Human Reproduction Update, Vol.17, No.2 pp. 184–196, 2011
Advanced Access publication on September 15, 2010 doi:10.1093/humupd/dmq041

human reproduction update

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

M.D. Sterrenburg [1,*], S.M. Veltman-Verhulst [1], M.J.C. Eijkemans [1,2], E.G. Hughes [3], N.S. Macklon [1,4], F.J. Broekmans [1], and B.C.J.M. Fauser [1]

[1]Department of Reproductive Medicine and Gynaecology, University Medical Centre Utrecht, Heidelberglaan 100, Utrecht 3584 CX, The Netherlands [2]Julius Centre for Health Sciences and Primary Care, University Medical Centre, Utrecht, The Netherlands [3]Department of Obstetrics and Gynaecology, McMaster University, Hamilton, Ontario, Canada [4]Department of Obstetrics and Gynaecology, University of Southampton, Southampton, UK

*Correspondence address. E-mail: m.d.sterrenburg@umcutrecht.nl

Submitted on March 9, 2010; resubmitted on July 21, 2010; accepted on July 28, 2010

TABLE OF CONTENTS

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* fertilization (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 randomized 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 recFSH, and between 150 versus 200 IU recFSH 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 recFSH 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.

Key words: RCT / IVF / recFSH / ovarian stimulation / meta-analysis

Introduction

In the UK, one in six couples is faced with the problem of infertility (Cahill and Wardle, 2002) and almost 45 000 cycles of *in vitro* fertilization (IVF) treatment are carried out annually. In order to compensate for inefficiencies in the process, and to allow for the selection of embryos for intrauterine 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 and Van Heusden, 1997).

For ovarian stimulation, recombinant follicle-stimulating hormone (recFSH) preparations are currently administered in dosages ranging from 100 to 600 IU/day (Nargund *et al*., 2007; Malizia *et al*., 2009). The induced multiple follicular development carries the risk of premature luteinization. Co-treatment with gonadotrophin-releasing hormone (GnRH) agonist or antagonist is normally instituted to prevent an untimely rise in the luteinizing hormone (LH) (Huirne and Lambalk, 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 recFSH-administered would significantly 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 is to identify the optimal daily starting dose of recFSH taking into account ovarian response, pregnancy chances, rate of cycle cancellation and the incidence of the potentially life-threatening complication of ovarian hyperstimulation syndrome (OHSS). Published randomized comparative dosage trials were searched in order to identify the recFSH dose 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 randomized 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 recFSH 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', 'recFSH', '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. 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/day versus higher, or 150 IU/day versus higher, two comparisons were made. Comparison A addressed 100 IU/day recFSH versus 200 IU/day recFSH, and comparison B 150 versus 200–250 IU/day recFSH. 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 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 and quantified by the I^2 statistic. This describes the percentage of the variability in the effect estimates that is due to heterogeneity rather than sampling variation. A value >50% is considered as moderate/high heterogeneity (Higgins *et al*., 2003). In case of statistically significant heterogeneity, univariate meta-regression was performed using the random effects method described by van Houwelingen *et al*. (2002), on the following study characteristics: mean age, mean BMI, duration of infertility, percentage of primary infertility and use of a GnRH analogue. All parameters were reported per started cycle, except for the number of oocytes which were calculated per ovum pick up and the number of frozen embryos which were stated per embryo transfer. All analyses were performed in Review Manager 5.

Results

We identified 11 relevant dosage RCTs reporting data on 1967 women undergoing a single IVF cycle. All trials had parallel design and in most studies the treatment was adequately concealed prior to allocation eight studies allocation score A (Out *et al*., 1999, 2000, 2001, 2004; Latin-American Puregon IVF Study Group, 2001; Wikland *et al*., 2001; Hoomans *et al*., 2002; Tan *et al*., 2005) and three studies allocation score B (de Jong *et al*., 2000; Pruksananonda *et al*., 2004; Cavagna *et al*., 2006). All but three studies were double-

