Article

Young patients with diminished ovarian reserve undergoing assisted reproductive treatments: a preliminary report

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Abstract

Young assisted-reproduction patients with diminished ovarian reserve (DOR) are one of the most challenging issues for IVF specialists. A retrospective study of 70 assisted reproduction patients younger than 35 years with DOR determined based on antral follicle count was conducted, investigating: (i) correlation of day 3 FSH measurement with antral follicle count; and (ii) cycle outcome of young DOR patients compared with 53 young assisted reproduction patients with normal ovarian reserve (NR). DOR was considered as antral follicle count of <6 per ovary. Day 3 FSH in the DOR group was significantly higher than in the NR group (8.3 and 6.6 mIU/ml respectively; $P < 0.05$). Implantation rates between the groups were similar (15% in DOR and 18% in NR). Pregnancy rate was 35.8% in the DOR group, significantly lower than that of the NR group, which was 54.7% ($P = 0.028$). Although the pregnancy rate was significantly lower in the DOR group compared with the NR group, the statistically insignificant difference in implantation rates demonstrated that the problem in young DOR patients was mainly the number of retrieved oocytes. Therefore, such couples should be informed that lower oocyte numbers will result in statistically lower, but still encouraging, pregnancy rates. Basal FSH should also be measured during evaluation as an adjunct to antral follicle count.

Keywords: *antral follicle count, assisted reproduction, basal FSH, diminished ovarian reserve, IVF outcome, young age*

Introduction

Although the chronological age of a woman undergoing an IVF cycle for infertility treatment is known to be an important factor affecting the outcome, another important factor demonstrated to affect success is biological age, i.e. ovarian age (Roseboom *et al*., 1995; Templeton *et al*., 1996). Many published reports state that ovarian age is an independent variable strongly affecting IVF outcome, while chronological age plays a less important prognostic role (Scott and Hofmann, 1995). Diminished ovarian reserve (DOR), occurring at any age, is a disappointing issue in reproductive medicine. A reduced number of ovarian follicles and probably poorer oocyte quality are the leading problems responsible for reduced pregnancy rates in DOR patients undergoing assisted reproduction. Recognition of patients with DOR prior to initiation of ovarian stimulation allows clinicians to inform patients about their increased risk for lower pregnancy success, and to optimize and individualize the stimulation protocol and adjust the starting dose of gonadotrophins (Fratterelli *et al*., 2000; Ubaldi *et al*., 2005).

Young DOR patients are assumed to have a depleted ovarian oocyte pool and present management challenges in IVF practice. Some investigators have proposed that the poor prognosis for IVF was not valid for young patients (≤30 years) with DOR, and a better outcome should be expected in these patients because their young age protects them from adverse effects of reduced ovarian reserve (Hanoch *et al*., 1998).

Basal ovarian antral follicle count performed by transvaginal sonographic examination has been suggested as the most reliable test of ovarian reserve, and therefore the best single predictor of poor ovarian response in assisted reproduction (Tomas *et al.*, 1997; Chang *et al.*, 1998; Fratterelli *et al.*, 2000; Ng *et al.*, 2000; Pohl *et al.*, 2000; Nahum *et al.*, 2001; Bancsi *et al.*, 2002; Loverro *et al.*, 2003).

Various hormonal markers have also been used to predict ovarian response. One of those markers suggested to signal declining ovarian function prospectively is cycle day 3 FSH concentration (Scott *et al.*, 1989; Barnhart *et al.*, 1998). Basal FSH concentration is an indirect estimate of ovarian reserve, being a measure of the magnitude of negative feedback exerted on the pituitary by ovarian inhibin and oestradiol secretion (Buckler *et al.*, 1991). Many investigators have demonstrated that an elevated cycle day 3 (basal) serum FSH concentration was a better predictor of poor IVF outcome than chronological age alone (Scott *et al.*, 1989; Toner *et al.*, 1991; Cahill *et al.*, 1994). Although basal FSH values were demonstrated to have intercycle variability, especially in poor responders, patients with any previous history of an elevated day 3 FSH measurement were observed to be associated with decreased ovarian responsiveness and poor outcome with subsequent stimulations (Scott *et al.*, 1990; Brown *et al.*, 1995; Esposito *et al.*, 2002).

