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The clinical significance of the retrieval of a low number of oocytes following mild ovarian stimulation for IVF: a meta-analysis

M.F.G. Verberg[1,4], M.J.C. Eijkemans[1,2], N.S. Macklon[1], E.M.E.W. Heijnen[1], E.B. Baart[1], F.P. Hohmann[3], B.C.J.M. Fauser[1], and F.J. Broekmans[1]

[1]Department of Reproductive Medicine and Gynaecology, University Medical Center, Utrecht, The Netherlands [2]Department of Public Health, Erasmus Medical Center, Rotterdam, The Netherlands [3]Department of Obstetrics and Gynaecology, Erasmus Medical Center, Rotterdam, The Netherlands

[4]Correspondence address. E-mail: m.f.g.verberg@umcutrecht.nl

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BACKGROUND: Milder ovarian stimulation protocols for *in vitro* fertilization (IVF) are being developed to minimize adverse effects. Mild stimulation regimens result in a decreased number of oocytes at retrieval. After conventional ovarian stimulation for IVF, a low number of oocytes are believed to represent poor ovarian reserve resulting in reduced success rates. Recent studies suggest that a similar response following mild stimulation is associated with better outcomes.

METHODS: This review investigates whether the retrieval of a low number of oocytes following mild ovarian stimulation is associated with impaired implantation rates. Three randomized controlled trials comparing the efficacy of the mild ovarian stimulation regimen (involving midfollicular phase initiation of FSH and GnRH co-treatment) for IVF with a conventional long GnRH agonist co-treatment stimulation protocol could be identified by means of a systematic literature search.

RESULTS: These studies comprised a total of 592 first treatment cycles. Individual patient data analysis showed that the mild stimulation protocol results in a significant reduction of retrieved oocytes compared with conventional ovarian stimulation (median 6 versus 9, respectively, $P < 0.001$). Optimal embryo implantation rates were observed with 5 oocytes retrieved following mild stimulation (31%) versus 10 oocytes following conventional stimulation (29%) ($P = 0.045$).

CONCLUSIONS: The optimal number of retrieved oocytes depends on the ovarian stimulation regimen. After mild ovarian stimulation, a modest number of oocytes is associated with optimal implantation rates and does not reflect a poor ovarian response. Therefore, the fear of reducing the number of oocytes retrieved following mild ovarian stimulation appears to be unjustified.

Key words: implantation oocyte quality / ovarian stimulation / poor response

Introduction

Ovarian stimulation is a key component of assisted reproductive treatment. Since the early days of *in vitro* fertilization (IVF), ovarian stimulation has been applied to compensate for inefficiencies in the IVF procedure by increasing the number of oocytes retrieved enabling the selection of the best quality embryos for transfer (Fauser *et al.*, 2005). Currently, long gonadotrophin-releasing hormone (GnRH) agonist pituitary

suppression combined with exogenous follicle-stimulating hormone (FSH) is the most frequently used stimulation protocol (Macklon *et al*., 2006). This conventional ovarian stimulation regimen is expensive, complex and associated with significant side effects and stress. There is increasing interest in the application of milder stimulation protocols which aim to render IVF treatment more patient-friendly, reduce the chance for complication (especially ovarian hyperstimulation syndrome) and lower cost (Fauser *et al*., 1999).

The use of GnRH antagonists allows the initiation of the IVF treatment cycle in a normal menstrual cycle with an undisturbed early follicular phase recruitment of a cohort of follicles. This enables the endogenous inter-cycle FSH rise to be utilized rather than suppressed. A moderate, but continued, elevation of FSH levels during the mid to late follicular phase (initiated on cycle Day 5) was shown to be sufficient to extend the FSH window, allowing multiple dominant follicle development to take place (Schipper *et al*., 1998; De Jong *et al*., 2000). As a consequence of the later initiation of exogenous FSH, the number of treatment days and the total amount of exogenous FSH required are substantially reduced (De Jong *et al*., 2000; Hohmann *et al*., 2003; Heijnen *et al*., 2007).