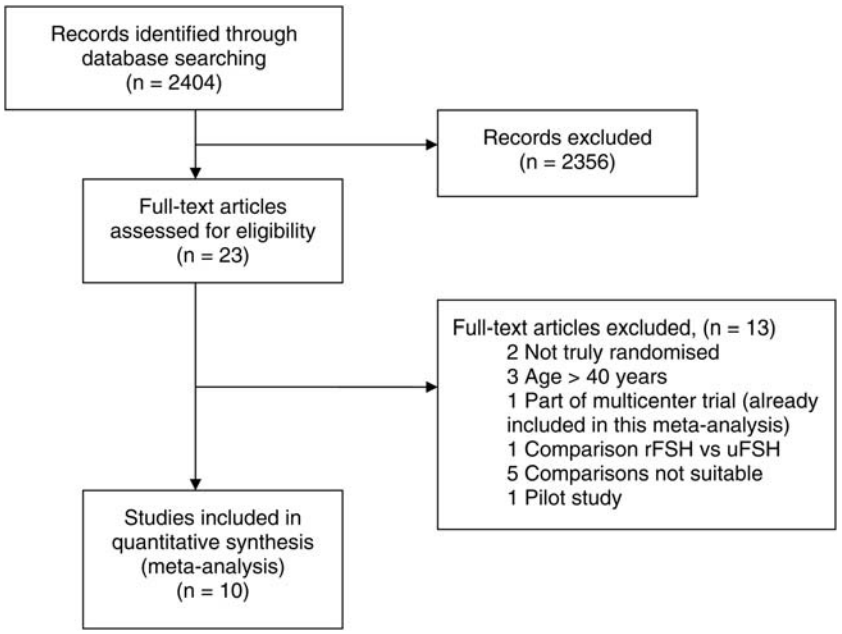

Figure 1 Flow chart of search strategy.

blinded (de Jong *et al.*, 2000; Wikland *et al.*, 2001; Cavagna *et al.*, 2006).

For comparison A (100 versus 200 IU/day recFSH) 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 five studies were analyzed in comparison B (150 versus 200–250 IU/day recFSH), involving 992 women. Table I 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 II 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 summarized in Figs 2–7. Figure 2 shows the number of oocytes obtained per oocyte retrieval. In comparison A, the 100 IU/day recFSH 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 recFSH users obtained 1.7 oocytes fewer than those applying higher dosages (mean difference −1.67; 95% CI −2.53, −0.81; $P = 0.0001$).

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; $P = 0.82$) (Fig. 3).

Figure 4 demonstrates the difference in the total amount of recFSH 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/day; 95% CI −860.26, −767.17; $P < 0.0001$ comparison B: mean difference −671.98 IU/day; 95% CI −896.85, −447.10; $P < 0.0001$).

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, $P = 0.38$] (Fig. 5).

Cancellation due to low ovarian response to stimulation in comparison A (Fig. 6) was observed to be more frequent in the 100 IU/day recFSH 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 7 illustrates the risk of OHSS in relation to dose. In comparison A, the risk was reduced in the 100 IU/day recFSH 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 recFSH 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 recFSH (comparison B $I^2 = 96\%$).

In meta-regression on the parameter number of oocytes, none of the study characteristics explained the heterogeneity. For the

Table I Inclusion-and exclusion criteria for all the included RCTs.

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 fertilization 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
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	
Comparison B (150 versus 200–250 IU/day)		

Continued

Table I *Continued*

Study	Inclusion criteria*	Exclusion criteria
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
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/m^2 and menstrual cycle in days.

Table II 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 Multicenter $n = 199$	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 Multicenter $n = 179$	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 Multicenter $n = 330$	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 center $n = 60$	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 Multicenter $n = 192$	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
Comparison B (150 versus 200–250 IU/day)							
Out *et al.* (2000)	Allocation A Multicenter $n = 138$	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 Bicenter $n = 117$	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 Multicenter $n = 404$	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

Continued

Table II *Continued*

Study	Design[a]	Comparison	Study protocol[b]	Population characteristics[c]			
				Mean age	Mean BMI	Mean duration of infertility	Number of primary infertility (%)
Out *et al.* (2004)	Allocation A	150 versus 200 IU/day	Antagonist	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
	Multicenter *n* = 131 versus *n* = 126 *n* = 257		Fixed 5 days, then flexible				
Cavagna *et al.* (2006)	Allocation B	150 versus 200 IU/day	Agonist	31.4 (2.8) versus 31.7 (2.8)	NA	6.1 (2.5) versus 6.7 (3.3)	73 versus 75
	Single center *n* = 76	*n* = 40 versus *n* = 36	Fixed				

NA, information not available.
[a]RCT, randomized control trial. Concealment of allocation: (A) adequate and (B) unclear.
[b]Use of GnRH analogue (agonist or antagonist), and use of recFSH fixed during treatment or flexible (increase or decrease of the dose after certain days).
[c]Mean (SD) or median (range).

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 recFSH 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 recFSH (Fig. 4).

Discussion

Until now, no consensus regarding the optimal starting dose of FSH for ovarian hyperstimulation in IVF/ICSI treatment cycles in presumed 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 centers within the same country. These differences in patient management may have major implications for IVF pregnancy rates, drug costs, complication rates and possibly also for patient discomfort.

The current meta-analysis represents a first attempt to provide objective information regarding the relationship between the applied daily FSH dose for ovarian stimulation in presumed normal responders in IVF/ICSI and the outcomes, cost and complications of the treatment. This study demonstrates that the average number of oocytes retrieved per pick-up is increased when higher FSH doses over 100 IU/day are given, whereas pregnancy rates do not differ across the dosage range tested (Fig. 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 recFSH have shown that the response to a 225 IU daily dose varies mainly according to the women's age and her ovarian reserve status (Karlsson *et al.*, 1997). This finding implies that a limitation exists under given circumstances in the number of follicles that can be stimulated to ongoing development. The comparison between 150 IU/day and higher dosages revealed that the increase in number of oocytes harvested is limited. This may indicate that the dose eliciting optimal stimulation of the ovaries in most patients may be somewhere between 150 and 200 IU/day. Using a dosage of 100 IU/day leads to a more pronounced reduction in oocyte number compared with higher doses, suggesting that in this dose range a dose–response relationship does exist. With current starting dosages of 150 IU/day or more in most centers, optimal or near optimal stimulation of the ovaries will usually be obtained.

