit (langer in de COH-groep) en aantal vrouwen dat tijdens de zwangerschap rookte (hoger in de COH-groep). Wij vonden geen verschillen in zwangerschapsduur, proportie prematuriteit en proportie laag geboortegewicht. Het aantal congenitale afwijkingen, opnames op de kinderafdeling en perinatale sterfte verschilde niet tussen de groepen.

De geboortegewichten in de MNC-groep waren vergelijkbaar met die van spontaan ontstane zwangerschappen en significant hoger dan in de COH-groep (267 gram verschil). Na correctie voor prognostische factoren (zwangerschapsduur, zwangerschapskarakteristieken, lifestyle factoren en basis patiëntenkarakteristieken), was het verschil 134 gram ten gunste van MNC-IVF ($P=0.045$).

Wij concludeerden dat, aangezien laboratoriumprocedures gelijk waren in beide groepen en er voor patiënten- en zwangerschapsfactoren gecorrigeerd is in deze studie, het mogelijk is dat ovariële stimulatie een oorzakelijke factor is in het ontstaan van lage geboortegewichten na COH-IVF.

Hoofdstuk 10 bevat een algemene discussie van de bevindingen uit onze studies. De mogelijke implementatie van MNC-IVF als standaardbehandeling en aanbevelingen voor toekomstig onderzoek worden hierin besproken.

MNC-IVF lijkt een zinvol alternatief voor standaard IVF-behandeling. Implementatie in de praktijk lijkt een realistische optie, op voorwaarde van een passend verzekeringssyteem en na bevestiging van de kosten-effectiviteit in nader onderzoek.

Publications

Rybouchkin A, Dozortsev D, Pelinck MJ, De Sutter P, Dhont M (1996) Analysis of the oocyte activating capacity and chromosomal complement of round-headed human spermatozoa by their injection into mouse oocytes. Human Reproduction 11: 2170-2175.

Pelinck MJ, De Vos M, Dekens M, Van der Elst J, De Sutter P, Dhont M (1998) In vitro-cultured embryos with multinucleated blastomeres have poor implantation potential in human IVF and ICSI. Human Reproduction 13: 960-963.

Pelinck MJ, Hoek A, Simons A, Arts E, Heineman MJ (2002) IVF-behandeling in de spontane cyclus met gebruik van een GnRH-antagonist in de laat-folliculaire fase en substitutie met recombinant FSH: voorlopige resultaten van een pilotstudy. Tijdschrift voor Fertiliteitsonderzoek 1: 16-18.

Pelinck MJ, Hoek A, Simons AH, Heineman MJ (2002) Efficacy of natural cycle IVF: a review of the literature. Human Reproduction Update 8: 129-139.

Pelinck MJ, Vogel N, Hoek A, Simons A, Arts E, Heineman MJ (2004) IVF-behandeling in de spontane cyclus. Tijdschrift voor Fertiliteitsonderzoek 1: 2-7.

Pelinck MJ, Vogel NE, Hoek A, Arts EG, Simons AH, Heineman MJ (2005) Minimal stimulation IVF with late follicular phase administration of the GnRH antagonist cetrorelix and concomitant substitution with recombinant FSH: a pilot study. Human Reproduction 20: 642-648.

van Veen AJ, Pelinck MJ, van Pampus MG, Erwich JJ (2005) Severe hypotension and fetal death due to tocolysis with nifedipine. British Journal of Obstetrics and Gynaecology 112: 509-510.

Pelinck MJ, Vogel NE, Hoek A, Simons AH, Arts EG, Mochtar MH, Beemsterboer S, Hondelink MN, Heineman MJ (2006) Cumulative pregnancy rates after three cycles of minimal stimulation IVF and results according to subfertility diagnosis: a multi-centre cohort study. Human Reproduction 21: 2375-2383.

van der Ploeg JM, van Lonkhuijzen LRCW, Pelinck MJ (2006) Evidence Based Medicine: bedreiging voor de geneeskunst? Nederlands Tijdschrift voor Obstetrie en Gynaecologie 119: 28-29.

van der Ploeg JM, Schutte JM, Pelinck MJ, Huisjes AJ, van Roosmalen J, de Vries JI (2007) Management of fetal death after 20 weeks of gestation complicated by placenta previa. Journal of Maternal-Fetal and Neonatal Medicine 20: 267-269.

Pelinck MJ, Vogel NE, Arts EG, Simons AH, Heineman MJ, Hoek A (2007) Cumulative pregnancy rates after a maximum of nine cycles of modified natural cycle and analysis of patient drop-out: a cohort study. Human Reproduction 22: 2463-2470.

Pelinck MJ, Knol HM, Vogel NE, Arts EG, Simons AH, Heineman MJ, Hoek A (2008) Cumulative pregnancy rates after sequential treatment with modified natural cycle IVF followed by IVF with controlled ovarian stimulation. Human Reproduction 23: 1808-1814.

Oonk MH, Pelinck MJ (2008) Diagnostic image (389). A woman with a multicystic vulvar mass. Nederlands Tijdschrift voor Geneeskunde 152: 2015.