The availability of GnRH antagonists has allowed the clinical development of milder ovarian stimulation protocols involving subtle interference with single dominant follicle selection (Fauser and van Heusden, 1997; Tarlatzis *et al*., 2006). By limiting the administration of FSH to the mid to late follicular phase, the total dose of FSH needed and the number of treatment days are reduced.

Following conventional ovarian stimulation, a low number of oocytes after follicle aspiration are associated with a poor clinical outcome and are believed to represent ovarian ageing (Beckers *et al*., 2002; Tarlatzis *et al*., 2003). Despite the lack of consensus regarding the exact definition of poor response (Klinkert *et al*., 2004), the relationship between the number of oocytes retrieved and success rates is well established in conventional IVF (Keay *et al*., 1997). In contrast, recent studies suggest that a similarly low number of oocytes in ovarian response following mild stimulation are associated with a distinctly higher chance of conceiving (Hohmann *et al*., 2003). This has led to the contention that a low number of oocytes obtained following mild stimulation represent a physiological response to the subtle interference with single dominant follicle selection and not a pathological reduction in ovarian response associated with ovarian ageing. The clinical implications of low oocyte numbers following mild stimulation may therefore be quite different from the poor ovarian response observed in conventional GnRH agonist suppression cycles. A mild treatment strategy in IVF has recently been shown to result in similar term live birth rates as conventional treatment within 1 year of treatment (Heijnen *et al*., 2007).

The purpose of the present review and meta-analysis was to investigate whether among women undergoing mild ovarian stimulation for IVF or intracytoplasmic sperm injection (ICSI), the retrieval of a low number of follicles following mild ovarian stimulation indeed impairs the implantation rate?

Materials and Methods

Search strategy and study selection

In April 2007, a computerized literature search was performed on the bibliographic databases EMBASE and MEDLINE from 1980. Additionally, references of retrieved articles were hand-searched. The search strategy aimed at identifying randomized-controlled trials (RCTs) on the basis of the following clinical question: among patients treated with mild ovarian stimulation, with FSH initiation on cycle Day 5 and GnRH antagonist co-treatment, are a lower number of oocytes associated with a reduced probability of pregnancy when compared with a similar response in a 'conventional' ovarian stimulation protocol, with a long GnRH agonist stimulation protocol and initiation of FSH on Day 2 of the subsequent menstrual bleed. Search terms used were 'mild ovarian stimulation', 'minimal ovarian stimulation', 'ovarian stimulation protocol', 'mild treatment strategy', 'low ovarian response', 'poor ovarian response' AND IVF or ICSI. The search was limited to RCTs in humans to allow comparison of the significance of low response in ovarian stimulation protocols.

Data collection and outcome measures

The following data for each patient were requested from all trials: method of ovarian stimulation, the number of oocytes retrieved, the number of embryos transferred, the ongoing pregnancy rate and when available the live birth rate. The primary outcome of interest was planned to be the live birth rate. However, due to differences between the studies regarding the number of embryos transferred, the primary outcome measure chosen was ongoing pregnancy rate per embryo transferred. This was defined as the number of ongoing pregnancies divided by the number of embryos transferred. Ongoing pregnancy was defined as the presence of fetal cardiac activity on ultrasonography at 9 weeks gestational age.

Data analysis

To examine the distribution of oocytes retrieved and the associated pregnancy rates, individual patient data (IPD) meta-analysis was performed. A non-parametric Mann–Whitney *U*-test was used to compare the distribution of oocytes retrieved following mild or conventional stimulation. In case individual data could not be obtained, weighted mean difference of oocyte number and peto odds for pregnancy rates was used.

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

The current study represents a combined multivariate analysis in order to adjust for potential confounding factors such as age, body mass index (BMI), duration of infertility and previous pregnancy. A robust correction for statistical dependence between embryos when more than one embryo was transferred was performed with a generalized estimating equation (GEE) approach in which study was included as a co-variate (Zeger *et al*., 1988).

