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Expected poor responders on the basis of an antral follicle count do not benefit from a higher starting dose of gonadotrophins in IVF treatment: a randomized controlled trial*

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BACKGROUND: The aim of this study was to evaluate the effect of doubling the starting dose of gonadotrophins on the ovarian response in IVF patients with a low antral follicle count (AFC). METHODS: Fifty-two patients with an AFC of <5 follicles of 2–5 mm diameter before starting their first IVF cycle participated in this randomized controlled trial. They were randomized by opening a sealed envelope, receiving either 150 IU (group I, $n = 26$) or 300 IU (group II, $n = 26$) of rFSH as a starting dose. The main outcome measures of the study were number of oocytes, poor response (<4 oocytes at retrieval or cancellation due to insufficient follicle growth) and ongoing pregnancy (12 weeks of gestation). RESULTS: The groups were comparable regarding patient characteristics and outcome of the IVF treatment. The median number of oocytes collected was 3 for both groups ($P = 0.79$). The difference in the mean number of oocytes was 0.3 oocytes in favour of group I ($P = 0.69$). Sixty-five per cent of the patients in group I experienced a poor response and 62% in group II. The ongoing pregnancy rate was 8% in group I and 4% in group II ($P = 0.55$). CONCLUSIONS: Expected poor response patients, defined as patients with an AFC <5, are likely not to benefit from a higher starting dose of gonadotrophins in IVF.

Key words: antral follicles/gonadotrophin dose/IVF/poor response/randomized study

Introduction

The purpose of ovarian hyperstimulation with gonadotrophins during IVF treatment is to obtain more than one embryo. The availability of several embryos makes it possible to select the best embryos for transfer leading to an improvement of the pregnancy rate (Wood *et al.*, 1985; Templeton *et al.*, 1996). Poor response to the hyperstimulation leads to a low number of oocytes at oocyte retrieval and fewer embryos available to choose from at transfer.

Patients who respond poorly during IVF treatment are known to have poorer pregnancy prospects than normal responders (Pellicer *et al.*, 1987), although poor responders without other evidence of diminished ovarian reserve have reasonable pregnancy rates (Lashen *et al.*, 1999; Biljan *et al.*, 2000). To improve the recruitment and development of the follicles, most clinicians increase the dose of gonadotrophins in the next cycle in case of a poor response. The debate as to whether or not a dose increase has any value in the treatment of poor responders is still ongoing (Land *et al.*, 1996; Lashen *et al.*, 1998; Khalaf *et al.*, 2002).

To optimize ovarian stimulation in a first IVF cycle, it is important to recognize potentially poor responders. Age and basal FSH are two commonly used markers to predict the ovarian response in IVF. Older patients and patients with elevated basal FSH levels are known to be at risk of developing a poor response during IVF treatment (Scott *et al.*, 1989; Marcus and Brinsden, 1996; Ron-El *et al.*, 2000). These patients can be designated as expected poor responders.

However, several studies have shown that the antral follicle count (AFC) is a better predictor of poor response than age and basal FSH, because it shows a better correlation with the number of oocytes at oocyte retrieval (Tomas *et al.*, 1997; Chang *et al.*, 1998; Frattarelli *et al.*, 2000; Nahum *et al.*, 2001; Hsieh *et al.*, 2001; Bancsi *et al.*, 2002). The AFC also provided the best reflection of reproductive age in normal fertile volunteers (Scheffer *et al.*, 2003) and a recent meta-analysis comparing the AFC with basal FSH confirmed the superiority of the former (Hendriks *et al.*, 2004).

Measuring the number of antral follicles on ultrasound just prior to the start of the stimulation with gonadotrophins is a simple procedure, with a good intra- and inter-observer reproducibility (Scheffer *et al.*, 2002). It provides important information on what to expect from the subsequent IVF

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treatment. When a low number of antral follicles is found, the patient is at high risk of developing a poor response in IVF.

Several publications have suggested that the AFC could be used to optimize stimulation protocols in IVF (Ng *et al.*, 2000; Kupesic *et al.*, 2003; Popovic-Todorovic *et al.*, 2003). In the present study the AFC was used to identify patients who were at risk of developing a poor response during their first IVF treatment. These expected poor responders were asked to participate in a randomized controlled trial comparing the standard starting dose of 150 IU of rFSH with the double dose of 300 IU. The aim of this trial was to determine whether this group of patients would benefit from a higher starting dose of gonadotrophins.

