Extended Assessment of Mild Ovarian Stimulation

Monique Sterrenburg

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Extended Assessment of Mild Ovarian Stimulation

Milde ovariële stimulatie: een beoordeling van nieuwe ontwikkelingen
(met een samenvatting in het Nederlands)

Proefschrift

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Ter nagedachtenis aan mijn vader
Voor mijn lieve ouders

Table of contents

Chapter 1

General introduction

Ovarian stimulation, by using exogenous FSH, aims at either restoring normal ovulatory cycles in women with oligo/amenorrhoea or at eliciting multiple follicle growth in normal cycling women for the purpose of intrauterine insemination (IUI) or in vitro fertilisation (IVF) treatment. In oligo/ amenorrhoeic women the cause for the anovulation can be a disruption of endogenous FSH production at the level of the hypothalamic-pituitary unit (WHO I anovulation) or a dysregulation of the ovarian paracrine milieu jeopardizing the monthly cyclic follicle recruitment (WHO II anovulation, among which the PCO syndrome).
Since the first IVF baby was born in 1978, the ovarian stimulation strategies key to achieving success, have undergone extensive change and development. Although the first IVF treatments were performed in the natural cycle, ovarian stimulation with exogenous gonadotrophins quickly became an established component of IVF treatment as it addressed the need to generate large numbers of oocytes to compensate for the limits of laboratory performance, in-vitro embryo development and selection.
While gonadotrophins remain the corner stone for today's treatment in anovulation and IVF patients, almost every aspect of their administration has changed. Their source, mode of production, means of administration, dose and even aim of treatment have altered considerably. However, the basic endocrine physiology which underpins the rational for current FSH treatment regimens remains unchanged.

Follicular development

The safe and effective use of the majority of drugs used in treating anovulation and infertility requires an understanding of the endocrinology of normal ovarian follicular development. While early follicle development is considered to be largely independent of gonadotrophins, late follicular development is follicle stimulating hormone (FSH) dependent. The great majority of human oocytes are destined to undergo atresia (Baker et al. 1963, Schwartzman et al. 1993). Only those follicles able to respond to stimulation by FSH will enter the final stage of development and ovulate (Gougeon et al. 1996, Macklon et al. 1998).
Each growing follicle possesses a threshold requirement for stimulation by circulating FSH (Brown et al. 1978, van der Meer et al., 1994). This threshold level should be passed to ensure ongoing pre-ovulatory development. In the normo-ovulatory cycle only one follicle will become responsive to FSH above this threshold. In response to negative feedback from rising estradiol and inhibin levels, FSH levels fall in the late follicular phase. The dominant follicle has increased sensitivity to the falling FSH levels and continues growing. Those follicles, which commence

the latter stages of development after FSH levels start to fall, will undergo atresia. The duration of this "FSH window" during which FSH levels are above the threshold required to stimulate ongoing development, determines the number of follicles which can develop to the pre-ovulatory stage (*Figure 1*) (Baird et al. 1987, Fauser et al. 1997). These advanced stages of follicular development are open to therapeutic intervention with exogenous FSH.

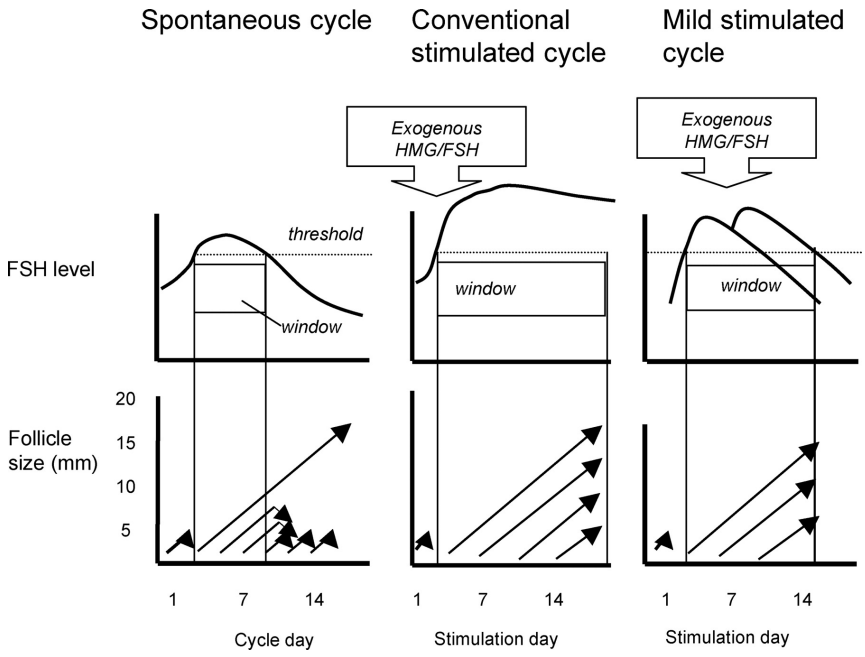

Figure 1 *The follicle-stimulating hormone (FSH) threshold and window concept for monofollicular selection (left panel), as conventionally applied to achieve multifollicular development (middle panel). Each arrow represents a developing follicle. The right panel represents the concept of extending the FSH window by administering exogenous FSH in the mid-follicular phase to maintain FSH levels above the threshold allowing multifollicular development (Fauser et al. 1993, Macklon et al. 2006).*

Conventional approaches of ovarian stimulation

The aim of ovulation induction in anovulatory women is the formation and ovulation of a single dominant follicle. In order to achieve this, specific treatment and monitoring protocols are needed. The first line of treatment is clomiphene citrate (Dickey et al. 1996, Yarali et al. 2004), a selective estrogen receptor modulator, due to low costs and minor side effects or complications. Multiple injections of exogenous gonadotrophins are considered the second line in treatment in case of failure to ovulate or conceive following clomiphene citrate (Kamrava et al. 1982,

Buvat et al. 1989, White et al. 1996, van Santbrink et al. 2005, Balen et al. 2007). A low-dose, step-up protocol (Franks et al. 1988) designed to allow the FSH threshold to be reached gradually has now become the most widely used regimen, reducing the risk of excessive stimulation and development of multiple preovulatory follicles. In this protocol, the initial subcutaneous or intramuscular dose of FSH is 50 to 75 IU/day; and the dose is increased if after 14 days, no response is observed on ultrasonography (and serum estradiol monitoring). Increments of 37.5 IU are then given at weekly intervals up to a maximum of 225 IU/day. The detection of an ovarian response is an indication to continue the current dose until human Chorionic Gonadotrophin (hCG) can be given to trigger ovulation.
In contrast to ovulation induction regimens, where the aim is to induce monofollicular development in an anovulatory woman, rFSH is applied in assisted conception to obtain large numbers of oocytes for IVF, and subsequent selection of embryos for intra-uterine transfer. Higher doses of gonadotrophins are therefore administered. The most commonly applied regimen today is what is sometimes termed the 'conventional' regimen with GnRH agonist as co-treatment in either a 'long' or 'short' protocol with high doses of gonadotrophins (Nargund et al. 2007). The GnRH agonist can either be started after OC pre-treatment or in the luteal phase of the menstrual cycle. The aim of this regimen is to retrieve 8-15 oocytes (Nargund et al. 2007). The disadvantage of the conventional protocol is that it is complex, time consuming, expensive, considerable patient discomfort, and chances for complications, especially ovarian hyperstimulation syndrome (OHSS) (Macklon et al. 2006). Until now, the optimal daily dose of gonadotrophins is still unclear, even in the conventional regimens.

Burden of IVF treatment

It is well known that IVF and ovulation induction are a demanding and stressful treatment for patients. High dropout rates are frequently encountered in IVF treatment. It is not only that patients withdraw from IVF treatment because of the withholding of further treatment because of poor prognosis or the inability to pay for further treatment (Cousineau et al. 2007), but also additional factors like age of the male, previously successful IVF treatment, parity of the female partner (Johnson et al. 2003), and pre-existing levels of anxiety and depression (Smeenk et al. 2004) are involved. Recent studies also reported a lower dropout rate after mild ovarian stimulation strategies (Heijnen et al. 2007).
The burden for patients undergoing IVF treatment has several aspects. The psychological aspect of higher levels of anxiety during IVF treatment, especially when undergoing follicle aspiration and the waiting period till the pregnancy test, is well

described (Boivin et al. 1996, de Klerk et al. 2006). Other aspects are more related to the treatment itself. One part is the daily injections patients need for the IVF treatment as well as the ovulation induction. There is still no oral drug available to stimulate the ovaries to grow more follicles, therefore patients still need daily hormone injections. Another option is the use of new compound, a long-acting FSH termed corifollitropin alfa. Corifollitropin alfa is a recombinant fusion protein composed of FSH and the carboxy terminal peptide (CTP) of the hCG ß-subunit *(Figure 2)* (Fares et al. 1992). This CTP is responsible for the longer half life time in hCG compared to FSH. It has an approximately 2-fold longer elimination half-life (T1/2) and an almost 4-fold extended time-interval (T_{max}) to peak serum levels (C_{max}) (Duijkers et al. 2002). Due to this pharmacokinetic profile, corifollitropin alfa can function as a sustained follicle stimulant with a similar pharmacodynamic profile as rFSH, but with the ability to initiate and sustain multiple follicle growth for an entire week. Therefore, corifollitropin alfa can replace the first seven injections of any daily rFSH injections in ovarian stimulation for IVF treatment, which will reduce the burden for patients as long as it is safe and with the same efficacy as rFSH.

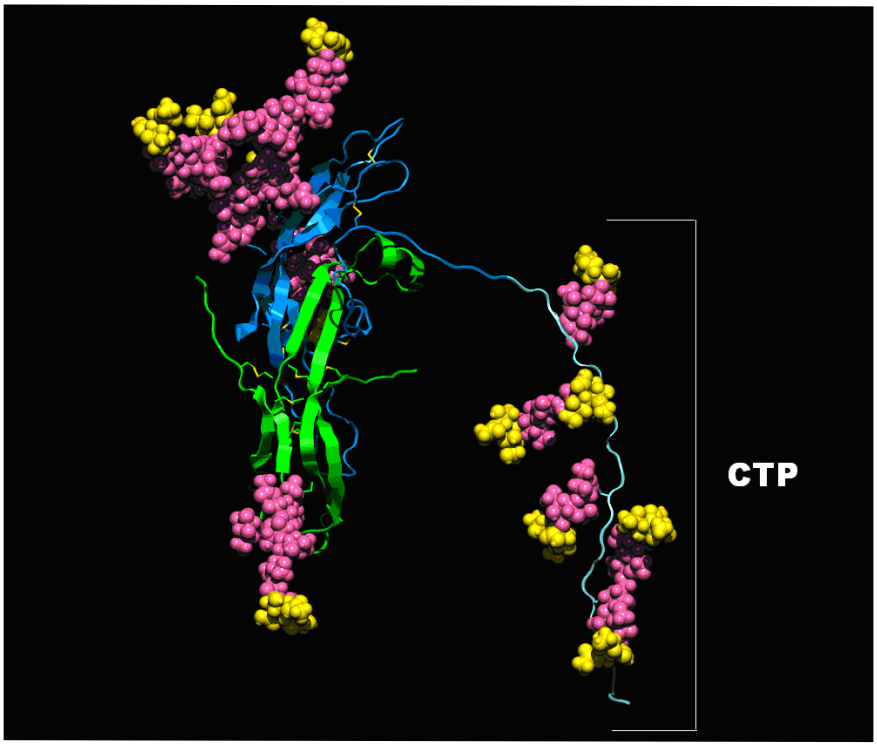

Figure 2 *The composition of corifollitropin alfa. CTP= carboxy terminal peptide.*

Another important aspect is the number of complications related to the treatment. The risk of OHSS for example is higher when more follicles will grow. Also, the costs of an IVF treatment are high, partly due to the medication used for ovarian stimulation and down regulation of the pituitary. Therefore it is essential to take the burden of the patient into consideration when developing new ovarian stimulation strategies and remaining the balance between outcome (pregnancy) and burden of the patient.

Mild ovarian stimulation

The clinical availability of GnRH antagonists opened the way to developing shorter and milder regimes of ovarian stimulation. GnRH antagonists cause an immediate and rapid, reversible suppression of gonadotrophin secretion, by competitive occupancy of the GnRH receptor (Klingmuller et al. 1993). Due to the acute gonadotrophin suppressive activity, GnRH antagonists may be administered at any time during the follicular phase of a treatment cycle to prevent a premature LH surge, providing a convenient alternative for agonists. In the multiple dose antagonist protocol 0.25 mg Cetrorelix or Ganirelix is given daily from the 6th or 7th day of gonadotrophin stimulation onward including the day of hCG (Albano et al. 1997, The ganirelix dose-finding study group 1998). With an antagonist, the duration of gonadotrophin treatment is shortened by 1-2 days and slightly fewer follicles are seen at the time of hCG injection compared with an agonist (Huirne et al. 2007). Therefore, the number of recovered oocytes tends to be lower. A likely explanation is that long agonist protocols extend the duration of the "FSH window" by suppressing the intercycle FSH rise (Huirne et al. 2007).

The first meta-analysis of five large randomised trials shows an overall significantly lower rate of pregnancy of 5% (OR 0.75, 95% CI; 0.62, 0.97) following GnRH antagonist versus co-treatment with GnRH agonist (Al-Inany et al. 2007, Kolibianakis et al. 2006). A number of possible reasons for this difference have been proposed, including the possibility of direct effects of antagonists on human embryos. This adverse effect however, was not observed on the freeze-thaw embryos of these cycles, suggesting that there is no direct negative effect of the GnRH antagonist on the quality of oocytes and embryos. As more experience is gained with the use of GnRH antagonists, and the protocol is further refined, it is anticipated that the difference in pregnancy rates observed in earlier studies will reduce. Indeed, in the most recently published meta-analysis, life birth rates did not differ significantly between the two modes of treatment.

Mild stimulation can be described as the administration of lower or fewer doses of exogenous gonadotrophins in GnRH antagonist co-treated cycles, and/or oral

compounds (like anti-estrogens, or aromatase inhibitors) for ovarian stimulation for IVF, aiming to limit the number of oocytes obtained to less than eight (Nargund et al. 2007, Fauser et al. 2010). The essential of mild stimulation is to remain as close as possible to the normal physiology of the ovary.

There are a number of advantages of the mild stimulation regimen. Since in the mild stimulation protocol is meant to apply lower doses and fewer days, the treatment will be less complex, may diminish patient distress (de Klerk et al. 2007) and complications such as OHSS (Heijnen et al. 2007), and the costs per cycle will be lower. Yet our group has previously demonstrated that in combination with a single embryo transfer strategy, the live birth rate after 1 year of treatment can be the same as following use of the conventional protocol in a strategy involving the transfer of two embryos (Heijnen et al. 2007). Moreover, several studies have demonstrated that the dropout rate from treatment decreases following milder treatment regimens (de Klerk et al. 2007, Hojgaard et al. 2001, Pelinck et al. 2007, Verberg et al. 2008, Dixon et al. 2008, Polinder et al. 2008).

However, milder stimulation protocols also have a number of disadvantages. The pregnancy rates per started cycle are lower, because of the higher cancellation rate (Heijnen et al. 2007). The cancellation criteria may need to be revised for mild ovarian stimulation, because the optimal number of oocytes is lower (Verberg et al. 2009). Therefore, there is less a margin for suboptimal laboratory performance. Also, the number of embryos available for cryopreservation may be lower and consequently the fewer added deliveries following thawing, so it could reduce the overall efficacy of a single stimulated cycle (Fauser et al. 2010). Cycle programming to avoid weekend oocyte retrievals may be more difficult, since the start of the cycle is determined by the patients menstruation which may be harder to program. While oral contraceptive pre-treatment offers a means of doing this, recent data indicate that their use with GnRH antagonists maybe associated with lower ongoing pregnancy rates (Griesinger et al 2010). A further potential drawback is the decreased ongoing implantation rate reported in high responders (Verberg et al. 2009), which was not observed in regimens with the GnRH agonist as co-treatment. The follicle growth dynamics may be different compared to the protocols with GnRH agonist and the window of scheduling the hCG seems narrower (Kolibianakis et al. 2005). There is evidence for a learning curve for clinicians when the switch is made from GnRH agonist to GnRH antagonist (Fauser et al. 2010).

So, despite the advances in ovarian stimulation protocols for IVF, many questions remain. In the era of transferring just one or two embryos, what is the optimum number of oocytes to be striven for? How can the burden of IVF treatment for patients be further decreased, without adversely affecting efficacy? How can further improvement be introduced in mild stimulation protocols?

Aims and outlines of the thesis

To address these issues, the aim of the work presented in this thesis was to look for strategies to improve the burden of ovarian stimulation for IVF and OVIN treatment without compromising the outcome. The specific focus will be on the following questions:

- Are ovarian response level and probability of pregnancy related?
- What is the clinical efficacy of a single dose, long acting FSH preparation in OVIN and IVF treatment?
- Can the mild stimulation antagonist protocol for IVF be improved by late start of rFSH?

The relation between number of oocytes and outcome in terms of pregnancy are explored in part 1. The drawback of decreased ongoing implantation rate in high responders in mild stimulation thereby is further investigated. Also the question will be analysed what the optimal daily stimulating dose would be in expected normal responders, based on a systematic review of the existing literature.

Part 2 describes the studies of a new compound, long-acting FSH, to find out if reducing the number of injections with this new medication would be as safe and efficient as rFSH in OVIN and IVF treatment.

In part 3 a mild stimulation protocol is studied to discern whether the burden for patients can be reduced while avoiding a detrimental effect on morphological markers of embryo quality. The prospective randomised study described in this chapter also investigates the endocrine characteristics, the follicular development, and the clinical applicability of a mild stimulation protocol (start rFSH on cycle day 5) compared with the regular protocol (start rFSH on cycle day 2) in IVF with GnRH antagonist as co-treatment.

Finally, in chapter 8 the final answers to the three questions will be discussed based on the conducted studies and the existing literature. The implications in the context of current practice and future research are discussed.

Chapter 2

Clinical outcomes in relation to the daily dose of recombinant follicle stimulating hormone for ovarian stimulation in In-Vitro Fertilisation in presumed normal responders younger than 39 years: a meta-analysis

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Abstract

Background

The optimal ovarian stimulation dose to obtain the best balance between the probability of pregnancy and the risk of complications, while maximizing cost-effectiveness of in vitro fertilisation (IVF) treatment, is yet to be established.

Methods

A systematic search of the electronic databases PubMed, EMBASE and Cochrane library, from 1984 until October 2009 for randomised controlled trials comparing different doses of recombinant FSH in IVF, was performed.

Results

Ten studies (totaling 1952 IVF cycles) were included in the present meta-analysis, comprising patients younger than 39 years with regular menstrual cycle, normal basal FSH levels and two normal ovaries. Comparison was made between studies using a daily dose of 100 versus 200 IU rFSH, and between 150 versus 200 IU rFSH or higher. Although oocyte yield was greater in the >200 IU/day dose group, pregnancy rates were similar compared with lower dose groups. The risk of insufficient response to ovarian stimulation was greatest in the 100 IU/day dose group. The risk of developing ovarian hyperstimulation syndrome was greater in the >200 IU/day dose group. The number of embryos available for cryopreservation was lowest in the 100 IU/day group, but similar comparing the 150 IU/day and the >200 IU/day dose groups.

Conclusions

This meta-analysis suggests that the optimal daily rFSH stimulation dose is 150 IU/day in presumed normal responders younger than 39 years undergoing IVF. Compared with higher doses, this dose is associated with a slightly lower oocyte yield, but similar pregnancy and embryo cryopreservation rates. Furthermore, the wide spread adherence to this optimal dose will allow for a considerable reduction in IVF costs and complications.

Introduction

In the United Kingdom, 1 in 6 couples are faced with the problem of infertility (Cahill et al. 2002) and almost 45,000 cycles of in-vitro fertilisation (IVF) treatment are carried out annually. In order to compensate for inefficiencies in the process, and to allow for the selection of embryos for direct intra-uterine transfer or cryopreservation, ovarian stimulation is usually performed by administering exogenous gonadotrophins.(Macklon et al. 2006) This approach results in the generation of multiple oocytes from a single treatment cycle, as opposed to the normal menstrual cycle which usually results in the ovulation of a single oocyte. (Fauser et al. 1997)

For ovarian stimulation, recombinant follicle-stimulating hormone (rFSH) preparations are currently administered in dosages ranging from 100 IU/day (IU/d) up to 600 IU/d.(Malizia et al. 2009, Nargund et al. 2007) The resultant multiple follicular development carries the risk of premature luteinisation. Co-treatment with gonadotrophin-releasing hormone (GnRH) agonist or antagonist is normally instituted to prevent an untimely rise in luteinising hormone (LH).(Huirne et al. 2001) The costs of the gonadotrophins represent a significant proportion (up to 30%) of the costs for an entire IVF treatment cycle.(Wechowski et al. 2009) Therefore, a reduction in the amount of rFSH administered would greatly affect the costs of an IVF treatment.

At present, the optimal starting dose for ovarian stimulation leading to the highest possibility of achieving a pregnancy, while minimizing the chances for major patient discomfort and complications, is not known. Studies reporting the dose effect relationship for this type of medication are scarce, and current practice is largely based on empirical considerations. The aim of the current systemic review was to identify the optimal daily starting dose of rFSH taking into account ovarian response, chance of pregnancy, rate of cycle cancellation, and the incidence of the potentially life-threatening complication of ovarian hyperstimulation syndrome (OHSS). Published randomised comparative dosage trials were searched in order to identify the rFSH dosage level with the best clinical efficacy, cost-effectiveness and safety profile.

Methods

Search strategy, selection criteria and data collection

In this meta-analysis, the Quality of Reporting of Meta-analyses (QUOROM) guidelines were adhered to. Prior to performance of the literature search, a number of inclusion criteria were established. Only randomised controlled trials were considered eligible. The reported methods of allocation concealment were critically assessed: (i) allocation was adequate, (ii) allocation was unclear and (iii) allocation was inadequate. In order to be included, it was necessary that the trials compared different starting dosages of rFSH for ovarian stimulation in women aged between 18 and 40 years undergoing IVF/ICSI treatment. We performed an electronic search of MEDLINE and EMBASE for English and non-English language publications from 1984 until October 2009. The following Medical Subject Headings (MeSH) search terms were used: 'IVF', 'ICSI', 'ART', 'ovarian stimulation', 'rFSH', 'gonadotrophin' and 'RCT'.

The MeSH strategy yielded 2404 publications in MEDLINE, EMBASE and the Cochrane library. Of those publications, 2356 (including duplicates) were excluded because it was clear from the title that they did not fulfil the selection criteria. From the remaining 48 articles, 25 could be excluded on the basis of the abstracts. Two reviewers (M.D.S. and S.M.V.-V.) independently reviewed the remaining 23 articles and extracted data from each study using a standardized form. Discrepancies were resolved by discussion and consensus. Finally, the bibliographies of identified studies were hand searched. *Figure 1* summarizes the flowchart of article selection and inclusion.

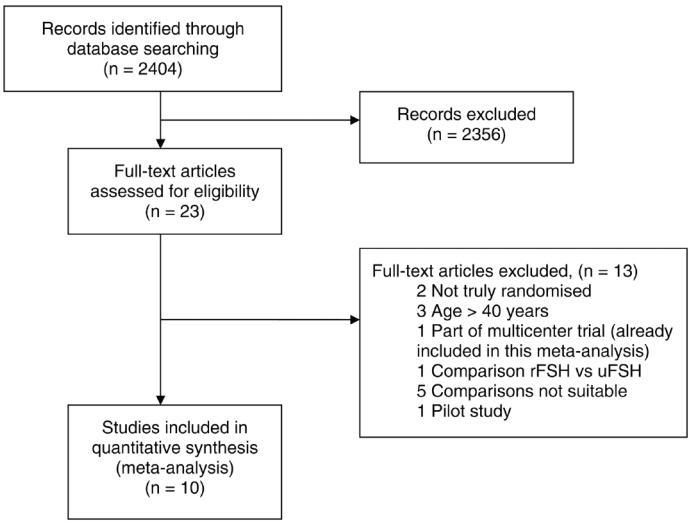

Figure 1 *Search strategy profile*

When clarification was required regarding an individual study, the first or senior author of the respective article was contacted. The parameter was stated not estimable if no further information could be obtained.

Statistical analysis

Since most of the studies compared either a dose of 100 IU/d versus higher, or 150 IU/d versus higher, two comparisons were made. Comparison A addressed 100 IU/d rFSH versus 200 IU/d rFSH, and comparison B 150 IU/d versus 200-250 IU/d rFSH. Further pooling of these groups was considered inappropriate since there was an overlap in dose (a high dose in one study could be the lower dose in another study).

When the outcome of interest was a continuous variable (e.g. number of oocytes), the difference in mean value between the two groups was calculated together with the standard error. These differences were pooled across studies, resulting in a weighted mean difference (WMD). Pooling was performed using the inverse of the variance as weight. For dichotomous outcome parameters (e.g. cancellation), the odds ratios (ORs) per study were calculated and pooled. Pooling was performed using the Mantel-Haenszel method. Statistical pooling was performed for the following outcome parameters: number of oocytes retrieved, clinical pregnancy rate, cancellation rate due to low response, amount of gonadotrophins in IU, OHSS rate, and number of cryopreserved embryos.

The 95% confidence intervals (CIs) were calculated for the WMD and pooled ORs, respectively, using both the directed weighted method and the random effects method. The random effect method is preferred because it remains valid even if true heterogeneity between studies is present, therefore we will only present random effects estimates. Statistical heterogeneity between studies was tested for all the outcome parameters. In case of statistically significant heterogeneity, univariate meta-regression was performed using the random effects method described by van Houwelingen et al.(van Houwelingen et al. 2002), on the following study characteristics: mean age, mean BMI, duration of infertility, percentage of primary infertility, and use of GnRH analogue. All parameters were reported per started cycle, except for number of oocytes which were calculated per ovum pick up (OPU) and the number of frozen embryos which were stated per embryo transfer (ET). All analyses were performed in Review Manager 5.

Results

We identified eleven relevant dosage RCT's reporting data on 1,967 women undergoing a single IVF cycle. All trials had parallel design and in most studies the treatment was adequately concealed prior to allocation (8 studies allocation score A (Out et al. 1999, Out et al. 2001, Hoomans et al. 2002, Tan et al. 2005, Out et al. 2000, Wikland et al. 2001, Latin-American Puregon IVF Study Group 2001, Out et al. 2004) and 3 studies allocation score B (de Jong et al. 2000, Pruksananonda et al. 2004, Cavagna et al 2006). All but three studies were double-blinded.(Wikland et al. 2001, de Jong et al. 2000, Cavagna et al. 2006)
For comparison A (100 IU/d versus 200 IU/d rFSH) six studies met the criteria for inclusion in the analysis. Unfortunately, as it was the only study eligible for comparison A which compared 100 versus 150 IU/day and used the GnRH antagonist as co-treatment, we had to exclude one pilot study (de Jong et al. 2000) because of the small numbers of patients (15 in total). Therefore, comparison A involved 960 women in total. The remaining 5 studies were analysed in comparison B (150 IU/d versus 200-250 IU/d rFSH), involving 992 women. *Table 1* summarizes the inclusion and exclusion criteria per included RCT. All the RCTs included presumed normal responders in their studies (age younger than 39 year, normal basal FSH, regular menstrual cycle and two normal ovaries). *Table 2* summarizes the characteristics of the included studies and the participants. In all studies the upper limit of included ages was at least 35 years.

The main outcome parameters are summarised in *figures 2 to 7*. *Figure 2* shows the number of oocytes obtained per oocyte retrieval. In comparison A, the 100 IU/day rFSH group yielded significantly fewer oocytes compared with the higher dose group (mean difference −3.56; 95% CI −4.86, −2.27; $P < 0.0001$). In comparison B, the 150 IU/day rFSH users obtained 1.7 oocytes fewer than those applying higher dosages (mean difference −1.67; 95% CI −2.53, −0.81; $P = 0.0001$).

Table 1 *Inclusion- and exclusion criteria for all the included RCT's*

Study	Inclusion criteria*	Exclusion criteria
Comparison A (100 versus 200 IU/day)		
Out et al. (1999)	Age: 18–39	Infertility caused by endocrine abnormalities such as hyperprolactinemia, PCOS, absence of ovarian function
	BMI: 18–29	Previous ovarian stimulation cycles after which less than three oocytes were retrieved
	Cycle: 24–35	Chronic cardiovascular, hepatic, renal or pulmonary disease
	Cause of infertility potentially solvable by IVF or ICSI	History of or current abuse of alcohol or drugs
	Good physical and mental health	Administration of non-registered investigational drugs within 3 months prior to screening
Out et al. (2001)	Age: 18–37	Female cause of infertility except mild endometriosis or mechanical factor
	BMI: 18–29	Previous IVF or ICSI cycle(s) after which less than three oocytes were retrieved
	Cycle: 24–35	Previous IVF or ICSI cycle(s) with hospitalization due to OHSS
	Male infertility solvable by ICSI	More than four previous IVF/ICSI cycles
	Presence of two ovaries	Total fertilisation failure in previous IVF or ICSI cycle
	Good physical and mental health	LH/FSH ratio at screening ≥3
		Chronic cardiovascular, hepatic, renal or pulmonary disease
		History of or current abuse of alcohol or drugs
		Administration of non-registered investigational drugs within 3 months prior to screening

*Age in years, BMI in kg/m2 and menstrual cycle in days.

Table 1 *Continued*

Study	Inclusion criteria*	Exclusion criteria
Hoomans et al. (2002)	Age: 18–39	Infertility caused by endocrine abnormalities such as hyperprolactinemia, PCOS, absence of ovarian function
	BMI: 18–29	Previous IVF or ICSI cycle(s) after which less than three oocytes were retrieved
	Cycle: 24–35	Previous IVF or ICSI cycle(s) with hospitalization due to severe OHSS
	Cause of infertility potentially solvable by IVF or ICSI	Chronic cardiovascular, hepatic, renal or pulmonary disease
	Good physical and mental health	History of or current abuse of alcohol or drugs
		Administration of non-registered investigational drugs within 3 months prior to screening
Pruksananonda et al. (2004)	Age: 25–38	Infertility caused by endocrine abnormalities such as hyperprolactinemia, PCOS, absence of ovarian function
	BMI: 18–29	Previous IVF or ICSI cycle(s) after which less than three oocytes were retrieved
	Cycle: 24–35	
	Good physical and mental health	
	Cause of infertility potentially solvable by IVF or ICSI	
Tan et al. (2005)	Age: 18–39	Infertility caused by endocrine abnormalities such as hyperprolactinemia, PCOS, absence of ovarian function
	BMI: 18–29	Previous IVF or ICSI cycle(s) after which less than three oocytes were retrieved
	Cycle: 24–35	Chronic cardiovascular, hepatic, renal or pulmonary disease
	Normal early follicular serum FSH concentration	History of or current abuse of alcohol or drugs
	Cause of infertility potentially solvable by IVF or ICSI	Administration of non-registered investigational drugs within 3 months prior to screening
	Good physical and mental health	

Table 1 *Continued*

Study	Inclusion criteria*	Exclusion criteria
Comparison B (150 versus 200–250 IU/day)		
Out et al. (2000)	Age: 30–39	Infertility caused by endocrine abnormalities such as hyperprolactinemia, PCOS, absence of ovarian function
	BMI: 18–29	One ovary or history of ovarian resection
	Cycle: 24–35	Severe endometriosis (Grade III)
	Cause of infertility potentially solvable by IVF or ICSI	Previous ovarian hyperstimulation cycles in which less than three oocytes were retrieved
	Good physical and mental health	Chronic cardiovascular, hepatic, renal or pulmonary disease
		History of or current abuse of alcohol or drugs
		Administration of non-registered investigational drugs within 3 months prior to screening
Wikland et al. (2001)	Age: 20–39	Not more than three previous ART attempts
	BMI: <30	No ovarian stimulation 3 months prior to study entry
	Cycle: 25–32	Previous history of severe OHSS
	Two normal ovaries	Previous failure of IVF or ICSI treatment due to poor response to gonadotrophin therapy (fewer than three mature follicles)
	Normal uterine cavity	ICSI failure
	Infertility treatment due to tubal, male or idiopathic factors or mild endometriosis	History of abnormal gynecological bleeding of undetermined origin
		Any contraindication to pregnancy
		Presence of clinically significant systemic disease

*Age in years, BMI in kg/m2 and menstrual cycle in days.