Heterogeneity between studies was tested for using the likelihood method described by Hardy and Thompson (1998), when at least three studies were available. *P*-values are two-sided, and $P < 0.05$ was considered the limit of statistical significance. Analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA, 1999) and SAS 8.1 (SAS institute, Cary, NC, USA).

Results

Search results

With the search strategy applied, 41 references were identified that could possibly contribute to solving the research question based on abstract reading. After reading the full papers, only three studies

finally met our inclusion criteria. The requested IPD could be obtained from all included studies. Characteristics of the included studies are listed in Table I. All studies were approved by the local Ethics Review Boards of participating centres and written informed consent was obtained from each participant. Inclusion criteria for patient characteristics were comparable for the three studies. All studies included infertile patients with a regular indication for IVF or ICSI, age below 38 years, regular menstrual cycles (mean 25–35 days), and no relevant systemic disease, severe endometriosis and uterine or ovarian abnormalities. One study focusing on clinical outcome excluded patients with a partner with a sperm count $\leq$5 million progressively motile sperm/ml (Baart *et al*., 2007). One study included a third study group in which a standard GnRH antagonist stimulation protocol was applied initiating FSH on cycle Day 2 and GnRH antagonist when the largest follicle had reached a diameter of 14 mm (Hohmann *et al*., 2003). As this study arm did not fulfil the search criteria for the present meta-analysis, this subgroup was excluded from our analyses.

Study design

In all three studies, the mild stimulation protocol consisted of a low dose of recombinant (r)FSH (Gonal-F®: Serono Benelux B.V., Amsterdam, The Netherlands; or Puregon®: N.V. Organon, Oss, The Netherlands) s.c. initiated on cycle Day 5, combined with a GnRH antagonist (ganirelix, Orgalutran®: N.V. Organon, 0.25 mg/day; or Cetrorelix, Cetrotide®: Serono Benelux, 0.25 mg/day) s.c. initiated when at least 1 follicle $\geq$14 mm was observed. The conventional stimulation protocol consisted of a standard long suppression protocol with a GnRH agonist (Leuproline, Lucrin®: Abbott B.V., Amstelveen, The Netherlands, 0.2 mg/day; or triptoreline, Decapeptyl®: Ferring B.V., Hoofddorp, The Netherlands, 0.1 mg/day) s.c. for ~2 weeks starting during the mid-luteal phase of the pretreatment cycle.

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

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

In one study, preimplantation genetic screening (PGS) of embryos was performed (Baart *et al*., 2007). Embryos were biopsied on Day 3 when at least six blastomeres were present. One or two cells were removed for a fluorescence *in situ* hybridization procedure. Detailed information on the process of biopsy, fixation and PGS has been described previously (Baart *et al*., 2004, 2007).

Table I Characteristics of included studies

Study	Design	Inclusion criteria	Study protocol	Control stimulation protocol
Hohmann *et al.* (2003)	Single centre RCT. Nov 1999–May 2001. Method of randomization: computer-generated randomization schedule, assigned via numbered sealed envelopes	Normo-ovulatory patients (cycles 25–35 days) with a regular indication for IVF (or IVF/ICSI). Age 20–38 years. BMI 19–29 kg/m^2. No more than three previous IVF cycles	Fixed FSH doses 150 IU/day from CD 5, GnRH antagonist from leading follicle 14 mm	1. Fixed FSH doses 150 IU/day from CD 2, GnRH antagonist from leading follicle 14 mm. 2. Long GnRH agonist protocol, fixed FSH doses after 2 weeks 150 IU/day
Heijnen *et al.* (2007)	Two centre RCT. Feb 2002–March 2004. Computer-generated randomization with random blocks of size 4 and 6, stratified by centre	Regular cycling patients (25–35 days), below 38 years, BMI 18–28 kg/m^2. Only couples with no previous IVF treatment or a healthy born child after a previous IVF treatment	Fixed FSH doses 150 IU/day from CD 5, GnRH antagonist from leading follicle 14 mm. Combined with single embryo transfer	Long GnRH agonist protocol, fixed FSH doses after 2 weeks 150 IU/day
Baart *et al.* (2007)	Two centre RCT. Dec 2002–Aug 2005. Computer-generated randomization schedule in a ration of 4:6, assigned via numbered sealed envelopes	Regular cycling patients, below 38 years, BMI 19–29 kg/m^2. Sperm count $>$5 mln/ml. No previous IVF cycles resulting in an embryo transfer	Fixed FSH doses 150 IU/day from CD 5, GnRH antagonist from leading follicle 14 mm	Long GnRH agonist protocol, fixed FSH doses after 2 weeks 225 IU/day