A retrospective, descriptive study of young assisted reproduction patients was conducted whose ovarian reserve was identified as diminished by transvaginal sonographic evaluation of ovarian follicles. This study was designed to investigate the correlation of cycle day 3 FSH values with the ovarian grade already assigned by the antral follicle count and the clinical value of basal FSH measurement in the management of young DOR patients. It was also intended to review the IVF outcome of young DOR patients and compare the clinical parameters with those of patients with a normal ovarian reserve.

Materials and methods

Seventy ovarian stimulation cycles in infertile patients younger than 35 years of age with diminished ovarian reserve who had undergone an assisted reproduction cycle and oocyte retrieval between January and December 2003 were analysed. Ovarian reserve was ascertained by transvaginal sonographic evaluation of the population of follicles ready to be recruited during the initial follicular phase of a natural cycle. Patients were initially examined with transvaginal ultrasound (Sonoline Adara SLC, 7.5 MHz vaginal probe; Siemens, Germany) and assigned ovarian grades based on total antral follicle counts per ovary; fewer than 4 antral follicles per ovary was classified as grade I, 4–6 as grade II, 6–10 as grade III, and more than 10 as grade IV and typical polycystic ovaries separately. Diminished ovarian oocyte reserve was established as ovarian grades I and/or II (Ng *et al.*, 2000). A control group of the same age range comprising 53 infertile patients with ovarian grades III and/or IV (NR patients) who had undergone an assisted reproduction cycle during the same time period was assigned. Couples who had had previous ovarian surgery, previously diagnosed ovarian endometriomas, polycystic ovarian syndrome and severe male infertility factors were excluded both from the study and control groups. Infertility aetiologies of the patients in the two groups are shown in **Table 1**.

Blood was drawn from all patients on day 3 of the preceding cycle for measurement of serum FSH. Serum FSH was analysed using a commercially available enzyme immunoassay kit (Abbott Laboratories, Abbott Park, IL, USA). The intra- and inter-assay coefficients of variation were 5.2 and 6.7% respectively. The upper limit of normal for FSH in the laboratory is 12 mIU/ml.

Ovarian stimulation was performed in young DOR patients with short gonadotrophin-releasing hormone (GnRH) agonist flare-up or antagonist protocols. The policy of this clinic is to use a short protocol in patients with low antral follicle counts and a long protocol in those with normal ovarian reserve determined during initial examination. In the short GnRH agonist flare-up protocol, the oral contraceptive pill was commenced on day 3 of the preceding cycle and continued for 21 days. On day 3 after the last pill, leuprolide acetate (Lucrin; Abbott, Cedex, France) at a dose of 40 μg daily was initiated and gonadotrophins were added 2 days later. GnRH agonist was administered at the same dose until human chorionic gonadotrophin (HCG) injection. In the GnRH antagonist protocol, gonadotrophins were started on day 3 of menstruation and GnRH antagonist at a dose of 0.25 mg daily was added when the leading follicle reached 14 mm in size and continued until HCG administration. Since increasing the dosage of gonadotrophins beyond six ampoules (450 IU) daily was demonstrated not to improve pregnancy rates, stimulation doses were also limited to six ampoules per day (Karande *et al.*, 1990). Mid-luteal phase GnRH agonist long protocol was used for ovarian stimulation of young NR patients. Women were given leuprolide acetate (Lucrin; Abbott, Cedex, France) at 0.1 mg daily dose starting on day 21 of the previous cycle for about 2 weeks, and the dose was halved and gonadotrophins were added when down-regulation was confirmed by ultrasound and serum oestradiol evaluation. Initial doses of gonadotrophins and adjustments thereafter were determined according to the patient's profile and ovarian response. When the leading follicle reached 20 mm in diameter, 10,000 IU of HCG was administered and transvaginal oocyte retrieval was performed 36 h later. IVF–intracytoplasmic sperm injection (ICSI) procedures were performed as described previously (Kahraman *et al.*, 1999). Embryos were graded (grade I being the best quality) according to the symmetry of the blastomeres and the presence or absence of fragmentation (Veeck, 1991) The luteal phase was supplemented with progesterone in oil 75 mg i.m. daily, starting on the day after oocyte retrieval, and was continued until a negative pregnancy test was obtained, or if pregnancy occurred, with vaginal progesterone (Progestan; Koçak, Turkey) 600 mg

Table 1. Distribution of causes of infertility by number and percentage. DOR = diminished ovarian reserve; NR = normal ovarian reserve. There were no statistically significant differences between the two groups.