Table 1 *Continued*

Study	Inclusion criteria*	Exclusion criteria
Latin-American Puregon IVF study group (2001)	Age: 30–39	Infertility caused by endocrine abnormalities such as hyperprolactinemia, PCOS
	BMI: 18–29	Absence of ovarian function
	Cycle: 24–35	One ovary or history of ovarian resection
	Cause of infertility solvable by IVF or ICSI	Severe endometriosis (Grades III and IV)
	Good physical and mental health	Previous ovarian hyperstimulation cycles in which less than three oocytes were retrieved
		Previous hospitalization due to OHSS
		Chronic cardiovascular, hepatic, renal or pulmonary disease
		History of or current abuse of alcohol or drugs
		Administration of non-registered investigational drugs within 3 months prior to screening
Out et al. (2004)	Age: 18–39	History of/or current endocrine abnormality
	BMI: 18–29	Elevated early follicular phase FSH and/or LH concentration
	Cycle: 24–35	Any clinically significant abnormal laboratory value
	Weight: 50–90 kg	One ovary
		Any ovarian and/or abdominal abnormality that would interfere with adequate ultrasound investigation
		Contra-indications for use of gonadotrophins
		Use of hormonal preparations within 1 month prior to date of signing consent
		Alcohol or drugs abuse, or history thereof
		Administration of investigational drugs within 3 months prior to screening
Cavagna et al. (2006)	Age: 18–35	Endocrine abnormalities
	BMI: 19–29	Previous ART cycle with poor response
	Cycle: 24–35	Systemic chronic disease
	FSH<10 mIU/ml	

*Age in years, BMI in kg/m2 and menstrual cycle in days.

Table 2 *Characteristics of included RCTs, involving a total of 1952 subjects.*

Study	Design[a]	Comparison	Study protocol[b]	Population characteristics[c]			
				Mean age	Mean BMI	Mean duration of infertility	Number of primary infertility (%)
Comparison A (100 versus 200 IU/day)							
Out et al. (1999)	Allocation A Multicentre n = 199	100 versus 200 IU/day n = 101 versus n = 98	Agonist Fixed	32.7 (3.41) versus 32.4 (3.05)	22.9 (2.87) versus 23 (2.83)	5.25 versus 5	62 versus 75
Out et al. (2001)	Allocation A Multicentre n = 179	100 versus 200 IU/day n = 91 versus n = 88	Agonist Fixed	27.5 (4.2) versus 27.5 (3.7)	22.7 (3.1) versus 23.2 (3.1)	3.9 (2.7) versus 4.1 (3)	69 versus 70
Hoomans et al. (2002)	Allocation A Multicentre n = 330	100 versus 200 IU/day n = 163 versus n = 167	Agonist Fixed	31.6 (3.6) versus 32.1 (3.8)	22.2 (2.9) versus 22.3 (2.9)	5.2 (2.8) versus 5.9 (3.5)	NA
Pruksananonda et al. (2004)	Allocation B Single centre n = 60	100 versus 200 IU/day n = 30 versus n = 30	Agonist Fixed	34.7 (3.14) versus 33.7 (6.87)	20.2 (1.97) versus 20.7 (2.22)	6 (3.2) versus 5.4 (2.3)	NA
Tan et al. (2005)	Allocation A Multicentre n = 192	100 versus 200 IU/day n = 97 versus n = 95	Agonist Fixed 4 days, then flexible	33.3 (3.1) versus 33.4 (3.3)	NA	4.7 (3.2) versus 4.8 (3.2)	80 versus 76

Table 2 *Continued*

Study	Design[a]	Comparison	Study protocol[b]	Population characteristics[c]			
				Mean age	Mean BMI	Mean duration of infertility	Number of primary infertility (%)
Comparison B (150 versus 200–250 IU/day)							
Out et al. (2000)	Allocation A Multicentre n = 138	150 versus 200 IU/day n = 67 versus n = 71	Agonist Fixed	35.1 (2.6) versus 34.5 (3.2)	23.8 (2.8) versus 23.5 (3.4)	7 (4.1) versus 7.69 (5.3)	60 versus 65
Wikland et al. (2001)	Allocation A Bicentre n = 117	150 versus 225 IU/day n = 58 versus n = 59	Antagonist Fixed 5 days, then flexible	32.7 (3.9) versus 32.2 (3.9)	22.9 (2.6) versus 22.9 (2.5)	3.6 (1.7) versus 3.7 (2.1)	43 versus 30
Latin-American Puregon IVF study group (2001)	Allocation A Multicentre n = 404	150 versus 250 IU/day n = 201 versus n = 203	Agonist Fixed	35.1 (3.1) versus 35.3 (2.9)	22.9 (2.7) versus 23.1 (2.7)	5.4 (3.3) versus 5.2 (3.5)	53 versus 61
Out et al. (2004)	Allocation A Multicentre n = 257	150 versus 200 IU/day n = 131 versus n = 126	Antagonist Fixed 5 days, then flexible	32.7 (3.6) versus 32.2 (3.5)	23.5 (2.9) versus 23.5 (2.7)	4.6 (2.7) versus 4.6 (2.5)	51 versus 62
Cavagna et al. (2006)	Allocation B Single centre n = 76	150 versus 200 IU/day n = 40 versus n = 36	Agonist Fixed	31.4 (2.8) versus 31.7 (2.8)	NA	6.1 (2.5) versus 6.7 (3.3)	73 versus 75

NA, information not available.

a *RCT, randomised control trial. Concealment of allocation: (A) adequate and (B) unclear.* **b** *Use of GnRH analogue (agonist or antagonist), and use of rFSH fixed during treatment or flexible (increase or decrease of the dose after certain days).* **c** *Mean (SD) or median (range).*

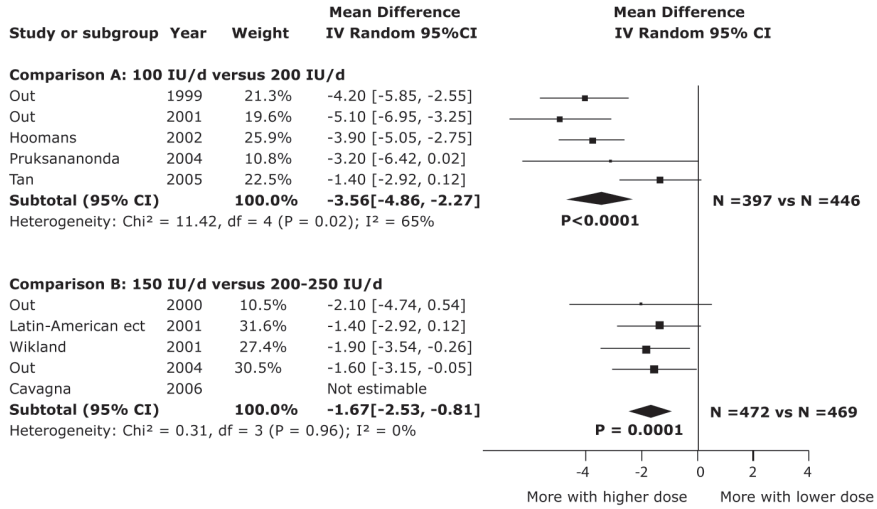

Figure 2 *Forest plot of mean difference of number of oocytes per oocyte pick-up. Forest plot: the area for each square is proportional to the weight of the corresponding study. The diamond represents the pooled WMD, and its width represents its 95% CI. A horizontal line represents each study, with its effect size and 95% CIs. The solid vertical line corresponds to no difference. df, degrees of freedom.*

In comparison A, the higher dose group obtained more cryopreserved embryos per embryo transfer (mean difference 1.40; 95% CI −2.32, −0.47; *P* = 0.003). No difference as observed for comparison B in the number of frozen embryos obtained (mean difference −0.05; 95% CI −0.49, –0.39; *P* = 0.82) (*Figure 3*).

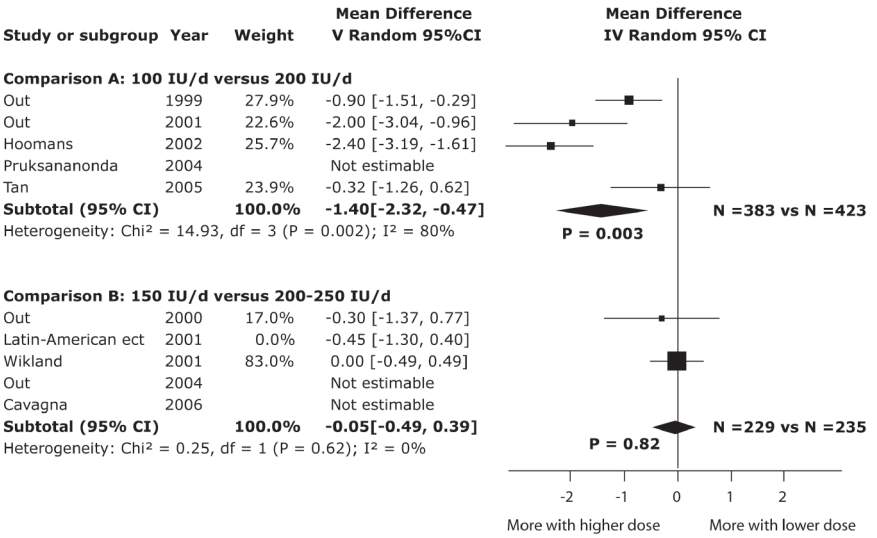

Figure 3 *Forest plot of mean difference of number of cryopreserved embryos.*

Figure 4 demonstrates the difference in the total amount of rFSH administered between the lower and the higher dose groups. The mean difference in both comparisons is very similar, but the CI differs (comparison A: mean difference −813.72 IU; 95% CI −860.26, −767.17; $P < 0.0001$ comparison B: mean difference −671.98 IU; 95% CI −896.85, −447.10; $P < 0.0001$).

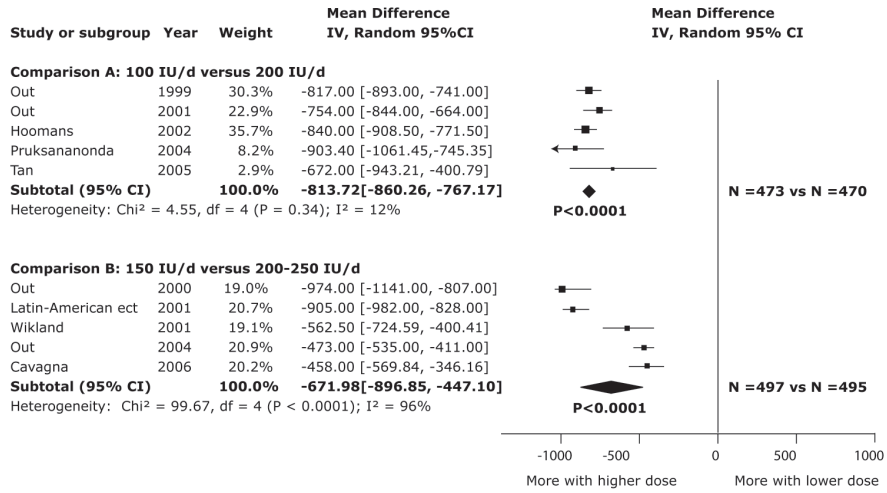

Figure 4 *Forest plot of mean difference of total amount of rFSH (IU).*

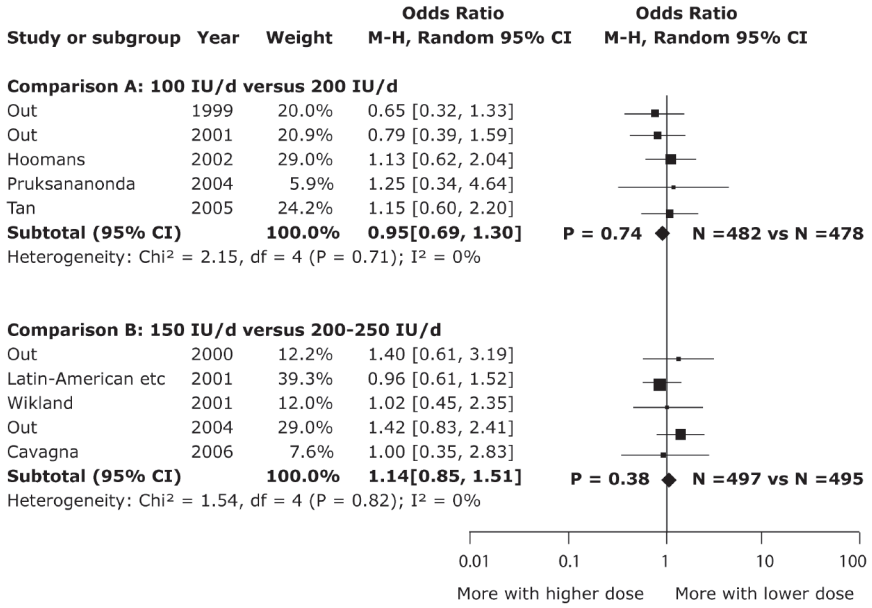

Figure 5 *Forest plots of ORs of clinical pregnancy rates.*

In both comparison groups A and B, the pregnancy rates per started IVF/ICSI cycle did not differ between lower and higher dosages [comparison A: OR 0.95 (calculated pooled estimates 19.5 and 20.3%, respectively), 95% CI 0.69–1.30, *P* = 0.74; comparison B: OR 1.14 (calculated pooled estimates 26.8 and 24.2%, respectively); 95% CI 0.85–1.51, *P* = 0.38] (*Figure 5*).

Cancellation due to low ovarian response to stimulation in comparison A (*Figure 6*) was observed to be more frequent in the 100 IU/day rFSH dose group [OR 5.02 (calculated pooled estimates 16.4 and 3.8%, respectively); 95% CI 2.19–11.51; *P* = 0.0001]. There was no difference in cancellation rate for low response in comparison B [OR 1.10 (calculated pooled estimates 4.4 and 4.0%, respectively); 95% CI 0.59–2.05; *P* = 0.76].

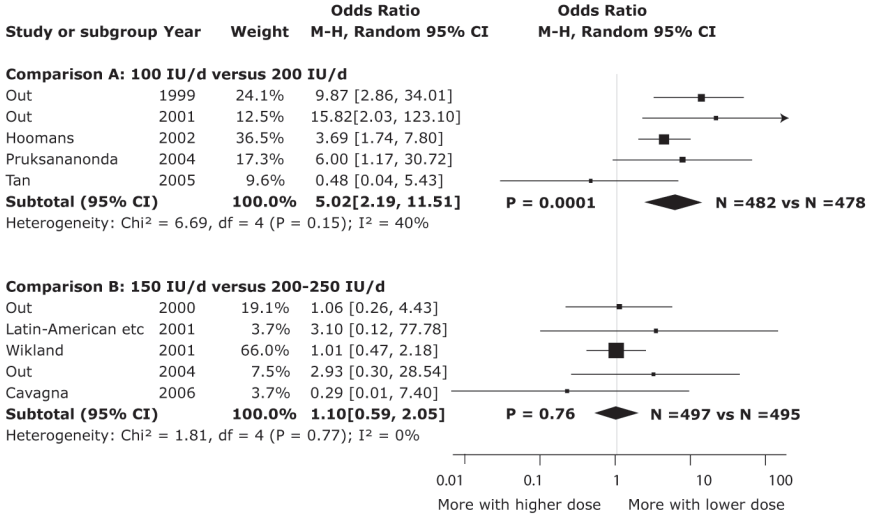

Figure 6 *Forest plots of ORs of cycle cancellation rates due to low ovarian response to stimulation.*

Figure 7 illustrates the risk of OHSS in relation to dose. In comparison A, the risk was reduced in the 100 IU/day rFSH dosage dose group by a factor of almost two [OR 0.58 (calculated pooled estimates 1.9 and 3.4%, respectively); 95% CI 0.18–1.90; *P* = 0.37]. In comparison B, the risk for OHSS was 33% lower in the 150 IU/day rFSH group compared with the higher dosage group [OR 0.67 (calculated pooled estimates 2.6 and 3.8%, respectively); 95% CI 0.33–1.37; *P* = 0.27]. However, for both comparison groups these ORs were not significantly different. Heterogeneity across comparisons was found for the parameters number of oocytes (comparison A I^2= 65%), number of frozen embryos (comparison A I^2= 80%) and total amount of rFSH (comparison B I^2= 96%).

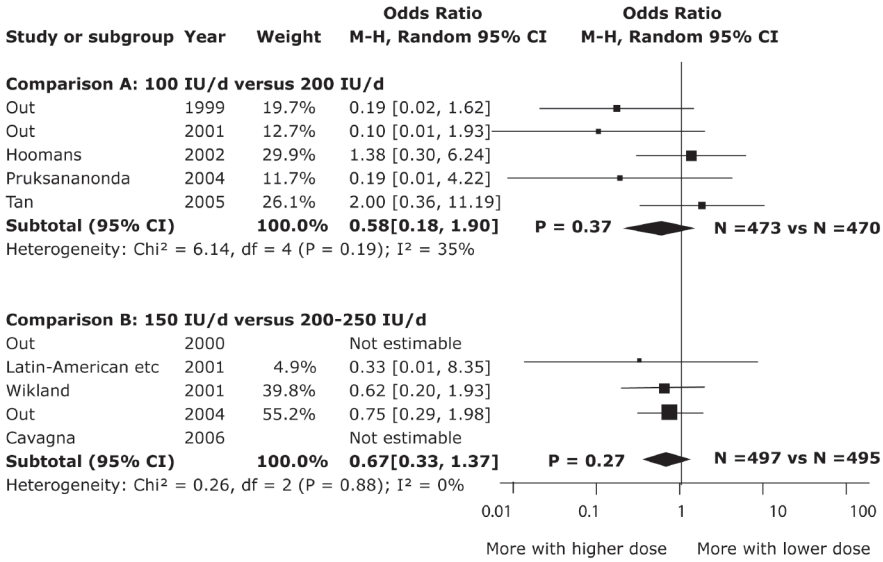

Figure 7 *Forest plots of ORs of OHSS rates.*

In meta-regression on the parameter number of oocytes, none of the study characteristics explained the heterogeneity. For the parameter number of frozen embryos, the body mass index (BMI) was the characteristic which could explain this heterogeneity. Women with a lower BMI showed a greater dose-related difference in the number of embryos available for freezing, and this was similarly evident in both the comparison groups.

In comparison B, heterogeneity in the total amount of rFSH was explained by the study characteristic age. The first two studies (Out *et al.* 2000; Latin-American Puregon IVF Study Group, 2001) have a mean age around 35 years and the rest of the studies (Wikland *et al.* 2001; Out *et al.* 2004; Cavagna *et al.* 2006), around 32 years. The studies with the 'older' patients have a larger difference in total amount of rFSH (*Figure 4*).

Discussion

Until now, no consensus regarding the optimal starting dose of FSH for ovarian hyperstimulation in IVF/ICSI treatment cycles in normal responders exists. Sufficiently powered dose-response studies providing useful information in relation to the preferred effective starting dose of exogenous gonadotrophins are scarce. Despite this lack of information, many clinicians have strong beliefs as to what constitutes the best dose regimen for their patients. However, this is based largely on personal experience and limited empirical research. Therefore, practices vary

throughout the world and even between IVF centres within the same country. These differences in patient management may have major implications for IVF pregnancy rates, drug cost, complication rates, and possibly also for patient discomfort.

The current meta-analysis is an attempt to provide objective information regarding the relation between the applied daily FSH dose for ovarian stimulation in normal responders in IVF/ICSI in relation to treatment outcomes, cost and complications. The study convincingly demonstrates that the average number of oocytes retrieved per pick-up is increased when higher FSH doses over 100 IU per day are given, whereas pregnancy rates do not differ across the dosage groups (*Figure 8*). Moreover, the number of frozen embryos available for subsequent transfer does not improve with dosages exceeding 150 IU/day, suggesting that cumulative pregnancy rates (including additional cryo embryo transfer cycles) will not become superior.

Pharmacodynamic studies of rFSH have shown that the response to a 225 IU daily dose varies mainly according to the women's age and her ovarian reserve.(Karlsson et al. 1997) This implies that there is a limitation in the number of follicles that can be stimulated to ongoing development. The comparison between 150 IU/d and higher dosages revealed that the increase in number of oocytes harvested is limited. This may indicate that the dose eliciting maximal stimulation of the ovaries in most patients may be somewhere between 150 and 200 IU/d. Using a dosage of 100 IU/d leads to a more pronounced reduction in oocyte number compared to higher doses, suggesting that in this dose range a dose-response relationship does exist. With current starting dosages of 150 IU/d or more in most centres, maximal or near maximal stimulation of the ovaries will usually be obtained.

In recent years, so called "mild" stimulation regimens have been proposed, aiming at harvesting more modest numbers of oocytes.(Nargund et al. 2007) Initial studies suggest that in comparison to conventional stimulation, milder ovarian stimulation protocols are associated with medical, health, economic, and psychological benefits.(Heijnen et al. 2007) The present meta-analysis demonstrates that even in conventional GnRH agonist stimulation regimes the use of lower daily dosages of rFSH (i.e. 100IU/day) produces more modest ovarian responses without undesirable effects on pregnancy rates. Consistent with these findings, a recent study in which rFSH dose adaptations were based on individualised patient profiles ranging from 75 up to 225 IU/d revealed that in ~30% of patients, a dose of 100 IU/d or less is sufficient to obtain moderate oocyte numbers with high pregnancy rates.(Olivennes et al. 2009) Milder ovarian responses may create equal numbers of good quality embryos compared to maximal stimulation approaches.(Hohmann

et al. 2003, Baart et al. 2007) The current paradigm of a standard dose which will work for the majority of women is therefore being increasingly questioned (Fauser et al. 2008).

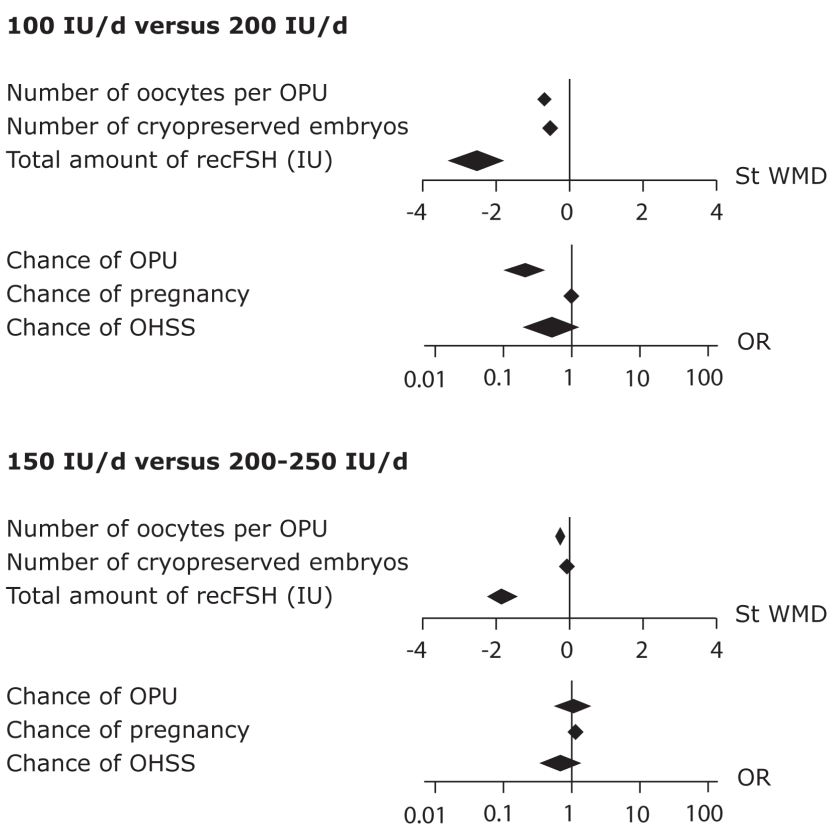

Figure 8 *Summary all parameters; (**A**) Comparison A: 100 versus 200 IU/day; (**B**) Comparison B: 150 versus 200–250 IU/day. St WMD, standardized weighted mean difference; OR, odds ratio; OPU, ovum pick up; OHSS, ovarian hyperstimulation syndrome.*

In the current meta-analysis, data were pooled in two comparison groups (A: 100 IU/d versus 200 IU/d; B: 150 IU/d versus 200-250 IU/d). Besides the dosages, the studies were different in the use of GnRH agonist and antagonist as co-treatment. We demonstrated that for the analysis of total amount of rFSH, number of oocytes, and number of cryopreserved embryos, heterogeneity could be explained by age and BMI. The validity of pooling studies with different co-treatment characteristics may be questioned. However, re-analysis excluding the studies with GnRH antagonist as co-treatment (Wikland et al. 2001, Out et al. 2004, de Jong et al.

2000) demonstrated no differences in pooled results of all outcome parameters compared to the original analysis.

A potentially negative outcome of giving a lower dose of rFSH is the risk of low response resulting in cycle cancellation. However, we have previously shown that the pregnancy rates following low dosage use are similar to conventional dosages. This indicates that doctors should not be unduly concerned if a low response is observed and can proceed to aspiration of the oocytes even when few follicles are present. When using 150 IU/d the probability of low response is not different from higher dosages, indicating that in general, 150 IU/d is likely to represent the optimal dosage.

Extreme responses to ovarian stimulation introduce the risk of developing the ovarian hyperstimulation syndrome (OHSS).(Delvigne et al. 2002, Aboulghar et al. 2003) Although early diagnosis and treatment may minimise the risk of catastrophic events (such as tromboembolism and multiple organ failure) preventing the occurrence of OHSS remains the corner stone of proper management. Alongside refraining from human chorionic gonadotrophin (hCG) for triggering of final oocyte maturation or the cryopreservation of all embryos obtained in very high responder patients, avoiding extreme ovarian response should be regarded as the primary approach in the prevention of OHSS. The tendency for lower OHSS rates in lower dose groups, as demonstrated in the present meta-analysis, further supports the approach of sub maximal ovarian stimulation.

Reduction in total rFSH dose would also considerably cut the cost of IVF treatment. As the total duration of stimulation (on average 12 days) is not affected by dosage changes, the use of a standard dose of 150 IU/d, instead of 225 IU/d, would reduce per-cycle costs of gonadotrophin medication by ~30%.(Wechowski et al. 2009) This would imply that every set of two IVF treatment cycles will save the amount of rFSH sufficient for a third stimulation cycle. The use of GnRH antagonist as co-treatment might lead to an even lower consumption of rFSH, mainly by a reduction in the duration of stimulation due to relatively high endogenous FSH concentrations early during the stimulation cycle.(Fauser et al. 1997, Heijnen et al. 2007, Baart et al. 2007)

Significant strengths of the current meta-analysis are that it was performed according to the QUORUM guidelines, that almost 2,000 IVF cycles were involved in the analysis, that heterogeneity was addressed and was explained by meta-regression. We had to split the dosage comparisons into two groups. An advantage of this split was that we could assess the dose at which there was no more gain of a higher dose and thus determine the optimal starting dose. Limitations of this study are that the patient groups were more restricted in age and BMI than is

seen in everyday practice. Furthermore, the most relevant clinical endpoint, cumulative live birth, was not available in most studies. We therefore had to restrict the analysis to ongoing pregnancy rates. The effect of OHSS is overestimated since in some studies no distinction was made between mild, moderate, and severe OHSS. Another limitation of this meta-analysis is that all included studies had number of oocytes as primary outcome parameter, therefore none of the studies were powered for differences in pregnancy rates. The pooling of data, however, has allowed for drawing valid conclusions on the effect of FSH dosage level on the clinical pregnancy rates. Finally, all underlying studies applied a so called "one-size fits all" approach, with no possibilities for patient tailored adjustments based on individual patient characteristics.(Popovic-Todorovic et al. 2003)

In conclusion, this meta-analysis suggests that the optimal starting dose of rFSH for IVF/ICSI is 150 IU daily. This dose is associated with a more modest oocyte yield, but an equal pregnancy rate to that achieved with higher dosages. Further benefits of this dose include the reduced OHSS risk, but production of sufficient numbers of oocytes to allow for cryopreservation of surplus embryos. In the future, the use of patient-tailored approaches to determine individual dose based on screening characteristics may further optimise the risk-benefit balance, increasing the proportion of women exhibiting an adequate ovarian response while further reducing the need for intense monitoring of ovarian response.(Olivennes et al. 2009, Fauser et al. 2008, Popovic-Todorovic et al. 2003)

Chapter 3

GnRH antagonist co-treatment in routine IVF: is high oocyte yield associated with reduced ongoing implantation rate?

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Abstract

Although GnRH antagonist co-treatment in IVF cycles reduces treatment duration and medication costs, overall efficacy is believed to be lower compared to GnRH agonist co-treatment. In a late start rFSH and flexible GnRH antagonist protocol a decrease in implantation rates was associated with over 10 oocytes being collected. The current study explores this association in the currently popular regimen of an early start rFSH combined with fixed day start of GnRH antagonist. Between September 2005 and June 2009, all patients in our department below 38 years of age undergoing such regimen in their first IVF cycle were included in a retrospective analysis associating the number of oocytes obtained with ongoing implantation rates.

In total 438 cycles were included. The overall ongoing implantation rate in this group was 27.4%. After adjusting for age, embryo quality, single or double embryo transfer, and the interaction between embryo number and quality, the OR for the relation between number of oocytes and ongoing implantation rate per oocyte was 0.96 (95% CI 0.92-0.99; p=0.046).

This analysis of standard stimulation cycles using GnRH antagonist co-treatment reveals that higher responses negatively affect ongoing implantation rates. This finding may have implications for stimulation dose assessment in GnRH antagonist cycles.