In recent years, so-called 'mild' stimulation regimens have been proposed, aimed at harvesting more modest numbers of oocytes (Nargund *et al.*, 2007). Initial studies suggest that in comparison with conventional stimulation, milder ovarian stimulation protocols

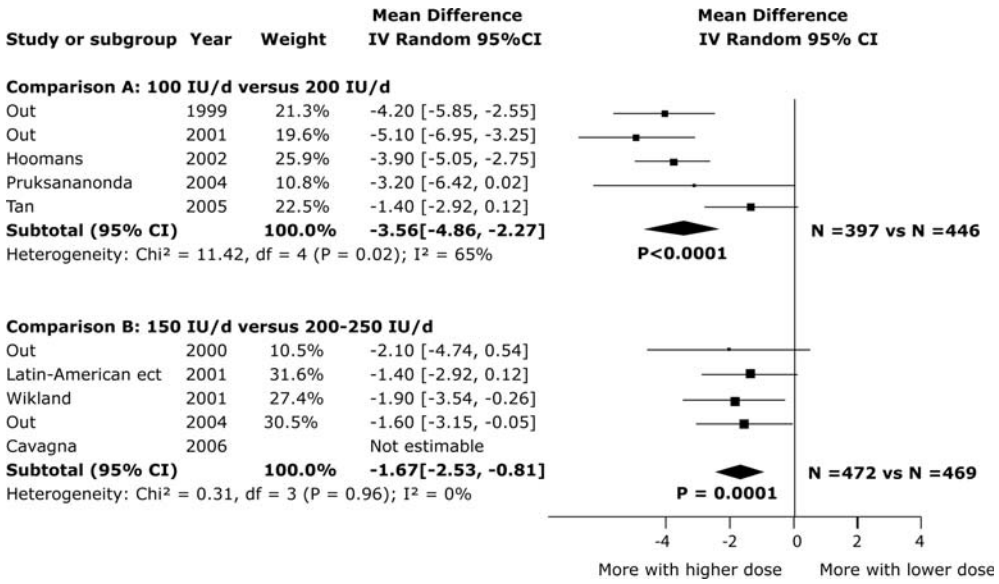

Figure 2 Forest plot of mean difference of number of oocytes per oocyte pick-up. Forest plot: the area for each square is proportional to the weight of the corresponding study. The diamond represents the pooled WMD, and its width represents its 95% CI. A horizontal line represents each study, with its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

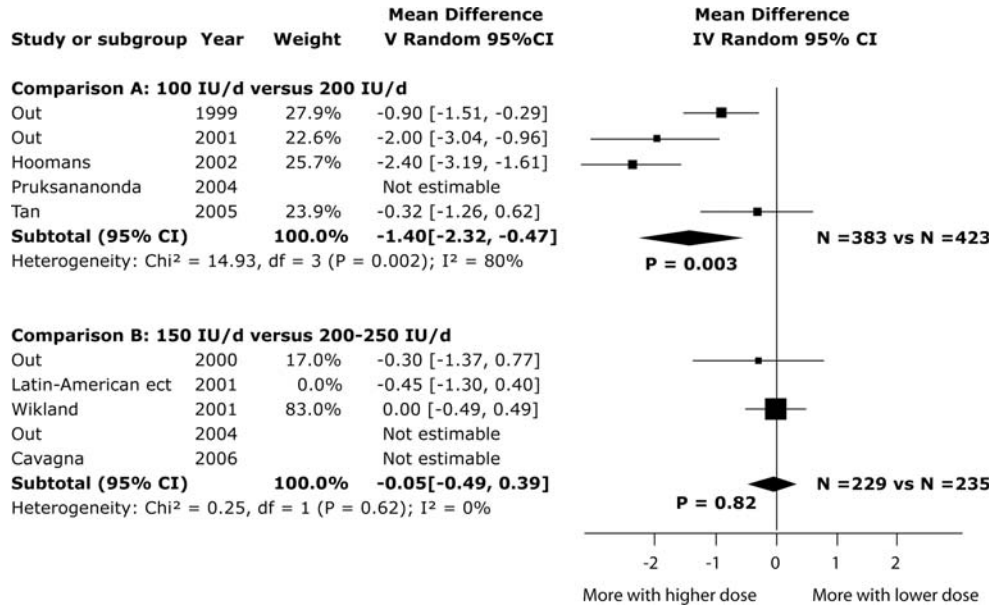

Figure 3 Forest plot of mean difference of number of cryopreserved embryos.