	Young DOR patients	*Young NR patients*
Tubal factor	14 (20)	11 (21)
Male subfertility	31 (44)	23 (43)
Unexplained infertility	21 (30)	14 (27)
Mixed	4 (6)	5 (9)

Values in parentheses are percentages.

daily until week 12 of gestation. Pregnancy was confirmed by a positive blood test for β-HCG 12 days after the transfer procedure. Stimulation cycle characteristics of patients in the two groups were also compared and clinically useful points were attested.

Data were analysed by using the Statistical Program for Social Sciences version 11.0 for Windows (SPSS Inc., Chicago, IL, USA). Student's *t*-test and chi-squared test were used when appropriate. $P < 0.05$ was considered significant.

Results

Assisted reproduction cycles of 70 young patients with diminished ovarian reserve and those of 53 young patients with normal ovarian reserve were analysed. Mean initial day 3 FSH concentration in young DOR patients was found to be significantly higher than that of young NR patients (8.3 and 6.6 mIU/ml respectively; $P < 0.05$). When stimulation cycle characteristics were inspected, significantly more ampoules of gonadotrophins were used in young DOR patients compared with young NR patients (3867 versus 2533 IU; $P < 0.0001$). Peak serum oestradiol concentrations (1834 versus 3051 pg/ml), total number of oocytes obtained (7.3 versus 16.6), MII oocyte ratio (68% versus 74%), number of embryos transferred (2.8 versus 3.8) and number of grade I embryos transferred (1.9 versus 3.2) were significantly lower in young DOR patients compared with young NR patients ($P < 0.05$). Similar fertilization rates were found between the two groups (76% in young DOR group and 78% in young NR group). Implantation rates between the groups were not significantly different (15% in young DOR and 18% in young NR patients). The pregnancy rate in young DOR patients was 35.8%, which is quite promising but significantly lower when compared with the value of control group, which was 54.7% ($P = 0.028$). For ongoing pregnancy rate and clinical abortion rate, no difference was found between the two groups (77.3 and 22.7% in young DOR patients and 85.7 and 14.3% in young NR patients respectively).

The results of ovarian stimulation and the IVF outcomes of young DOR and young NR patients are presented in **Table 2**.

Assisted reproduction cycle characteristics of young patients with diminished ovarian reserve were stratified by the number of total oocytes retrieved and by basal FSH concentrations. Although the patient numbers are very limited, subanalyses of those patients with ≤4 total oocytes retrieved and with basal FSH values ≥12 mIU/ml are demonstrated in **Table 3**.

Table 2. Assisted reproduction treatment cycle characteristics of patients with decreased ovarian reserve (DOR) and normal ovarian reserve (NR). Values are means ± SD, NS = not significant; BMI = body mass index; HMG = human menopausal gonadotrophin, HCG = human chorionic gonadotrophin.

	Young DOR patients (n = *70)*	*Young NR patients* (n = *53)*	P-*value*
Age (years)	31.0 ± 2.8	29.4 ± 3.5	NS
Infertility duration (years)	6.9 ± 3.5	7.2 ± 3.3	NS
BMI (kg/m^2)	23.8 ± 3.4	25.0 ± 4.2	NS
Basal FSH concentration (mIU/ml)	8.3 ± 4.3	6.6 ± 1.8	<0.05
Total HMG/FSH used (IU)	3867 ± 1358	2533 ± 822	<0.0001
HCG day	12.3 ± 2.5	12.1 ± 1.2	NS
Oestradiol on HCG day (pg/ml)	1834 ± 1002	3051 ± 1094	<0.0001
No. of oocytes retrieved	7.3 ± 4.0	16.6 ± 5.2	<0.0001
No. of MII oocytes	5.0 ± 2.9	12.3 ± 4.6	<0.0001
MII oocyte rate (%)	68 ± 20	74 ± 14	0.04
Fertilization rate (%)	76 ± 24	78 ± 17	NS
No. of embryos transferred	2.8 ± 1.4	3.8 ± 1.1	<0.0001
No. of grade I embryos transferred	1.9 ± 1.3	3.2 ± 1.4	<0.0001
Implantation rate (%)	15	18	NS
Pregnancy rate/embryo transfer (%)	35.8	54.7	<0.05
Clinical abortion rate (%)	22.7	14.3	NS
Ongoing pregnancy rate (%)	77.3	85.7	NS

Table 3. Patients stratified according to total oocyte number and basal FSH concentration. Values are means.