Introduction

In order to prevent premature luteinisation, ovarian stimulation for IVF treatment is usually combined with treatment with a GnRH analog (Albano et al. 1997, Hughes et al. 1992, MacLachlan et al. 1989, Tan et al. 1994, Zorn et al. 1987). In contrast to GnRH agonists which act by down regulating the expression of GnRH receptors at the pituitary, GnRH antagonists block the GnRH receptor competitively enabling rapid suppression of gonadotrophin secretion by the pituitary (Gregory et al. 1982, Hazum et al. 1985). The use of GnRH antagonists therefore allows for the initiation of IVF treatment in a normal menstrual cycle with an undisturbed early follicular phase recruitment of a cohort of follicles. The endogenous inter-cycle FSH rise remains intact rather than being suppressed, as occurs in GnRH agonist, leading to follicle development in the early follicular phase of the menstrual cycle. (Fauser et al. 1997, Fauser et al. 2010). The GnRH antagonist approach leads to a shorter treatment duration and lower burden to the patient (Huirne et al. 2001). The disadvantage compared to GnRH agonists is the smaller therapeutic range causing escape LH peaks in some cases (Kolibianakis et al. 2004). This may result in lower pregnancy rates and, indeed, the debate continues regarding this issue (Al-Inany et al. 2006, Banga et al. 2010, Kolibianakis et al. 2006). The GnRH antagonist co-medication in the treatment cycle can be initiated according two distinct protocols. In the fixed protocol, the GnRH antagonist is initiated in the middle-late follicular phase, mostly on stimulation day 6 (Olivennes et al. 1998). To reduce the number of injections, a flexible protocol has been developed, where the initiation of administration is dependent on the follicle size (as soon as the follicles reach a size of ≥14 mm) (Escudero et al. 2004, Hohmann et al. 2003, Klipstein et al. 2004, Kolibianakis et al. 2003a, Ludwig et al. 2002, Macklon et al. 2000, Mochtar et al. 2004). Half of the patients will then start with the GnRH antagonist beyond stimulation day 6 (Escudero et al. 2004, Kolibianakis et al. 2003a, Mochtar et al. 2004). In this group, reported pregnancy rates were reduced, probably due to higher LH, estradiol and progesterone levels (Kolibianakis et al. 2003a). Recent studies from our group, combined in a meta-analysis (Verberg et al. 2009a), have shown decrease in implantation rates in case more than 10 oocytes were collected in GnRH antagonist cycles. The studies included in this meta-analysis commenced rFSH on cycle day 5 and used a flexible start of GnRH antagonist in a ”mild” stimulation protocol. A possible explanation for the decreased implantation rates in high responders was the late, flexible start of the GnRH antagonist, resulting in escape LH rises before the GnRH antagonist had been initiated due to early and rapid rising estradiol levels (Verberg et al. 2009a). An early, fixed start of the GnRH antagonist might solve this problem. In this present retrospective study, the IVF database was reviewed after the introduction of the GnRH antagonist in our standard protocol, using early initiation of FSH (cycle day 2), with a standard dose 150 IU stimulation dosage, and a fixed start of the antagonist (stimulation day 5). The hypothesis was that using

this approach the previously demonstrated reduction of ongoing implantation rates in high responders would not be observed.

Material & Methods

Subjects and study design

In this retrospective study, all consecutive patients undergoing in vitro fertilisation (IVF), with or without intracytoplasmatic sperm injection (ICSI) from September 2005 till June 2009, at the Department of Reproductive Medicine and Gynaecology of the University Medical Centre Utrecht (Utrecht, The Netherlands) were included. Only first treatment cycles using GnRH antagonist as co-treatment were included. Patients with PCOS, age ≥ 38 years, and patients who participated in any prospective study, which implied the use of an altered medication scheme, were excluded. In view of the research question, cycles not leading to embryo transfer were also excluded (*Figure 1*).

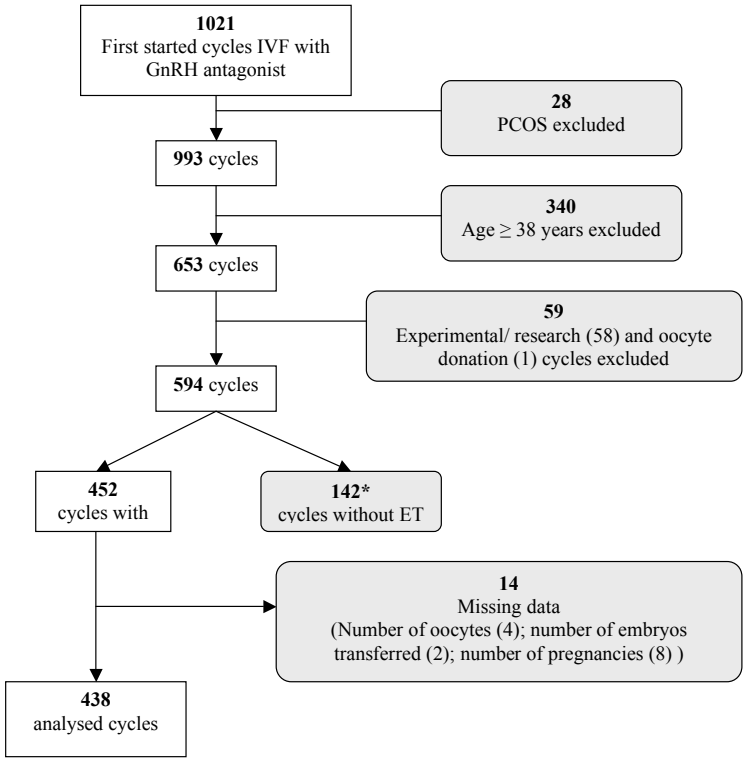

Figure 1 *Flowchart of included cycles*
*IVF = in vitro fertilisation; PCOS = polycysteus ovarian syndrome; ET = embryo transfer; sET= single embryo transfer; dET= double embryo transfer; * 50 cycles with no OPU and 92 cycles with no ET*

All included patients commenced rFSH medication on cycle day 2 or 3. The standard starting dosage was 150 IU rFSH. In some cases, a dose adjustment was applied or the dose was adjusted during stimulation, with a maximum dosage used of 300 IU rFSH/day. On stimulation day 5, GnRH antagonist co-medication was started. Follicle growth was monitored by ultrasound, starting from stimulation day 6 or 7. When at least 3 follicles reached a diameter of 17 mm or more, a bolus of 10.000 IU of hCG was administered for final oocyte maturation. Oocyte pick-up (OPU) was scheduled 36 hours later. Subsequently, IVF with or without ICSI was performed and 1 or 2 embryos were transferred 3 or 4 days thereafter. Luteal support using intravaginal progesterone (Progestan®, 200 microgram three times daily) was given form the day of oocyte pick-up until 12 days later. In our clinic patients less than 36 years of age had the option to transfer 1 embryo or 1 or 2 embryos depending on the morphological embryo quality. Patients of 36 years of age or older had the extra option to transfer a standard number of 2 embryos, irrespective of quality. The aim for this procedure is to prevent multiple pregnancies.

Statistical analysis

The patient and treatment characteristics were compared between ongoing pregnant or not (ongoing pregnant defined as pregnancy developing beyond 12 weeks) using t-tests and chi squared test for continuous and categorical variables, respectively. Also, the outcome parameter live birth delivery rate, defined as the birth of a newborn, irrespective of the duration of gestation that exhibits any sign of life, was evaluated. The statistical analysis for our primary aim, the association between number of oocytes and embryo implantation rate, included binary logistic regression, with embryo implantation (yes/no) as the outcome. This analysis was adjusted for the number of embryos transferred, (sET or dET), female age, embryo quality and the interaction between number of embryos transferred and embryo quality. The reason for this approach was that the embryo transfer policy depended on age and morphological quality of the embryos, characteristics that are clearly predictive of embryo implantation. However, within the sET group, there were patients of young age with good quality embryos, but also patients in which there was only one poor quality embryo available, making sET the only option, often with poor outcome. Therefore, the prognostic effect of embryo quality may be stronger in the sET cycles than in the dET cycles, leading to statistical interaction. Since all these factors may also be related to the number of oocytes, adjustment in the analysis was required for embryo quality (defined as the presence of at least one morphological top embryo (day 3: 6-10 blastomeres, <20% fragmentation, and equal blastomere size) and for female age. To investigate whether there was a non-linear (or curvilinear) relationship between the number of oocytes and implantation in the logistic regression, a restricted cubic spline function was applied, similar as applied in the study by Verberg et al (Verberg et al. 2009a).

Finally, the ongoing implantation of the first cycle of frozen embryos after the fresh cycle was analysed, to test whether the effects found in the fresh cycle were also present in the frozen cycle. Missing values were included in the analysis using multiple imputations from the aRegImpute function (library HMisc) in R version 2.9.0 (www.r-project-org). More elementary analyses were performed using SPSS version 15.0 (SPSS, Chicago, IL).

Results

During the period of the study, 1,021 first IVF treatment cycles with GnRH antagonist as co-treatment were carried out. *Figure 1* shows the numbers and reason for excluding cycles from this analysis; 427 cycles were excluded due to PCOS, age ≥ 38 years, and experimental and research cycles. Also, 142 cycles not leading to embryo transfer were excluded, Of these 142 cycles, 50 cycles (8.4%) were cancelled before oocyte pick up and 92 cycles (15.4%) had no embryo transfer related to absent oocyte yield or fertilisation failure. Finally, 438 cycles could be analysed of which 263 were sET and 175 dET cycles.

The overall ongoing pregnancy rate was 120/438 (27.4%) with obvious differences in the sET (23.6%) and the dET (33.1%) group. *Table 1* shows the demographic and infertility characteristics of the included patients. The only notable difference between the group with or without ongoing pregnancy rate was the cause of infertility. In *Table 2* an overview of the baseline characteristics of the treatment is given. The number of oocytes retrieved was higher in the group without ongoing pregnancy than in the group with ongoing pregnancy, without reaching the statistical significance level (8.9 (SD 5.4) and 8.4 (SD 8.7), respectively; p= 0.37).

The number of top-embryos was significantly higher in the ongoing pregnancy

Table 1 *Demographic and infertility characteristics of the included patients.*

Patients characteristics	Ongoing pregnancy		Total	p-values
	No (N=318)	Yes (N=120)	N=438	
Mean age in years (SD)	33.8 (3.1)	33.7 (2.7)	33.8(3.0)	0.50
Mean duration of infertility in years (SD)	4.2 (2.2)	4.2 (2.2)	4.2 (2.2)	0.93
Cause of infertility (%)				
Tubal pathology	26.4	9.7	22.0	
Male factor	30.8	38.9	33.0	
Unexplained	28.4	31.9	29.3	0.03
Failed insemination	11.9	15.3	12.8	
Other	2.5	4.2	2.9	
Number of women with primary infertility (%)	60.2	56.9	59.3	0.55

SD= standard deviation

Table 2 *Treatment characteristics of the included patients. The number of top-embryos and cryo-embryos are higher in the sET group due to the selection procedure at this centre, aimed at prevention of multiple pregnancies (patients < 36 years: 1 embryo or 1 or 2 embryos depending on morphological quality; patients ≥ 36 years: same as patients < 36 years with extra option to transfer 2 embryos).*

Treatment characteristics	Ongoing pregnancy		Total	p-values
	No (N=318)	Yes (N=120)	N=438	
Duration stimulation in days (SD)	9.6 (4.6)	10.0 (4.9)	9.7 (4.7)	0.44
Number of oocytes retrieved (SD)	8.9 (5.4)	8.4 (4.8)	8.7 (5.3)	0.37
Proportion of top-embryos	52.9%	64.3%	56.2%	0.04
sET	65.1%	93.4%	71.9%	0.0001
dET	29.3%	31.5%	30.1%	0.78
Number of cryo-embryos (SD)	1.19 (2.16)	1.23 (2.22)	1.20 (2.17)	0.90
sET (SD)	1.69 (2.47)	2.05 (2.67)	1.78 (2.52)	0.33
dET (SD)	0.32 (0.95)	0.34 (1.07)	0.33 (0.99)	0.90

sET= single embryo transfer; dET= double embryo transfer; SD= standard deviation

group (64.3% versus 52.9%, p=0.04). The sET group showed the highest percentage of top embryos in case of ongoing pregnancy rate (93.4%) compared to the other groups (sET without ongoing pregnancy, dET without ongoing pregnancy, and dET with ongoing pregnancy). This also applied to the number of cryoembryos, which was the highest in the sET group with ongoing pregnancy (2.05 (SD 2.67)).

The overall ongoing implantation rate was 23.0% and did not differ very much in sET (23.6%) and dET (22.0%) group. The relation between number of oocytes and the probability of ongoing implantation per oocyte was expressed by an Odds Ratio (OR) of 0.98 (95% CI 0.95-1.02; p=0.38). After adjusting for age, top embryo, sET/dET, and the interaction between sET/dET and top embryo, the OR for the relation between number of oocytes and ongoing implantation was 0.96 (95% CI 0.92-0.99) and statistically significant (p=0.046) (*Table 3*). This implies that with every additional oocyte obtained, the chance of ongoing implantation was reduced by 4%.

Table 3 *The OR for ongoing implantation per number of oocytes. In the multivariate analysis, the number of oocytes was adjusted for age, top embryo, sET/dET, and the interaction between sET and dET on top embryo.*

Variable	OR	95% CI	P-value
Univariate			
Number of oocytes per oocyte	0.98	0.95-1.02	0.38
Multivariate			
Number of oocytes per oocyte, adjusted for age	0.98	0.94-1.02	0.30
Number of oocytes per oocyte, adjusted for top embryo	0.98	0.94-1.02	0.28
Number of oocytes per oocyte, adjusted for sET/dET	0.98	0.95-1.02	0.39
Number of oocytes per oocyte, adjusted age, top embryo, sET/dET, and interaction sET/dET on top embryo	0.96	0.92-1.00	0.046

sET= single embryo transfer; dET= double embryo transfer; OR = odds ratio; CI = confidence interval

The probability of ongoing implantation in relation to the number of oocytes after adjusting for age, top embryo, sET/dET, and the interaction between sET/dET and top embryo was lower when a higher number of oocytes was collected (*Figure 2*). Top = the presence of at least one morphological top embryo

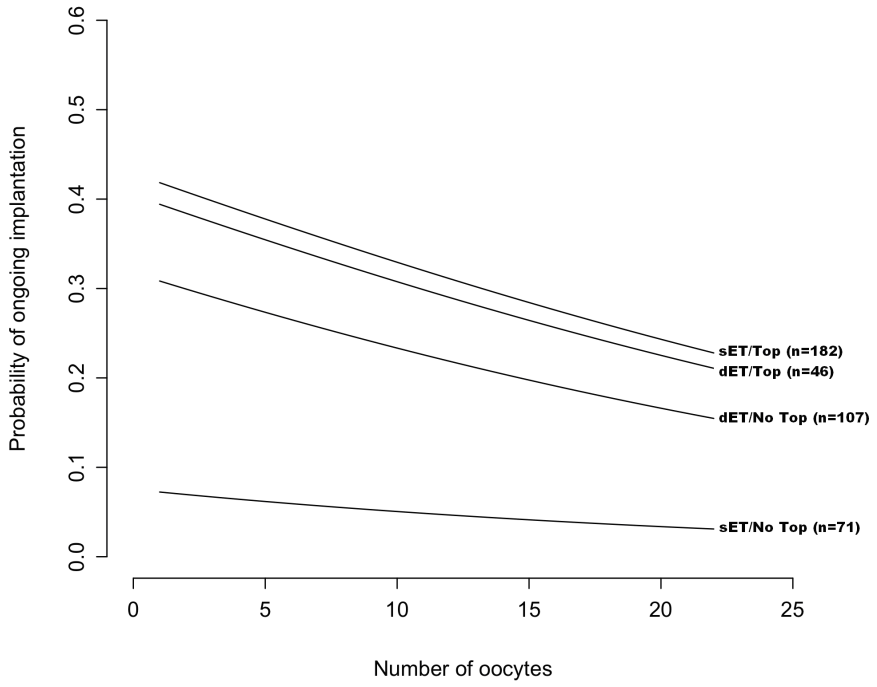

Figure 2 *The probability of ongoing implantation in the sET and dET group with or without a top embryo. There is a decrease in ongoing implantation probability with higher numbers of oocytes yielded.*

Figure 3 is a detail of the uppermost line in *Figure 2* to take a closer look at the 95% CI to find out if there is an optimum of number of oocytes where the ongoing implantation rate is the highest and if there is a decrease in implantation rate in the patients with low number of oocytes. The probability of ongoing implantation in the group who underwent sET with a top quality embryo is demonstrated since this is the group with the highest number of cycles and the highest ongoing implantation rate. However, because of the wide confidence interval at the beginning and at the end of the figure, it is not possible to identify an optimum.

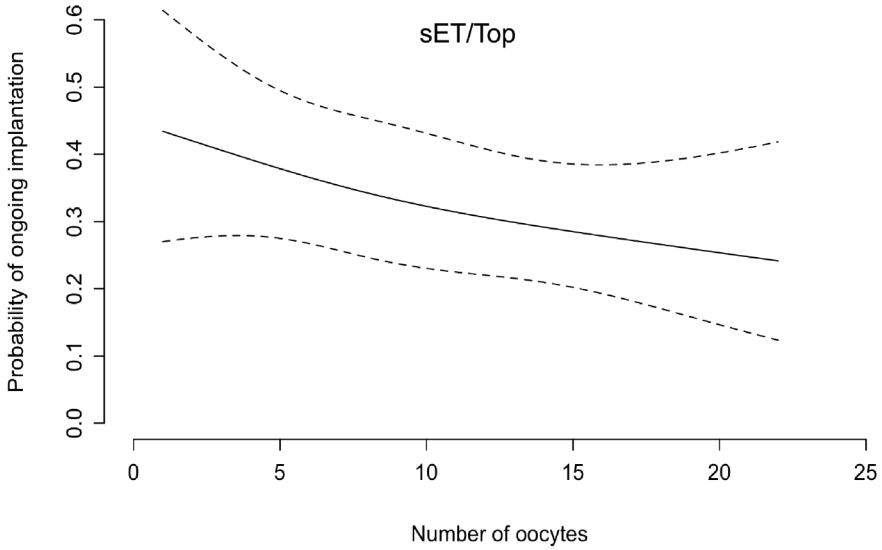

Figure 3 *The probability of ongoing implantation in the sET group with a top embryo. The decrease in ongoing implantation probability with increasing oocyte number is significant. Due to the wide confidence interval at the beginning and the end of the range of number of oocytes, it is not possible to conclude whether there is a peak in the number of oocytes with the best implantation potential.*

Of the included cycles, 112 cycles delivered embryos that could be cryopreserved. *Figure 4* shows the probability of implantation of the first transfer of a frozen embryo after the fresh cycle, adjusted for age and number of transferred cryoembryos. Patients who became pregnant after the fresh cycle were further excluded from this analysis. There was no decrease in the probability of ongoing implantation in case of a high number of oocytes yielded at the fresh cycle, although the wide confidence interval does not completely exclude such an effect.

The overall live birth delivery rate, including cryo transfers, was 119/438 (27.2%). The relation between number of oocytes and live birth rate was also analysed. The OR for the relation between number oocytes and live birth was 0.98 (95% CI 0.95-1.03; p=0.53). After adjusting for the factors as described before, the OR was 0.96 (95% CI 0.92-1.004; p=0.082)

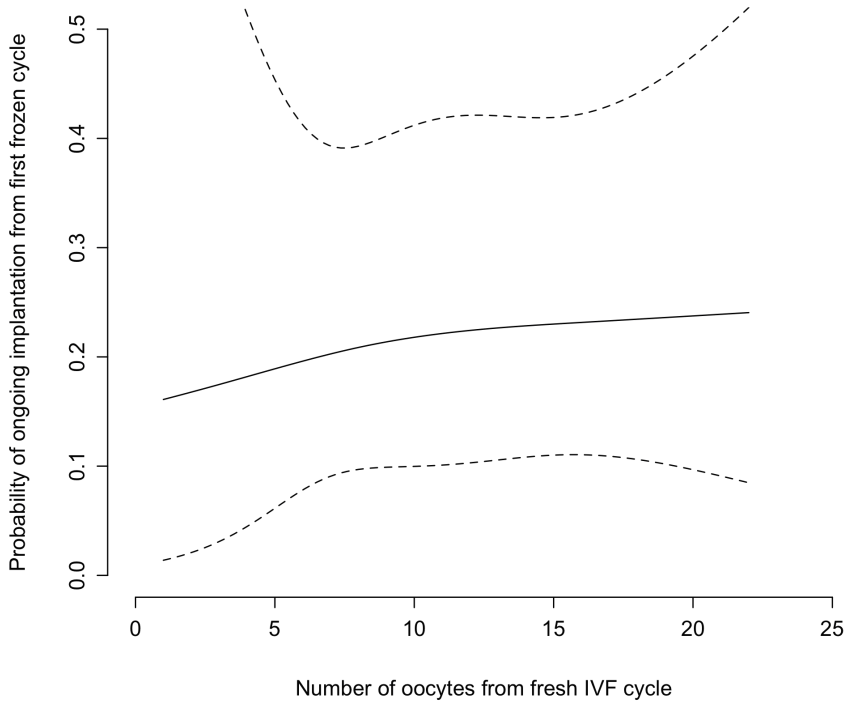

Figure 4 *The probability of ongoing implantation after the first transfer cycle of a frozen embryo.There is no significant change in the probability of ongoing implantation with more oocytes retrieved in fresh cycle pick up.*

Discussion

This retrospective study shows that high responders to a fixed GnRH antagonist protocol with FSH stimulation starting at cycle day 2 or 3 demonstrate a decrease in ongoing pregnancy rate compared to normal responders. Apparently, the reduction in ongoing implantation rate with higher ovarian response is not limited to the use of a flexible GnRH antagonist, late start mild stimulation protocol, as was published in the meta-analysis by Verberg (Verberg et al. 2009a).

Two meta-analyses (Al-Inany et al. 2006, Kolibianakis et al. 2006) included randomised studies where the GnRH agonist was compared with the GnRH antagonist. Most of the included studies show a higher implantation and pregnancy rates in the GnRH agonist group. The decrease in ongoing implantation rate in high responders in this study may be an explanation for the lower implantation rates observed following GnRH antagonist versus agonist in early comparative studies (Al-Inany et al. 2006, Kolibianakis et al. 2006).

The potentially negative effects of ovarian stimulation level on oocyte and embryo quality have been reported in several human and animal studies.After the exposure

to high doses of gonadotrophins during *in vitro* maturation of oocytes, increased incidences have been reported of morphological and chromosomal abnormalities (Eppig et al. 1998, Haaf et al. 2009, Roberts et al. 2005, Van Blerkom et al. 2001). Patients undergoing ovarian stimulation have higher estradiol concentrations compared to the follicular phase of a normal menstrual cycle. High estradiol levels have been reported to be associated with a negative effect on embryo developmental and implantation potential (Ertzeid et al. 2001, Santos et al. 2010, Valbuena et al. 1999, Van der Auwera et al. 2001) as well as their chromosomal constitution (Katz-Jaffe et al. 2005). Finally, in a randomised controlled trial, a reduction has been demonstrated in the number of oocytes retrieved with a concomitant higher proportion of chromosomally normal embryos in a mild stimulation protocol compared to the conventional GnRH agonist protocol (Baart et al. 2007).

The impaired implantation rates in patients with a higher response in this retrospective study could also be related to an early rise of LH, even before the antagonist medication is initiated. The early rise of LH may caused by early rises in estradiol levels due to higher number of antral follicles responding to exogenous FSH (Borm et al. 2000). In these patients, the start of the GnRH antagonist might be too late to prevent overexposure to LH, leading to luteinisation or other unknown effects on follicle/oocyte quality. Higher levels of mid-follicular LH during down-regulation in IVF cycle have observed to be negatively associated with the chance of ongoing pregnancy rates (Kolibianakis et al. 2004, Humaidan et al. 2002, Kolibianakis et al. 2003b).

Another explanation for the untoward effects of high ovarian responses in antagonist cycles may be that endometrial receptivity is negatively influenced by supra-physiological estradiol concentrations(Macklon et al. 2000, Simon et al. 1995). This may be due to advanced postovulatory endometrial maturation and defective induction of progesterone receptors (Devroey et al. 2004). The endometrium is probably advanced for more than 3 days after ovarian stimulation with exogenous gonadotrophins with co-treatment of GnRH agonist or antagonist (Devroey et al. 2004). The LH concentration at the initiation of gonadotrophin stimulation and the duration of stimulation before start of the GnRH antagonist are predictive of endometrial advancement (Kolibianakis et al. 2003b). Therefore, both the production of LH and estradiol in the early follicular phase should be controlled to prevent this advancement of the endometrium (Devroey et al. 2004). The possibility of unfavourable effects of high estradiol levels on the endometrium may be supported by the present finding, that no decrease was observed in ongoing implantation rates in first frozen/thawed embryo cycles. This

supports the above theory, as in a frozen-thaw cycle the estradiol levels are very similar to the levels in the natural cycle.

In an earlier meta-analysis an optimum number of oocytes was observed. In this retrospective study there was no optimum number of oocytes as shown in *Figure 4*. The retrospective nature of this study means that the findings should be interpreted with some caution. However, the principle goal of this retrospective study was to find out whether the previously reported relation between higher number of oocytes and lower implantation rates observed following the use of a late FSH start, flexible start GnRH antagonist protocol (Verberg et al. 2009a), was absent in a more widely used fixed start GnRH antagonist. A shortcoming of this retrospective study is the lack of endocrine data to confirm the incidence of LH rises in our patient group.

The question remains what we can do to prevent lower implantation rates in high responders when the GnRH antagonist has been used to prevent premature luteinisation. One possible option is to start earlier with GnRH antagonist or apply dose adjustments in case of an expected high responder in order to normalise response and maximise LH control. Early initiation of the antagonist indeed has demonstrated a more consistent suppression of LH rises, but the effect on outcome in terms of implantation rates remains to be established (Kolibianakis et al. 2006). Studies on dose adjustments based on response prediction algorythms have been performed in agonist cycles, showing the feasibility of such an approach (Olivennes et al. 2009, Popovic-Todorovic et al. 2003). Studies focussing on response prediction in antagonist cycles with subsequent dose adjustment are lacking at present (Jurema et al. 2003, Verberg et al. 2009b). One other option is to cryopreserve embryos in case of high response. The implantation rate after cryopreservation remains the same when compared to the fresh transfer (D'Angelo et al. 2007).

In summary, the ongoing implantation rate was observed to be negatively correlated to the number of oocytes obtained in mild ovarian stimulation protocols with GnRH antagonist co-treatment in IVF. The decrease in ongoing pregnancy rate in high responders may be a possible explanation for the lower implantation rates observed in early comparative studies of GnRH agonist and GnRH antagonist. The question remains why this phenomenon occurs. Patients with a higher number of oocytes have a larger pool of antral follicles and therefore higher estradiol levels. The start of the GnRH antagonist might be too late to prevent luteinisation in this group and might have a detrimental effect on the endometrium. Whether an earlier start of GnRH antagonist would address this remains to be shown.

Chapter 4

A double-blind, non-inferiority RCT comparing corifollitropin alfa and recombinant FSH during the first seven days of ovarian stimulation using a GnRH antagonist protocol

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Abstract

Background

Corifollitropin alfa, a fusion protein lacking LH activity, has a longer elimination half-life and extended time to peak levels than recombinant FSH (rFSH). A single injection of corifollitropin alfa may replace seven daily gonadotrophin injections during the first week of ovarian stimulation.

Methods

In this large, double-blind, randomised, non-inferiority trial the ongoing pregnancy rates were assessed after one injection of 150 µg corifollitropin alfa during the first week of stimulation compared with daily injections of 200 IU rFSH using a standard GnRH antagonist protocol.

Results

The study population comprised 1506 treated patients with mean age of 31.5 years and body weight of 68.8 kg. Ongoing pregnancy rates of 38.9% for the corifollitropin alfa group and 38.1% for the rFSH group were achieved, with an estimated non-significant difference of 0.9% (95% confidence interval (CI): -3.9, 5.7) in favour of corifollitropin alfa. Stratified analyses of the pregnancy rates confirmed robustness of this primary outcome by showing similar results regardless of IVF or ICSI, or number of embryos transferred. A slightly higher follicular response with corifollitropin alfa resulted in a higher number of cumulus-oocyte-complexes compared with rFSH [estimated difference 1.2 (95% CI: 0.5;1.9)] whereas median duration of stimulation was equal (9 days) and incidences of (moderate/severe) ovarian hyperstimulation syndrome was the same (4.1 and 2.6, respectively p=0.15).

Conclusion

Corifollitropin alfa is a novel and effective treatment option for potential normal responder patients undergoing ovarian stimulation with GnRH antagonist co-treatment for IVF resulting in a high ongoing pregnancy rate, equal to that achieved with daily rFSH.

Introduction

Corifollitropin alfa is the first hybrid molecule with sustained follicle-stimulating activity. Corifollitropin alfa is a recombinant fusion protein composed of FSH and the carboxy terminal peptide (CTP) of the hCG ß-subunit (Fares et al. 1992). Like recombinant FSH (rFSH), corifollitropin alfa interacts only with the FSH-receptor and lacks LH activity (LaPolt et al. 1992, Fauser et al. 2009). However, corifollitropin alfa has an approximately 2-fold longer elimination half-life ($t_{1/2}$) and an almost 4-fold extended time-interval (t_{max}) to peak serum levels (C_{max}) (Duijkers et al. 2002). Due to this pharmacokinetic profile, corifollitropin alfa can function as a sustained follicle stimulant with a similar pharmacodynamic profile as rFSH, but with the ability to initiate and sustain multiple follicular growth for an entire week. Consequently, a single subcutaneous injection of the recommended dose of corifollitropin alfa can replace the first seven injections of any daily FSH preparation in an ovarian stimulation treatment cycle prior to IVF.

The need for simplified treatment approaches which will lessen the treatment burden of IVF is self-evident. The IVF treatment process itself is increasingly recognized as contributing to the physical, psychological, and emotional burden on infertility patients (Boivin et al. 1996, Cousineau et al. 2007). Infertile patients experience high levels of distress and their level of anxiety and depression is equivalent to that experienced by women with cancer or heart disease (Domar et al. 1993). A number of IVF studies in which treatment costs were reimbursed have nevertheless reported drop-out rates well above 50% before completing their covered number of cycles (Land et al. 1997, Olivius et al. 2002, Schroder et al. 2004), largely due to the psychological impact of treatment (Olivius et al. 2004, Rajkhowa et al. 2006). Thus, the primary reason for treatment discontinuation is not based on physician recommendation, but is because patients are too distressed to continue (Hammarberg et al. 2001).

In GnRH antagonist co-treatment stimulation protocols, the duration of stimulation is reduced compared to GnRH agonist protocols and less FSH is used to reach the same criteria for administering hCG (Tarlatzis et al. 2006). Interestingly, a prospective cohort study comparing a GnRH antagonist protocol with a conventional long GnRH agonist protocol demonstrated a significantly reduced drop-out rate in the antagonist group, indicating that the impact of the treatment strategy is an important factor determining the risk of drop-out (Verberg et al. 2008). Clearly, simple treatment regimens that lessen the burden of IVF improve the overall patient experience, and encourage lower drop-out rates (Olivennes, 2003, Heijnen et al. 2004, Pennings et al. 2007). Last but not least, fewer injections to be given may improve drug compliance and/or prevent errors during drug administration.

Developing corifollitropin alfa in a short GnRH antagonist protocol may add to the further reduction of the treatment intensity experienced by patients undergoing ovarian stimulation for IVF. Following the dose-finding trial (Corifollitropin Alfa Dose-finding Study Group, 2008) and subsequent PK/PD modelling (de Greef et al. 2007) it was concluded that the recommended dose of corifollitropin alfa was 100 µg for subjects with body weight ≤ 60 kg and 150 µg for subjects with body weight > 60 kg. Based on simulations, these two dosages of corifollitropin alfa will provide the same exposure and the same degree of ovarian response in the recommended body weight groups. After a single injection of corifollitropin alfa on menstrual cycle day 2 or 3, treatment may be continued with a daily dose of rFSH from stimulation day 8 onwards if needed. Patients who reach the criteria of triggering final oocyte maturation prior to day 8 of stimulation do not require any daily FSH to be administered.