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 recFSH (i.e. 100 IU/day) produces more modest ovarian responses without undesirable effects on pregnancy rates. Consistent with these findings, a recent study in which recFSH dose adaptations were based on individualized patient profiles, ranging from 75 up to 225 IU/day, revealed that in ~30% of patients, a dose of 100 IU/day 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 with conventional 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). In the current meta-analysis, data were pooled in two comparison groups (A: 100

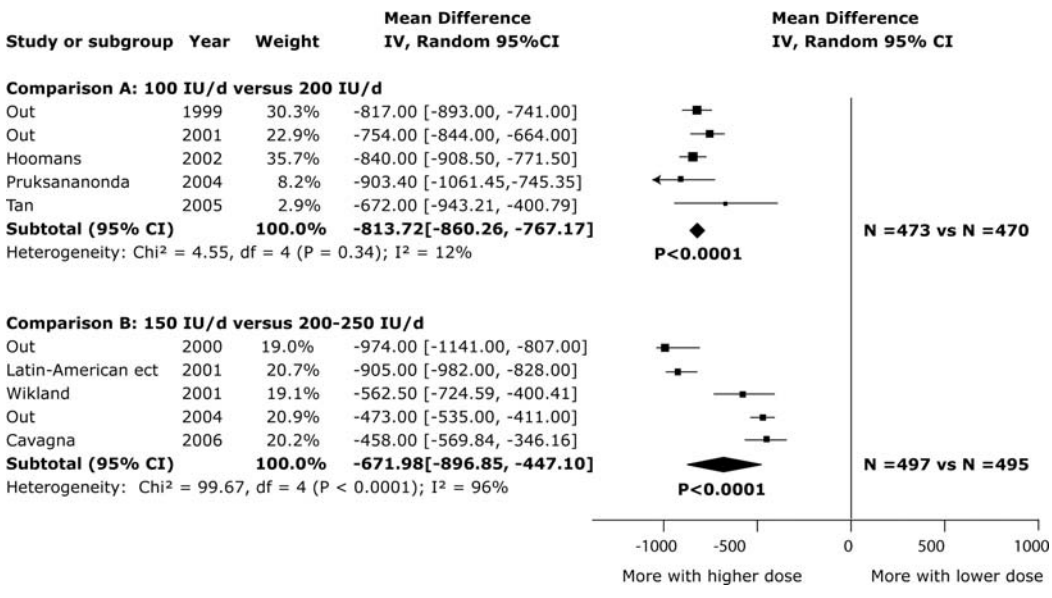

Figure 4 Forest plot of mean difference of total amount of recFSH (IU).

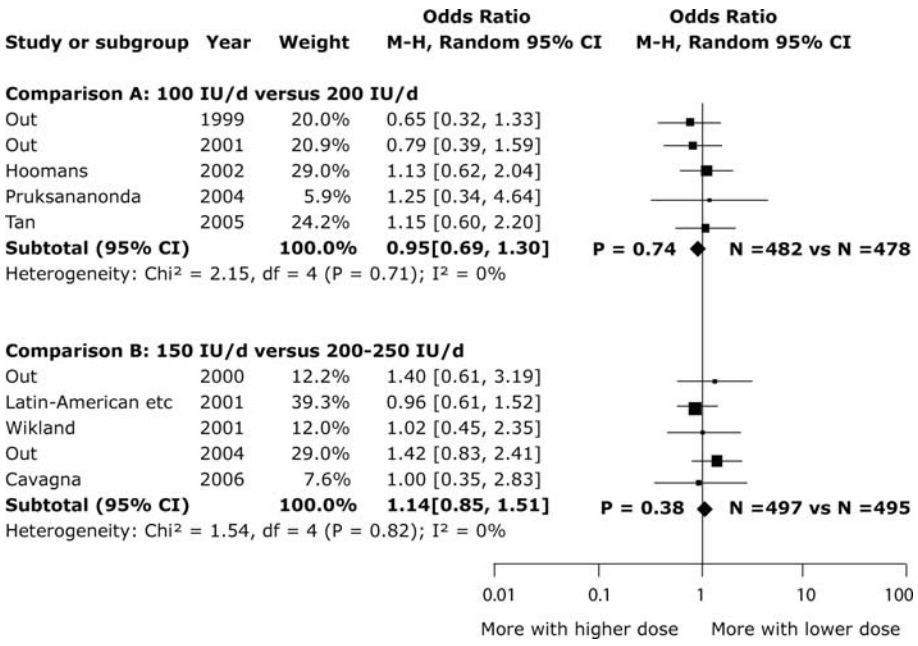

Figure 5 Forest plots of ORs of clinical pregnancy rates.

versus 200 IU/day; B: 150 versus 200–250 IU/day). Besides the dosages, the studies were different in the use of GnRH agonist and antagonist co-treatment. We demonstrated that for the analysis of total amount of recFSH, 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) demonstrated no differences in pooled results of all outcome parameters compared with the original analysis.