Table II Included studies and number of first IVF treatment cycles (total = 592), progress to embryo transfer and treatment outcome

		Started treatment cycles (all first cycles)	Retrieval procedures	Embryo transfers	Ongoing pregnancy rate*	Live birth rate
Hohmann *et al.* (2003)	Mild	49	32	28	16%	n.a.
	Conventional	45	38	26	18%	n.a.
Heijnen *et al.* (2007)	Mild	201	147	124	13%	13%
	Conventional	193	176	160	33%	31%
Baart *et al.* (2007)	Mild	63	56	41	21%	n.a.
	Conventional	41	40	33	18%	n.a.
Total	Mild	313	235	193	15%	n.a.
	Conventional	279	254	219	29%	n.a.

*Ongoing pregnancy rate per started cycle.
n.a., not available.

Subjects and oocyte distribution

Data of 592 first treatment cycles were included in the analysis. Table II shows the number of patients in whom oocyte retrieval and embryo transfer were performed in the first treatment cycle. Figure 1 shows a bar chart of the distribution of retrieved number of oocytes per stimulation protocol. The range of the number of retrieved oocytes per patient was similar for both protocols (range 0–28). However, the distribution of oocyte numbers was significantly different following mild and conventional ovarian stimulation [median oocyte number (25–75 percentile), 6 (3–10) and 9 (6–12), respectively ($P < 0.001$). The mean (SD) of the total dosage of FSH was 1183 (223) IU in the mild group versus 1836 (657) IU for the conventional treatment arms ($P < 0.001$, *t*-test with unequal variances).

Table III shows the ongoing pregnancy rate per embryo transferred as a function of the number of retrieved oocytes for each stimulation protocol. Figure 2 shows a visualization of these numbers by means of fitted curves. Following mild ovarian stimulation, the retrieval of a low number of oocytes is associated with the highest chance of ongoing pregnancy per embryo transferred. Optimal outcomes following mild stimulation (30.7%) are observed in those oocyte retrievals where five oocytes were obtained (median value). The retrieval of more than eight oocytes following mild stimulation is associated with a sharp decrease in implantation rates. In the conventional ovarian stimulation protocol, the highest ongoing pregnancy rate per embryo transferred (28.5%) is associated with a median of 10 oocytes. In contrast to the mild stimulation protocol, low numbers of oocytes are associated with a compromised outcome. The difference in pattern of the ongoing pregnancy rate per embryo transferred as a function of the number of retrieved oocytes between the two stimulation regimens was statistically significant ($P = 0.045$) (Fig. 2). No significant heterogeneity was detected between studies.

Correction for clustering of embryos when more than one embryo was transferred and correction for potential confounding factors including age, BMI, duration of infertility, previous pregnancy and the study did not change the observed difference in the relationship between oocyte number and ongoing pregnancy rate per embryo transferred for both protocols (*P*-value 0.045).

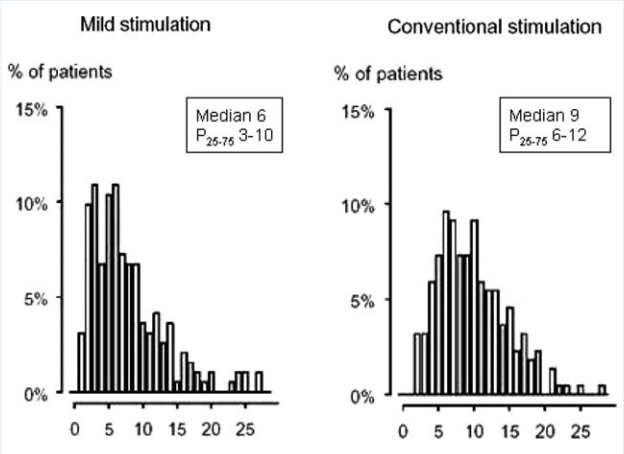

Figure 1 Distribution of the number of retrieved oocytes per patient following mild ($n = 235$) or conventional ($n = 254$) ovarian stimulation for IVF. The number of patients is expressed as a percentage of total.