First and foremost the question that needs to be addressed is whether the new corifollitropin alfa regimen results in the same success rates as a daily FSH regimen with GnRH antagonist co-treatment. To this end, the aim of the ENGAGE trial was to investigate whether the ongoing pregnancy rates of the new corifollitropin alfa regimen were comparable to a daily rFSH regimen in patients undergoing ovarian stimulation prior to IVF. The sample size required to sufficiently power this trial for ongoing pregnancy rate as a primary end-point renders this the largest double-blind randomised trial in the field of Assisted Reproductive Technology (ART) to date.

Materials and Methods

The ENGAGE trial was a multi-centre, randomised, double-blind double-dummy, non-inferiority clinical trial involving 14 centres in North America (13 centres in USA and one in Canada) and 20 centres in Europe (three in Spain and The United Kingdom; two in Belgium, Czech Republic, Finland, France, Norway, and Sweden; one in Denmark and The Netherlands) and conducted between June 2006 and January 2008.

The study was approved by the Independent Medical Ethics Committee or Institutional Review Board for each centre as well as by the responsible Health Authority and was conducted in accordance with the Declaration of Helsinki, International Conference on Harmonization guidelines for Good Clinical Practice, and local regulatory requirements. An Independent Data Safety Monitoring Board was appointed to monitor the safety of subjects participating in the trial, and written informed consent was provided by all patients.

Study population

Women aged 18-36 years with a body weight of more than 60 kg up to and including 90 kg, a body mass index of 18-32 kg/m^2, a menstrual cycle length of 24-35 days, access to ejaculatory sperm, and an indication for controlled ovarian stimulation (COS) before IVF or intracytoplasmic sperm injection (ICSI) were eligible to enrol in the study. Patients who had a (history of) an endocrine abnormality, an abnormal outcome of blood chemistry or haematology, an abnormal cervical smear, a chronic disease, relevant ovarian- , tubal- or uterine-pathology that could interfere with the COS treatment (e.g. endometrioma > 10 mm or fibroids ≥ 5 cm), embryo implantation or pregnancy were not to be included in the trial. Patients who had a history of ovarian hyperresponse (more than 30 follicles ≥ 11 mm) or ovarian hyperstimulation syndrome (OHSS), polycystic ovary syndrome (PCOS) or a basal antral follicle count (AFC) of more than 20 on ultrasound (< 11 mm, both ovaries combined) were excluded from participation for reason of safety. Other exclusion criteria included a previously low ovarian response to FSH or human menopausal gonadotrophin (hMG) treatment (i.e. cycle cancelled due to insufficient ovarian response or less than four oocytes obtained), an FSH or LH over 12 IU/L in the early follicular phase, more than three unsuccessful IVF cycles since the last ongoing pregnancy, a history of recurrent miscarriage (three or more), or currently smoking more than five cigarettes per day.

Study design

The trial was designed as a randomised, double-blind, double-dummy, active-controlled, non-inferiority trial to compare the efficacy of a single injection of corifollitropin alfa during the first week of stimulation with 7 daily injections of rFSH for inducing and sustaining multifollicular growth during COS. Randomisation to one of the two treatment arms (1:1 ratio) was done per centre and stratified by age (< 32 and ≥ 32 years) by central remote allocation using randomly permutated blocks with an undisclosed fixed block size of four.

Stimulation regimen and ART procedures

All patients were to start their treatment cycle on menstrual cycle day 2 or 3 as depicted in *Figure 1*. Patients started stimulation with a single s.c. injection of 150 µg (0.5 mL) corifollitropin alfa (NV Organon, The Netherlands) or matching placebo. Injections could be done by the patient herself, her partner or the medical staff. To conceal treatment allocation all patients also started daily s.c. injection of 200 IU rFSH (follitropin beta, Puregon® / Follistim® AQ Cartridge, NV Organon, The Netherlands) or matching placebo on the same day (Stimulation Day 1) using

the Puregon® / Follistim Pen®. Daily active or placebo ("dummy") rFSH injections were continued through the first seven days of stimulation. The chosen reference dose of 200 IU rFSH daily was considered the optimal choice for the included patient population weighing over 60 kg in a global trial combining European and North American sites. This dose was fixed for the first five days of stimulation. This is in line with the suggestion by Arce *et al.* (Arce *et al.* 2005) who recommended a fixed starting dose for at least 5-7 days in all efficacy trials as only after such period the impact of the administered FSH dose can be adequately evaluated. Moreover, this is considered appropriate because daily FSH only reaches steady state after 3-5 days of dosing (Mannaerts et al 1993). A reduction of the rFSH dose was allowed from stimulation day 6 onward in case of too high an ovarian response at the discretion of the investigator. When no follicle ≥ 11 mm was visible on ultrasound scan (USS) before injection on stimulation day 8 the cycle was to be cancelled due to insufficient ovarian response. From stimulation day 8 onwards treatment in both groups was continued with a daily s.c. dose of (active) rFSH up to and including the day of hCG administration. The maximum rFSH dose to continue treatment after the first 7 days was 200 IU but the dose could be reduced when desired. For normal responders, the recommended daily dose of rFSH was 150 IU. Whenever deemed required by the investigator, rFSH administration could be withheld for a maximum of three days (coasting) up to and including the day of hCG administration. In case there was a too high ovarian response as per the investigator's opinion, the cycle could be cancelled at any time.

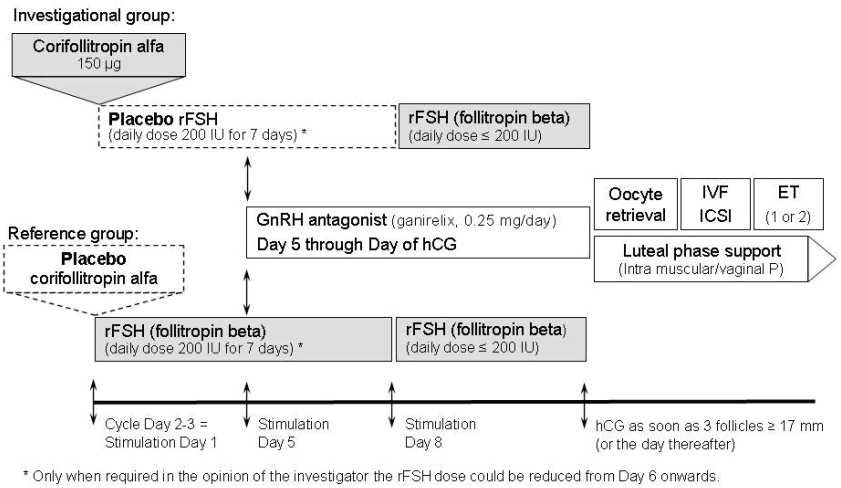

Figure 1 *Graphical illustration of the treatment regimens applied in this trial. Upper panel depicts investigational group (corifollitropin alfa), lower panel depicts reference group (rFSH). rFSH: recombinant FSH, P: progesterone*

However, in case of a risk for OHSS, defined as more than 30 follicles ≥ 11 mm on USS, hCG was to be withheld and the treatment cycle was to be cancelled per protocol. The maximum total duration of stimulation was 19 days.
To prevent premature LH surges the GnRH antagonist ganirelix (0.25 mg, Orgalutran® / ganirelix acetate injection, NV Organon, The Netherlands) was administered once daily s.c. starting on stimulation day 5 up to and including the day of hCG. Urinary hCG (10,000 IU) was administered to induce final oocyte maturation as soon as at least three follicles ≥ 17 mm were observed by USS. Investigators were allowed to delay hCG administration for one day when preferred for practical reasons. In case there was a too high ovarian response in the opinion of the investigator, a lower dose (5,000 IU hCG) could be used. About 34-36 hours thereafter, oocyte retrieval followed by standard IVF or ICSI was to be performed. Embryo quality was evaluated for all available embryos on day 3 of culture by the local embryologist using a protocol-defined guideline based on the following parameters: number of blastomeres, degree of fragmentation, blastomere size uniformity and presence or absence of multinucleation. Embryos graded as grade 1 (6-10 cells, no fragmentation and equal blastomere size) or grade 2 (allowing up to 20% fragmentation) were qualified as good quality embryos. The quality of embryos continued in culture after day 3 was reassessed on the day of transfer or freezing using grading criteria appropriate for the stage of embryo culture. At embryo transfer (ET), 3 or 5 days after oocyte retrieval, one or two embryos were to be transferred. The decision on the day of transfer and number of embryos to be transferred was made by the investigator. To support implantation and early pregnancy, luteal phase support with progesterone (at least 600 mg/day vaginally or at least 50 mg/day intramuscularly, to be prescribed locally) was started on the day of oocyte retrieval and continued for at least 6 weeks, or either up to menses or up to a negative pregnancy test performed at least 14 days after embryo transfer.

Assessments

Before the start of ovarian stimulation, pregnancy was excluded by means of an hCG test, a blood sample was obtained for hormone assessments, and USS was performed to measure and count visible follicles. Local tolerance parameters (pain, itching, swelling and redness) were assessed by the clinical staff 30 minutes after injection for both (placebo) corifollitropin alfa and (placebo) rFSH injection sites. Patients returned to the clinic for USS and blood sampling on stimulation days 5 and 8, and then daily up to and including the day of hCG administration for USS only. Additional blood samples were collected on the day of embryo transfer and two weeks after embryo transfer. Patients who left the study prior to embryo

transfer were sampled for hormones and antibody assessments at the day of discontinuation and two weeks thereafter.
Validated immunoassays were performed at a central laboratory (Schering-Plough, Oss, The Netherlands and Waltrop, Germany) to measure serum levels of FSH, LH, estradiol (E_2), progesterone, inhibin-B and antibodies against corifollitropin alfa (Devroey et al. 2004).

Endpoints

Ongoing pregnancy, defined as presence of at least one foetus with heart activity at least 10 weeks after embryo transfer as assessed by USS or Doppler, or confirmed by live birth was the primary endpoint for this trial. This can be considered as the best and closest estimate of the ultimate treatment success (delivery of a healthy baby) and excludes subjects with a miscarriage during the first ten weeks of pregnancy (Arce et al. 2005). In addition, the number of retrieved oocytes was considered as co-primary endpoint in this trial being more proximately related to the pharmacological effect of the two treatment regimens which are compared in this trial. Other clinical outcome parameters evaluated included dose of rFSH required, duration of stimulation, number and size of follicles, serum hormone levels, fertilisation rate, number and quality of embryos obtained and pregnancy rates. The ultimate live birth rate, the health of the offspring, and outcomes achieved with spare, frozen embryos will be reported separately when follow up has been completed.
Occurrence of (serious) adverse events, including moderate and severe OHSS as per World Health Organization criteria (WHO, 1973), outcome of local tolerance and immune response assessments were evaluated as safety end-points.

Statistical analysis

The sample size needed for this trial was largely determined by the chosen predefined non-inferiority margin (i.e. smaller margin requires larger sample size to maintain the same power) but also depended on the anticipated ongoing pregnancy rate (i.e. higher pregnancy rate requires larger sample size) to be expected in a combined trial comprising sites in Europe, USA and Canada. For this global trial a non-inferiority margin of 8% was considered appropriate. This implies that if the lower bound of the 95% confidence interval (CI) for the estimated difference in ongoing pregnancy rates between treatment groups (corifollitropin alfa minus rFSH) was determined to be above -8%, corifollitropin alfa could be considered non-inferior to rFSH. Although such difference would be relevant for an individual subject seeking to become pregnant after IVF treatment it should be considered

in the context of existing differences in routine pregnancy rates between centres, countries and regions (Gleicher et al. 2006, Gleicher et al. 2007). A sample size of at least 1380 subjects was calculated to be the minimum required to demonstrate non-inferiority with a power of 90%, using a -8% non-inferiority margin for the lower limit of the two-sided 95% CI, and assuming an ongoing pregnancy rate of 30%. Based on these data, a minimum of 700 subjects per group, in total at least 1400 subjects, were to be randomised which makes this the largest double-blind comparative randomised trial performed to date.

For analysis of the primary end-point, ongoing pregnancy, the treatment groups were formally compared with a generalized linear model for the ongoing pregnancy rate including covariates treatment group, age (< 32 yrs, ≥ 32 yrs) and region (Europe, North America). The difference between the two treatment groups (corifollitropin alfa - rFSH) and its associated two-sided 95% likelihood-based CI was estimated and a pre-defined non-inferiority margin of 8% was applied. Additional explorative analyses were performed to investigate robustness of the primary end-point results. No adjustment for multiplicity to correct for repeated testing was performed.

The number of cumulus-oocyte-complexes retrieved was defined as co-primary end-point in this trial. As there is an optimal range of oocytes obtained in response to ovarian stimulation, below and above which the success rates of IVF are compromised (van der Gaast et al. 2006) equivalence testing was deemed appropriate. Therefore, equivalence margins for the difference in the number of oocytes retrieved were predefined to be -3 and +5 oocytes. If the new corifollitropin alfa regimen resulted in three or more oocytes less than the reference treatment, such difference was considered as clinically relevant because three oocytes usually result in one good quality embryo for transfer or freezing. Anticipating obtaining an average of 12 to 13 oocytes with the applied rFSH doses in the reference group, an excess of more than five oocytes would be undesirable as patients with more than 18 oocytes retrieved are described to be at increased risk to develop OHSS (Papanikolaou et al. 2006, Verwoerd et al. 2008). Hence, an upper margin of +5 oocytes is applied for the difference in the number of oocytes retrieved between the treatment groups. The treatment groups were formally compared using analysis of variance for the number of oocytes including covariates treatment group, age (< 32 yrs vs. ≥ 32 yrs) and centre. The estimate of the difference between the two treatment groups and its associated two-sided 95% CI was given. In case the 95% CI of the difference exceeded -3 or +5 oocytes corifollitropin alfa treatment was not considered equivalent to the rFSH treatment. Efficacy analyses are based on the intention-to-treat (ITT) population entailing

all patients randomised and treated, analyzing them according to their allocated treatment (i.e. "as randomised"). Both ITT and per-protocol (PP) were analyses were performed (see Supplementary Data), but as the results were very similar, only the ITT results are presented here. The main efficacy analyses were performed "per started cycle" (i.e., including all subjects who started treatment regardless whether they discontinued) as this provides the most conservative efficacy estimates as it also accounts for any unintended interference of premature cycle cancellations. For safety endpoint analyses patients are grouped "as treated" which implied that three patients (one randomised to corifollitropin alfa and two patients to rFSH) treated inadvertently with the wrong (comparator) medication type are analyzed according to the treatment they actually received.

Results

A total of 1696 patients signed informed consent for eligibility evaluation and participation in this trial (*Figure 2*). Subsequently a total of 187 patients failed screening or dropped out due to personal reasons prior to treatment allocation. Eventually, 1509 patients were randomised to one of the two treatment groups of which 1506 patients actually started stimulation. The remaining three patients where discontinued prior to the start of treatment (one for personal reasons and two were found to violate entry criteria after randomisation but before commencing treatment).

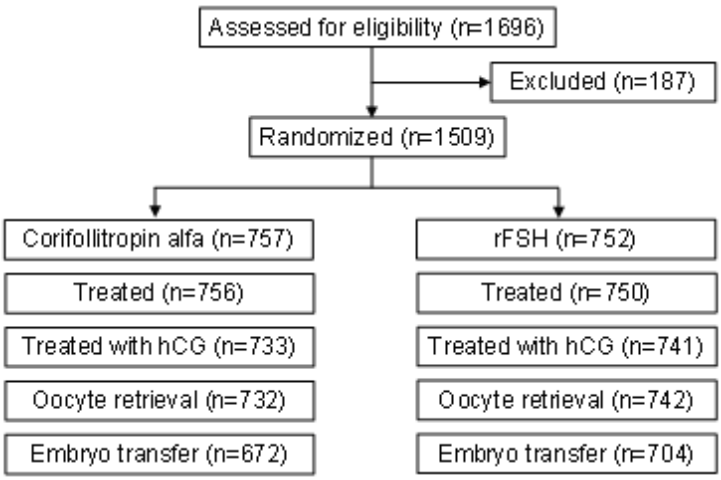

Figure 2 *Flow-chart showing the number of participants at each stage of the clinical trial. rFSH, recombinant follicle stimulating hormone; hCG, human chorionic gonadotrophin*

Demographics of the ITT population as well as relevant fertility characteristics, USS findings and hormone profiles were comparable in the two groups (*Table 1*). Mean age (SD) of the patients included in this trial was 31.5 (3.3) years, mean body weight was 68.6 (7.5) kg and mean body mass index was 24.8 (2.7) kg/m^2. The average duration of infertility was 3.3 (2.3) years and for 74% of the patients this was their first treatment cycle. On stimulation day 1, no differences were observed between the treatment groups in the basal antral follicle counts or baseline serum hormone levels (*Table 1*).

Table 1 *Demographics, fertility characteristics and baseline (stimulation day 1) USS and serum hormone levels per treatment group (Intent-to-Treat population). Numbers represent mean (standard deviation) unless otherwise indicated.*

	Corifollitropin alfa (n=756)	rFSH (n=750)
Demographics		
Age (years)	31.5 (3.3)	31.5 (3.2)
Body weight (kg)	68.8 (7.6)	68.4 (7.3)
Body mass index (kg/m²)	24.8 (2.8)	24.8 (2.7)
Race, n (%)		
Asian	21 (2.8%)	21 (2.8%)
Black	33 (4.4%)	28 (3.7%)
Caucasian	643 (85.1%)	650 (86.7%)
Other	59 (7.8%)	51 (6.8%)
Fertility characteristics		
Primary infertility, n (%)	403 (53.3%)	393 (52.4%)
Duration of infertility (years)	3.3 (2.4)	3.2 (2.2)
Cause of infertility [a], n (%)		
Male factor	388 (51.3%)	347 (46.3%)
Tubal factor	198 (26.2%)	191 (25.5%)
Endometriosis	109 (14.4%)	115 (15.3%)
Other/unexplained	128 (26.2%)	226 (30.2%)
First IVF cycle, n (%)	569 (75.3%)	552 (73.6%)
Stimulation day 1		
Total ovarian volume (mL)[b]	13.2 (8.1)	13.2 (7.1)
Basal antral follicles (< 11 mm)	12.3 (4.6)	12.4 (4.4)
FSH (IU/L)	6.7 (2.1)	6.6 (1.9)
LH (IU/L)	4.8 (2.0)	4.7(1.8)
Estradiol (pmol/L)	126.1 (39.3)	124.8 (37.4)
Progesterone (nmol/L)	1.8 (1.3)	1.8 (1.4)

[a] A patient can have multiple causes of infertility. [b] According to the formula for a polate ellipsoid: 0,523x longitudinal x antero-posterior x transverse diameters as measured per transvaginal ultrsound.
AFC: antral follicle count; E2: estradiol; rFSH: recombinant FSH.

Primary end-point: ongoing pregnancy rate

Results of the primary end-point analyses are presented in *Table 2*. High ongoing pregnancy rates of 38.9% for the corifollitropin alfa group and 38.1% for the rFSH group were obtained per started cycle. The estimated treatment difference, adjusted for age group (< 32 years, ≥ 32 years) and region (Europe, North America), was +0.9% (95% CI: [-3.9; 5.7]) in favour of corifollitropin alfa.

Table 2 *Primary endpoints of the ENGAGE trial: Ongoing pregnancy rate (assessed at least 10 weeks after embryo transfer) and the mean (standard deviation) number of cumulus-oocyte-complexes retrieved (Intent-to-Treat population).*

	Corifollitropin alfa	rFSH	Estimated difference[a)]
	(n=756)	(n=750)	[95% CI], p-value
Ongoing pregnancies (n)	294	286	
Per started cycle (%)	38.9%	38.1%	0.9 [-3.9; 5.7], p=0.71
Per embryo transfer (%)	43.8%	40.6%	3.1 [-2.0; 8.2], p=0.24
Cumulus-oocyte-complexes			
Per started cycle	13.7 (8.2)	12.5 (6.7)	1.2 [0.5; 1.9], p=0.001
Per oocyte retrieval	14.1 (7.9)	12.7 (6.7)	1.6 [0.8; 2.3], p<0.001

a) Estimated treatment difference (corifollitropin alfa - rFSH) adjusted for covariates; CI, confidence interval. P-value corresponds to the test whether the treatment difference equals zero.

Co-primary end-point: number of cumulus-oocyte-complexes retrieved

The number of cumulus-oocyte-complexes retrieved was defined as co-primary end-point in this trial. The mean (SD) number of oocytes retrieved in the corifollitropin alfa group was 13.7 (8.2) which was higher than the mean of 12.5 (6.7) obtained in the rFSH group (*Table 2*). The estimated treatment difference was 1.2 oocytes in favour of corifollitropin alfa (P=0.001), while the 95% CI was (0.5; 1.9). This indicates that the number of oocytes retrieved in the two treatment groups was at least equivalent based on the pre-defined equivalence range.

Other clinical outcome parameters

The median duration of stimulation was 9 days for both treatment groups, which implied patients treated with corifollitropin alfa needed on average 2 days of rFSH to complete their treatment cycle (*Table 3*). Per protocol, the dose of (placebo) rFSH could be reduced (not increased) from stimulation day 6 onwards. Dose decreases on stimulation days 6 or 7 were recorded slightly more often in the corifollitropin alfa group (85/750, 11.3%) than in the rFSH group (62/741, 8.4%). Coasting by withholding rFSH for 2 or more days was applied in 1.6% [12/773, 95% CI: (0.7;2.6)] of patients in the corifollitropin alfa group and in 2.2% [16/741, 95% CI (1.0;3.3)] of patients in the rFSH group. A fixed dose regimen from stimu-

lation day 8 up to but not including the day of hCG was used in 72.1% (413/573) of the corifollitropin alfa group and 81.0% (430/531) of the rFSH group. After a single injection of corifollitropin alfa, 249 out of 756 patients (32.9%) reached the criteria for giving hCG before or on stimulation day 8: the ongoing pregnancy rate for this subgroup of patients was 44%.

Table 3 *Clinical parameters from stimulation phase up to embryo transfer (Intent-to-Treat population). Numbers represent mean (standard deviation) unless otherwise indicated.*

	Corifollitropin alfa (n=756)	**rFSH** (n=750)
Stimulation characteristics [a]	median (range)	median (range)
Total dose of rFSH (IU)	400 (0-2000)	1800 (400-2800)
Total dose of rFSH from day 8 onwards (IU)	400 (0-2000)	400 (0-1400)
Total duration of stimulation (days) [b]	9 (6-18)	9 (6-15)
Follicles, day of hCG [a]		
≥ 11 mm	16.0 (7.0)	13.9 (6.1)
≥ 15 mm	9.6 (4.8)	8.7 (4.0)
≥ 17 mm	5.7 (3.2)	5.6 (2.9)
Serum parameters, day of hCG [a]		
FSH (IU/L)	12.5 (3.3)	11.6 (2.8)
LH (IU/L)	1.4 (1.8)	1.9 (1.6)
E2 (pmol/L)	5508.8 (3469.8)	5165.3 (2998.2)
Inhibin-B (pg/mL)	610.3 (492.3)	614.8 (435.6)
Progesterone (nmol/L)	3.0 (2.1)	3.2 (1.5)
Clinical outcome per started cycle		
Oocytes retrieved, ICSI only	13.8 (7.6)	12.1 (6.3)
Metaphase II oocytes (ICSI only), % of total	10.8 (6.5), 78.9 (18.9)	9.2 (5.1), 77.4 (18.1)
Fertilisation rate (%) [c,d]	66.0 (23.4)	67.6 (22.9)
Total number of embryos obtained (day 3) [c]	8.3 (5.6)	7.4 (4.8)
Excellent (top) quality embryos (grade 1) [c]	2.6 (3.4)	2.5 (3.4)
Good quality embryos (grade 1+2) [c]	4.6 (4.3)	4.4 (3.9)
Single embryo transfer (%) [d]	25.7%	27.0%
Embryos transferred [e]	1.7 (0.4)	1.7 (0.4)
Embryos cryopreserved [f]	4.3 (3.6)	3.9 (2.7)

[a] Restricted to patients with hCG injection; [b] Number of days up to and including the day of hCG administration; [c] Restricted to patients with IVF and/or ICSI; [d] Defined as 100 times the number of mature oocytes (with two pronuclei) obtained divided by the number of oocytes used for fertilisation; [e] Restricted to patients with embryo transfer; [f] Restricted to patients with cryopreserved embryos.

Eventually, 97.0% of the patients in the corifollitropin alfa group and 98.8% of the rFSH group received hCG to induce final oocyte maturation. The vast majority of them received 10,000 IU hCG [76.9% (581/756) and 85.6% (642/750) of the patients in the corifollitropin alfa and rFSH group, respectively], while a dose of 5,000 IU hCG was used for 19.8% (150/756) and 13.1% (98/750) of the respec-

tive groups. Only the most frequent reasons for discontinuation prior to hCG are mentioned. In total, 23 (3.0%) of the patients in the corifollitropin alfa group and 7 (0.9%) of the patients in the rFSH group discontinued prior to hCG. In the rFSH group, 2 patients discontinued after the oocyte retrieval procedure, but it appeared that they had not received hCG. Taking this into account, totals add up correctly to the number of subjects treated with hCG (733=756-23, 741=750-7-2).

On the day of hCG, the mean (SD) number of follicles measuring ≥ 11 mm on USS was 16.0 (7.0) for the corifollitropin alfa group and 13.9 (6.1) for the rFSH reference group (*Table 3*). When comparing serum hormones on the day of hCG administration comparable levels were observed in both treatment groups. Oocyte retrieval was performed in 96.8% of the patients in the corifollitropin alfa group and 98.9% in the rFSH group. A higher mean number of cumulus-oocyte-complexes was retrieved in the corifollitropin alfa group (*Table 2*). Oocyte maturity was assessed for ICSI patients and also showed a higher number and percentage of mature oocytes (*Table 3*) in the corifollitropin alfa group (mean 10.8, 78.9%) compared to the rFSH group (mean 9.2, 77.4%). A comparable fertilisation rate was observed between the groups resulting in a mean (SD) of 8.3 (5.6) embryos obtained on day 3 of culture in the corifollitropin alfa group and 7.4 (4.8) embryos in the rFSH group for patients with IVF and/or ICSI. Embryo quality, as assessed by the local embryologist, was similar in both groups.

Ultimately, 672 patients (88.9%) in the corifollitropin alfa group and 704 patients (93.9%) in the rFSH group had embryo transfer in this trial. Between hCG administration and embryo transfer 39 out of 756 patients (5.2%) in the corifollitropin alfa group and 34 out of 750 (4.5%) patients in the rFSH group were discontinued as a result of too few or too low quality oocytes, lack of fertilisation or poor embryo development. During this period 6/756 patients (0.8%) in the corifollitropin alfa group and none in the rFSH group were discontinued due to a too high ovarian response or risk of OHSS. Although no embryo transfer was performed, fertilized oocytes or embryos were cryopreserved for all these six patients. When embryo transfer was performed, single embryo transfer was carried out in slightly more than a quarter of all transfers (*Table 3*). On average, 1.7 (0.4) embryos were transferred in both treatment groups. For 51.7% and 53.2% of the patients in the corifollitropin alfa and rFSH groups, a mean (SD) of 4.3 (3.6) and 3.9 (2.7) supernumerary embryos have been cryopreserved, respectively.

In the corifollitropin alfa group 29.2% (221/756), and in the rFSH 30.3% (227/750), received only i.m. luteal phase progesterone support, whereas respectively 52.0% (393/756) and 54.9% (412/750) received only intravaginal progesterone support.

Other subjects received combination of routes.
Pregnancy rates (confirmed by positive hCG test) of 48.1% and 46.9% were achieved in the corifollitropin alfa and rFSH groups, respectively, followed by a comparable number of early pregnancy losses during the first 10 weeks after embryo transfer. Accordingly, similar ongoing pregnancy rates were observed in both groups (*Table 4*). There was no relevant difference in the ongoing pregnancy rates between subjects who received the hCG injection on the same that three follicles ≥ 17 mm were observed (40.0 and 37.8% for corifollitropin alfa and rFSH, respectively) versus subjects who received hCG one day later (38.9% and 41.8% for corifollitropin alfa and rFSH, respectively).

Table 4 *Clinical efficacy outcomes per started cycle (Intent-to-Treat population).*

	Corifollitropin alfa (n=756)	**rFSH** (n=750)	**p-value**[a)]
Positive hCG test [b)], n (%)	364 (48.1%)	352 (46.9%)	0.64
Clinical pregnancy [c)], n (%)	322 (42.6%)	308 (41.1%)	0.57
Vital pregnancy [d)], n (%)	302 (39.9%)	293 (39.1%)	0.75
Ongoing pregnancy [e)], n (%)	294 (38.9%)	286 (38.1%)	0.71
Multiple pregnancy [f)], n (%)	83 (28.2%)	66 (23.1%)	0.18
Early miscarriage [g)], n (%)	27 (8.4%)	21 (6.8%)	0.55

[a)]P-values are based on Fisher' exact test, except for ongoing pregnancy where the p-value is based on the likelihood ratio test corresponding to the generalised linear model with covariates treatment group, age class (<32 years, ≥32 years), and region (Europe, North America). [b)]Positive hCG test at least 14 days after embryo transfer or USS with at least one gestational sac. [c)] Clinical pregnancy: gestational sac on USS. [d)] Vital pregnancy: gestational sac + fetal heartbeat. [e)] Ongoing pregnancy: vital foetus at least 10 weeks after embryo transfer or live birth. [f)] Per ongoing pregnancy. [g)] Per clinical pregnancy.

The robustness of this primary efficacy outcome was explored in specific subsets of patients grouped based on the ART procedure-related factors, i.e. undergoing IVF or ICSI, having single or double embryo transfer and having embryo transfer on day 3 or day 5 (*Figure 3*). The ongoing pregnancy rates in each subset were similar for corifollitropin alfa and rFSH and there was no significant interaction with the type of treatment factor (IVF or ICSI), nor for the number of embryos transferred (one or two). A borderline significant interaction with the type of treatment was only observed for the factor day of transfer (P=0.045) as a result of higher pregnancy rates for day 3 transfers with corifollitropin alfa whereas, for day 5 transfers, the rFSH group showed marginally higher pregnancy rates (*Figure 3*). The subset analyses together confirm the robustness of the observed difference in pregnancy rates, independent of ART procedure-related parameters.