A potentially negative outcome of giving a lower dose of recFSH is the risk of low response resulting in cycle cancellation. However, we have previously shown in this meta-analysis 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

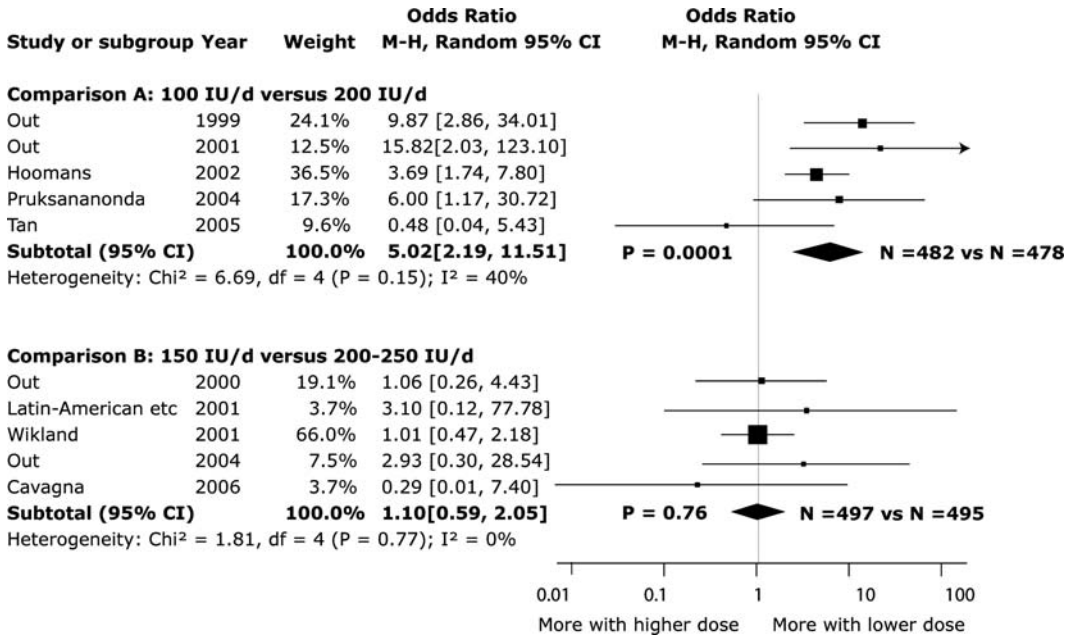

Figure 6 Forest plots of ORs of cycle cancellation rates due to low ovarian response to stimulation.

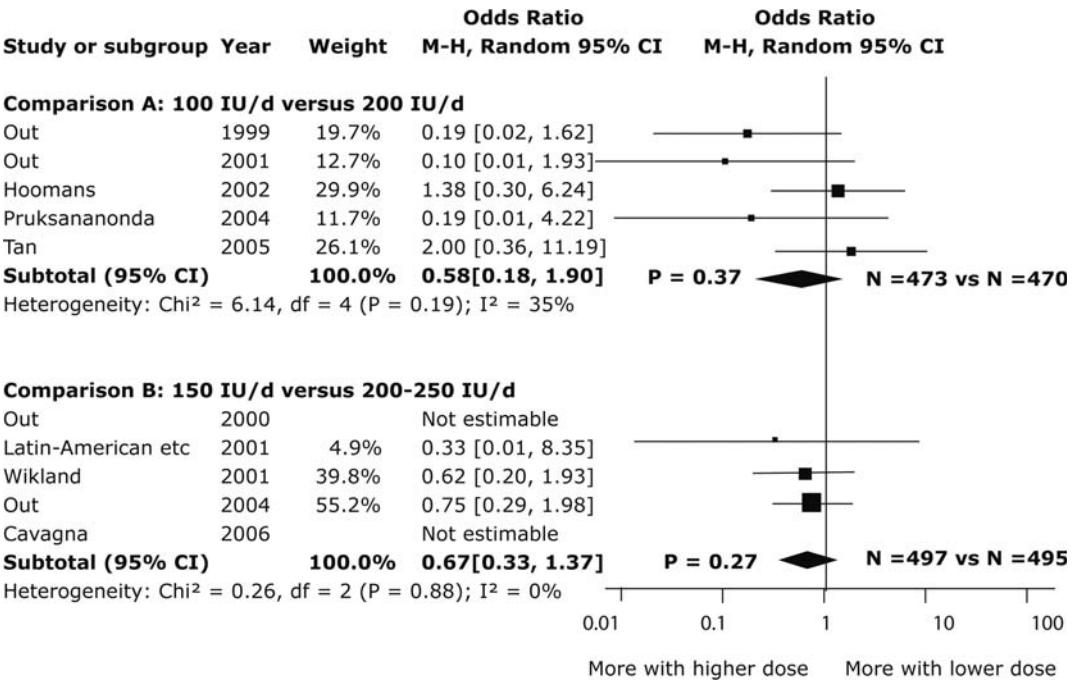

Figure 7 Forest plots of ORs of OHSS rates.

even when few follicles are present. When using 150 IU/day the probability of low response is not different from higher dosages, indicating that in general, 150 IU/day is likely to represent the optimal dosage.