Materials and methods

Subjects

All 520 women who started their first IVF treatment in our centre between May 2001 and November 2002 underwent an AFC just prior to the start of the hyperstimulation with gonadotrophins. For the assessment of the number of follicles a Voluson 530D (Kretz Technik, Austria) with a 7.5 MHz vaginal transducer was used. The three-dimensional data were stored for later analysis. In order to obtain a reliable follicle count, both ovaries had to be clearly visible on ultrasound. Patients with large ovarian cysts (>30 mm) were excluded from this study. All AFC were performed by one investigator (E.K.). The intra-observer variability in the AFC is very modest (intra-class correlation coefficient of 0.99 with a mean difference between two counts of −0.02), as was reported previously (Scheffer *et al.*, 2002).

The AFC was used for the selection of patients, because it was shown to be the best single predictor of poor ovarian response (Bancsi *et al.*, 2002). Addition of basal FSH to the prediction model slightly improves the error rate (87% correct predictions versus 80% when only the AFC is used). However, we wished to screen all first cycle IVF patients for a low AFC in order to assess their eligibility for the study, without deviating from our standard protocol. As a baseline ultrasound before initiation of stimulation is part of our routine protocol and measurement of basal FSH is not, the expectation of poor response was based on the AFC alone.

Patients with <5 antral follicles prior to the start of the IVF stimulation were considered to be at risk of developing a poor response during their first IVF treatment. When <5 antral follicles of 2–5 mm were present, a recount was done by a second investigator who was not involved in this trial. After confirmation of the low count by the second investigator, the patient was asked to participate in the trial.

The threshold between 4 and 5 antral follicles of 2–5 mm was based on the study by Tomas *et al.* (1997) where patients with <5 follicles measuring 2–5 mm were classified as patients with inactive ovaries. After studying different threshold levels in our own database of AFC, a threshold of <5 follicles appeared to have the lowest error rate for the prediction of poor response (Bancsi *et al.*, 2004).

Poor response was defined as the collection of <4 oocytes at oocyte retrieval or cancellation due to an insufficient reaction to the stimulation with gonadotrophins (<3 follicles on ultrasound). This definition is based on the assumption that at least four oocytes are needed to have an average of two embryos available for transfer, given a mean fertilization rate of 50–60% in IVF (Bancsi *et al.*, 2002). Although there are other possible definitions, many studies use the same definition (Dor *et al.*, 1992; van Hooff *et al.*, 1993; Hanoch *et al.*, 1998; Hugues and Cedrin, 1998; Surrey *et al.*, 1998; Pinkas *et al.*, 2000; El Toukhy *et al.*, 2002). It appears to be the most widely used definition of poor response.

Also patients who were normally excluded from IVF treatment at our centre because of age (41–46 years) or basal FSH (>15 IU/l) could enter this study provided they had <5 follicles on ultrasound. The other inclusion criteria were: a regular spontaneous menstrual cycle of 25–35 days, the presence of both ovaries and written informed consent.

Although there are indications that the bioavailability of gonadotrophins is lower in obese women (Chan *et al.*, 2003), there is no conclusive evidence that body mass index (BMI) is a predictive factor of ovarian response (Popovic-Todorovic *et al.*, 2003). Therefore patients with a high BMI were not excluded from participation in the present study.

Fifty-three women were recruited for this study (Figure 1). In one patient the second investigator could not confirm the poor response AFC category and this patient was not randomized. All patients who were eligible for the study agreed to participate. From these patients, baseline parameters such as age, basal FSH, BMI, cause and duration of infertility were collected from their medical record. They were randomized by opening a sealed envelope that contained information on the starting dose: 150 IU of rFSH (standard dose, group I) or 300 IU of rFSH (study dose, group II). This study was approved by the Institutional Review Board.

Treatment protocol

The stimulation protocol that has been used was a long suppression protocol. In the midluteal phase, pituitary desensitization was started by leuprolide acetate injections (Lucrin; Abbott, The Netherlands). After menstruation, the ovarian stimulation was started with either the standard dose of 150 IU (group I) or the double dose of 300 IU (group II) of follitropin alpha (Gonal-F; Serono Benelux BV, The Netherlands).