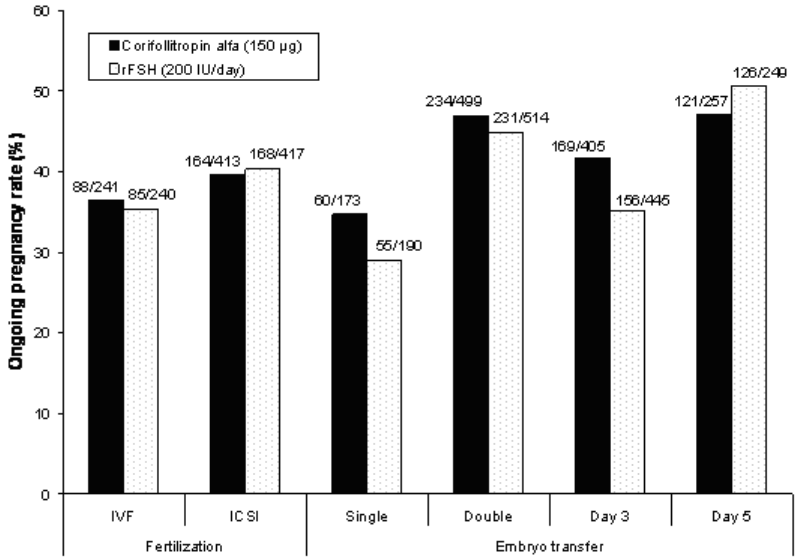

Figure 3 *Ongoing pregnancy rates stratified for fertilisation procedure, number of embryos transferred and day of transfer. Subset with double embryo transfer includes three patients with three embryos transferred.*

Multiple pregnancy rates per ongoing pregnancy were 28.2% and 23.1% in the corifollitropin alfa group and the rFSH group, respectively (*Table 4*). Although this difference is not significant, it is line with the slightly higher implantation rate defined as 100 times the maximum number of gestational sacs divided by the number of embryos transferred per subject for corifollitropin alfa [observed means (SD) were 36.2% (41.6%) versus 32.2% (40.1%)].

Safety

In total, 16 subjects (2.1%) in the corifollitropin alfa group discontinued due to a (serious) adverse event [SAE, two (0.3%) before and 14 (1.9%) following oocyte retrieval] as compared with three subjects (0.4%) in the rFSH group [two (0.3%) before and one (0.1%) after oocyte retrieval]. A total of 53 patients in the corifollitropin alfa-treated group (7.0%) and 47 patients in the rFSH-treated group (6.3%) developed OHSS in this trial. The incidences of (moderate/severe) OHSS were 4.1% [31/755, 95% CI: (2.6; 5.6)] and 2.7% [20/750, 95% CI: (1.5; 3.9)] for the corifollitropin alfa and rFSH group, respectively, which was not significant (Fisher exact P=0.15).

An equal number of 37 SAEs was reported for the corifollitropin alfa and the rFSH-treated groups. Most frequently reported SAEs were OHSS [14 patients (1.9%) treated with corifollitropin alfa and 9 patients (1.2%) treated with rFSH] and (ruptured) ectopic pregnancy [7 patients (0.9%) and 9 patients (1.2%), respectively] while, respectively, the most frequently reported AEs were procedural pain (22.3% and 20.1%), pregnancy-related events including (missed) abortion (13.8% and 11.2%), pelvic pain (12.1% and 12.3%), pelvic discomfort (11.5% and 11.6%) and headache (10.5% and 15.2%). There were no drug-related immune responses or moderate or severe local tolerance reactions observed in this trial.

Discussion

The current ENGAGE trial was a double-blind, double-dummy trial initiated to investigate whether the new corifollitropin alfa regimen provides similar success rates compared with the current care. The double-dummy approach guaranteed the blinding of the medication during the trial and prevented any bias in terms of treatment decisions. Owing to the fact that the trial was powered to enable comparison of the ongoing pregnancy rates as the primary study end-point, this is the largest double-blind efficacy trial IVF performed to date. The outcome of the trial provides compelling evidence of equal efficacy in terms of ongoing pregnancy rates, because the point estimates i.e. 38.9 and 38.1% for ongoing pregnancy rates were very close for the two treatment groups and the 95% power limit of the difference was only -3.9%. Subset analyses of subjects undergoing IVF or ICSI and of subjects who had single or double embryo transfer reveal consistently high ongoing pregnancy rates, similar between the treatment groups, which confirm the robustness of the primary endpoint. Even though an equal number and quality of embryos were replaced in both treatment groups, the multiple pregnancy rate in corifollitropin alfa group tended to be higher (+4.4% absolute risk increase) than in the reference group, which may be related to the slightly higher implantation rate. This increase, although not significant, cannot be ruled out and may prove to be clinically relevant, given the low power of the study to detect such a difference (c.22%). In this context, it should be emphasized that both treatment arms used a "protocolised" fixed dose treatment regimen applying GnRH antagonist (ganirelix) co-treatment, which confirms the successful outcome of a patient-friendly short GnRH antagonist protocol in corifollitropin alfa, as well as rFSH, treatment cycles. In addition, one-third of the patients treated with a single injection of corifollitropin alfa reached the criteria for hCG injection prior to or on stimulation day 8, omitting the need for any additional FSH injections. The ongoing pregnancy

rate of this subset of good responder patients was 44.0%, thus 5.9% higher than the overall group of subjects treated with corifollitropin alfa.

A hybrid molecule composed of human FSH and the CTP of hCG was first described by Boime and colleagues (Fares et al. 1992). In subsequent clinical trials the new recombinant fertility hormone exhibited a slower absorption (and subsequent rise to peak levels) and an approximately 2-fold longer t1/2 compared to rFSH (Duijkers et al. 2002, Bouloux et al. 2001). After corifollitropin alfa injection, peak levels of FSH activity are reached within 2 days whereas steady state levels with daily FSH are reached only after 4 to 5 days (Mannaerts et al. 1996). These pharmacokinetic properties of corifollitropin alfa create an opportunity to further simplify ovarian stimulation protocols for IVF by omitting the need for daily gonadotrophin injections during the first week of stimulation (Fauser et al. 2009). In the current trial, the difference in exposure during the first days of stimulation may have resulted in a slightly higher ovarian response in the corifollitropin alfa treated patients, as previous trials with rFSH have shown that the number of follicles recruited increases with the starting dose of rFSH given (Wikland et al. 2001, Out et al. 2004). In view of the equal pregnancy rates, the current trial does not suggest that the higher exposure to FSH immunoactivity during the first days of stimulation interferes with the endometrial receptivity.

In this trial hormonal pre-treatment with oral contraceptives or supplementation with LH or hCG during the treatment phase was not allowed per protocol. The efficacy results in this large, global, multi-centre trial suggest that the amount of endogenous LH during treatment with either corifollitropin alfa or daily rFSH in a standard GnRH antagonist protocol is sufficient to support high success rates in terms of ongoing pregnancies (Cedrin-Durnerin et al. 2004). Because of concerns that the relatively high exposure to corifollitropin alfa during the first days of stimulation might initiate an early rise of LH (Devroey et al. 2004), all patients in this trial started treatment with ganirelix on stimulation day 5. This fixed start of GnRH antagonist co-treatment was considered advantageous from an efficacy perspective as this may result in higher pregnancy rates, as well as from a methodological point of view as this reduces variability in the applied treatment regimens (Arce et al. 2005, Al-Inany et al. 2005, Kolibianakis et al. 2004).

The corifollitropin alfa regimen used in this trial comprised of a single dose of corifollitropin alfa followed by daily doses of rFSH (as needed up to a daily maximum of 200 IU). This corifollitropin regimen was compared with a fixed dose of 200 IU/day rFSH for the first 7 days, but with the option to decrease the daily FSH dose in cases when hyper-response was observed. Thus there was a difference in patient management options, with no option to adjust the dosing during the first week

of stimulation in the corifollitropin arm. After corifollitropin alfa injection, serum FSH activity declines from stimulation day 3 (C_{max}) onwards, but further reduction of exposure during the first week of stimulation cannot be attained.

The validity of the claimed efficacy and safety data of this trial are limited to the study population only. Patients with known risk factors for hyper-response, such as patients with history of OHSS, PCOS or with a high AFC (>20) were excluded from the current trial. In addition, patients with history of low ovarian response in previous IVF cycle were excluded. However, it should be noted that 74% of the patients included in this trial underwent their first IVF cycle for which the ovarian response is less predictable and will still include low- and high responder patients. In this large cohort of (potential) normal responder patients, corifollitropin alfa has been shown to be well-tolerated and non-immunogenic. In line with the higher ovarian response, the observed incidence of OHSS was higher in the corifollitropin alfa group, but the difference was not significant. Our study was not sufficiently powered to detect an underlying difference in the incidence of OHSS, and therefore its actual presence cannot be excluded. In line with the recruitment of slightly more follicles by corifollitropin alfa, dose reductions during stimulation were more frequently made in the corifollitropin alfa group than in the reference group. Clearly, a minority of subjects required a dose reduction on stimulation day 6 or 7, which may have affected the ovarian response in the rFSH group rather than in the corifollitropin alfa group in which only the placebo was reduced.

Appropriate clinical monitoring and lowering or withholding the daily rFSH dose to complete the cycle, as well as lowering or withholding the dose of hCG to trigger final oocyte maturation, or cryopreservation of all embryos obtained are options which can be considered as part of patient management to minimize the risk of OHSS (Delvigne et al. 2002).

Owing to the double-blind design of the trial, requiring all patients to be treated with an equal number of injections, no comparative data could be collected on perceived patient convenience or preference for the corifollitropin alfa regimen. Since a single injection of corifollitropin alfa replaces the first seven daily injections of rFSH during ovarian stimulation, the intuitive advantages of such simpler treatment regimen are obvious, but need to be confirmed in clinical practice. In addition to patients preference, future controlled trials may examine the efficacy of corifollitropin alfa in (potential) poor responding patients as well as the safety in (potential) high responders.

In conclusion, in this study we tested the efficacy (in terms of ongoing pregnancy rates) of substituting the first 7 daily doses of rFSH with a single injection of corifollitropin alfa in women undergoing ovarian stimulation for IVF/ICSI using rFSH and GnRH antagonists. Our data demonstrate that a single corifollitropin alfa injection results in an ongoing pregnancy rate which is equal to that of a daily rFSH regimen. Combined with appropriate patient selection and state-of-the-art clinical management during the stimulation phase of the treatment cycle, corifollitropin alfa potentially offers an attractive new treatment option for patients undergoing ovarian stimulation during ART.

Chapter 5

Low-dose corifollitropin alfa followed by low-dose rFSH or hCG for ovulation induction in WHO II anovulatory infertility; Results of a pilot study

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Abstract

The aim of this study was to evaluate whether a single or repeated low dose of corifollitropin alfa followed by a low dose of recombinant follicle-stimulating hormone (rFSH) or human chorionic gonadotrophin (hCG) can induce monofollicular growth in women with WHO II group anovulatory infertility.

Patients received a single or repeated injection of a low dose of 15 to 30 mg corifollitropin alfa. As soon as the largest follicle reached a diameter beyond 12 mm the subject started with daily injections of either 50 IU rFSH or 200 IU hCG. In total 8 subjects were included. Three subjects received hCG for final oocyte maturation and had ovulation confirmed. One subject became pregnant. Five subjects were cancelled during the trial. None of the subjects showed monofollicular growth.

In conclusion, a single or repeated low dose of corifollitropin alfa followed by a low daily dose of either rFSH or hCG did not induce monofollicular development in women with WHO group II anovulatory infertility. The results of this pilot trial indicate that the tested treatment regimen is not suitable for the induction of monofollicular growth in these women.

Introduction

Corifollitropin alfa is a recombinant fusion protein consisting of the common glycoprotein a–subunit and a hybrid β-subunit composed of the sequence of the b–subunit of human follicle-stimulating hormone (FSH) and the carboxy-terminal peptide (CTP) part of the b–subunit of human chorionic gonadotrophin (hCG) (Fares et al. 1992, Fauser et al. 2009). The latter extension provides corifollitropin alfa with a prolonged half-life and slower absorption to serum peak levels. Therefore, corifollitropin alfa can initiate and sustain multifollicular growth for 7 days and replace daily injections of FSH during the first week of ovarian stimulation in in-vitro fertilisation (IVF) treatments. Peak levels are reached after a mean of 44 hours (compared with 10 hours after recombinant FSH) and the terminal half-life is approximately 69 hours (Fauser et al. 2010). In world-wide phase I, II and III trials, more than 2100 normo-ovulatory subjects have been safely treated with corifollitropin alfa for ovarian stimulation in combination with a GnRH antagonist in IVF treatments (Devroey et al. 2009, Fauser et al. 2009).

Approximately 20–30% of women seeking fertility treatment present with anovulation (Healy et al. 1994). Clomiphene citrate (CC) is generally applied as first-line treatment in these women (Dickey et al. 1996, Yarali et al. 2004), due to low costs and minor side effects or complications. Multiple injections of exogenous gonadotrophins are considered second-line treatment in case of failure to ovulate or conceive following CC (Balen et al. 2007, Buvat et al. 1989, Kamrava et al. 1982, van Santbrink et al. 2005, White et al. 1996). The aim of treatment of anovulatory infertility is to approach normal ovarian physiology as closely as possible; i.e. maturation and ovulation of single dominant follicle and subsequent singleton pregnancy (ACOG Committee on Practice Bulletins-Gynecology, 2002, Homburg et al. 1999).

Corifollitropin alfa allows the development of new treatment regimens requiring fewer injections. A previous trial testing low dosages (7.5, 15, 30, or 60 µg) of corifollitropin alfa in women with WHO group II anovulatory infertility showed follicle growth up to pre-ovulatory sizes. However, the incidence of subsequent ovulation appeared to be low (Balen et al. 2004). It was concluded that a single dose of corifollitropin alfa was unable to restore follicle growth up to ovulation since a single dose too low resulted in follicular growth arrest and a single dose too high resulted in multiple follicular growth.

Each patient has her own threshold above which monofollicular growth may occur (Macklon et al. 2006). Therefore, patients may benefit when starting with a very low dose of corifollitropin alfa and, if needed, step up to a higher dose. Adjuvant low dosages of recombinant FSH (rFSH) may be required later during the cycle to sustain the growth of a single dominant follicle. A disadvantage of the use of subsequent daily injections of low-dose rFSH would be the risk of multiple follicular growth, as smaller follicles will also be stimulated by rFSH as a result of extending the FSH 'window' (Fauser et al. 1993, Fauser et al. 1997).

Recent studies have established that growth of dominant follicles in ovarian stimulation can be maintained by the late follicular phase administration of a low dose of hCG instead of low-dose rFSH (Blockeel et al. 2009, Filicori et al. 2005). This is only possible once the luteinising hormone (LH)/hCG receptor has been expressed by the granulosa cells, which occurs when the follicle reaches a diameter of approximately 10 mm (Hillier et al. 1994). Since the goal of ovulation induction is monofollicular growth, replacing rFSH with hCG as soon as a dominant follicle is present, might serve to reduce the risk of multifollicular development to the pre-ovulatory stage, and therefore improve the clinical efficacy and safety of a corifollitropin alfa ovulation induction protocol for treating anovulation.
The present study was designed to evaluate whether a single or repeated low dose of corifollitropin alfa followed by a low dose of rFSH or hCG can induce monofollicular growth in women with WHO II group anovulatory infertility.

Subjects and methods

This phase II trial was approved by the local ethical committee of the UMC Utrecht participating centre and each subject provided written informed consent before participation. The study was conducted in compliance with the current vision of the Declaration of Helsinki, and according to the European Community note on Good Clinical Practice for trials on medicinal products in the European Community and local requirements (CPMP Working Party on Efficacy of Medicinal Products, Pharmol Toxicol, 1990).
Inclusion criteria were; (1) oligomenorrhea (cycle length 35 days or longer) or amenorrhea (no menstrual period for more than 6 months), (2) age of at least 18 and at most 39 years, (3) body mass index (BMI) ≥18 and ≤30 kg/m^2, (4) normal serum FSH (<12 IU/litre), and (5) progestagen-induced withdrawal bleeding. This trial was designed in two separate stages; (I) a dose confirmation stage, stage I (N = 5) and (II) a clinical feasibility stage, stage II (N = 40).

In stage Ia (*Figure 1*) a small cohort of women (N = 5) were included to explore whether the intended single or repeated injections (maximum 3) of 15 µg corifollitropin alfa (Elonva®, NV Organon, The Netherlands) followed by daily rFSH (50 IU, Puregon®, NV Organon, The Netherlands) provides an appropriate response, i.e. one follicle with a diameter of ≥18 mm and in total no more than 2 follicles with a diameter of ≥15 mm. The treatment outcome of the first 5 subjects would give an indication of the validity of the anticipated dose to be used in stage II. After careful evaluation the dose used in stage Ia was not considered valid to be used in stage II. Therefore, the protocol was amended and a stage Ib phase was included.

In stage Ib (*Figure 1*) the intention was to include a small cohort of women (N = 5) to explore whether the adjusted dosing regimen of corifollitropin alfa, i.e. one intended single injection of 30 µg, followed by injections of 20 µg in case of no response or insufficient response (maximum of 3 injections) followed by daily urinary hCG (200 IU, Pregnyl©, NV Organon, The Netherlands) would provide an appropriate response (one follicle with a diameter of ≥18 mm and in total no more than 2 follicles with a diameter of ≥15 mm).

The aim of stage II was to assess the clinical feasibility of a single or repeated dose of corifollitropin alfa followed by daily administration of 200 IU hCG (N = 20) versus a single or repeated dose of corifollitropin alfa followed by daily administration of 75 IU rFSH (N = 20) to induce monofollicular growth in women with WHO group II anovulatory infertility.

All subjects started the treatment with an injection of a low dose of corifollitropin alfa on day 1, 2 or 3 after the onset of a progestagen-induced withdrawal bleeding. Follicular development was monitored by ultrasound and hormone samples every other day. If 4 days after the first injection of corifollitropin alfa follicle growth was insufficient (no follicle ≥12 mm), a second injection of corifollitropin alfa was given. If after another 4 days follicle growth was insufficient, the subject received a third administration of corifollitropin alfa. In subjects with insufficient follicular growth 4 days after the third injection (stimulation day 13), or multifollicular growth (if 3 or more follicles of ≥15 mm were observed), treatment was discontinued (*Figure 1*).

As soon as the largest follicle reached a size of ≥12 mm (day 5, 9 or 13), the subject started the same day with daily injections of 50 IU rFSH or 200 IU hCG. Ultrasound monitoring was performed every other day. A bolus injection of 5000 IU hCG was administered if at least one follicle reached ≥18 mm and in total no more than 2 follicles ≥15 mm were observed. Treatment was discontinued when

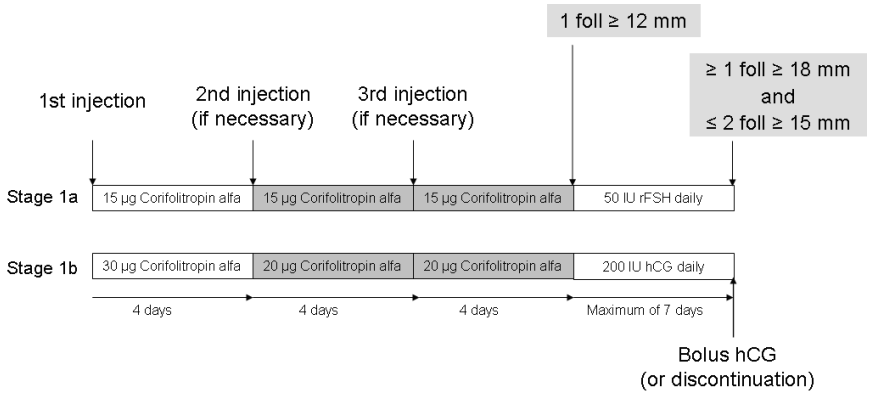

Figure 1 *Flow chart of pilot study.*

3 or more follicles of ≥15 mm developed or when after 7 days of treatment with rFSH or hCG no follicle of ≥18 mm was measured by ultrasound. Ovulation was confirmed by a mid-luteal serum progesterone level of ≥15 nmol/L. A pregnancy test (urinary hCG) was performed prior to administration of progestagens and 2 or 3 weeks after bolus injection of hCG.

The primary endpoint in this trial was incidence of monofollicular development, defined as the number of subjects with monofollicular response (1 follicle ≥18 mm and no other follicle ≥15 mm on the day of the bolus injection of hCG) divided by the number of treated subjects. The secondary endpoints were ovulation rate (the number of subjects with confirmed ovulation divided by the number of treated subjects), monofollicular ovulation rate (the number of subjects with monofollicular response and confirmed ovulation divided by the number of treated subjects), cancellation rate, number and sizes of follicles, endocrine profile (FSH, LH, estradiol, progesterone, and inhibin-B, and hCG were measured every other day from stimulation day 1 up to and including day of bolus hCG) and ongoing pregnancy rate.

Validated immunoassays (Devroey et al. 2004) were performed at a central laboratory (Schering-Plough, Oss, The Netherlands and Waltrop, Germany) to measure serum levels of FSH, LH, estradiol, progesterone and inhibin-B.

The primary efficacy parameter of the trial was the incidence of monofollicular development. It was considered a priori that a monofollicular rate of 10% or below was unacceptably low. In order to show with at least 80% power that the lower limit of the two-sided 95% confidence interval for monofollicular rate is above 10%, 19 subjects per treatment group are needed in stage II. It was assumed that the true monofollicular rate equals 40%. With 20 subjects per treatment group, an observed monofollicular response in (at least) 6 subjects is needed to exclude the unacceptable rate of 10% at significance level of 5%. No interim analysis was performed. All analyses were performed using SAS under Windows XP.

Results

Patient characteristics

In total 8 subjects were included in stage I; 5 in stage Ia and 3 in stage Ib. The clinical results of stage Ia and Ib resulted in the decision to stop the trial during stage Ib without proceeding to stage II. Patient demographic and infertility characteristics are shown in *Table 1*. Their overall mean age was 30 years and 7 out of the 8 patients had polycystic ovaries. Three subjects suffered from amenorrhea and five subjects from oligomenorrhea. None of the subjects reported suffering from hirsutism, acne, hyperandrogenism and/or hyperinsulinemia.

Table 1 *Patient demographic and infertility characteristics.*

Characteristic		rFSH Stage Ia N = 5	hCG Stage Ib N = 3	Total N = 8
Age (years)	Mean (SD)	30.8 (5.9)	28.7 (2.5)	30.0 (4.8)
Body weight (kg)	Mean (SD)	66.5 (6.8)	69.1 (5.4)	67.5 (6.0)
BMI (kg/m^2)	Mean (SD)	22.7 (1.9)	23.9 (4.4)	23.1 (2.8)
Primary infertility n (%)		3 (60.0)	3 (100.0)	6 (75.0)
Duration of infertility (months)	Mean (SD)	36.2 (47.8)	38.0 (25.0)	36.9 (38.5)
Cycle abnormality n (%)	Amenorrhea	1 (20.0)	2 (66.7)	3 (37.5)
	Oligomenorrhea	4 (80.0)	1 (33.3)	5 (62.5)
Polycystic ovaries n (%)		5 (100.0)	2 (66.7)	7 (87.5)
Previous treatment CC n (%)		4 (80.0)	3 (100.0)	7 (87.5)
Previous treatment gonadotropins n (%)		3 (60.0)	2 (66.7)	5 (62.5)

BMI = body mass index; CC = clomiphene citrate; SD = standard deviation.

Simulation selected dose

Figure 2 shows simulated pharmacokinetic profile and FSH activity of a low dose of corifollitropin alfa (15 µg) followed by 50 IU rFSH versus the standard treatment to induce monofollicular growth in women with WHO group II anovulatory infertility, i.e., daily 50 IU rFSH (75 IU from day 15 onwards). The dosages selected for stage I, the dose confirmation stage, were based on this simulation figure.

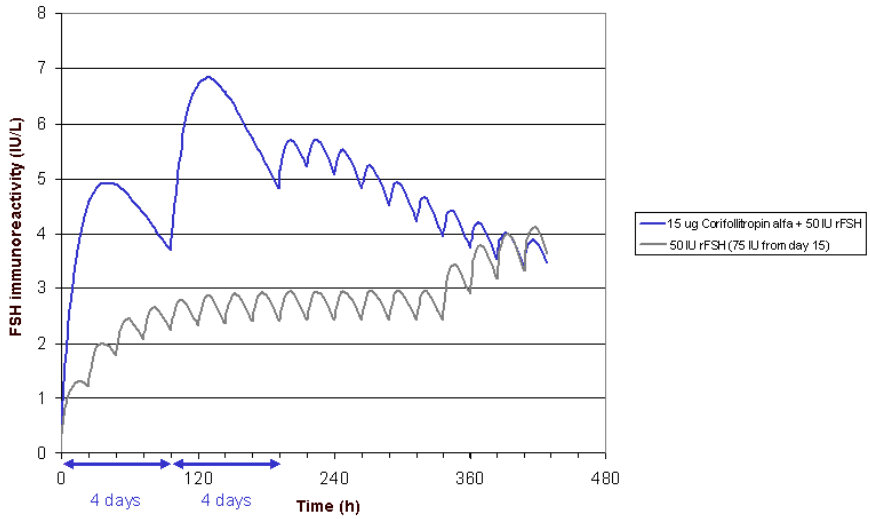

Figure 2 *The simulated FSH immunoreactivity of a dose of 15 µg corifollitropin alfa followed by daily 50 IU rFSH (back line) in comparison to a fixed starting dose of 50 IU rFSH increased to 75 IU daily (grey line) from day 15 onwards.*

Treatment outcome

The treatment results per subject are summarised in *Table 2* and the treatment characteristics are shown in *Table 3*. Three subjects received hCG for final oocyte maturation and had ovulation confirmed. One subject (stage Ib) achieved an ongoing twin pregnancy. Five subjects had their treatment cancelled during the trial. One subject (patient suffering from amenorrhea) had no follicle ≥ 12 mm after 3 injections with corifollitropin alfa, 3 subjects had an arrest of follicle growth after start with rFSH/hCG, and one subject was cancelled due to multifollicular growth.

Table 2 *Treatment results.*

Treatment results	rFSH Stage Ia	hCG Stage Ib	Total
	N = 5	N = 3	N = 8
Monofollicular development n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Ovulation rate n (%)	2 (40.0)	1 (33.3)	3 (37.5)
Monofollicular ovulation n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Cancellation n (%)	3 (60.0)	2 (66.6)	5 (62.5)
Insufficient ovarian response after 3 injections corifollitropin alfa n (%)	0 (0.0)	1 (33.3)	1 (12.5)
Multifollicular growth n (%)	1 (20.0)	0 (0.0)	1 (12.5)
Arrest follicle growth after start rFSH/hCG n (%)	2 (40.0)	1 (33.3)	3 (37.5)

Table 3 *Treatment characteristics.*

Subject	Number of injections of corifollitropin alfa	Number of days rFSH/ hCG	Bolus hCG	Reason cancelled	Pregnancy
1	2	3	-	Multifollicular growth	No
2	2	2	+	-	No
3	1	6	-	Arrest growth dominant follicle	No
4	2	4	-	Arrest growth dominant follicle	No
5	2	7	+	-	No
6	1	3	+	-	Yes
7	3	0	-	Insufficient ovarian response	No
8	2	6	-	Arrest growth dominant follicle	No

The patients with multifollicular growth (subject 1) and the patients with bolus hCG (subject 2, 5, and 6) had a normal hormonal response; estradiol and inhibin-B levels were increasing as well as the FSH, like the simulation figure. The patients with an arrest of growth of the follicle (subject 3, 4 and 8) and patients with insufficient ovarian response (subject 7) showed an increase in estradiol and inhibin-B levels after the corifollitropin alfa injection(s), but a decrease as soon as they started with rFSH or hCG. For detailed information per subject see *Figure 3*. Subjects 1-5 injected 15 µg of corifollitropin alfa (maximum 3 injections) and started with daily injections of rFSH after a dominant follicle occurred. Subjects 6–8 injected 30 µg of corifollitropin alfa (if necessary second or third injection of 20 µg of corifollitropin alfa) and started with daily injections of hCG after a dominant follicle occurred.

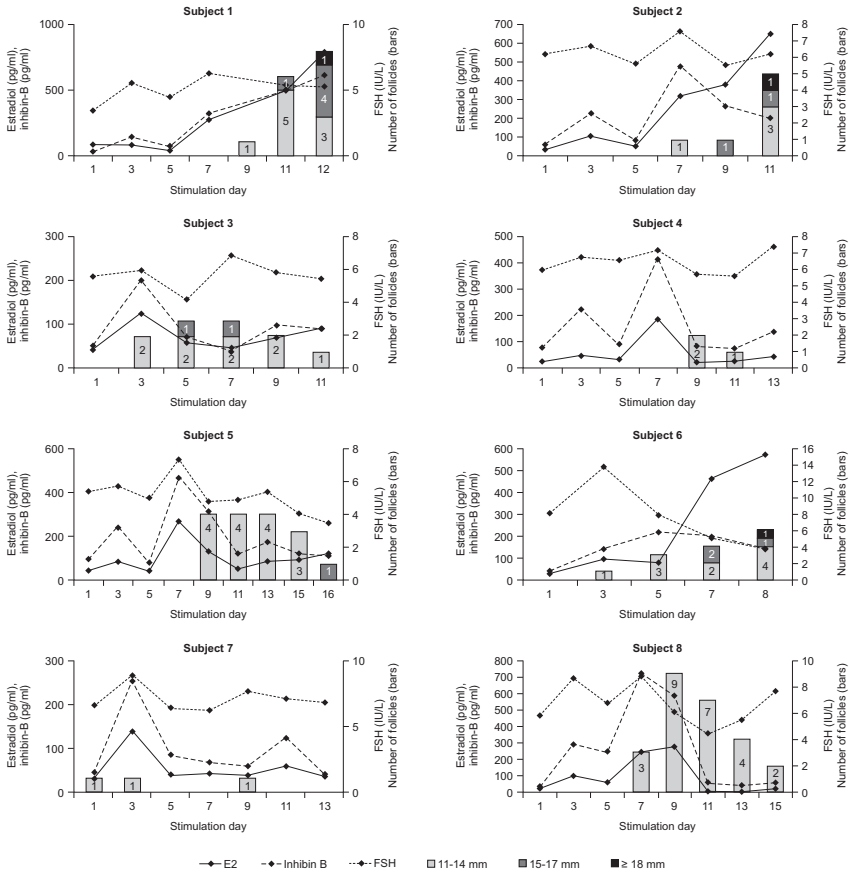

Figure 3 *Number and sizes of follicles, estradiol (pg/ml), inhibin-B (pg/ml), and FSH (IU/L) levels per stimulation day per patient.*

Subject 1: multifollicular growth; subject 2: bolus hCG, not pregnant; subject 3: arrest growth dominant follicle; subject 4: arrest growth dominant follicle; subject 5: bolus hCG, not pregnant; subject 6: bolus hCG, pregnant; subject 7: insufficient ovarian response; and subject 8: arrest growth dominant follicle.

Discussion

A total of 8 subjects were treated with at least one injection of a low dose of corifollitropin alfa. None of the subjects showed monofollicular growth. The study was prematurely cancelled since the collected data after a limited number of patients clearly did not support monofollicular growth in women with WHO II anovulatory infertility.

In comparison to ovarian stimulation prior to IVF, the doses of corifollitropin alfa tested in the current ovulation induction trial were 5 to 10-fold lower. Previously, a single dose of 100 or 150 mg corifollitropin alfa has shown to be very suitable for the stimulation of multifollicular development prior to IVF or ICSI (Devroey et al. 2004, The corifollitropin alfa Ensure study group, 2010). It should be noted that the dosages tested for ovarian stimulation in IVF treatment are much higher compared with the dosages tested in the current trial in anovulatory women (Devroey et al. 2009).