Extreme responses to ovarian stimulation introduce the risk of developing the OHSS (Delvigne and Rozenberg, 2002; Aboulghar and Mansour, 2003). Although early diagnosis and treatment may minimize the risk of catastrophic events (such as thromboembolism and multiple organ failure) preventing the occurrence of OHSS remains the cornerstone of proper management. Alongside refraining from human chorionic gonadotrophin for triggering of final oocyte maturation or the cryopreservation of all embryos obtained in very high responder patients, avoiding extreme ovarian response should

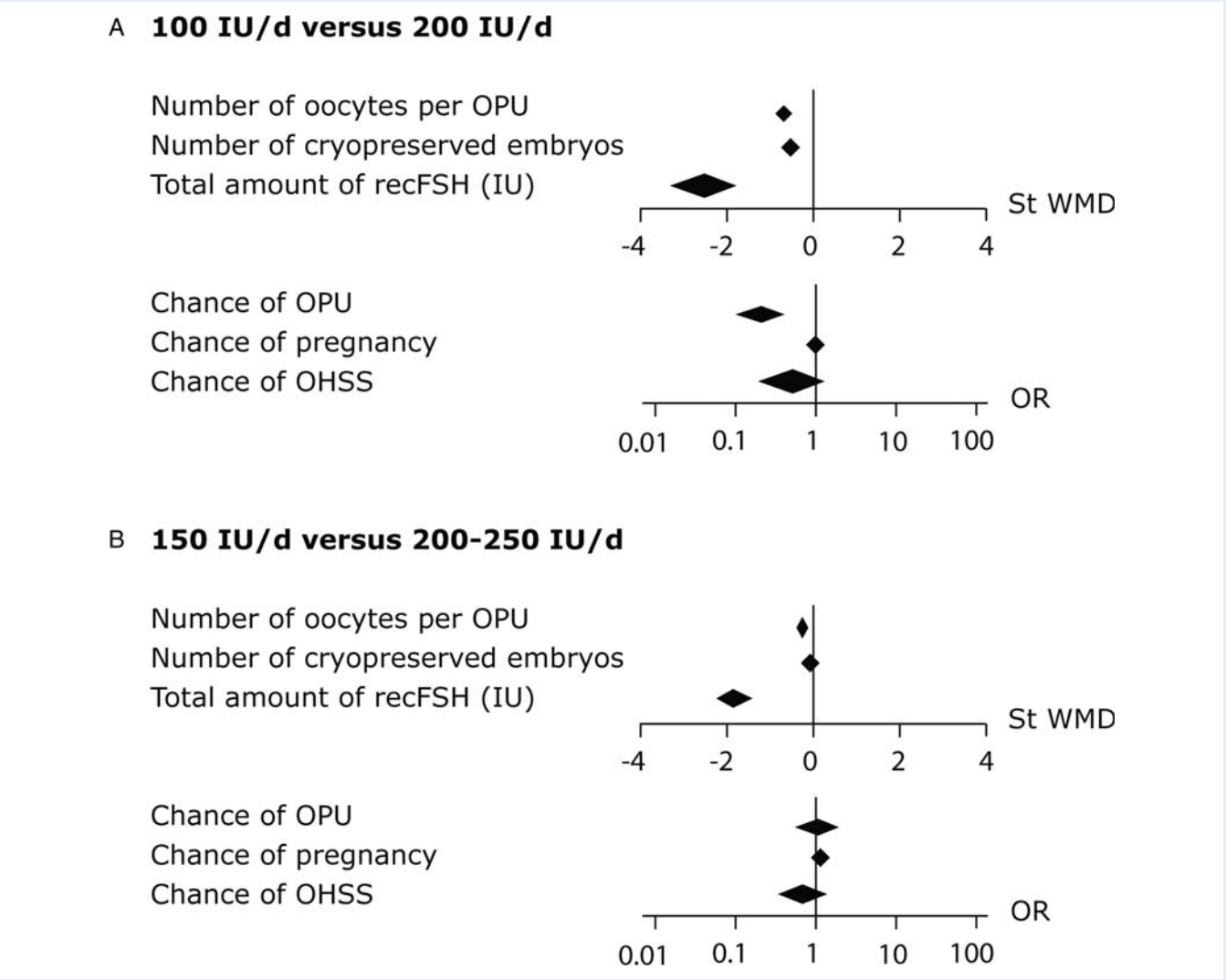

Figure 8 Summary all parameters; (**A**) Comparison A: 100 versus 200 IU/day; (**B**) Comparison B: 150 versus 200–250 IU/day. St WMD, standardized weighted mean difference; OR, odds ratio; OPU, ovum pick up; OHSS, ovarian hyperstimulation syndrome.

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 submaximal ovarian stimulation.