Discussion

This review and meta-analysis and subsequent IPD-analysis involving three RCTs is the first to establish that the optimal number of oocytes retrieved in IVF is dependent on the stimulation regimen used. The retrieval of a modest number of oocytes following mild ovarian stimulation is associated with the optimal chance of achieving a pregnancy. These findings are in striking contrast to the well-established relationship between a poor ovarian response and poor clinical outcome related to ovarian ageing (Tarlatzis *et al.*, 2003). Our current findings imply that the fear of obtaining low numbers of oocytes, which drives current practice in ovarian stimulation, is unjustified. Additionally, these findings show that in both stimulation protocols, a moderate ovarian response leads to a higher chance of pregnancy than a hyperresponse as in both protocols the number of pregnancies following the retrieval of 18 oocytes or more was very low. As expected, the current analysis does not shown better pregnancy rates following mild ovarian stimulation. The main principle of

Table III Ongoing pregnancy rate per embryo transferred as a function of the number of retrieved oocytes following mild or conventional ovarian stimulation for IVF

Number of retrieved oocytes	Conventional stimulation			Mild stimulation		
	Implantation failure	Ongoing pregnancy/ embryo transferred		Implantation failure	Ongoing pregnancy/ embryo transferred	
	n	*n*	%	*n*	*n*	%
1–3	21	4	16	38	15	28
4–6	64	21	25	46	19	29
7–9	69	26	27	43	10	19
10–12	58	27	32	22	3	12
13–15	41	12	23	14	3	18
16–18	20	10	33	9	2	18
19–21	11	0	0	4	0	0
22–24	1	3	75	4	0	0
25–27	2	0	0	5	0	0
28–30	1	0	0	0	0	0
Total	288	103	26	185	52	22

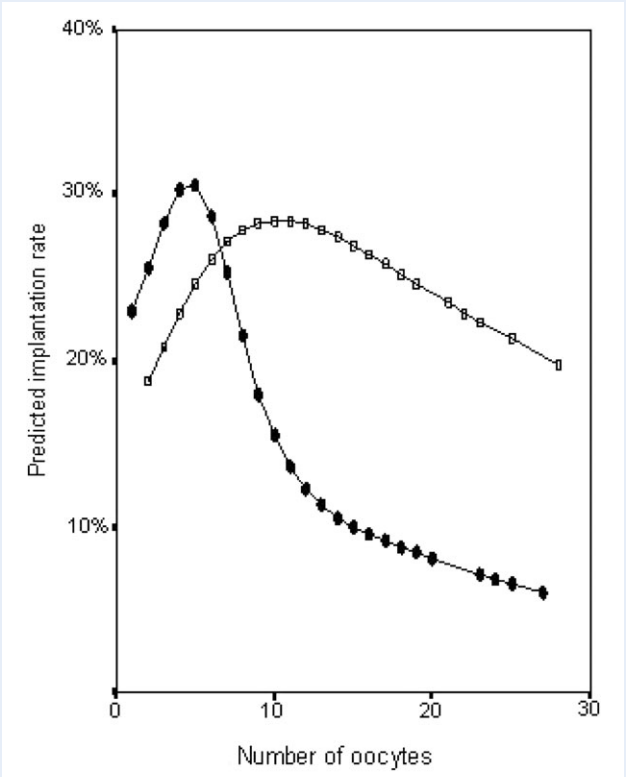

Figure 2 Ongoing pregnancy rate per embryo transferred (implantation rate) according to the number of oocytes retrieved following mild or conventional ovarian stimulation for IVF ($P = 0.045$).