Finding an appropriate corifollitropin alfa treatment regimen sufficient to induce and sustain monofollicular development in women with WHO group II anovulatory infertility, seems challenging due to the relatively high FSH activity (C_{max}) two days (t_{max}) after corifollitropin alfa administration. Reason for discontinuation ranged from insufficient ovarian response to multifollicular growth. It has been previously demonstrated that the effective and safe therapeutic window for exogenous FSH to treat anovulation is small (Fauser et al. 1993). Serum FSH levels should be sufficiently high to support the leading follicle up to ovulation (van Santbrink et al. 1997) but exceeding the FSH threshold too long may result in multifollicular development. Another complicating factor is the individual variability in sensitivity of the ovary to respond to FSH, especially in anovulatory women (Schipper et al. 1998).

Figure 3 shows the hormone levels of all included subjects. In almost all the patients there is a rise in FSH after the corifollitropin alfa injections, comparable to the simulating diagram. One subject, whose treatment was cancelled because of multifollicular growth, demonstrates an excessive rise in both estradiol and inhibin-B (subject 1). The other subjects without bolus hCG (subject 3, 4, 7, and 8) show a decline in estradiol and inhibin-B levels because of insufficient follicular growth.

Sixty to 85% of anovulatory women ovulate in response to CC (most typically WHO II patients). Of those who ovulate, approximately 50% do so at a dose of 50 mg (Neveu et al. 2007). Of those who ovulate, 30–40% conceive. In CC treatment predictors for ovulation include the free androgen index (FAI), BMI, presence of oligomenorrhea or amenorrhea and ovarian volume (Eijkemans et al. 2003, Imani

et al. 1998). After the use of a low-dose step-up protocol with gonadotrophins, the rates of ovulation are 72% with pregnancy rates of 45% (White et al. 1996). Individualising the starting dose might result in higher ovulation and pregnancy rates. However, clear predictors for monofollicular growth following treatment with gonadotrophins are elusive (Imani et al. 2002, van Wely et al. 2005), making the prediction of the response to stimulation of women with WHO group II anovulatory infertility difficult.

Several studies have shown that the low-dose step up protocol is effective and reduces the risk of ovarian hyperstimulation syndrome (OHSS). Weekly increments of 25 IU of rFSH in the daily dose were more effective and efficient than 50 IU increments (Lan et al. 2009, Leader et al. 2006). In order to reduce the risk of over-response, the rFSH starting dose is usually maintained for 14 days before being increased. Since patients have to inject themselves daily during that period, the use of a long-acting preparation such as corifollitropin alfa could offer the advantage of a decrease in the number of injections required.

However, in the present study a single or repeated low dose of 15-30 µg corifollitropin alfa followed by a low daily dose of either rFSH or hCG did not induce monofollicular development in women with WHO group II anovulatory infertility. The results of this pilot trial indicate that the tested treatment regimen is not suitable for ovulation induction in anovulatory women.

Chapter 6

Follicular phase endocrine characteristics during ovarian stimulation and GnRH antagonist co-treatment for IVF; RCT comparing rFSH initiated on cycle day 2 or 5

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Abstract

Context

Strategies involving mild ovarian stimulation protocols for IVF might lessen discomfort to the patient and substantially lower complication rates.

Objective

To compare the follicular phase endocrine characteristics and follicular development in patients who started rFSH on cycle day (CD) 2 or CD5 in IVF treatment, using GnRH antagonist as co-medication.

Design

Prospective randomised controlled trial in 2 university centres in Belgium and the Netherlands.

Patients

Seventy-six IVF/ICSI patients were included.

Interventions

The control group (CD2) received a standard treatment with 150 IU rFSH from cycle day 2, whereas in the study group (CD5) stimulation was started on day 5 of the cycle. The GnRH antagonist was administered daily from CD 6 onwards in both treatment arms.

Main outcome measure

Endocrine follicular phase profile during ovarian stimulation.

Results

Follicular phase patterns of gonadotrophin and steroid concentrations were found to be comparable in both treatment groups, except for serum estradiol being significantly higher in the CD2 group on day 6 of the cycle (295.6 ± 202.5 ng/L in the CD2 vs 102.5 ± 47.9 ng/L in the CD5 group; $p < 0.01$) and LH being significantly higher in the CD5 group on day 6 of the cycle (1.7 ± 0.7 IU/L in CD2 vs 5.0 ± 2.1 IU/L in CD5 group; $p < 0.01$). With regard to follicular development, there was no difference in the numbers of small follicles (< 10 mm), intermediate follicles (10-12 mm and >12-14 mm) and large follicles (>14mm) in both groups.

Conclusions

This study shows that the administration of rFSH starting on day 2 or day 5 of the cycle in a GnRH antagonist protocol for IVF/ICSI patients yields a comparable endocrine profile and follicular development. Future studies should focus on the design of more patient tailored ovarian stimulation protocols.

Introduction

For 25 years, ovarian stimulation – a key component of assisted reproductive technologies (ART) – has been applied with the aim of increasing the number of oocytes retrieved in order to generate multiple embryos to improve selection for embryo transfer (Templeton et al. 1998, Fauser et al. 2005, Macklon et al. 2006). At the present time, a long gonadotrophin-releasing hormone (GnRH) agonist pituitary suppression regimen with high doses of exogenous follicle-stimulating hormone (FSH) remains the most frequently applied stimulation strategy (Macklon et al. 2006).

The introduction of GnRH antagonists has provided the opportunity for different and potentially milder ovarian stimulation protocols (Fauser et al. 1999, Tarlatzis et al. 2006). A wide variety of GnRH antagonist protocols have been proposed, aiming to diminish patient discomfort and reduce side effects. Some mild ovarian stimulation protocols aim for multiple dominant follicle selection by widening the natural FSH window by administering exogenous FSH during the mid- to late follicular phase (Schipper et al. 1998, Hohmann et al. 2001).

Reduced drug administration does have the advantage of being less expensive and may be more patient-friendly (Macklon et al. 2006, Nargund et al. 2007, Heijnen et al. 2007). A potential concern regarding the wide spread application of milder stimulation protocols in routine clinical practice is a decreased ovarian response following mild stimulation which may increase cancellation rates resulting in decreased pregnancy chances (Heijnen et al. 2007, Hohmann et al. 2003). However, acceptable pregnancy rates have been demonstrated in recent studies because improved embryo quality may compensate for fewer embryos being generated (Heijnen et al. 2007, Hohmann et al. 2003). Moreover, recent evidence suggests that mild stimulation protocols lead to lower blastomere aneuploidy rates compared to conventional treatment regimens (Baart et al. 2007). Furthermore, the potentially negative effect of supraphysiological steroid levels on endometrial receptivity may also suggest that limited ovarian stimulation is beneficial (Simon et al. 1995, Devroey et al. 2004).

Another drawback of mild ovarian stimulation protocols is the observed decreased implantation rates in high responders when a flexible start of the GnRH antagonist is being performed (Verberg et al. 2008). This may – next to decreased embryo quality or compromised endometrial receptivity – also be due to escaping follicular phase LH levels in high responders caused by late initiation of the GnRH antagonist (Kolibianakis et al. 2003).

Little information is currently available regarding follicular phase endocrinology during mild ovarian stimulation protocols. The purpose of this randomised con-

trolled trial was to compare endocrine characteristics and follicular development of IVF/ICSI cycles in which stimulation was started either on cycle day 2 or 5, and to evaluate its clinical applicability. The hypothesis was put forward that in late start of the stimulation combined with early start of the GnRH antagonist, a mild ovarian response would be accompanied by a more favourable estradiol rise and LH suppression profile.

Material and Methods

Subjects and study design

The study presented here is a prospective randomised controlled trial and was conducted in 76 women between October 2008 and August 2009 with a standard indication for IVF, with or without intracytoplasmic sperm injection (ICSI). All the patients were recruited from the IVF outpatient clinic of the Department of Reproductive Medicine and Gynaecology of the University Medical Centre Utrecht and from the Centre of Reproductive Medicine of the Universitair Ziekenhuis Brussel. Randomisation took place at the outpatient clinic, when the results of the pre-treatment hormonal analyses were discussed with the patient. A computer-generated list was used for randomisation, assigned via numbered sealed envelopes. Each patient was enrolled into the study only once. This study was approved by the local ethics review committee in both centres and by the Central Committee on Research involving Human Subjects as a competent authority. All participating patients gave written informed consent. The study was registered with the Clinical Trial web (www.clinicaltrials.gov, number NCT00823472).
Inclusion criteria were; 1) regular indication for IVF and first treatment cycle of IVF; 2) age between 18-36 years; 3) body mass index between 18-29 kg/m^2; 4) history of regular menstrual cycle (25-35 days); 5) normal FSH serum levels on CD 2 (< 12 U/L); 6) no major uterine or ovarian abnormalities; 7) no endocrine or metabolic abnormalities; 8) no polycystic ovary syndrome (PCOS); and 9) no severe endometrioses (≥ grade 3).

Stimulation protocol

The trial was designed to compare two protocols for ovarian stimulation with GnRH antagonists co-treatment. In one group of patients a standard antagonist protocol was applied involving exogenous follicle-stimulating hormone (FSH) starting on CD2 (Group CD2). Alternatively patients underwent a mild stimulation protocol involving the initiation of exogenous stimulation on CD5 (Group CD5) as described previously (Heijnen et al. 2007). In the CD2 group, daily injections of

recombinant FSH (Puregon®,Schering Plough, Oss, The Netherlands) were initiated on CD 2 of the menstrual cycle at a fixed dose of 150 IU per day. In the CD5 group, rFSH was initiated on CD 5 of the cycle at a similar daily dose of 150 IU. The dose of rFSH was maintained fixed throughout the whole stimulation period. In both groups, subcutaneous administration of the GnRH antagonist ganirelix (Orgalutran®, Schering Plough) was started on CD 6, at a daily dose of 0.25 mg.
Final oocyte maturation was induced by the administration of a single subcutaneous bolus dose of 10.000 IU hCG (Pregnyl®, Schering Plough), as soon as three follicles ≥ 17 mm in diameter were present on ultrasonography. In case of the presence of less than 3 follicles, hCG was administered as soon as 2 follicles ≥ 17 mm were observed. In case of less than 2 follicles, the cycle was cancelled or rescue intrauterine insemination was performed. Oocyte-pick up (OPU) was performed 36 hours after hCG administration. Subsequently, IVF with or without ICSI was performed, and a single embryo was transferred 3 or 5 days thereafter. Luteal phase support in the form of intravaginal progesterone (Utrogestan®, Besins International, Paris, France) 200 mg, three times daily was given from the day of OPU for 12 days.

Assessments and hormone assays

In order to investigate the impact of both protocols on the endocrinology in the follicular phase, blood samples for endocrine monitoring were collected every other day during the stimulation phase starting on CD 2. Blood samples were drawn by vein puncture, allowed to coagulate at least 30 minutes and centrifuged for 10 min at 1800g, after which serum was decanted avoiding erythrocytes. Serum was stored individually at -20°C or colder at the clinic for a maximum of twenty weeks before analysis. Automated immunoanalysis was done by the hormone laboratory at Universitair Ziekenhuis Brussel 'UZBrussel' (Brussels, Belgium) by validated laboratory immunoassay methods (electrochelimuminescence from Roche on Cobas 6000 ®) , selected for their sensitivity and reproducible proficiency profile to increase the precision of the measurements in serum.
Transvaginal ovarian ultrasound scans to assess follicular growth were also performed every other day. In the case of a positive urine pregnancy test, an ultrasound scan was carried out 6-7 weeks after oocyte retrieval to determine the viability of the pregnancy. Clinical pregnancy was defined by the observation of fetal cardiac activity on ultrasonography at seven weeks of gestation.

Outcome measures and data analysis

The primary endpoint of the study is the hormonal follicular response. Secondary endpoints include duration of stimulation; total cumulative dose of rFSH consu-

med; cancellation rate; number of cumulus-oocyte complexes (COC), number of M II and 2-PN oocytes, number of top embryos per started cycle; and clinical pregnancy rates in each treatment group. It is important to note that the implantation rate equals the pregnancy rate since only one embryo was transferred.
Demographic and clinical characteristics such as age, weight, and height were also collected.

Data analysis

Data are presented as means with standard deviation for continuous data and percentages for count data. Between group statistical comparisons were performed with t-tests for continuous and chi squared tests for count data. Two-sided P values < 0.05 are considered statistically significant.
The clinical trial comparing both treatment regimens was designed to detect a difference of at least 20% in top quality embryos obtained comparing the CD5 and CD2 start of stimulation. Two hundred and thirty four patients should be included to achieve this aim. The current analysis is an interim analysis of a still ongoing trial, involving the intense endocrine assessment in a subset of women undergoing frequent blood sampling included in the onset of the trial.

Results

Subjects and characteristics

There were no significant differences between the groups with regard to demographic characteristics, as shown in *Table 1*.
In the CD2 group, 3 patients did not get to undergo oocyte retrieval, as compared to 5 patients in the CD5 group. The reasons for cancelling the intended treatment cycle are shown in *Figure 1*. In the CD2 group, 5 patients did not reach the stage

Table 1 *Patient demographics and baseline characteristics of 76 patients undergoing an IVF/ICSI treatment in a GnRH antagonist protocol, randomised for two different strategies of ovarian stimulation.*

	CD 2 Group (n= 36)	CD 5 Group (n= 40)	P-value[b)]
Age (y)[a)]	30.4 ± 3.4	30.6 ± 3.9	0.813
Weight (kg)[a)]	61.9 ± 11.4	66 ± 10.8	0.112
Height (cm)[a)]	165 ± 2	167 ± 6	0.061
BMI (Kg/m^2)[a)]	22.8 ± 3.5	23.7 ± 3.5	0.267
FSH, IU/L	6.4 ± 1.8	6.7 ± 1.5	0.431
LH, IU/L	4.8 ± 2.5	5.5 ± 1.8	0.162
E2, pg/mL	40.2 ± 18.3	42.9 ± 17.4	0.512
P, ng/mL	0.7 ± 0.3	0.7 ± 0.4	1.0

[a)] Mean ± standard deviation; [b)] P value for between-group difference from t-tests
BMI: Body Mass Index; E2: estradiol; P: Progresterone

of embryo transfer due to failed fertilisation or failed blastocyst development, and one patient developed the ovarian hyperstimulation syndrome (OHSS). In the CD5 group, there was no blastocyst development in 5 patients and no fertilisation in 2 patients (*Figure 1*).

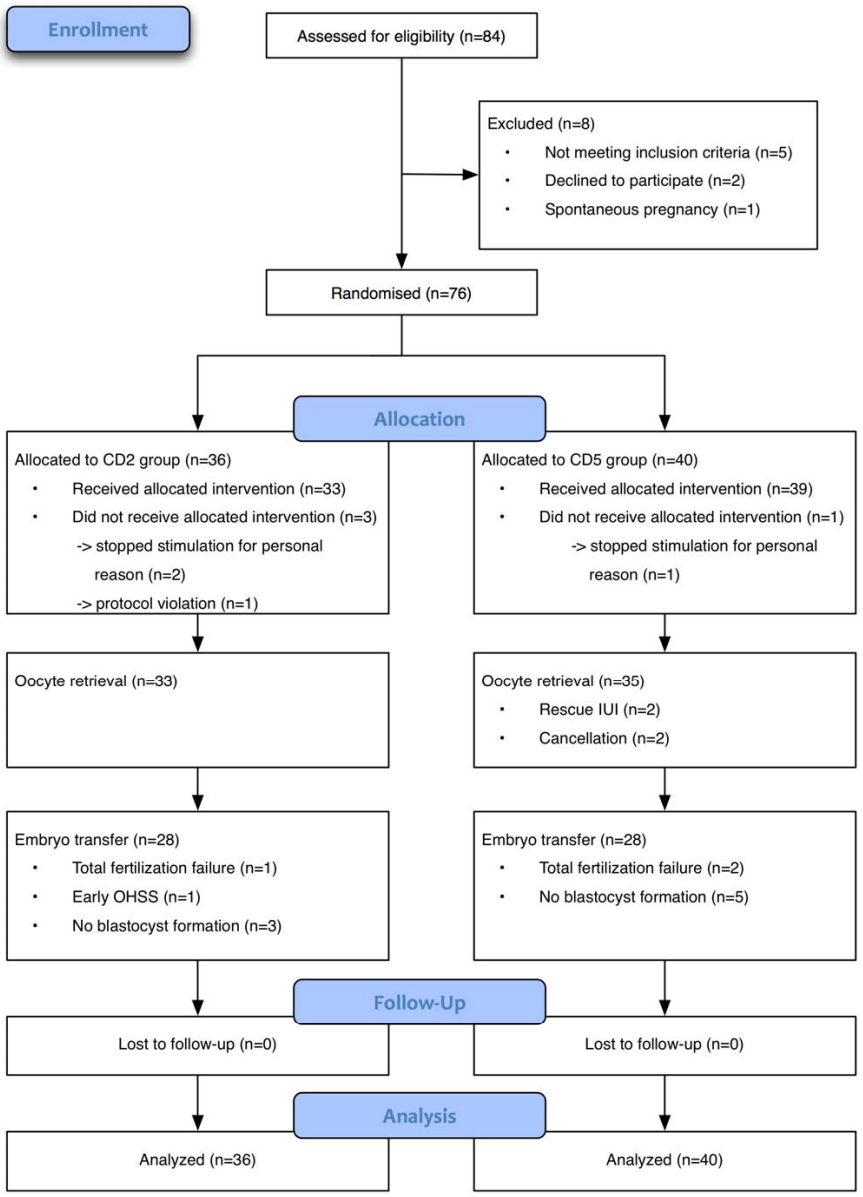

Figure 1 *Trial profile with the phases of the study*
OHSS: Ovarian Hyperstimulation Syndrome

Details referring to the stimulation are summarized in *Table 2*. Overall duration of rFSH administration (days of stimulation) in the CD5 group was significantly lower than in the rFSH-group. The total dose of rFSH consumed was significantly lower in the CD5 group: 1,364 IU (SD 226) versus 1,177 IU (SD 295), $p < 0.01$. The number of oocyte-cumulus complexes (OCC) obtained at retrieval was similar in both groups. The fertilisation rate, the implantation rate, the pregnancy rate and abortion rate were comparable in both groups. The ongoing pregnancy rate per started cycle was 28% (10 out of 36 patients) in the CD2 group and 25% (10 out of 40 patients) in the CD5 group ($P = 0.78$) (*Table 3*).

Table 2 *Follicular characteristics and cycle outcome measures.*

	CD 2 Group (n= 33)	**CD 5 Group (n= 39)**	**Between-group difference (95% confidence limits)**	**P-value[b)]**
Total dose of rFSH[a)] (IU)	1364 ± 226	1177 ± 295	187.0 (58.7, 315.3)	< 0.01
rFSH duration[a)] (days)	9.1 ± 1.5	7.8 ± 2.0	1.30 (0.44, 2.16)	< 0.01
Duration of the follicular phase[a)] (days)	10.1 ± 1.5	11.9 ± 2.0	-1.80 (-2.66, -0.94)	< 0.01
	CD 2 Group (n= 33)	CD 5 Group (n= 35)		
Number of COCs[a)]	8.9 ± 4.7	10.3 ± 6.2	-1.40 (-4.24, 1.44)	0.30

[a)] Mean ± standard deviation; [b)] P value for between-group difference from t-tests

COC: cumulus-oocyte complexes

Table 3 *Clinical outcome measures[a)]*

	CD 2 Group	**CD 5 Group**	**Between-group difference (95% confidence limits)**	**P-value[b)]**
Number of patients with positive hCG	13	10		
Per started cycle % (n)	13 / 36 (36%)	10 / 40 (25%)	11.1% (-9.5%, 31.8%)	0.29
Per pickup % (n)	13 / 33 (39%)	10 / 36 (28%)	11.6% (-10.6%, 33.8%)	0.31
Per embryo transfer % (n)	13 / 28 (46%)	10 / 28 (36%)	10.7% (-14.9%, 36.3%)	0.42
Number of ongoing pregnancies				
Per started cycle % (n)	10 / 36 (28%)	10 / 40 (25%)	2.8% (-17.1%, 22.6%)	0.78
Per pickup % (n)	10 / 33 (30%)	10 / 36 (28%)	2.5% (-18.9%, 24.0%)	0.82
Per embryo transfer % (n)	10 / 28 (36%)	10 / 28 (36%)	0.0% (-25.1%, 25.1%)	1.0

[a)]Data are presented as number of cases including nominator and denominator values (percentages in parenthesis). [b)] P value for between-group difference from Chi squared tests. Absolute between-group difference = (CD 5-group value [third column]) - (CD 2-group value [second column]). Between-group differences not including zero.

Endocrinology

Endocrine profiles of all patients per randomised group are depicted in box and whisker plots (*Figure 2*). Serum hormone levels were measured on day 2, day 6 and day 8 of the cycle, and immediately before triggering final oocyte maturation. Follicular phase patterns of both gonadotrophin and steroid levels did not demonstrate clear differences between both treatment groups, except for the serum estradiol and FSH level being significantly higher and the serum LH level being significantly lower on day 6 of the cycle in the CD2 group. Also, LH levels appeared significantly higher in the CD5 group at the time of hCG. *Table 4* shows serum concentrations of gonadotrophins and gonadal steroids across the stimulation cycle.

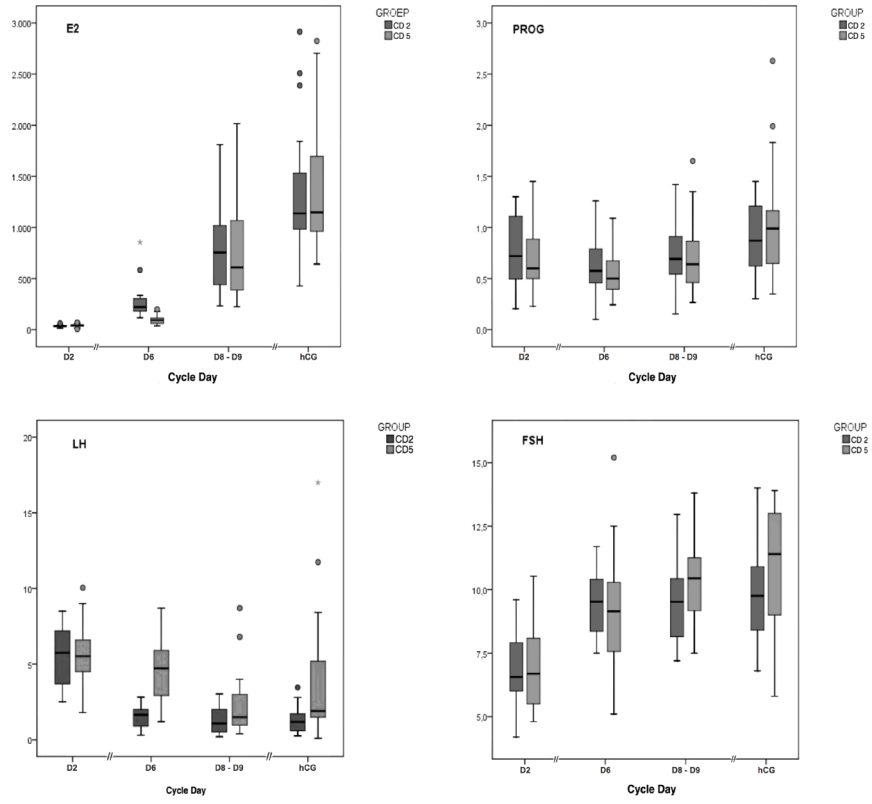

Figure 2 *Box (median values and 25th and 75th percentiles) and whisker (P_5 and P_{95}) plots representing FSH (IU/L), LH (IU/L), E_2 (ng/L) and P (ng/L) serum concentrations of both treatment groups. On the X-axis, the days of blood sampling are given (day 2, day 6, day 8 of the cycle and day of hCG administration).*
CD 2 group; d2 (n=36), d6 (n=33), d8 (n=33), d of hCG (n=33)
CD 5 group; d2 (n=40), d6 (n=39), d8 (n=39), d of hCG (n=39)

Table 4 *Serum hormone levels throughout the follicular phase*

Serum hormone levels on day 2 of the cycle			
	CD2	**CD5**	**p-value**
FSH (IU/L)	6.9 ± 2.3	7.0 ± 1.6	0.825
LH (IU/L)	5.0 ± 2.3	5.2 ± 2.3	0.706
E2 (pg/mL)	35.6 ± 12.2	39.4 ± 15.1	0.235
P (ng/mL)	0.7 ± 0.3	0.7 ± 0.3	1.0
Serum hormone levels on day 6 of the cycle			
	CD2	**CD5**	**p-value**
FSH (IU/L)	9.6 ± 1.2	8.6 ± 1.0	< 0.01
LH (IU/L)	1.7 ± 0.7	5.0 ± 2.1	< 0.01
E2 (pg/mL)	295.6 ± 202.5	102.5 ± 47.9	< 0.01
P (ng/mL)	0.6 ± 0.3	0.5 ± 0.2	0.096
Serum hormone levels on day 8 of the cycle			
	CD2	**CD5**	**p-value**
FSH (IU/L)	10.1 ± 1.9	9.5 ± 1.6	0.150
LH (IU/L)	1.5 ± 1.0	1.9 ± 1.3	0.154
E2 (pg/mL)	868.2 ± 404.6	776.4 ± 483.2	0.390
P (ng/mL)	0.7 ± 0.3	0.7 ± 0.4	1.0
Serum hormone levels before final oocyte maturation			
	CD2	**CD5**	**p-value**
FSH (IU/L)	10.4 ± 1.3	10.8 ± 1.8	0.291
LH (IU/L)	1.4 ± 1.1	3.7 ± 3.8	< 0.01
E2 (pg/mL)	1405.5 ± 728.1	1402.5 ± 605.9	0.985
P (ng/mL)	1.0 ± 0.4	1.0 ± 0.5	1.0

FSH: Follicle Stimulating Hormone; LH: luteinising hormone; E_2: Estradiol; P: progesterone

Ultrasound findings

The numbers and sizes of follicles were investigated during the stimulation in both treatment groups. With regard to the ultrasound findings before administration of hCG, the numbers and sizes of pre-ovulatory follicles were investigated in both groups. There was no difference in the numbers of small follicles (< 10 mm), intermediate follicles (10-12 mm and >12-14 mm) and large follicles (>14mm) in both groups.

Discussion

To our knowledge, the current study represents the first randomised trial comparing hormonal profiles and follicle development characteristics in IVF/ICSI cycles where ovarian stimulation is initiated on different days of the menstrual cycle. Surprisingly, this study shows that the administration of rFSH starting on CD 2 or 5 of the cycle in a GnRH antagonist co-treatment protocol for IVF/ICSI patients, yields only subtle differences in the endocrine profile and follicular development. Since the daily doses of rFSH were fixed at a relative low dose of 150 IU for the entire stimulation period, the differences observed between the hormonal levels and the follicular development of the two groups compared are not biased. In addition, estradiol levels were not considered in deciding on hCG administration. As a result, duration of stimulation reflects only follicular development characteristics.

In the currently tested so called 'mild' stimulation protocol, the stimulation of a cohort of ovarian follicles (also referred to as secondary recruitment) is initiated by the endogenous inter-cycle release of FSH (Fauser et al. 1999, Fauser et al. 1997). The FSH window is defined as the time during which the circulating FSH concentrations are above the threshold required for follicular development (van Santbrink et al. 1995). As previously described, by combining the rise in endogenous FSH and the administration of exogenous FSH only during the mid- to late-follicular phase, FSH levels will remain above the threshold keeping the FSH window open (Macklon et al. 2006). This protocol may result in significantly decreased consumption of exogenous gonadotrophins, may be associated with shorter treatment duration, and promotes a more targeted interference with single dominant follicle selection resulting in ongoing multi-follicular development.

In the current study, the stimulation is initiated 3 days later in the CD5 study group compared to the CD2 control group, which implies a significant longer follicular phase in the CD5 group (10.1 days in the CD2 group versus 11.9 days in the CD5 group, $p < 0.01$). The duration of the stimulation differs by 1.3 days only (*Table 2*). This finding suggests indeed that less administered FSH is needed when starting with stimulation later during the follicular phase and a significant decrease in total consumption of rFSH (1,364$\pm$226 (SD) IU versus 1,177$\pm$295 IU; $p < 0.01$). More specifically, the need for exogenous FSH during the first days of ovarian stimulation can be questioned, as the relatively high endogenous FSH concentrations during this period could be sufficient. In contrast to the cost reduction of FSH, the consumption of the GnRH antagonist is increased since a fixed starting day of GnRH antagonist has been applied. This implies a discrete additional medication cost. Maybe GnRH antagonist could be commenced later in the cycle when exogenous FSH is started on CD5.

The day of triggering with hCG, the serum hormone levels of estradiol, FSH and progesterone are similar. Although not expected, the LH levels are significantly higher on the day of hCG in the CD5 group compared with the CD2 group (1,4 ± 1,1 versus 3,7 ± 3,8 IU/L, p< 001). To evaluate whether this increased value of LH, observed in the CD5 group, is not attributed to chance, would require a much larger study. Nevertheless, a possible explanation is that LH release depends on the duration of GnRH antagonist administration (Banga et al. 2010). There are 1.7 days more of GnRH antagonist administration in the CD5 group compared to the CD2 group (*Table 2*).

Although the administration of rFSH on day 5 of the cycle can overrule single dominant follicle selection in the majority of women, more than ten per cent of the patients (4 out of 39) developed mono-follicular growth, probably due to closure of the FSH window (Fauser et al. 1993). These subjects add the element of time to the FSH threshold theory and emphasize the importance of a transient increase of FSH above the threshold concentration for single dominant follicle selection (Schipper et al. 1998, Zeleznik et al. 1985). As described previously, the endogenous rise and fall in FSH occurs earlier in women with more advanced ovarian aging (Klein et al. 2002). Under those conditions, the initiation of exogenous FSH on cycle day 5 may be too late.

Our results suggest that ovarian stimulation is feasible in the majority of patients when initiated on cycle day 5, with minimal impact on the length of the follicular phase. These results suggest that by selecting the appropriate day to initiate ovarian stimulation (namely on cycle day 2, 3, 4 or 5) oocyte retrievals on weekends can be largely avoided and could serve as a additional planning tool to schedule IVF treatment cycles at the patient's and the centre's convenience.

The development of a standardized protocol most suitable for ovarian stimulation for all women seems to be elusive due to important ovarian response differences among patients. Considering the risks, side effects and the high costs of ovarian stimulation and multiple gestation, mild approaches should be taken serious. Moreover, patient tailored regimens based on individual patient characteristics should be developed further. In other words, mild response rather than mild stimulation needs further scrutiny (Fauser et al. 2010).

In conclusion, this study shows that the endocrine profile and follicular development of IVF/ICSI cycles, in which stimulation was started either on day 2 or day 5 of a GnRH antagonist protocol, are comparable. Whether there is a difference in embryo quality, pregnancy rate and live birth rate, remains to be determined in a larger trial.

Chapter 7

Comparison between start of ovarian stimulation on cycle day 2 versus cycle day 5 with GnRH antagonist in IVF treatment: a randomised controlled trial

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Abstract

Background

Milder stimulation protocols have the advantage of being less expensive and more patient-friendly. The aim of this study was to compare the clinical applicability of a mild stimulation (start stimulation at cycle day 5)/fixed GnRH antagonist protocol with a regular (start stimulation at cycle day 2) GnRH antagonist protocol in terms of embryo quality.