Reduction in total recFSH 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/day, instead of 225 IU/day, 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 recFSH sufficient for a third stimulation cycle. The use of GnRH antagonist as co-treatment might lead to an even lower consumption of recFSH, mainly by a reduction in the duration of stimulation due to relatively high endogenous FSH concentrations early during the stimulation cycle (Fauser and Van Heusden, 1997; Baart *et al.*, 2007; Heijnen *et al.*, 2007).

Significant strengths of the current meta-analysis are that it was performed according to the QUOROM guidelines, that almost 2000 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 end-point, 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 the 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*., 2003b).

In conclusion, this meta-analysis suggests that the optimal starting dose of recFSH for IVF/ICSI is 150 IU daily in presumed normal responders younger than 39 years. This dose is associated with a more modest oocyte yield, but an equal pregnancy rate compared with higher doses. Further benefits of this dose include a possible reduction in the risk for OHSS, in the face of sufficient numbers of oocytes to allow for cryopreservation of surplus embryos. In the future, the use of patient-tailored approaches may further optimize 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 (Popovic-Todorovic *et al*., 2003a; Fauser *et al*., 2008; Olivennes *et al*., 2009).

Authors' roles

M.D.S., S.M.V.-V., M.J.E., F.J.B. and B.C.J.M.F. involved in study design, analysis, manuscript drafting and critical discussion; E.G.H. involved analysis, manuscript drafting and critical discussion; N.S.M. participated in study design, manuscript drafting and critical discussion.

Acknowledgements

We thank the corresponding authors for their additional information regarding their articles.

Conflict of interest: M.D.S, S.M.V.-V. and M.J.E. declare that they have no conflict of interest. N.S.M. has received fees and grant support from the following companies (in alphabetic order); Ferring, Organon, Merck Serono and Schering Plough. E.G.H. has received fees and grant support from the following companies (in alphabetic order); Ferring, Organon, Merck Serono and Schering Plough. F.J.B. is a member of the external advisory board for Ferring Pharmaceuticals, Hoofddorp, The Netherlands. He receives no monetary compensation. B.C.J.M.F. has received fees and grant support from the following companies (in alphabetic order); Andromed, Ardana, Ferring, Genovum, Merck Serono, Organon, Pantharei Bioscience, PregLem, Schering, Schering Plough, Serono and Wyeth.

Funding

There was no external funding involved in the conception or development of this study. The first author (M.D.S.) and senior author (B.C.J.M.F.) had full access to all the data used for this meta-analysis and took final responsibility for submission for publication.

References

Aboulghar MA, Mansour RT. Ovarian hyperstimulation syndrome: classifications and critical analysis of preventive measures. *Hum Reprod Update* 2003;**9**:275–289.

Baart EB, Martini E, Eijkemans MJ, Van Opstal D, Beckers NG, Verhoeff A, Macklon NS, Fauser BC. Milder ovarian stimulation for *in vitro* fertilization reduces aneuploidy in the human preimplantation embryo: a randomized controlled trial. *Hum Reprod* 2007;**22**:980–988.

Cahill DJ, Wardle PG. Management of infertility. *BMJ* 2002;**325**:28–32.

Cavagna M, Dzik A, Freitas GC, Soares JB, De Pawn K, Sales ALM, Andrade PC, Mantese JC, Gebrim LH. Comparison of 150 and 225 IU of follitropin-(beta) in a fixed-dose regimen for ovarian stimulation using a depot formulation of GnRH agonist: a prospective randomized clinical trial. *J Bras Reproducao Assistida* 2006;**10**:21–24.

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

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

Fauser BC, Van Heusden AM. Manipulation of human ovarian function: physiological concepts and clinical consequences. *Endocr Rev* 1997;**18**:71–106.

Fauser BC, Diedrich K, Devroey P, Evian Annual Reproduction Workshop Group 2007. Predictors of ovarian response: progress towards individualized treatment in ovulation induction and ovarian stimulation. *Hum Reprod Update* 2008;**14**:1–14.

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

Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003;**327**:557–560.

Hohmann FP, Macklon NS, Fauser BC. A randomized comparison of two ovarian stimulation protocols with gonadotropin-releasing hormone (GnRH) antagonist cotreatment for *in vitro* fertilization commencing recombinant follicle-stimulating hormone on cycle day 2 or 5 with the standard long GnRH agonist protocol. *J Clin Endocrinol Metab* 2003;**88**:166–173.

Hoomans EH, Mulder BB, Asian Purgeon Study Group. A group-comparative, randomized, double-blind comparison of the efficacy and efficiency of two fixed daily dose regimens (100- and 200-IU) of recombinant follicle stimulating hormone (rFSH, Puregon) in Asian women undergoing ovarian stimulation for IVF/ICSI. *J Assist Reprod Genet* 2002;**19**:470–476.

Huirne JA, Lambalk CB. Gonadotropin-releasing-hormone-receptor antagonists. *Lancet* 2001;**358**:1793–1803.