Curves were fitted to the observed implantation rates using a flexible 4-knotted spline function. Black dots, mild ovarian stimulation; open squares, conventional ovarian stimulation.

mild approaches is raising less costs and patient discomfort without reducing the overall success rates of the IVF treatment (Fauser *et al*., 1999).

The observed relationship between low oocyte numbers and a high chance of achieving an ongoing pregnancy following mild stimulation suggests that when few oocytes are obtained, they are likely to represent a homogenous group of good quality oocytes. This could be the result of the subtle interference with the natural selection of good quality oocytes or the minimized exposure of growing follicles to the potentially negative effects of ovarian stimulation. In contrast to the conventional stimulation protocol with GnRH agonist co-treatment resulting in pituitary desensitisation, ovarian stimulation with GnRH antagonist co-treatment enables the endogenous inter-cycle FSH rise to occur (Tarlatzis *et al*., 2006). Therefore, cyclic follicle recruitment and initial stages of gonadotrophin-dependent growth of the recruited cohort of follicles can proceed undisturbed (Fauser and van Heusden, 1997; McGee and Hsueh, 2000). By postponing the initiation of ovarian stimulation to the mid-follicular phase, exogenous FSH may only stimulate the most mature follicles to ongoing development up to the Graafian stage giving rise to the best quality oocytes (Keay *et al*., 1997).

Supportive evidence regarding the potentially negative effects of ovarian stimulation comes from several human and animal studies reporting detrimental effects of ovarian stimulation on oocyte and embryo quality. Increased incidences of morphological and chromosomal abnormalities have been observed in oocytes after exposure to high doses of gonadotrophins during *in vitro* maturation of oocytes (Eppig *et al*., 1998; van Blerkom and Davis, 2001; Roberts *et al*., 2005). Ovarian stimulation and concurrent high estradiol levels were shown to have a negative impact on the developmental and implantation potential (Valbuena *et al*., 1999; Ertzeid and Storeng, 2001; Van der Auwera and D'Hooghe, 2001) as well as the chromosomal constitution of embryos (Katz-Jaffe *et al*., 2005). Moreover, ovarian

stimulation might disrupt mechanisms involved in maintaining accurate chromosome segregation (Munne *et al*., 1997; Hodges *et al*., 2002). A randomized trial concerning the chromosomal constitution of human embryos following mild ovarian stimulation for IVF showed a significantly higher proportion of euploid embryos compared with conventional stimulation, suggesting that through maximal stimulation the surplus of obtained oocytes and embryos are of lower quality (Baart *et al*., 2007).

Following conventional stimulation, a low number of retrieved oocytes are associated with compromised outcomes. Optimal outcomes were observed when 10 oocytes were obtained. These findings are consistent with most previous studies on this subject. A low number of oocytes following conventional stimulation are related to ovarian ageing caused by the depletion of the primordial follicle pool, leading to a decreased number of developing follicles and diminished oocyte quality (Tarlatzis *et al*., 2003; Broekmans *et al*., 2007). Few reported studies have addressed the issue of the optimal number of oocytes in a conventional stimulation protocol (Timeva *et al*., 2006; van der Gaast *et al*., 2006). Timeva *et al*. (2006) report an optimum between 5 and 15, and van der Gaast *et al*. (2006) found an optimum median number of oocytes of 13. Indeed, most previous studies on this subject report a steady increase in pregnancy rates with increasing numbers of oocytes which eventually levels off (Devreker er al., 1999; Sharma *et al*., 2002). This confirms the concept that in conventional stimulation increasing oocyte numbers augment the ability to select the best embryo(s) for transfer. Beyond a certain point however, pregnancy rates decrease due to the aforementioned detrimental effects of the development of large quantities of follicles (and concomitant supraphysiological hormone levels) on oocyte and embryo quality (Simon *et al*., 1995; Ertzeid and Storeng, 2001; Pena *et al*., 2002) as well as endometrial receptivity (Devroey *et al*., 2004; Kodaman and Taylor, 2004). In the mild stimulation group, this effect was clearly demonstrated when more than nine oocytes were obtained.