Methods

This prospectively randomised, bi-centre, clinical study in 147 women undergoing IVF treatment evaluated whether cycle day 5 start of stimulation will lead to better quality of embryos, based on morphology, than cycle day 2 start, in IVF treatment with GnRH antagonist co-treatment started on fixed day (cycle day 6). Randomization to one of the two treatment groups was performed according to a computer-generated randomisation schedule, assigned via numbered sealed envelopes.

Results

A total of 147 patients signed informed consent for eligibility evaluation and participation in this trial. The proportion of morphological top embryos, was 51.1% in the CD2 start group and 41.2% in the CD5 start group (p=0.15). The ongoing pregnancy rate per started cycle, was 28% in the CD2 start group and 16% in the CD5 start group (p=0.096).

Conclusions

The late start of ovarian stimulation by rFSH in an antagonist cycle seems not to be more effective in creating a mild response with high quality embryo's, compared to early, standard start of the rFSH and may even produce poorer outcomes in terms of ongoing pregnancies. Further studies should focus on individualised dosing based on ovarian response testing prior to initiating stimulation with rFSH.

Introduction

Conventional GnRH agonist ovarian stimulation protocols in in-vitro fertilisation (IVF) aim for the development of multiple embryos to improve selection for transfer (Templeton et al. 1998). The introduction of GnRH antagonists has provided the opportunity for milder stimulation protocols (Tarlatzis et al. 2006, Fauser et al. 1999). These protocols aim for limited dominant follicle selection by widening the natural FSH window through administering exogenous FSH in the mid- to late follicular phase (Schipper et al. 1998, Hohmann et al. 2001)

Milder stimulation protocols have the advantage of being less expensive and more patient-friendly (Macklon et al. 2006, Heijnen et al. 2007, Nargund et al. 2007). Moreover, recent evidence suggests that mild stimulation protocols lead to lower embryo aneuploidy rates compared to conventional treatment regimens (Baart et al. 2007). Also, a high yield of oocytes after ovarian stimulation is associated with an increased chromosome error rate (Haaf et al. 2009).

Although with mild stimulation protocols the expected number of oocytes retrieved will be lower, pregnancy rates have shown to be similar possibly because embryo quality out favours embryo quantity (Hohmann et al. 2003).

A disadvantage of mild stimulation is the higher cancellation rate for low response because fewer follicles will develop (Hohmann et al. 2003). Also mild ovarian stimulation protocols have resulted in decreased implantation rates in high responders when using a flexible start GnRH antagonist protocol (Verberg et al. 2009). The possible cause for this phenomenon may be insufficient LH suppression by the GnRH antagonist in high responders by both the late initiation of the GnRH antagonist and/or early response of the ovaries to rFSH (Pelinck et al. 2007).

The purpose of this study was to compare the clinical applicability of a mild stimulation (start stimulation at cycle day 5)/fixed GnRH antagonist protocol with a regular (start stimulation at cycle day 2) GnRH antagonist protocol in terms of embryo quality.

Material and methods

This trial was a bi-centre, prospective, randomised controlled clinical trial conducted between October 2008 and September 2010.

The study was approved by the local medical ethics committee of both centres and by the Central Committee on Research involving Human Subjects as a competent authority. The trial was conducted in accordance with the Declaration of Helsinki, International Conference on Harmonization Guidelines for Good Clinical Practice, and local regulatory requirements. An Independent Data Safety

Monitoring Board was appointed to monitor the safety of subjects participating in the trial, and written informed consent was provided by all patients.

Study population

Women aged 18-39 years with a body mass index of 18-29 kg/m2, a menstrual cycle length of 25-35 days, no major uterine or ovarian abnormalities, normal FSH serum levels on cycle day 2 (<12 U/L), and undergoing the first IVF or ICSI treatment cycle were eligible to enrol in the study. Patients who had a (history of) endocrine abnormalities, PCOS, endometriosis ≥ grade 3, or oocyte donation were not to be included in the trial. Patients were recruited from the outpatient clinic of the Department of Reproduction and Gynaecology of the University Medical Centre Utrecht, Utrecht, The Netherlands and VU Brussels, Brussels, Belgium

Study design

The study was designed as a randomised controlled clinical trial to compare the start of rFSH on cycle day 2 or cycle day 5 in the GnRH antagonist protocol. Randomization to one of the two treatment arms (1:1 ratio) was done per centre. Randomization took place after the final outpatient clinic visit. A computer-generated list was used for randomisation, assigned via numbered sealed envelopes. Each patient was enrolled into the study only once.

Stimulation regimen and ART procedures

All patients started with 150 IU of rFSH (Follitropin beta, Puregon®, Schering-Plough, Oss, The Netherlands) on cycle day 2 or cycle day 5 depending on the arm they were randomised for. This dose of rFSH was fixed for the whole stimulation period. To prevent premature LH surges, both treatment arms started with a daily injection of GnRH antagonist (0.25 mg Ganirelix, Orgalutran®, Schering-Plough, Oss, The Netherlands) on cycle day 6 up to and including the day of bolus hCG. A single bolus of urinary hCG (10.000 IU Pregnyl®, Schering-Plough, Oss, The Netherlands) was administered to induce final oocyte maturation as soon as at least three follicles of ≥ 17 mm were observed by ultrasound. In case of presence of less than 3 follicles, hCG was administered as soon as 2 follicles of ≥ 17 mm were observed. In case of less than 2 follicles, the cycle was cancelled or a rescue intrauterine insemination was performed. Oocyte-pick up (OPU) was performed 36 hours after Pregnyl administration. Subsequently, IVF with or without ICSI was performed. Embryo quality was evaluated for all available embryos on day 3 of culture by the local embryologist using a protocol-defined guideline based on the following parameters: number of blastomeres, degree of fragmentation,

blastomere size uniformity, and presence or absence of multinucleation. Embryos graded on day 3 of culture as grade 1 (≥ 7 cells, <20% fragmentation, and equal blastomere size in accordance with the cleavage stage) were qualified as top quality embryos. Embryos graded as grade 2 (≥ 7 cells, allowing up to 20% fragmentation) were qualified as good quality embryos. At embryo transfer, in UMC Utrecht at day 3 and in VU Brussels at day 5 after oocyte retrieval, a single embryo was transferred. The remaining embryos were cryopreserved or lost depending on their quality. To support implantation and early pregnancy, luteal phase support with intravaginal progesterone (Utrogestan®, Besins International, Paris, France, 200 mg, three times daily) was started on the day of OPU and continued for 12 days. In case of no menses after 14 days after embryo transfer a pregnancy test could be performed. An outline of both protocols is presented in *Figure 1*.

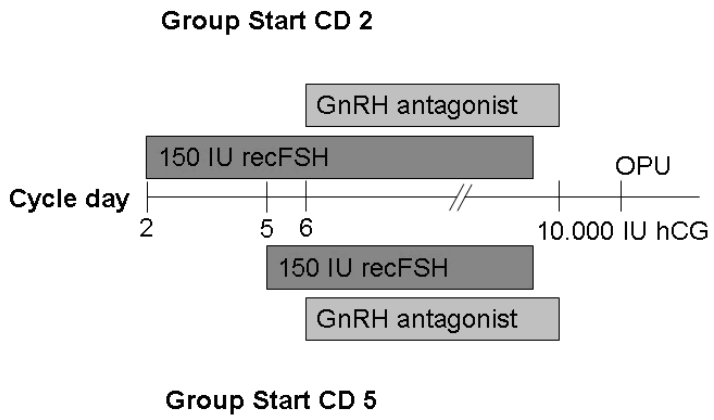

Figure 1 *Graphical illustration of the treatment regimens applied in this trial. Upper panel depicts reference group (start rFSH on cycle day 2), lower panel depicts investigational group (start rFSH on cycle day 5). CD = cycle day; GnRH = Gonadotrophin-Releasing Hormone; rFSH = recombinant Follicle Stimulating Hormone OPU = Oocyte Pick-Up; hCG = human Chorionic Gonadotrophin*

Assessments

Patients returned to the clinic for blood samples (FSH, LH, estradiol, progesterone, AMH) on CD21 of the preceding cycle, CD2, CD6, and day of bolus hCG. Ultrasound were performed on CD21 of the preceding cycle, CD2, CD6, and from CD6 onwards every other day up to and including the day of bolus hCG.

Outcome measures

The primary outcome of this study was the proportion of morphologically top embryos per OPU. Other clinical evaluated parameters were duration of stimulation, cancellation rate, fertilisation rate, number of cumulus oocyte complexes obtained, number of mature oocytes obtained, number of top embryos per started cycle, amount of IU rFSH, implantation rates high responders, endocrine changes (FSH, Oestradiol, Progesteron, LH, AMH) and clinical pregnancy rate.
Occurrence of (serious) adverse events was evaluated as safety end point.

Statistical analyses

The aim of the study was to determine whether cycle day (CD) 5 start of stimulation will lead to better quality of embryos, based on morphology, than CD 2 start, in IVF with GnRH antagonist co-treatment started on a fixed day. The main outcome variable was the presence of at least one morphological top embryo per oocyte retrieval. The Hohmann study (Hohmann et al. 2003), using a flexible start of the antagonist co-treatment, found that 37% of the CD2 cycles with oocyte retrieval produced at least one top embryo versus 61% of the CD5 cycles. This difference would certainly be clinically relevant. We needed to include 2 x 75 = 150 patients with oocyte retrieval to have 80% power that such a difference would be statistically significant at alpha = 0.05 (2-sided). Assuming that 90% of cycles will have oocyte retrieval, the number of included patients needs to be 150 / .90 = 166. For a difference of 40% versus 60%, 2 x 105 = 210 oocyte retrievals are required, implying 234 patients. Both clinics had to randomize 117 patients.
Sub analyses of an extended group of these data were in press in JCEM. These sub analyses were more focused on the endocrinology of the stimulation phase rather than the clinical data.

Results

A total of 147 patients signed informed consent for eligibility evaluation and participation in this trial. Subsequently a total of 19 patients were discontinued prior to the start of the treatment (8 for personal reasons, 4 spontaneously pregnant, 4 were found to violate entry criteria after randomisation, one had abnormal laboratory measurements, and 2 because of other reasons). Finally, a total of 129 patients started stimulation in to one of the two treatment groups (*Figure 2*). At last, 97 patients completed the treatment and underwent a single embryo transfer.

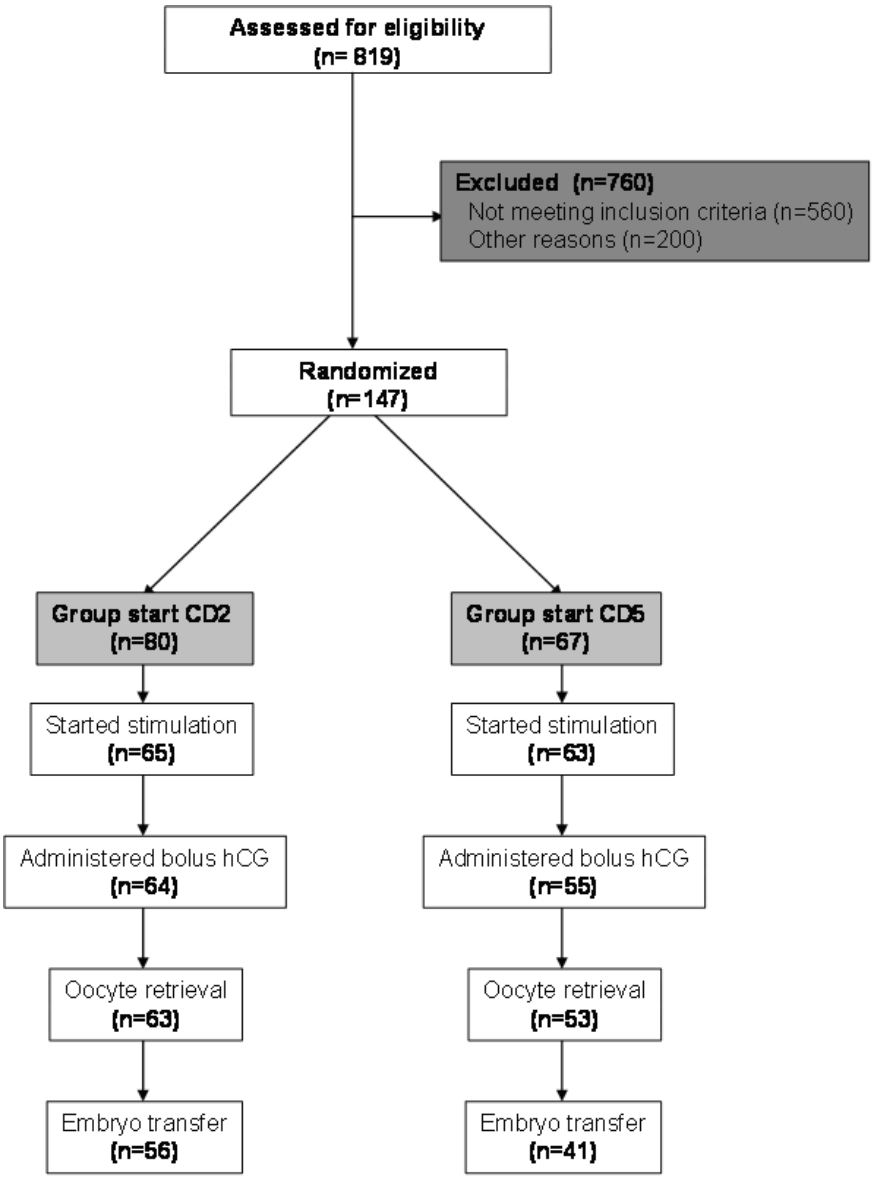

Figure 2 *Flow chart showing the number of participants at each stage of the clinical trial.*

Demographics of the ITT (intention-to-treat) population as well as relevant fertility characteristics, and USS findings were comparable in the two groups (*Table 1*). There were no differences observed in the baseline serum hormone levels on cycle day 2 between the two groups (*Table 4*).

Table 1 *Demographics and fertility characteristics per treatment group at cycle day 2. There are no differences between the two randomised groups. Numbers are mean (SD) unless otherwise indicated*

	Start cycle day 2	Start cycle day 5	Difference	p-value
	n=80	n=67		
Demographics				
Age (years)	31.0 (3.5)	31.1 (4.4)	0.09	0.9
Body weight (kg)	64.4 (9.0)	64.3 (9.9)	0.10	0.9
BMI (kg/m2)	23.0 (2.6)	22.5 (2.8)	0.54	0.2
Fertility characteristics				
Primary infertility (%)	71	76	5.00	0.5
Duration of infertility (years)	2.7 (1.7)	2.8 (1.7)	0.09	0.7
Cause of infertility[a], n (%)				
Male factor	61.3	56.7	4.6	
Tubal factor	8.8	17.9	9.1	
Endometriosis	1.3	0.0	1.3	
Unexplained	26.3	28.4	2.1	
Other	6.3	1.5	4.8	

[a] *A patient can have multiple causes of infertility*

Clinical outcome parameters

Table 2 shows the treatment characteristic per started group. The primary outcome parameter, proportion of morphological top embryos, was 51.1% in the CD2 start group and 41.2% in the CD5 start group (p=0.15). Another important outcome parameter in IVF treatment, the ongoing pregnancy rate per started cycle, was 28% in the CD2 start group and 16% in the CD5 start group (p=0.096) (*Table 3*).

The total cancellation per started cycle in the CD2 start group was 13.8% (9 of 65 patients) and 34.9% (22 of 63 patients) in the CD5 start group (p=0.21). There was a higher cancellation due to monofollicular growth in the CD5 start group (9.5%) compared to the CD2 start group (1.5%) (p=0.17). Cancellation because of hyperresponse was the same in the CD2 start group and the CD5 start group (1.5%) (p=0.17). The percentage of patients without an ET due to total fertilisation failure or no good quality embryo to transfer in the CD2 start group and the CD5 start group was 4.6% versus 1.6%, respectively (p=0.08).

Table 2 *Clinical parameters from stimulation phase up to embryo transfer per started cycle. Numbers are mean (SD) unless otherwise indicated.*

	Start cycle day 2	Start cycle day 5	Difference	p value
	n=64	n=55		
Primary outcome parameter				
Proportion excellent quality embryos (%)	51	41	10.17	0.1
Stimulation characteristics [a]				
Total dose of rFSH (IU)	1495.3 (265.9)	1292.7 (274.6)	202.59	<0.0001*
Total duration of stimulation (days) [b]	10.0 (1.8)	8.6 (1.8)	1.35	<0.0001*
Total duration of follicular phase (days) [b]	10.0 (1.8)	11.6 (1.9)	1.59	<0.0001*
Total number of injections of GnRH antagonist [b]	6.1 (1.7)	7.6 (1.9)	1.45	<0.0001*
Follicles, day of hCG [a]				
11-14 mm	4.9 (3.8)	4.2 (3.6)	0.73	0.3
15-16 mm	2.6 (2.5)	2.2 (2.1)	0.40	0.4
≥ 17 mm	4.7 (2.6)	3.7 (1.9)	0.99	0.03*
Clinical outcome per started cycle				
Oocytes retrieved	9.7 (5.4)	9.5 (5.6)	0.25	0.8
Metaphase II oocytes (ICSI only)	7.8 (4.9)	7.6 (4.4)	0.26	0.8
Fertilisation rate (%) [c]	57	58	1	0.8
Total number of embryos obtained (day 3) [c]	5.7 (3.7)	5.9 (3.9)	0.11	0.9
Excellent (top) quality embryos (grade 1) [c]	3.1 (2.9)	2.3 (2.3)	0.72	0.2
Good quality embryos (grade 2+3) [c]	1.2 (1.3)	1.7 (1.7)	0.54	0.07
Embryos cryopreserved [d]	2.0 (2.6)	1.4 (1.8)	0.63	0.2

[a]Restricted to patients with bolus hCG injection; [b] number of days up to day of bolus hCG; [c] restricted to patients with IVF and/or ICSI; [d] restricted to patients with cryopreserved embryos;
** statistically significant*

Safety

In both groups there were minor adverse events (presence per adverse event <5%) reported and no serious adverse event. Only one patient in the CD2 group did not have an embryo transfer due to the risk of OHSS. No other cases of severe OHSS were observed.

Table 3 *Clinical efficacy outcomes*

	Start cycle day 2	Start cycle day 5	p-value
	(n=80)	(n=67)	
Clinical pregnancy rate			
per randomised treatment, n (%)	25 (31)	11 (16)	0.017
Ongoing implantation/pregnancy rate			
per started cycle, n (%)	19 (29%)	10 (16%)	0.053
per OPU, %	30%	19%	0.1
per ET, %	34%	24%	0.2
Follow-up pregnancy			
Early miscarriage, n (%)	5 (7.6)	1 (1.6)	0.053
Multiple pregnancies, n (%)[a)]	1 (5.3)	1 (10.0)	0.5
Cumulative ongoing pregnancy rate			
Fresh cycle and cryo-thawcycle(s), n (%) per started cycle	32 (40)	15 (22)	0.007*

[a)] *Restricted to patients with clinical pregnancy*
* *Statistically significant*

Table 4 *Hormone levels of the patients on cycle day 2, cycle day 6, and day hCG. Numbers are mean (SD) unless otherwise indicated restricted to patients with bolus hCG injection*

	Start cycle day 2	Start cycle day 5	Difference	p-value
	(n=80)	(n=67)		
Hormone levels cycle day 2				
FSH (IU/L)	7.2 (2.2)	7.4 (2.1)	0.22	0.6
LH (IU/L)	5.3 (2.8)	4.9 (1.7)	0.38	0.4
Estradiol (pmol/L)	130.7 (44.4)	140.1 (51.6)	9.37	0.3
Progesterone (nmol/L)	2.4 (1.0)	2.6 (2.2)	0.22	0.5
AMH (ng/ml)	3.3 (3.1)	3.1 (2.7)	0.21	0.8
Hormone levels cycle day 6 a)				
FSH (IU/L)	11.0 (3.0)	10.0 (2.7)	1.08	0.042*
LH (IU/L)	2.0 (1.9)	4.2 (1.9)	2.27	<0.0001*
Estradiol (pmol/L)	1167.2 (779.9)	389.0 (310.1)	778.2	<0.0001*
Progesterone (nmol/L)	2.1 (0.8)	1.9 (1.2)	0.16	0.4
AMH (ng/ml)	2.7 (2.9)	3.3 (3.4)	0.53	0.6
Hormone levels day bolus hCG a)				
FSH (IU/L)	11.2 (2.4)	11.4 (2.5)	0.17	0.7
LH (IU/L)	1.6 (1.6)	2.9 (2.9)	1.30	0.005*
Estradiol (pmol/L)	5612,6 (3651.4)	5147.4 (2880.1)	465.17	0.5
Progesterone (nmol/L)	3.2 (1.2)	3.2 (1.7)	0.07	0.8
AMH (ng/ml)	1.6 (1.3)	1.9 (2.3)	0.32	0.5

* *statistically significant*

Discussion

The current trial aimed to investigate whether the proportion of morphological top embryos would be higher in a late start, mild stimulation approach and built upon previous observations, suggesting higher proportions of good quality embryo's in mild regimens (Baart et al. 2007, Hohmann et al. 2003). The present results failed to demonstrate a higher proportion of morphological top embryos in the late start group. Moreover, in terms of final embryo performance, ongoing pregnancy rates after the single embryo replacement were clearly better in the early start group. This implies that this mild stimulation regimen is not suitable for all expected normal responders.

The duration of the "FSH window" during which FSH levels are above the threshold required to stimulate ongoing development, determines the number of follicles which can develop to the pre-ovulatory stage (Baird, 1987, Fauser et al. 1997). Therefore, in the mild stimulation protocol lower number of oocytes are retrieved compared to the conventional regimen (Heijnen et al. 2007). The start of rFSH on CD5 extends the " FSH window", so only the more mature follicles will grow. The start on CD5 might be too late for a group of patients. The next step in improving milder stimulation protocols might be a prediction model to forecast the ovarian response.

A randomised controlled trial demonstrated that mild stimulation regimens have a higher number of chromosomally normal embryos (Baart et al. 2007). Also, the potentially negative effects of ovarian stimulation on oocyte and embryo quality have been reported in several human and animal studies. After the exposure to high doses of gonadotrophins during in vitro maturation of oocytes, increased incidences have been reported of morphological and chromosomal abnormalities (Haaf et al. 2009, Eppig et al. 1998, Roberts et al. 2005, Van Blerkom et al. 2001). For that reason it is important to further investigate milder ovarian stimulation regimens.

In this randomised controlled trial the pregnancy rates in the CD5 start group (16%) are decreased compared to the CD2 start group (29%). In *Table 5* the study performed until now about mild stimulation from our group are summarized. The overall pregnancy rates in CD2 start group are higher in the present study compared to the CD2 start group in the Hohmann study (Hohmann et al. 2003). It looks like there is a learning curve in working with the GnRH antagonist and the CD2 start of rFSH with GnRH antagonist as co-treatment seems to be improving. The experience in starting with rFSH on CD5 is less than with CD2. This might be an explanation for the lower pregnancy rates. Also, in the CD5 start group more cycles were cancelled and previous performed studies showed that the

Table 5 *Comparison of the RCTs by Hohmann (2003), Heijnen (2007), and the present RCT.*

Parameter	Hohmann et al 2003			Heijnen et al 2007			Sterrenburg et al 2011		
	CD2 start n = 47	CD5 start n = 49	p-value	Standard n = 444	CD5 start n = 325	p-value	CD2 start n = 80	CD5 start n = 67	p-value
Study protocol									
Dose per day of rFSH (IU)	150	150		150	150		150	150	
GnRH co-treatment	Antagonist (flex)	Antagonist (flex)		Agonist (long protocol)	Antagonist (flex)		Antagonist (fixed)	Antagonist (fixed)	
Number of embryo transferred	sET	sET		dET	sET		sET	sET	
Total duration of stimulation (days)	9.5 (2.1)	7.6 (2.1)	<0.0001	11.5 (3)	8.3 (2.2)	<0.0001	10.0 (1.8)	8.6 (1.8)	<0.0001
Total dose rFSH (IU)	NA	NA	NA	1832 (758)	1307 (529)	<0.0001	1495 (266)	1293 (275)	<0.0001
Cancellation of started cycle (till oocyte retrieval)	13 (27%)	18 (37%)	NA	27 (8.3%)	80 (18%)	<0.0001	17 (21%)	14 (21%)	NA
Number of oocytes retrieved	8.6 (5.8)	8.4 (6.3)	0.1	8.5 (4.3)	6.9 (4.8)	<0.0001	9.7 (5.4)	9.5 (5.6)	0.8
Total number of embryos obtained	4.5 (3.8)	4.4 (4.2)	0.07	3.8 (2.9)	2.8 (2.7)	0.0002	5.7 (3.7)	5.9 (3.9)	0.9
Excellent (top) quality embryos (grade 1), %	29	40	0.2	NA	NA	NA	51	41	0.1
Embryos cryopreserved	NA	NA	NA	0.6 (1.4)	0.9 (1.8)	0.04	2.0 (2.6)	1.4 (1.8)	0.2
Ongoing pregnancy rate (per started cycle)	8 (17.8%)	8 (17.0%)	0.9	93 (28.6%)	78 (17.6%)	<0.0001	29%	16%	0.053
Cumulative pregnancy rate (per started cycle)	NA	NA	NA	97 (29.8%)	84 (18.9%)	NA	32 (40%)	15 (22%)	0.007

Flex = flexible start of GnRH antagonist (start depending on follicle size); Fixed = fixed start of GnRH antagonist (on stimulation day 5)

cumulative pregnancy rates per year of treatment were comparable between the two groups (Heijnen et al. 2007). To further investigate this idea in the milder stimulation regimens with GnRH antagonist as co-treatment, one year treatment with CD2 start of rFSH should be compared with one year of treatment with CD5 start of rFSH.

The duration of the "FSH window" (Macklon et al. 2006) seems to differ between patients and the start of rFSH on CD5 was too late for patients with monofollicular growth. On the other hand, if patients had an OPU, the number of retrieved oocytes was the same. One of the secondary outcome parameters, number of follicles on the day of hCG, was significantly higher in the CD2 start group (CD2: 4.7 (SD 2.6) versus CD5: 3.7 (SD 1.9); p=0.034). However, the number of oocytes between the two group was not statically significant different (CD2: 9.7 (SD 5.4) versus CD5: 9.5 (SD 5.7); p=0.8). Apparently in the CD 5 start group the smaller follicles contained oocytes as well.

Another important outcome parameter in IVF treatment is the number of cancellations. Cancellations can be divided in different categories; drop out before start stimulation, cancellation before OPU due to hypo- or hyperresponse, and cancellation because of no embryo transfer. The first category in this study shows a significantly difference in favour of the CD5 start group (CD2: 65/80, 81%; CD5: 63/67, 94%; p=0.021). Previous studies have shown a lower drop out rate in milder stimulation protocols (Heijnen et al. 2007). For most of the patients the reason not to start with the stimulation phase was because of personal reasons (8 patients in CD2 start group and none in the CD5 start group). In the second part of the treatment, only 2 patients were cancelled in the CD2 start group (one patients because of monofollicular growth and one patient due to hyperresponse) and 11 patients in the CD5 start group (8 patients due to monofollicular growth, 1 patients because of hyperresponse, and 3 because of other reasons). The risk of cancellation was higher in the CD5 start group as showed before in other studies comparing mild stimulation protocols (Heijnen et al. 2007, Hohmann et al. 2003). After OPU 7 patients were cancelled in the CD2 start group and 12 patients in the CD5 start group, most of the patients due to total fertilisation failure.

The strengths of the present study were that it was a randomised controlled trial and that it was well monitored. It is the first comparison of CD2 versus CD5 start since Hohmann in 2003 focussed on the clinical usability of this protocol.

Some weaknesses of this study should be mentioned. The study was conducted in two different IVF centres (UMC Utrecht and VU Brussels). Therefore, there was a differences between the 2 centres in the number of ICSI performed (UMCU 53% and VUB 90%), the day of embryo transfer (day 3 in UMCU and day 5 in VUB), and difference in the IVF laboratory. These factors were all of influences of the results.

Also the aimed number of included patients was not reached, due to problems of recruitment in both centres.
In conclusion, the late start of ovarian stimulation by rFSH in an antagonist cycle seems not to be more effective in creating a mild response with high quality embryo's, compared to early, standard start of the rFSH and may even produce poorer outcomes in terms of ongoing pregnancies. Further studies should focus on individualised dosing based on ovarian response testing prior to initiating stimulation with rFSH.

Chapter 8

General discussion

After more than 30 years of IVF treatment, only recently has research begun to focus on the burden of infertility treatment (Boivin et al. 1996, Olivius et al. 2004, de Klerk et al. 2007, Heijnen et al. 2007, Verberg et al. 2008). Not only do women suffer from the psychological consequences of the diagnosis of infertility, psychological stress is also the most common reason given for discontinuing IVF treatment. Physical, psychological and emotional discomfort experienced during treatment may be related to the necessity to use hormonal injections, to undergo a potentially painful oocyte aspiration procedure and the waiting period which follows the intense activity and attention given to the woman up to the time of embryo transfer and continues until the result of a pregnancy test is known (Boivin et al. 1996).

Our group has shown that women's anxiety levels during IVF treatment may be related to the type of stimulation protocol employed (Heijnen et al. 2007). Following milder stimulation regimens, women were less likely to drop out of further treatment, than those who had been unsuccessful following conventional hyperstimulation protocols (Verberg et al. 2008). This thesis addresses various methods which may facilitate more 'patient friendly' ovarian stimulation protocols, for both ovulation induction in PCOS patients as well as ovarian stimulation for IVF in infertile couples. The work presented provides data on the appropriate starting dose to optimize the balance between efficacy and burden, presents studies of long acting FSH compound which reduces the number of injections necessary and assesses novel mild stimulation strategies. In this chapter, the key elements of ovarian stimulation which have led to the development of these approaches are considered, and other strategies which may further reduce the burden of IVF and render it more patient 'centred' are considered.

GnRH Antagonists

A key step forward in developing milder strategies for IVF stimulation was the introduction of GnRH antagonists. In contrast to GnRH agonists which rely on pituitary desensitisation, GnRH antagonists cause immediate gonadotrophin suppression by competitive occupancy of the GnRH receptor. As outlined in the introduction, only follicles that are sensitive to a FSH rise within a certain time frame, the so called FSH-window, will develop further (*Figure 1, Chapter 1*). The aim of conventional ovarian stimulation protocols is to extend this FSH window to facilitate the growth of multiple dominant follicles. Manipulation of this FSH window has only become possible by the application of the GnRH antagonist as an alternative means of preventing premature luteinisation. GnRH antagonist regimens allow for greater flexibility in influencing the FSH window by choosing

the start of the rFSH closer or further away from the first day of the menstrual cycle. Moreover, in GnRH antagonist protocols the endogenous FSH production is supplemented by exogenous FSH, thus lower doses are sufficient. The most obvious impact on patient burden is the shorter stimulation cycle, resulting in fewer injections (Devroey et al. 2009). Moreover, side-effects and risk of OHSS and cycle cancellation are reduced in antagonist cycles (Heijnen et al. 2007, Al-Inany et al. 2007, Kolibianakis et al. 2006b).