	Total oocyte number retrieved ≤4 (n = 17)	*Basal FSH ≥12 mIU/ml (n = 13)*
Age (years)	31.9	32.5
Basal FSH concentration (mIU/ml)	9.2	15.1
Total HMG/FSH used (IU)	3119	3955
No. of oocytes retrieved	3	6.3
No. of MII oocytes	1.8	3.9
Fertilization rate (%)	81	78
No. of embryos transferred	1.5	2.1
Implantation rate (%)	20	18.5
Pregnancy rate/embryo transfer[a] (%)	23.5	30

[a]Two spontaneous pregnancies were seen during the 6 months following the treatment cycle in each of the two patient groups.

Discussion

The aim of this study was to draw attention to one of the most challenging issues of assisted reproduction, which is young patients with reduced ovarian reserve. In those young DOR patients, the number of follicles ready to respond to gonadotrophins is limited. Therefore, using different stimulation regimens or increasing the dosage of gonadotrophins beyond 450 IU/day (six ampoules daily) to improve the response of those DOR patients was proposed by some as unsuccessful (Karande *et al*., 1990; Land *et al*., 1996).

Total antral follicle count determination by transvaginal ultrasound has been suggested as the best single marker of ovarian reserve in predicting poor response to IVF treatment (Pache *et al*., 1990; Tomas *et al*., 1997; Chang *et al*., 1998; Ng *et al*., 2000; Bancsi *et al*., 2002). Chang *et al*. (1998) reported a positive correlation between the number of antral (2–5 mm) follicles and IVF outcome. It was suggested that the antral follicle number was a good predictor of the ovarian response in IVF, although not of the pregnancy rate (Chang *et al*., 1998; Ng *et al*., 2000). In contrast, patients with lower antral follicle counts in this study were found to have a lower pregnancy rate when compared with those with normal ovarian reserve, which is also the result of lower ovarian response to ovulation induction.

Some authors revealed that antral follicle determination might reliably be performed before or after down-regulation (Hansen *et al*., 2003). Intercycle variations in antral follicle counts might also exist as for basal FSH concentrations, but it was shown that repeated antral follicle count determinations to identify the optimal cycle for stimulation did not improve the predictive ability of this parameter (Bancsi *et al*., 2004; Kwee *et al*., 2004).

With ovarian reserve determinations, it is best to estimate egg quantity but not egg quality, which cannot be evaluated beforehand and is an important determinant of pregnancy rate. In one study, it was stated that young patients with low antral follicle counts might be regarded as having diminished ovarian reserve, but they should not be restricted from treatment because they probably still had good oocytes (van Rooij *et al*., 2003). It was found the MII oocyte rate was statistically lower in the young DOR group ($P = 0.04$), but probably again as the result of lower total oocyte numbers retrieved. Galey-Fontaine *et al*. (2005) also proposed that even if the ovarian response to stimulation was low, patients younger than 36 years should proceed to oocyte retrieval. The present study agrees with the above-mentioned investigators that taking into account the pregnancy rate of 35% in young DOR patients, it is better for them to continue with assisted reproduction since their young age is an advantage, although they are disadvantaged by their poor ovarian response. In support, Van Rooij *et al*. (2003) illustrated that once eggs were retrieved from younger women with reduced ovarian reserve, they had near-normal implantation and pregnancy rates. Poe-Zeigler *et al*. (1994) and Lashen *et al*. (1999) also previously drew attention to the same point. It was reported that the main determinant of IVF success in young poor responders was the number of oocytes retrieved, and hence the number of embryos available for transfer (De Sutter and Dhont, 2003; Ulug *et al*., 2003). Contrarily, some investigators proposed that young age did not protect against the adverse effects of reduced ovarian reserve and their poor outcome with IVF resulting from their age-independent decline of ovarian reserve should be emphasized (Hanoch *et al*., 1998; Nasseri *et al*., 1999; Akande *et al*., 2002; El-Toukhy *et al*., 2002).