Alternatively, the impaired pregnancy rate in patients with a more pronounced ovarian response in the mild stimulation protocol group could be related to the occurrence of premature LH rises (The ganirelix dose-finding study group, 1998; Borm and Mannaerts, 2000). The occurrence of an untimely LH rise (a consequence of the high estradiol concentrations developed early during ovarian stimulation) has a negative impact on the chance of achieving an ongoing pregnancy (Humaidan *et al*., 2002). In the mild stimulation protocols used in the three studies, a flexible GnRH antagonist protocol was applied to limit the number of days of usage (initiating the GnRH antagonist dependent on the size of the follicle) (Al-Inany *et al*., 2005). Under conditions of a profound ovarian response with an early rise in estradiol and subsequent elevated LH levels, the ultrasound criteria used to initiate GnRH antagonist co-treatment may no longer be valid (Albano *et al*., 1997; The ganirelix dose-finding study group, 1998; Borm and Mannaerts, 2000). This observation might explain the observed lower efficacy of the flexible protocol compared with a fixed protocol [OR 0.70 (95% CI 0.47–1.05)] in a meta-analysis on four studies (Tarlatzis *et al*., 2006). For patients with a profound ovarian response, early initiation of the GnRH antagonist is worth evaluation (Kolibianakis *et al*., 2006). An important weakness of the present analysis is the lack of endocrine data to confirm the occurrence of LH rises or surges in our patient group.

A relative shortcoming of the present analysis is the heterogeneity regarding the number of embryos transferred per patient in the different studies. In the Heijnen *et al*. (2007) paper, the mild stimulation group had a single embryo transfer while the conventional had two embryos transferred. This was overcome by using the implantation rate (ongoing pregnancy rate per embryo transferred) as the primary outcome measure of this study. Even though the number of transferred embryos was independent of the ovarian response (unless only one embryo was available), the use of implantation rates introduces a new potential bias. When embryo implantation is used, outcomes are clustered within each woman. Thus, variability in implantation status of individual embryos comes from two sources, within-women variation and between-woman variation (Hogan and Blazar, 2000). Consequently, a statistical method is required to compensate for the clustering of data. In this study, a validated method for correction for statistical dependence between embryos was performed (GEE approach) (Eppig *et al*., 1998). Because the difference between outcomes in both protocols remained statistically significant after this analysis, it was assumed that the clustering of data could not be held responsible for the observed effect.

A further potential confounding factor is the heterogeneity of the three studies incorporated into the meta-analysis. In one study, PGS of embryos was performed (Baart *et al*., 2007), the day of embryo transfer differed between studies and inclusion criteria varied between studies as one study permitted up to three prior failed cycles, while one only included treatment of naïve patients (Hohmann *et al*., 2003) and one study excluded couples with a sperm count $<5 \times 10^6$/ml (Baart *et al*., 2007). However, the aim of the analysis was to study the effect of the difference in the number of oocytes retrieved following the two different stimulation protocols. The ovarian response was unlikely to be influenced by the factors outlined and therefore their role as confounding factors may be limited to the occurrence of pregnancy. The ongoing pregnancy rate, due to randomization, will be affected in an equal way in both arms of each study. As such, the comparison of the relation between oocyte yield and implantation rate is valid for this specific study and therefore will not confound the overall outcome of our analysis, as this focuses on differences and not the absolute effects.

Finally, the fact that the current analysis is based on data derived from only three studies from one study group leads to a loss of generalizability. However, this overcomes the common criticism of meta-analysis about the clinical heterogeneity arising from different centres and countries. Additionally, data of individual studies all pointed in the same direction and the observations regarding the conventional protocol are in accordance with the existing literature which confirms the authenticity of the observed findings.

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

pregnancy rates compared with a conventional stimulation protocol with GnRH agonist co-treatment, the benefits of mild stimulation should be balanced with the potential slight decrease in pregnancy rate per cycle. The lower implantation rates in high responders in the mild stimulation protocol warrant further study as to its cause.

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