Karlsson MO, Wade JR, Loumaye E, Munafo A. A population model for the follicular growth in women treated with follicle stimulating hormone. *Clin Pharmacol Ther* 1997;**62**:665–674.

Latin-American Puregon IVF Study Group. A double-blind clinical trial comparing a fixed daily dose of 150 and 250 IU of recombinant follicle-stimulating hormone in women undergoing *in vitro* fertilization. *Fertil Steril* 2001;**76**: 950–956.

Macklon NS, Stouffer RL, Giudice LC, Fauser BC. The science behind 25 years of ovarian stimulation for *in vitro* fertilization. *Endocr Rev* 2006;**27**:170–207.

Malizia BA, Hacker MR, Penzias AS. Cumulative live-birth rates after *in vitro* fertilization. *N Engl J Med* 2009;**360**:236–243.

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

Olivennes F, Howles CM, Borini A, Germond M, Trew G, Wikland M, Zegers-Hochschild F, Saunders H, Alam V, CONSORT study group. Individualizing FSH dose for assisted reproduction using a novel algorithm: the CONSORT study. *Reprod Biomed Online* 2009;**18**:195–204.

Out HJ, Lindenberg S, Mikkelsen AL, Eldar-Geva T, Healy DL, Leader A, Rodriguez-Escudero FJ, Garcia-Velasco JA, Pellicer A. A prospective, randomized, double-blind clinical trial to study the efficacy and efficiency of a fixed dose of recombinant follicle stimulating hormone (Puregon) in women undergoing ovarian stimulation. *Hum Reprod* 1999;**14**:622–627.

Out HJ, Braat DD, Lintsen BM, Gurgan T, Bukulmez O, Gokmen O, Keles G, Caballero P, Gonzalez JM, Fabregues F *et al.* Increasing the daily dose of recombinant follicle stimulating hormone (Puregon) does not compensate for the age-related decline in retrievable oocytes after ovarian stimulation. *Hum Reprod* 2000;**15**:29–35.

Out HJ, David I, Ron-El R, Friedler S, Shalev E, Geslevich J, Dor J, Shulman A, Ben-Rafael Z, Fisch B *et al.* A randomized, double-blind clinical trial using fixed daily doses of 100 or 200 IU of recombinant FSH in ICSI cycles. *Hum Reprod* 2001;**16**:1104–1109.

Out HJ, Rutherford A, Fleming R, Tay CC, Trew G, Ledger W, Cahill D. A randomized, double-blind, multicentre clinical trial comparing starting doses of

150 and 200 IU of recombinant FSH in women treated with the GnRH antagonist ganirelix for assisted reproduction. *Hum Reprod* 2004;**19**:90–95.

Popovic-Todorovic B, Loft A, Bredkjaeer HE, Bangsboll S, Nielsen IK, Andersen AN. A prospective randomized clinical trial comparing an individual dose of recombinant FSH based on predictive factors versus a 'standard' dose of 150 IU/day in 'standard' patients undergoing IVF/ICSI treatment. *Hum Reprod* 2003a;**18**:2275–2282.

Popovic-Todorovic B, Loft A, Lindhard A, Bangsboll S, Andersson AM, Andersen AN. A prospective study of predictive factors of ovarian response in 'standard' IVF/ICSI patients treated with recombinant FSH. A suggestion for a recombinant FSH dosage normogram. *Hum Reprod* 2003b; **18**:781–787.

Pruksananonda K, Suwajanakorn S, Sereepapong W, Virutamasen P. Comparison of two different fixed doses of follitropin-beta in controlled ovarian hyperstimulation: a prospective randomized, double blind clinical trial. *J Med Assoc Thai* 2004;**87**:1151–1155.

Tan SL, Child TJ, Cheung AP, Fluker MR, Yuzpe A, Casper R, Leung P, Cadesky K, Davis VJ. A randomized, double-blind, multicenter study comparing a starting dose of 100 IU or 200 IU of recombinant follicle stimulating hormone (Puregon) in women undergoing controlled ovarian hyperstimulation for IVF treatment. *J Assist Reprod Genet* 2005;**22**:81–88.

van Houwelingen HC, Arends LR, Stijnen T. Advanced methods in meta-analysis: multivariate approach and meta-regression. *Stat Med* 2002;**21**:589–624.

Wechowski J, Connolly M, Schneider D, McEwan P, Kennedy R. Cost-saving treatment strategies in *in vitro* fertilization: a combined economic evaluation of two large randomized clinical trials comparing highly purified human menopausal gonadotropin and recombinant follicle-stimulating hormone alpha. *Fertil Steril* 2009;**91**:1067–1076.

Wikland M, Bergh C, Borg K, Hillensjo T, Howles CM, Knutsson A, Nilsson L, Wood M. A prospective, randomized comparison of two starting doses of recombinant FSH in combination with cetrorelix in women undergoing ovarian stimulation for IVF/ICSI. *Hum Reprod* 2001;**16**:1676–1681.