Pelinck MJ, Keizer HH, Hoek A, Simons AHM, Schelling K, Middelburg KJ, Heineman MJ (2009) Perinatal outcome in singletons born after modified natural cycle IVF and standard IVF with ovarian stimulation. European Journal of Obstetrics & Gynecology and Reproductive Biology; in press

Pelinck MJ, Hoek A, Vogel NEA, Simons AHM, Heineman MJ, van Echten-Arends J, Arts EGJM (2009) Embryo quality and impact of specific embryo characteristics on ongoing implantation in unselected embryos derived from modified natural cycle IVF. Fertility and Sterility; in press

Abstracts and presentations related to this thesis

Pelinck MJ, Hoek A, Simons AHM, Heineman MJ (2001) IVF-behandeling in de spontane cyclus met gebruik van een GnRH-antagonist in de laat-folliculaire fase en substitutie met recombinant FSH: resultaten van een pilot studie. VFS najaarsvergadering; Maastricht, the Netherlands (presentation prize). TFO 4: 98-99.

Pelinck MJ, Arts EGJM, Hoek A, Simons AHM, Vogel NEA, Heineman MJ (2002) IVF-behandeling in de spontane cyclus met gebruik van een GnRH-antagonist in de laat-folliculaire fase en substitutie met recombinant FSH. Resultaten volgens indicatie voor IVF. VFS voorjaarsvergadering; Brussel, België. TFO 2: 44-45.

Pelinck MJ, Arts EGJM, Hoek A, Simons AHM, Heineman MJ (2002) Natural cycle IVF with late follicular phase GnRH antagonist administration: a pilot study. ESHRE, 18th annual meeting; Vienna, Austria. Hum Reprod 17, Abstract Book 1: 18.

Pelinck MJ, Hoek A, Arts E, Simons A, Vogel N, Heineman MJ (2002) Natural cycle IVF/ICSI with late follicular phase GnRH-antagonist administration: results according to subfertility diagnosis. ASRM, 58th annual meeting; Seattle, USA. Fertil Steril 2002, 78 (3): S42-43.

Pelinck MJ, Simons AHM, Arts EGJM, Hoek A, Vogel NEA, Heineman MJ (2003) Modified natural cycle IVF: characteristics of the luteal phase. 7th International symposium on GnRH Analogues in Cancer and Human Reproduction; Amsterdam, the Netherlands. Gynecol Endocrinol 17, S1: 19.

Vogel NEA, Pelinck MJ, Arts EGJM, Hoek A, Simons AHM, Heineman MJ (2003) Effectiveness of the modified natural cycle ICSI: results of a pilot study. ASRM, 59th annual meeting; San Antonio, Texas, USA. Fertil Steril 80 (S3): P-7.

Keizer M, Middelburg K, Vogel N, Pelinck MJ, Simons A, Hoek A (2004) Birth weight of singletons born after modified natural cycle IVF compared with singletons born after IVF with ovarian hyperstimulation. ASRM, 60th annual meeting; Philadelphia, USA. Fertil Steril 82 (S2): S48.

Pelinck MJ, Vogel N, Arts E, Hoek A, Simons A, Heineman MJ (2004) Cumulative pregnancy rates after a maximum of nine cycles of modified natural cycle IVF. ASRM, 60th annual meeting; Philadelphia, USA. Fertil Steril 82 (S2): S133-134.

Pelinck MJ, Vogel NEA, Arts EGJM, Hoek A, Simons AHM, Heineman MJ (2004) Modified natural cycle IVF: results according to indication. ESHRE, 20th annual meeting; Berlin, Germany. Hum Reprod 19 (S1): i135.

Knol G, Pelinck MJ, Arts EGJM, van Echten J, Hoek A, Vogel N, Simons A, Heineman MJ (2005). Embryo quality in modified natural cycle IVF. ESHRE, 21st annual meeting; Copenhagen, Denmark. Hum Reprod 20 (S1): i77.

Pelinck MJ, Van Leeuwen A, Vogel NEA, van Echten J, Arts EGJM, Hoek A, Simons AHM, Heineman MJ (2005) Implantation potential of unselected embryos with multinucleated blastomeres. ESHRE, 21st annual meeting; Copenhagen, Denmark. Hum Reprod 2005, 20 (S1): i152.

Groen H, Pelinck MJ, Hoek A, ten Vergert EM (2005) Cost-effectiveness of in vitro fertilisation (IVF) in the manipulated natural cycle versus conventional IVF. HTAi, 2nd annual meeting; Rome, Italy. Ital J Public Health 3 (S1): 181.

Keizer M, Schelling K, Pelinck M, Hoek A, Simons A, Heineman M (2005) Larger birth weight in singletons born after minimal stimulation IVF compared to singletons born after COH-IVF. Conjoint annual meeting of ASRM and CFAS; Montreal, Canada. Fertil Steril 84 (S1): S81-82.