The stimulation was monitored using transvaginal ultrasonography and estradiol measurements. In patients who were stimulated with the standard dose of 150 IU of rFSH, the dose was doubled after 7 days of stimulation if the estradiol level was <200 pmol/l or after 10 days if the estradiol level was <500 pmol/l, based on our clinical practice. In group II (starting dose of 300 IU) the dose

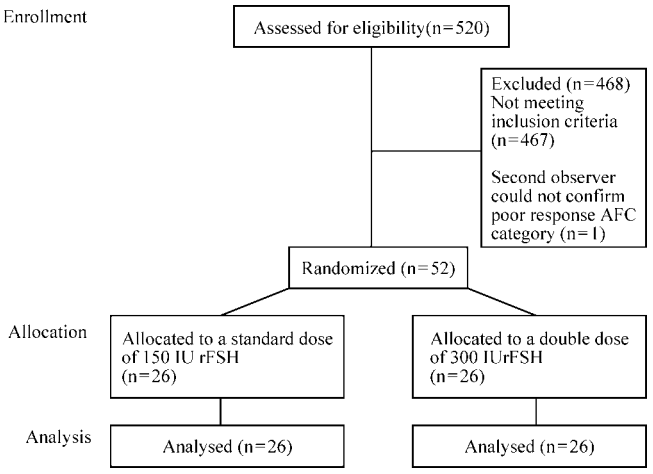

Figure 1. CONSORT flow diagram of the progress of participants through each state of the randomized trial.

remained fixed. hCG [10 000 IU; Profasi; Serono Benelux] was administered 36 h before the transvaginal oocyte collection. If the follicular growth was considered to be insufficient or not present at all, the cycle was cancelled.

The maximum number of embryos replaced was two in women aged <38 years and three in older women. The luteal phase was supported by hCG [Profasi; Serono Benelux] or micronized progesterone (Progestan; Nourypharma BV, The Netherlands). A more detailed description of the protocol used in our centre was published previously (van Kooij *et al.*, 1996).

Statistical analysis

The primary endpoint of this study was the number of oocytes collected at retrieval. Secondary outcome measures were poor response and ongoing pregnancy. We defined ongoing pregnancy as the presence of fetal heart activity on ultrasound at 12 weeks of gestation.

The two treatment groups were compared using the Mann–Whitney *U*-test and the χ^2-test. $P < 0.05$ was considered statistically significant. The difference and 95% confidence interval (CI) of the difference between the number of oocytes per group was estimated with linear regression. In the actual analysis the *P*-value for this test closely resembled the result of the Mann–Whitney *U*-test, meaning that there was no great violation of the assumption of normal distribution. Statistics Package for Social Sciences for Windows, version 10.0 (SPSS Inc., USA) was used for data analysis.

The study was designed to detect a difference of two oocytes with a standard deviation of 3.5 oocytes. For this purpose a total number of 50 cases was needed (power of 80% and a significance level of 5%).

Results

All 52 patients included in this study were eligible for analysis. More than half of the patients were aged >40 years ($n = 28$), three of them had basal FSH levels >15 IU/l. In two patients the basal FSH level was not known. Twenty-four patients were aged <41 years, 10 of them had basal FSH levels >15 IU/l.

Most patients underwent a conventional IVF treatment. Five patients were indicated for an IVF/ICSI procedure. Twenty-six patients received 150 IU (group I) and 26 received 300 IU of rFSH at the start of the stimulation (group II). The two groups were comparable regarding patient characteristics (Table I). In nine of the patients who started with 150 IU, the dose had to be increased to 300 IU due to an insufficient response. Despite this dose adjustment, all these patients remained poor responders according to the definition we applied.

Table II shows the outcome of the IVF treatment. The median number of oocytes collected was 3 for both groups. With linear regression the difference in the mean number of oocytes between the two groups was estimated at 0.3 oocytes in favour of group I (95% CI −1.8 to 1.2; $P = 0.69$). The proportion of low responders did not differ between the two groups.

There were only four pregnancies, three in group I (150 IU) and one in group II (300 IU). These patients had 2, 2, 4 and 6 oocytes at oocyte retrieval. Ongoing pregnancy rates per cycle were 8% in group I and 4% in group II (difference not significant). All pregnant women were aged >40 years, one of them had an elevated basal FSH level of 21.9 IU/l. There were no multiple pregnancies.

There were no cases of ovarian hyperstimulation syndrome in the study group. The highest number of oocytes collected was 10, in two patients in group I. The highest estradiol level on the day of the hCG administration was 12 750 pmol/l (group II).