Although the antagonist has much to offer in terms of patient-friendliness, early reports demonstrated that GnRH agonist cycles showed higher implantation and pregnancy rates (Al-Inany et al. 2007, Kolibianakis et al. 2006b). The current thesis together with other studies (Verberg et al. 2009, Griesinger et al. 2005, Kolibianakis et al. 2006a) challenge these findings of earlier studies (**Chapter 6 & 7**; Verberg 2009). The decrease in implantation and pregnancy rates might be caused by the group of high responders in the antagonist cycle. The hypothesis is that the decrease in implantation and pregnancy rates in high responders is due to an early LH rise due to higher estradiol levels. The detrimental effect of high LH levels, however, is poorly understood. High LH levels have been associated with infertility and increased risk of miscarriage (Regan et al. 1990). The current finding that implantation rates in frozen/thawed embryo cycles after an initial high response are equal to those after normal stimulation supports the theory that the high response itself is responsible for the observed lower implantation and pregnancy rates (**Chapter 3**, (D'Angelo et al. 2007)). Consequences of the current finding are that in an expected high response it may be necessary to start antagonist treatment earlier. At best, prevention of excessive response by adopting individualized stimulation protocols or mild stimulation regimens should be advocated. Nevertheless, even with such regimens OHSS does occur. Future studies should address if it may be advisable to freeze all adequate embryos after a high response and perform frozen/thawed cycles when the patient has recovered. In the meta-analysis in **chapter 2** we have shown that a lower starting dose of rFSH does not necessarily mean a lower pregnancy rate, although the response can be lower. Therefore, one should not fear to start with a lower dose of rFSH at the start of the treatment.

Another regimen which is more patient friendly is IVF in the natural or modified natural cycle. IVF in the natural cycle is when IVF carried out with oocytes collected from a woman's ovary or ovaries in a spontaneous menstrual cycle without administration of any medication at any time during the cycle. The aim of this cycle is to collect a naturally selected single oocyte at the lowest possible cost. (Nargund et al. 2007). The aim in treatments with modified natural cycles

is the same, but with medication to reduce the chance of cycle cancellation (Nargund et al. 2007). Treatment strategies for modified natural cycle include FSH stimulation, clomiphene citrate, and use of GnRH antagonist (Kolibianakis et al. 2004, Pelinck et al. 2006, Teramoto et al. 2007). These protocols are more patient friendly due to fewer injections and less complications, and also the costs of IVF treatment would be lower. Also, an advantage of these regimes are the prevention of multiple pregnancies, since mostly sET are performed. However, the laboratory performance should be excellent to compensate for the fewer oocytes retrieved. Another disadvantage can be the higher cancellation rates, although the drop out rates are lower.

Different drug-administration methods

The discovery of ovarian stimulatory drugs like clomiphene and hMG date from before the first IVF cycle in 1978 which was performed in a natural cycle. Clomiphene citrate, a non-steroidal estrogen antagonist, was discovered in the late 1950's for the treatment of breast cancer and endometrial hyperplasia. Women suffering from secondary amenorrhoea reinitiated menstrual cycles after administration of clomiphene citrate (KISTNER et al. 1961). Evidence of the pituitary-gonadal axis stems from the early 20th century, but extraction and clinical application of FSH and LH from postmenopausal urine samples, known as human menopausal gonadotrophin, only started in the early 1960s. Because of increased demands by the growing application of IVF and concerns regarding batch-to-batch variation in bioavailability and urinary contaminants, the production of recombinant FSH was realized around (Keene et al. 1989). After about 30 years of service in IVF, rFSH was only recently modified by the fusion with the carboxy terminal peptide (CTP) of the hCG β-subunit (Fares et al. 1992). This fusion protein is now known as corifollitropin alfa or Elonva®.

From a patient's perspective oral compounds, like clomiphene citrate, would be preferred, as it was shown that injecting drugs may augment patient's anxiety levels and thus treatment burden (Hojgaard et al. 2001). Moreover, Højgaard et al showed that patients on oral compounds were less inclined to perceive stress associated with hormone treatment, side effects, or cycle cancellation and were more inclined to accept a higher number of treatment cycles.

Another option to reduce the burden and stress of frequent injections is to look at personalised regimens reducing the number of injections, as shown in **chapter 7** of this thesis. In a randomised clinical trial it was shown that starting rFSH on day 2 or day 5 of an antagonist cycle yields comparable results in terms of endocrine profile and follicular development. Apparently, the endogenous production of

FSH is sufficient to start follicular maturation and cannot be accelerated by the addition of exogenous FSH. Moreover, the ability to flexibly start the rFSH dose in an antagonist cycle enables patients and centres to plan follicle aspirations. Originally, this flexibility was only attributed to GnRH agonist cycles. This flexibility could invite more centres to start using GnRH antagonist cycles, requiring far less injections and treatment time and lessening treatment burden. However, so far the start of rFSH on cycle day 2 had better outcome parameters than start on cycle day 5. Also comparing the different studies so far, as discussed in **chapter 7**. Since most treatment regimens involve the injection of drugs, the development of the long-acting FSH corifollitropin alfa is likely to reduce distress during IVF treatment. This thesis shows in a non-inferiority trial that corifollitropin alfa is as effective and safe as current rFSH treatment regimens (**Chapter 4**). Moreover, one-third of patients needed only the injection of corifollitropin alfa, without the need for additional rFSH injections. It was already demonstrated that fewer injections would lead to less impact on daily life since no mandatory injections need to be taken everyday at a standard time (Huisman et al. 2009). However, in this thesis Elonva® is only investigated with the GnRH antagonist as co-treatment. Only one trial (Fatemi et al. 2010) so far observed the effect of Elonva® with GnRH agonist as co-treatment. This study showed that the current available dosages of Elonva®, 100ug and 150 ug, are able to support follicular growth (Fatemi et al. 2010). Larger, prospective, controlled trials are needed to further investigate the efficacy and safety of Elonva®, as well as in patients with higher risk of OHSS.

Future studies investigating the possibility to administer rFSH orally, or studies focussing on a long acting antagonist could alleviate the burden of IVF treatments even further.

Chapter 9

Summary
Nederlandse samenvatting

Ovarian stimulation, by using exogenous FSH, aims at either restoring normal ovulatory cycles in women with oligo/amenorrhoea or at eliciting multiple follicle growth in normal cycling women for the purpose of intrauterine insemination (IUI) or in vitro fertilisation (IVF) treatment. In oligo/ amenorrhoeic women the cause for the anovulation can be a disruption of endogenous FSH production at the level of the hypothalamic-pituitary unit (WHO I anovulation) or a dysregulation of the ovarian paracrine milieu jeopardizing the monthly cyclic follicle recruitment (WHO II anovulation, among which the PCO syndrome).
Since the first IVF baby was born in 1978, the ovarian stimulation strategies key to achieving success, have undergone extensive change and development. Although the first IVF treatments were performed in the natural cycle, ovarian stimulation with exogenous gonadotrophins quickly became an established component of IVF treatment as it addressed the need to generate large numbers of oocytes to compensate for the limits of laboratory performance, in-vitro embryo development and selection.
While gonadotrophins remain the corner stone for today's treatment in anovulation and IVF patients, almost every aspect of their administration has changed. Their source, mode of production, means of administration, dose and even aim of treatment have altered considerably. However, the basic endocrine physiology which underpins the rational for current FSH treatment regimens remains unchanged.

Part I of this thesis discusses and investigates the optimal daily starting dose of rFSH for ovarian stimulation in IVF treatment in relation to the most important outcome parameter, pregnancy rates. In **chapter 2** a meta-analysis is performed to obtain the balance between probability of pregnancy and the risk of complications, while maximizing cost-effectiveness of IVF treatment. This meta-analysis suggests that the optimal daily rFSH stimulation dose is 150 IU/day in presumed normal responders younger than 39 years undergoing IVF. Compared with higher doses, this dose is associated with a slightly lower oocyte yield, but similar pregnancy and embryo cryopreservation rates. Furthermore, the wide spread adherence to this optimal dose will allow for a considerable reduction in IVF costs and complications.
Although GnRH antagonist co-treatment in IVF cycles reduces treatment duration and medication costs, overall efficacy is believed to be lower compared to GnRH agonist co-treatment. In a late start rFSH and flexible GnRH antagonist protocol a decrease in implantation rates was associated with over 10 oocytes being collected. The study in **chapter 3** explores this association in the currently

popular regimen of an early start rFSH combined with fixed day start of GnRH antagonist. This analysis of standard stimulation cycles using GnRH antagonist co-treatment reveals that higher responses negatively affect ongoing implantation rates. This finding may have implications for stimulation dose assessment in GnRH antagonist cycles.

The studies in **part 2** describe the implementation of a new compound, the long-acting rFSH fusion protein, corifollitropin alfa. This compound, also known as Elonva®, has a longer elimination half-life and extended time to peak levels than rFSH. In IVF treatment a single injection of corifollitropin alfa replaces 7 daily injections during the first week of ovarian stimulation, as described in **chapter 4**. In a protocol with GnRH antagonist as co-treatment, ovarian stimulation with corifollitropin alfa results in a high ongoing pregnancy rate, equal to that achieved with daily rFSH.
The aim of the study described in **chapter 5** was to evaluate whether a single or repeated low dose of corifollitropin alfa followed by a low dose of recombinant follicle-stimulating hormone (rFSH) or human chorionic gonadotrophin (hCG) can induce monofollicular growth in women with WHO II group anovulatory infertility. In this pilot study a single or repeated low dose of corifollitropin alfa followed by a low daily dose of either rFSH or hCG did not induce monofollicular development in women with WHO group II anovulatory infertility. The results of this pilot trial indicate that the tested treatment regimen is not suitable for the induction of monofollicular growth in these women.

In **part 3** we evaluate how to further improve the mild ovarian stimulation protocol. Mild stimulation can be described as the administration of lower or fewer doses of exogenous gonadotrophins in GnRH antagonist co-treated cycles, and/or oral compounds (like anti-estrogens, or aromatase inhibitors) for ovarian stimulation for IVF, aiming to limit the number of oocytes obtained to less than eight. The essential of mild stimulation is to remain as close as possible to the normal physiology of the ovary. There are a number of advantages of the mild stimulation regimen. Since in the mild stimulation protocol is meant to apply lower doses and fewer days, the treatment will be less complex, may diminish patient distress and complications such as OHSS, and the costs per cycle will be lower.
A randomised trial was performed in two centres to compare the initiation of rFSH cycle day 2 versus cycle day 5 with the GnRH as co-treatment in terms of embryo quality. In **chapter 6** the follicular phase endocrine characteristics and follicular development were compared. This study shows that the administration of rFSH

starting on day 2 or day 5 of the cycle in a GnRH antagonist protocol for IVF/ICSI patients yields a comparable endocrine profile and follicular development. Future studies should focus on the design of more patient tailored ovarian stimulation protocols. The clinical data of this RCT are described in **chapter 7**. The late start of ovarian stimulation by rFSH in an antagonist cycle seems not to be more effective in creating a mild response with high quality embryo's, compared to early, standard start of the rFSH and may even produce poorer outcomes in terms of ongoing pregnancies. Further studies should focus on individualised dosing based on ovarian response testing prior to initiating stimulation with rFSH.

Finally, in the last chapter of this thesis, **chapter 8**, the conclusions that can be drawn from this thesis and also the implications in the context of current practice and future research are discussed.
This thesis addresses various methods which may facilitate more 'patient friendly' ovarian stimulation protocols, for both ovulation induction in PCOS patients as well as ovarian stimulation for IVF in infertile couples. The work presented provides data on the appropriate starting dose to optimize the balance between efficacy and burden, presents studies of long acting FSH compound which reduces the number of injections necessary and assesses novel mild stimulation strategies. Although the antagonist has much to offer in terms of patient-friendliness, early reports demonstrated that GnRH agonist cycles showed higher implantation and pregnancy rates. The current thesis together with other studies challenge these findings of earlier studies. The decrease in implantation and pregnancy rates might be caused by the group of high responders in the antagonist cycle. The hypothesis is that the decrease in implantation and pregnancy rates in high responders is due to an early LH rise due to higher estradiol levels. Future studies should address if it may be advisable to freeze all adequate embryos after a high response and perform frozen/thawed cycles when the patient has recovered.
From a patient's perspective oral compounds, like clomiphene citrate, would be preferred, as it was shown that injecting drugs may augment patient's anxiety levels and thus treatment burden. Another option to reduce the burden and stress of frequent injections is to look at personalised regimens reducing the number of injections. Since most treatment regimens involve the injection of drugs, the development of the long-acting FSH corifollitropin alfa is likely to reduce distress during IVF treatment. Future studies investigating the possibility to administer rFSH orally, or studies focussing on a long acting antagonist could alleviate the burden of IVF treatments even further.

Chapter 9

Summary
Nederlandse samenvatting

Het stimuleren van de ovaria, met exogeen FSH, heeft als doel de normale cyclus te herstellen bij vrouwen met een oligo-/amenorroe of het produceren van meerdere follikels bij vrouwen met een normale cyclus bij intra-uteriene inseminatie (IUI) of In-Vitro Fertilisatie (IVF) behandelingen. De oorzaak van anovulatie bij vrouwen met een oligo-/amenorroe kan een verstoring van de endogene FSH productie zijn op het niveau van hypothalamus-hypofyse as (WHO I anovualtie) of een verstoorde hormonale wisselwerking tussen hypofyse en ovaria (WHO II anovulatie, waaronder polycysteus ovarium syndroom (PCOS)).
Sinds de geboorte van de 1e IVF baby zijn er veel ontwikkelingen geweest in de ovariële stimulatie protocollen, een van de belangrijkste stappen in een IVF behandeling. Nadat de eerste IVF cycli alleen werden verricht in een natuurlijke cyclus, kwamen al snel de stimulaties met exogene gonadotrofinen zodat meer eicellen verkregen werden om de laboratorium prestaties te compenseren ten aanzien van de embryo-ontwikkeling en de embryoselectie.
Nog steeds zijn gonadotrofinen een belangrijk onderdeel van de IVF behandeling. Ondanks dat de productie, wijze van toedienen en dosering in de loop van de tijd zijn veranderd, is de endocriene fysiologie die ten grondslag ligt aan ovariumstimulatie ongewijzigd.

In het **eerste deel** van dit proefschrift wordt de optimale start dosering van rFSH in combinatie met de belangrijkste uitkomstmaat van IVF, het aantal zwangerschappen, onderzocht en bediscussieerd. In **hoofdstuk 2** wordt een meta-analyse beschreven waarin onderzocht wordt hoe de balans is tussen de kans op zwangerschap en de kans op een complicatie, met daarbij in achtneming van de kosten van de behandeling. Deze meta-analyse laat zien dat de optimale stimulatiedosering 150 IU/dag rFSH is bij patiënten jonger dan 39 jaar waarbij een normale response op stimulatie kan worden verwacht. Vergeleken met hogere doseringen, zie je bij deze dosering een lager aantal eicellen, maar gelijke kans op zwangerschap en kans op het invriezen van een embryo om later in een natuurlijke cyclus terug te plaatsen. Daarbij nemen de kosten van een IVF behandeling af als er minder rFSH per behandeling gebruikt hoeft te worden.
GnRH antagonisten zorgen er voor dat de IVF behandeling korter is en daardoor minder kosten met zich meebrengt. Helaas lijkt de kans op zwangerschap kleiner als de behandeling vergeleken wordt met een behandeling met GnRH agonist. Wanneer er met rFSH laat gestart wordt, dat wil zeggen op cyclusdag 5, en daarnaast een flexibel antagonist protocol gebruikt wordt, is de kans op zwangerschap bij een hyperresponse (meer dan 10 eicellen) kleiner. In **hoofdstuk 3** wordt onderzocht of dit fenomeen ook gezien wordt bij een vroege start van

rFSH, op cyclusdag 2, met daarbij een fixed protocol van de GnRH antagonist. Uit deze analyse blijkt dat hoge aantal eicellen negatief geassocieerd is met kans op zwangerschap. Deze bevinding kan gevolgen hebben voor de dosering van rFSH bij stimulatie van de ovaria in GnRH antagonist cycli.

De studies beschreven in het **tweede deel** van dit proefschrift onderzoeken de invoering van een nieuw langwerkend rFSH preparaat, corifollitropin alfa. Dit medicament, ook bekend onder de naam Elonva®, heeft langere halfwaardetijd dan rFSH. Bij een IVF behandeling kan een enkele injectie van corifollitropin alfa de eerste 7 dagelijkse injecties van rFSH vervangen bij ovariële stimulatie, zoals beschreven in **hoofdstuk 4**. Wanneer corifollitropin alfa met GnRH antagonist gebruikt wordt, is de kans op zwangerschap even hoog als bij dagelijkse injectie met rFSH.
In **hoofdstuk 5** wordt onderzocht of eenmalige of herhaalde lage dosering van corifollitropin alfa, bij ovulatie-inductie behandelingen, gevolgd door een dagelijkse lage dosering rFSH of hCG monofolliculaire groei kan bewerkstelligen bij vrouwen met een WHO II anovulatie. In deze pilot studie kon geen monofolliculaire groei bewerkstelligd worden. Deze pilot studie laat zien dat het protocol zoals getest, niet geschikt is voor vrouwen met een WHO II anovulatie om monofolliculaire groei te bewerkstelligen.

In het **derde deel** van dit proefschrift wordt onderzocht hoe het milde stimulatieprotocol verbeterd kan worden. Van milde stimulatie kan worden gesproken wanneer lagere dosering of minder injecties worden gegeven in vergelijking met het conventionele protocol. Tevens wordt er bij milde stimulatie schema's gebruik gemaakt van GnRH antagonist i.p.v. GnRH agonist met als doel om minder dan 8 eicellen te verkrijgen. De essentie van mild stimuleren is om zo dicht als mogelijk bij de fysiologische situatie van de ovaria te blijven. Er zijn een aantal voordelen bij het gebruik van het milde stimulatie schema, o.a. is het schema minder moeilijk voor patiënten omdat het korter is, waardoor tevens de stress, de kans op complicaties als OHSS en de kosten per cyclus lager zullen zijn.
Een gerandomiseerd onderzoek werd opgezet in twee centra, VU Brussel en UMC Utrecht, waarbij vergeleken werd wat de invloed op de morfologische embryokwaliteit was wanneer gestart werd op cyclus dag 2 of cyclusdag 5 met rFSH in een GnRH antagonist protocol. In **hoofdstuk 6** worden de endocrinologische karakteristieken en follikel ontwikkeling in de folliculaire fase belicht. Dit onderzoek laat zien dat er weinig verschil is tussen het endocrinologisch profiel en de ontwikkeling van de follikels wanneer er wordt gestart op cyclusdag 2 of cyclus-

dag 5 met rFSH. De klinische data van dit gerandomiseerde onderzoek worden besproken in **hoofdstuk 7**. De late start van rFSH in GnRH antagonist protocol lijkt niet effectiever te zijn in het creëren van een milde response met hogere morfologische kwaliteit van embryo's dan de vroege, standaard start van rFSH en leidt zelfs tot slechtere uitkomsten zoals lagere zwangerschapscijfers. Toekomstige studies zullen gericht moeten zijn op ontwikkelen van meer geïndividualiseerd stimulatieprotocol met gegevens die voorafgaand aan de stimulatiefase bekend zijn zoals leeftijd van de patiënte, FSH op cyclusdag 2, BMI en cyclusduur.

In het laatste hoofdstuk van dit proefschrift, **hoofdstuk 8**, worden de conclusies en implicaties van dit proefschrift besproken in de context van de huidige praktijk en toekomstig onderzoek.
Dit proefschrift behandeld verschillende methodes waardoor ovarium stimulatie protocollen patiënt vriendelijker zouden kunnen worden, voor zowel ovulatie inductie bij PCOS patiënten als IVF behandelingen. Het gepresenteerde werk geeft informatie over de geschikte startdosering om de balans tussen werkzaamheid en lasten voor de patiënt, te optimaliseren. Tevens presenteert dit proefschrift onderzoeken naar een nieuw langwerkend rFSH medicament waardoor het totaal aantal injecties omlaag gaat en onderzoekt het nieuwe milde stimulatie protocollen. Hoewel het GnRH antagonist protocol veel heeft te bieden op het gebied van patiënt vriendelijkheid, laten een aantal meta-analyses zien dat de uitkomst niet beter is dan bij het gebruik van GnRH agonist waarbij hoger implantatie en zwangerschapkansen worden gezien. Het onderzoek in dit proefschrift gaat op zoek naar de oorzaak van dit verschil en de uitdaging om het GnRH antagonist protocol te optimaliseren waardoor deze discrepantie in zwangerschapskansen zullen verdwijnen. De daling in implantatie en zwangerschapskansen kan verklaard worden door de groep high responders in de GnRH antagonist cycli. De hypothese is dat de daling van de implantatie en het percentage zwangerschappen in high responders is te wijten aan een vroege LH stijging als gevolg van hogere oestradiol gehalte. Toekomstige studies zullen moeten onderzoeken of het verstandig is om bij zogenaamde high responders de eicellen in te vriezen en in een latere natuurlijke cyclus terug te plaatsen.
Vanuit het oogpunt van de patiënte hebben orale medicamenten zoals clomifeen citraat de voorkeur, omdat aangetoond is dat het injecteren van medicatie angstverhogend werkt en daardoor de behandeling als zwaarder wordt ervaren. Een andere manier om het aantal injecties te verminderen is om de behandeling van de patiënte te individualiseren. Aangezien de meeste behandelingen nog steeds gebruik maken van injecties, zal de invoering van het nieuwe langwerkende pre-

paraat Elonva®, zorgen voor een verlaging van de stress die ervaren wordt door patiënten bij een IVF behandeling.
Toekomstige onderzoeken zullen gericht moeten worden op de ontwikkelingen van orale rFSH medicatie en de ontwikkeling van een langwerkend GnRH antagonist wat de behandeling minder stress vol zullen maken. Tot die tijd zal de nadruk moeten liggen op individualisering van de huidige behandelingsschema's.

Chapter 9

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Chapter 9

List Abbreviations

ART	assisted reproductive techniques
BMI	body mass index
CC	Clomiphene Citrate
CD	cycle day
CI	confidence interval
CTP	carboxy terminal peptide
dET	double embryo transfer
E2	17ß-estradiol
ET	embryo transfer
FSH	follicle stimulating hormone
GnRH	gonadotrophin-releasing hormone
hCG	human chorionic gonadotrophin
ICSI	intracytoplasmic sperm injection
IU	international unit
IUI	intra uterine insemination
IVF	in vitro fertilisation
LH	luteinising hormone
NA	not available
OC	oral contraceptive
OHSS	ovarian hyperstimulation syndrome
OI	ovulation induction
OR	odds ratio
P	progesterone
PCOS	polycysteus ovarian syndrome
r	recombinant
RCT	randomised controlled trial
s.c.	subcutaneously
SD	standard deviation
sET	single embryo transfer

Chapter 9

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Chapter 9

List of Publications and Presentations

Publications

Alpha-synuclein expression in the developing human brain.
R. Raghavan, L. Kruijff, M.D. Sterrenburg, B.B. Rogers, C.L. Hladik, C.L. White 3rd.
Pediatr Dev Pathol. 2004 Sep-Oct;7(5):506-16

A double-blind, non-inferiority RCT comparing corifollitropin alfa and recombinant FSH during the first seven days of ovarian stimulation using a GnRH antagonist protocol
P. Devroey, R. Boostanfar, N.P. Koper, B.M.J.L. Mannaerts, P.C. Ijzerman-Boon, B.C.J.M. Fauser, ENGAGE Investigators.
Hum Reprod. 2009 Dec; 24 (12): pp 3063-3072

Clinical outcomes in relation to the daily dose of recombinant follicle-stimulating hormone for ovarian stimulation in in vitro fertilisation in presumed normal responders younger than 39 years: a meta-analysis
M.D. Sterrenburg, S.M. Veltman-Verhulst, M.J.C. Eijkemans, E.G. Hughes, N.S. Macklon, F.J. Broekmans, B.C.J.M. Fauser.
Hum Reprod Update. 2011 Mar-Apr; 17 (2): pp 184-96

Follicular Phase Endocrine Characteristics during Ovarian Stimulation and GnRH Antagonist Cotreatment for IVF: RCT Comparing rFSH Initiated on Cycle Day 2 or 5.
C. Blockeel, M.D. Sterrenburg, F.J. Broekmans, M.J.C. Eijkemans, J. Smitz, P. Devroey, B.C.J.M. Fauser.
J Clin Endocrinol Metab. 2011 Feb 9. [Epub ahead of print]

GnRH antagonist co-treatment in routine IVF: is high oocyte yield associated with reduced ongoing implantation rate?
M.D. Sterrenburg, M.J.C. Eijkemans, N.S. Macklon, M.F.G. Verberg, B.C.J.M. Fauser.
Submitted for publication

Low dose corifollitropin alfa followed by low dose rFSH or hCG for ovulation induction in WHO II anovulatory infertility; Results of a pilot study
M.D. Sterrenburg, E.M.E.W. Heijnen, N.S. Macklon, B.M.J.L. Mannaerts, B.C.J.M. Fauser.
Submitted for publication

Comparison between start of ovarian stimulation on cycle day 2 versus cycle day 5 with GnRH antagonist in IVF treatment: a randomised controlled trial
M.D. Sterrenburg, C. Blockeel, M.J.C. Eijkemans, S.L. Broer, F.J. Broekmans, N.S. Macklon, P. Devroey, B.C.J.M. Fauser.
Submitted for publication

Presentations

Transport-PGD in the Netherlands
M.D. Sterrenburg, G.C. Page-Christiaens, N.S. Macklon
Oral presentation, ESHRE consortium PGD, Barcelona 2008

Is there an optimum dose of rFSH for controlled ovarian stimulation in in-vitro fertilisation (IVF) or intracytoplasmatic sperm injection (ICSI) treatment?; a meta-analysis
M.D. Sterrenburg, S.M. Verhulst, F.J. Broekmans, M.J.C. Eijkemans, B.C.J.M. Fauser.
Poster presentation, SGI, Glasgow 2009

The Optimal Daily Dose of rFSH for Ovarian Stimulation in IVF; a Meta-Analysis
M.D. Sterrenburg, S.M. Verhulst, M.J.C. Eijkemans, E.G. Hughes, N.S. Macklon, F.J. Broekmans, B.C.J.M. Fauser
Oral presentation, ESHRE, Amsterdam 2009

Corifollitropin alfa followed by a low dose of rFSH or hCG to induce monofollicular growth in women with WHO group II anovulatory infertility; a pilot study
M.D. Sterrenburg, E.M.E.W. Heijnen, N.S. Macklon, B.M.J.L. Mannaerts, B.C.J.M. Fauser.
Oral presentation, ASRM, Atlanta, Georgia 2009

Chapter 9

Words of Appreciation

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Lieve gynaecologen, verpleegkundigen, secretaresses uit Gelre ziekenhuis: onwijs bedankt voor alle leuke en leerzame momenten in Apeldoorn bij het eerste deel van mijn opleiding tot gynaecoloog!

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Beste Prof. van den Tweel, Lieve Jan, mijn eerste wetenschappelijke stappen waren, dankzij jou, in Dallas bij de afdeling neuropathologie in Dallas, Texas. Tot jouw grote spijt heb ik niet voor een carrière binnen de pathologie gekozen. Ik ben erg vereerd dat je voorzitter bij mijn promotie kunt zijn. Ik hoop dat we van onze etentjes een traditie kunnen maken!

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ErNi tours & MoRo tours bedankt, jullie deden het niet voor jezelf!

Lieve Wim, hartelijk dank voor het helpen bewerken van de foto op de voorzijde van mijn proefschrift, ik heb weer veel geleerd van photoshoppen!

Lieve Yvette & Karel, we zien elkaar nu wat minder nu de afstand groter is, maar de vriendschap is er zeker niet minder om geworden! Fijn dat ik altijd bij jullie terecht kon voor advies, gezelligheid en afleiding.

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Marije & Yvette, ik vind het ontzettend fijn dat jullie naast me staan op 28 april!

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Lieve papa, net als bij elk belangrijk moment in mijn leven, zal ik je ook nu weer gigantisch missen! Ik weet dat je trots op me geweest zou zijn en hoop stiekem dat je vanaf een afstand met me mee kijkt.

Chapter 9

Curriculum Vitae

Monique Sterrenburg werd geboren op Koninginnedag 1976 te Dordrecht. Nadat ze in 1994 slaagde voor haar VWO eindexamen, begon ze aan de studie geneeskunde aan de Universiteit van Utrecht. Het lukte haar om een wetenschappelijke stage te regelen in Dallas aan de University of Texas Southwestern Medical Center en vertrok voor een half jaar naar de VS. Daar deed ze neuropathologisch onderzoek naar het eiwit alfa-synuclein dat betrokken is bij de ziekte van Parkinson en Alzheimer. Naast haar studie werkte Monique op de afdeling pathologie waar ze professor van den Tweel assisteerde bij de organisatie van het internationale congres voor pathologen (IAP 2002).

Tijdens haar co-assistentschap gynaecologie in 2003 in het Medisch Spectrum Twente werd haar interesse voor de gynaecologie gewekt. Ze was zo enthousiast dat ze ook haar keuze co-assistentschap in dit specialisme volgde, dit keer in het UMC Utrecht. Na het behalen van haar diploma als basisarts kon zij direct aan de slag als ANIOS gynaecologie en obstetrie in het Sint Antonius ziekenhuis te Nieuwegein. In 2006 kreeg ze een baan als fertiliteitarts in het UMC Utrecht en startte tegelijkertijd haar promotieonderzoek, wat geleid heeft tot dit proefschrift. Tevens was zij in die periode nauw betrokken bij het opzetten van het transport PGD (Pre-implantatie Genetische Diagnostiek) programma. Eind 2009 begon zij met de opleiding tot gynaecoloog in Gelre ziekenhuizen te Apeldoorn met als opleider Peter van de Weijer. Momenteel werkt ze als AIOS gynaecologie in het WKZ/UMC Utrecht waar ze opgeleid wordt door Gerard Visser. In haar vrije tijd staat Monique graag op het (beach) volleybalveld en tovert ze met veel liefde de lekkerste gerechten op tafel voor familie en vrienden.

Monique Sterrenburg was born in Dordrecht on the Queen's birthday in 1976. After graduating in 1994, she started studying medicine at the University of Utrecht. As part of her medical training she completed a traineeship at the University of Texas Southwestern Medical Center (Dallas, USA) during which she performed neuropathological research on alpha-synuclein, a protein that is involved in Parkinson's and Alzheimer's disease. As a medical student, she also worked at the Department of Pathology where she assisted Professor van den Tweel in organizing an international conference for pathologists (IAP 2002).
During her internship at the Medical Spectrum Twente she discovered a strong interest in the field of Gynaecology and therefore decided to complete another Gynaecology internship at the UMC Utrecht.
After receiving her Medical Degree she became a junior doctor in Gynaecology and Obstetrics at the Sint Antonius Hospital in Nieuwegein. In 2006 she started working as a fertility physician and PhD student at the UMC Utrecht. In that same period she was closely involved in setting up and running the transport PGD (Preimplantation Genetic Diagnosis) program. In the winter of 2009 she started her residency in gynaecology at the Gelre Hospital in Apeldoorn under supervision of Peter van de Weijer. At present, she is continuing her residency at the WKZ/UMC Utrecht, supervised by Gerard Visser.
In her time off, Monique is a fanatical (beach) volleyball player and enjoys preparing the most delicious and amazing food for family and friends.

Marije Appelhof & Yvette Snuif, paranimfen