Some studies have concluded that antral follicle count evaluation predicted IVF outcome more reliably than basal FSH concentration (Nahum *et al*., 2001; Bancsi *et al*., 2002). From this observation, it was intended to investigate the correlation of basal FSH determination with antral follicle evaluation. To decide whether a young woman has a low chance of success with IVF treatment before initiation of the cycle, and to manage her treatment accordingly, needs strong evidence, and thus

combining two proposed predictors of outcome, antral follicle count assessment and basal FSH determination, would be fair. It was suggested that basal FSH concentration should not be a tool to select patients for IVF treatment; instead, it should be used as additional information to counsel patients appropriately (Bancsi *et al*., 2003; Abdalla and Thum, 2004). Significantly higher FSH concentrations were found ($P < 0.05$) , despite being within the normal range, in the young DOR patient group. Thus, a correlation was shown between ovarian grade, i.e. antral follicle counts, and FSH concentrations, the clinical value of which might be to provide additional information in the management and counselling of young patients with low antral follicle counts.

Several investigators have demonstrated that even within the normal range, pretreatment day 2–4 serum FSH concentrations in IVF patients, independent of age, was closely related to the inverse of the number of follicles ready to be stimulated, as well as to the number of oocytes obtained (Toner, 1993; Cahill *et al*., 1994; Fanchin *et al*., 1994; Hansen *et al*., 1996; Kim *et al*., 1997; Sharif *et al*., 1998). Therefore, it has been argued that the lack of success of IVF in women with high basal FSH concentrations is largely due to low numbers of available embryos, from which selection for transfer is limited, whilst the age-related effect is due to declining implantation rates (Toner, 1993). In the present study, significantly lower ($P < 0.0001$) numbers of embryos were transferred in young DOR patients because of fewer available embryos, but implantation rates were found to be not statistically different from those of NR patients.

Biological ageing of the ovaries can occur independently of chronological age. Akande *et al*. (2002) demonstrated that biological ageing of ovaries was indicated by rising basal FSH concentrations, and even while still within the normal range, this rise was associated with declining oocyte quality, as indicated by reduced implantation. In agreement, El-Toukhy *et al*. (2002) proposed that young patients with elevated FSH concentrations had lower implantation rates after IVF and thus performed as poorly as older patients with normal FSH, that is their young age did not protect against the adverse effects of reduced ovarian reserve. In contrast to those proposals, in another study, it was concluded that an elevated basal FSH concentration did not indicate deterioration of oocyte and embryo quality, and high FSH therefore did not reflect ageing oocytes; it was just that fewer were produced (Abdalla *et al*., 2004). This has also been suggested in several other studies (Check *et al*., 2002; Esposito *et al*., 2002; van Rooij *et al*., 2003). The present study confirms the findings of Abdalla *et al*. (2004) and contradicts the findings of studies mentioned above (Akande *et al*., 2002; El-Toukhy *et al*., 2002). The results of the present study clearly show that young (<35 years) IVF patients with decreased ovarian reserve have higher basal FSH values when compared with their peers with normal ovarian reserve, and young DOR patients have similar implantation rates to NR patients and encouraging pregnancy rates, although they have a low response to stimulation. Some other published reports also indicate that young women with elevated FSH concentrations can achieve reasonable pregnancy rates with assisted reproduction (van Montfrans *et al*., 2000; van Rooij *et al*., 2001).

In conclusion, this study has found that: (i) although not suggested as a sensitive tool, basal FSH determination is an informative test in counselling young IVF patients with DOR. In fact, it should be used to complement sonographic antral follicle evaluation of such young patients and should be used as an adjunct in presenting information regarding the IVF outcome frankly to the patient; (ii) young DOR patients have a low response to stimulating drugs, but these women have extremely encouraging pregnancy rates. Therefore, this should be kept in mind while counselling and managing young IVF patients.

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