Table I. Characteristics of patients who received 150 IU rFSH/day after randomization and patients who received 300 IU rFSH/day

Characteristic	Group I 150 IU Gonal-F ($n = 26$)	Group II 300 IU Gonal-F ($n = 26$)	*P*
Age (years)	40.4 (36.6–44.5)	42.2 (33.7–44.6)	0.77[a]
Duration of cycle (days)	28 (24–30)	27 (23.7–28.6)	0.18[a]
Body mass index (kg/m^2)	21.2 (19.4–30.5)	21.8 (19.1–28.1)	0.64[a]
Smoking			0.40[b]
Never	19 (73.1)	12 (54.5)	
Quit	1 (3.8)	1 (4.5)	
Now	6 (23.1)	9 (40.9)	
Duration of infertility (years)	3.0 (1.0–5.0)	3.0 (1.0–5.5)	0.58[a]
Primary infertility	10 (38.5)	10 (38.5)	1.00[b]
Cause of infertility			0.46[b]
Tubal	5 (19.2)	9 (34.6)	
Male	12 (46.2)	10 (38.5)	
Unexplained	9 (34.6)	7 (26.9)	
Basal FSH (IU/l)	9.3 (5.5–22.6)	12.0 (5.8–20.8)	0.24[a]
Antral follicle count	3.0 (2.0–4.0)	3.0 (0.7–4.0)	0.70[a]
ICSI cycles	3 (11.5)	2 (7.7)	0.64[b]

Values are median (10th–90th percentiles) or number (percentage).
[a]Mann–Whitney *U*-test.
[b]χ^2-Test.

Table II. Comparison of the ovarian response and outcome of the IVF treatment of patients who received 150 IU rFSH/day after randomization and patients who received 300 IU rFSH/day

Characteristic	Group I 150 IU Gonal-F ($n = 26$)	Group II 300 IU Gonal-F ($n = 26$)	*P*
Cancels (low response)	5 (19)	6 (23)	0.73[b]
Dose adjustment	9 (35)	NA	NA
Day of hCG	14.0 (9.4–18.2)	13.0 (11.0–17.0)	0.34[a]
Maximum estradiol level (pmol/l)	2706 (200–5889)	2470 (200–6983)	0.48[a]
No. of follicles ≥10 mm	4 (1–8)	3 (0–8)	0.18[a]
Total FSH dose (IU)	2100 (1455–4440)	3600 (3000–4800)	NA
No. of oocytes	3 (1–9)	3 (1–6)	0.79[a]
No. of embryos	2 (0–6)	2 (0–6)	0.86[a]
Fertilization rate (%)	63 (3–100)	50 (0–100)	0.78[a]
No. of embryos transferred	1 (1–3)	2 (1–2)	0.45[a]
Low response	17 (65)	16 (62)	0.77[b]
Clinical pregnancies	3 (12)	1 (4)	0.30[b]
Ongoing pregnancies	2 (8)	1 (4)	0.55[b]

Values are median (10th–90th percentiles) or number (percentage)
[a]Mann–Whitney *U*-test.
[b]χ^2-Test.

Discussion

In this randomized controlled trial it is shown that doubling the starting dose of gonadotrophins in expected poor responders, defined as patients with an AFC <5, does not lead to

an improvement of the response during IVF treatment. The poor response rate in the group treated with a double starting dose did not differ from the group treated with the standard dose. The mean number of oocytes was 0.3 oocytes lower in group II (95% CI of −1.8 to 1.2).

This study was designed to detect at least a difference of 2 oocytes in favour of the high stimulation group, but in fact we found slightly fewer oocytes in this group. To detect this difference, 25 oocyte retrievals in each arm were needed. Because 11 low responders did not proceed to oocyte retrieval, there is a risk that this study became underpowered and conclusions only speculative. However, eight of the 11 patients who were cancelled (three in group I and five in group II) showed absolutely no follicular growth or estradiol rise in response to prolonged stimulation. We can therefore assume that an oocyte retrieval in these patients would have led to the collection of 0 oocytes. If we include these patients in the analysis, the mean number of oocytes is 0.5 lower in group II ($n = 25$) compared to group I ($n = 24$), 95% CI of −2.0 to 0.9. Since there was no tendency at all towards a better response in the high dose group, extension of the sample size was not meaningful in our opinion. We believe that the power of the present study may still be sufficient to prove that the use of a higher starting dose is very unlikely to be beneficial in the type of patients we investigated.