Pelinck M, Groen H, Vogel N, Simons A, Heineman M, Hoek A (2005) Cost-effectiveness of minimal stimulation IVF compared to COH-IVF. Conjoint annual meeting of ASRM and CFAS; Montreal, Canada. Fertil Steril 84 (S1): S240.

Arts EGJM, Knol GJ, Pelinck MJ, van Echten-Arends J, Hoek A, Vogel NEA, Simons AHM, Heineman MJ (2006) Embryo quality and implantation in modified natural cycle IVF. First world congress on natural cycle/minimal stimulation IVF; London, UK.

Pelinck MJ, Vogel NEA, Arts EGJM, Simons AHM, Heineman MJ, Hoek A (2006) Modified natural cycle IVF. Cumulative pregnancy rates after a maximum of nine cycles and analysis of patient drop-out. First world congress on natural cycle/minimal stimulation IVF; London, UK.

Middelburg KJ, Haadsma ML, Nijhuis WL, Kiewiet SM, Hoek A, Heineman MJ, Bos AF, Hadders-Algra M (2007) Are worse perinatal outcomes of singletons conceived with IVF related with the ART-procedure or with subfertility itself? ESHRE, 23d annual meeting; Lyon, France. Hum Reprod 22 (S1): i7.

Pelinck MJ, Knol HM, Vogel NEA, Arts EGJM, Simons AHM, Heineman MJ, Hoek A (2007) Cumulative pregnancy rates after sequential treatment with modified natural cycle IVF followed by IVF with controlled ovarian stimulation. ESHRE, 23d annual meeting; Lyon, France. Hum Reprod 22 (S1): i128.

Arts EGJM, van Echten-Arends J, Vogel NEA, Hoek A, Simons AHM, Heineman MJ, Pelinck MJ (2008) Impact of specific embryo characteristics on ongoing implantation in unselected embryos derived from modified natural cycle IVF. Alpha, 7th Biennial Conference; Istanbul, Turkey

Pelinck MJ, Vogel N, Arts E, Simons A, Hoek A (2007) Cumulative pregnancy rates after a maximum of nine cycles of modified natural cycle and analysis of patient drop-out. 5th World Congress on Ovulation Induction; Rome, Italy.

Pelinck MJ, Vogel N, Arts E, Simons A, Hoek A (2007) Cumulative pregnancy rates after a maximum of nine cycles of modified natural cycle and analysis of patient drop-out. World Congress on In Vitro Fertilization and In-Vitro Maturation; Montréal, Canada.

Pelinck M, Arts E, Hoek A, Simons A, van Echten-Arends J (2008). Impact of specific embryo characteristics on implantation and abortion rate in unselected embryos derived from modified natural cyle IVF. XII Reinier de Graaf Symposium; Groningen, the Netherlands.

Pelinck MJ, Keizer HH, Hoek A, Simons AHM, Schelling K, Middelburg K, Heineman MJ (2008) Perinatal outcome in singletons born after modified natural cycle IVF and standard IVF with ovarian stimulation. 11th World Congress on Controversies in Obstetrics, Gynecology & Infertility; Paris, France.

Pelinck MJ (2008) Modified natural cycle IVF-feasibility and results. Basics and Beyond in Obstetrics and Gynecology; Bangalore, India.

Pelinck MJ (2009) Modified natural cycle: the best of both worlds? ESHRE Campus course; Maribor, Slovenia.

Pelinck MJ (2009) Modified natural cycle IVF: why and how. 3rd International IVI Congress; Madrid, Spain.

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Curriculum vitae

Marie-José Pelinck werd geboren op 12 maart 1969 in Apeldoorn. In 1986 behaalde zij het diploma Gymnasium-ß aan het Jacobus College te Enschede. Daarna verbleef zij een jaar in Zwitserland en een jaar in Wageningen. Vervolgens startte zij de studie Geneeskunde aan de Katholieke Universiteit van Leuven, België.

Na het behalen van het artsexamen was zij werkzaam als IVF-arts in het Universitair ziekenhuis te Gent, als AGNIO Verloskunde en Gynaecologie in het St Elisabeth Ziekenhuis te Tilburg en als AGNIO Verloskunde en Gynaecologie in het Martini Ziekenhuis te Groningen.

In 1999 startte zij als IVF-arts in het Academisch Ziekenhuis te Groningen, alwaar een start werd gemaakt met het in dit proefschrift beschreven onderzoek.

De opleiding tot gynaecoloog werd gevolgd in het Academisch Ziekenhuis Groningen (opleiders prof. dr. J.G. Aalders, prof. dr. M.J. Heineman en prof. dr. M.J.E. Mourits), het Martini Ziekenhuis te Groningen (opleider dr. A.J. van Loon) en het Medisch Centrum Leeuwarden (opleiders dr. J.G. Santema (†) en dr. T. Spinder).

Naast de opleiding tot gynaecoloog volgde zij de Masteropleiding Epidemiologie aan het EMGO-instituut, Vrije Universiteit te Amsterdam en ontving het diploma Master of Epidemiology in 2006.

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