To our knowledge this is the first published randomized controlled trial to compare two different starting doses of gonadotrophins in expected poor responders. There is one randomized study on doubling the dose of hMG in poor responders during the course of an IVF treatment cycle (van Hooff *et al.*, 1993). In this study the hMG dose of 225 IU was continued or doubled after 5 days of stimulation in 47 randomized patients with estradiol levels <500 pmol/l. Doubling the hMG dose did not alter the IVF outcome. Also in our study, increasing the dose from 150 to 300 IU had no effect. In our opinion a strategy of increasing the dose during stimulation is therefore not useful.

Theoretically the follicular response might only improve when expected poor responders are stimulated with a high dose of gonadotrophins right from the start of the stimulation, thereby ensuring stimulation of the entire follicular cohort. The results of our study show that neither response nor the probability of pregnancy improved.

The conclusion of the present study is in line with the outcome of several retrospective studies on dose increase in patients with a poor response in the previous cycle (Karande *et al.*, 1990; Pantos *et al.*, 1990; Manzi *et al.*, 1994; Land *et al.*, 1996; Lashen *et al.*, 1998). In a retrospective analysis performed in our centre, it was shown that expected poor responders have poor cumulative pregnancy rates in IVF (Klinkert *et al.*, 2004). In that study, expected poor responders were defined as patients aged >41 years and/or patients with elevated FSH levels >15 IU/l. Despite a dose increase after the poor results in the first cycle, most of these patients remained poor responders in the subsequent cycles. Stimulation with high doses of gonadotrophins seems to be unrewarding in these patients, since the outcome of the treatment remains poor. However, since poor responders who achieved a pregnancy in their first treatment cycle by definition were not included in these retrospective studies, definite conclusions are impossible.

In two randomized trials comparing low and high starting doses of gonadotrophins in an unselected IVF population, it was concluded that only young patients benefit from a higher starting dose (Out *et al.*, 2000; Yong *et al.*, 2003). Since the ovarian reserve in these patients is usually sufficient, their ovaries have the capability to respond to a higher dose by increasing the number of growing follicles in the FSH-sensitive cohort. In contrast, patients of advanced age or with elevated FSH levels or with a low AFC are likely to have a diminished ovarian reserve. These patients respond poorly to ovarian hyperstimulation, because the number of FSH-sensitive follicles is limited. In such patients aggressive stimulation is not expected to increase the number of follicles because there are simply too few. Several alternative stimulation protocols, such as the flare-up protocol, have been suggested for the treatment of poor responders in IVF. However, the efficacy of such alternative regimes has never been established in large-scale prospective randomized trials (Surrey and Schoolcraft, 2000; Tarlatzis *et al.*, 2003) and in our opinion any strategy in patients with a diminished ovarian reserve is bound to fail.

The patients participating in the present study are expected to respond poorly during their first IVF stimulation because they have a low AFC. But although the AFC appears to be the best predictor of ovarian response during IVF treatment that is available to date, this test is obviously not perfect. Despite the low AFC, one-third of the patients had a normal response to the ovarian hyperstimulation, according to the definition used. Apparently not all recruited FSH-sensitive follicles were already visible in these patients at the time the AFC was performed. However, the median number of oocytes collected in the so-called normal responders was only 5, ranging from 4 to 10, indicating that the response in most of these patients was very moderate. Moreover, it is unlikely that the presence of patients with a normal response despite a low AFC has a predominant effect on the outcome of this study. Since it is a randomized trial, the distribution of these patients over the two groups is only determined by chance.

Basal FSH values obtained during the infertility work-up were significantly higher in the poor responders, therefore we wondered whether addition of basal FSH and/or age to the AFC would have improved the prediction of poor response in our study group. To evaluate this we performed a *post hoc* regression analysis. Univariate analysis of these two variables showed no significant association with poor response [odds ratio for FSH 1.10 (CI 0.98–1.22; $P = 0.10$) and for age 1.02 (0.87–1.20; $P = 0.85$)]. In a multivariate analysis they were both not selected, indicating that their additional value probably would have been limited.

In conclusion, this study suggests that using a high dose of gonadotrophins in patients with an expected poor response in IVF is unrewarding, since both response and ongoing pregnancy rate cannot be expected to improve. Although difficult to perform, a larger study is recommended to confirm these conclusions.

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