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OVARIAN RESPONSE

Poor ovarian response in patients younger than 35 years: Is it also a qualitative decline in ovarian function?

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Abstract

Objective. To investigate whether poor response to controlled ovarian stimulation (COS) is due to a qualitative decline in ovarian function.

Methods. This retrospective cohort study included 436 patients younger than 35-years old, undergoing COS for intracytoplasmic sperm injection (ICSI). Patients with four or fewer MII oocytes after COS (poor-responder group, PR, $n = 52$) were age-matched with normoresponder patients (NR, $n = 364$).

Results. Although similar duration of stimulation (10.5 ± 0.4 and 9.3 ± 0.8 days; $p = 0.1358$), increased doses of gonadotrophins (2510 ± 865 and 2253 ± 572 IU; $p = 0.0061$) were used in the PR. The results show a increased chance of cycle ending of PR (PR: 26.9% and NR: 3.1%; $p < 0.0001$). Although the lower total number of oocytes retrieved (2.4 ± 1.4 and 16.2 ± 9.3; $p < 0.0001$), equal rate of fertilization (70.2% and 72.0%, $p = 0.1190$) and high quality embryos were obtained (50.0% and 45.2%; $p = 0.4895$), resulting in similar implantation (14.5% and 19.7%; $p = 0.2246$) and abortion (10.0% and 15.4%; $p = 1.00$) rates, respectively. A trend towards increased pregnancy rate per embryo transfer in NR group was noted (PR: 26.3% and NR: 42.2%; $p = 0.0818$).

Conclusions. Low ovarian response could be associated mainly with a quantitative rather than a qualitative decline in ovarian function. Therefore, even if the ovarian response to stimulation is low, patients aged $\leq$35 years should process to oocyte retrieval.

Keywords: *ICSI, ovarian stimulation, oocyte retrieval*

Introduction

Women have a finite number of germ cells whose number progressively diminishes through an irreversible process of follicular atresia. The rate of follicular attrition is not constant but rather follows an exponential pattern (Faddy et al., 1992). Premature reduction of ovarian follicle number has been demonstrated to significantly affect the success of assisted reproduction techniques (ART), despite the chronological women age (Templeton et al., 1996).

A dynamic assessment of the ovarian reserve could be associated with the way a woman's ovaries respond to stimulation with gonadotrophins during *in vitro* fertilization (IVF) treatment (Nikolaou et al., 2002; Ubaldi et al., 2005). It has been estimated that among patients undergoing IVF treatment the prevalence of poor ovarian response is 9–24% (Keay et al., 1998).

Garcia et al. (1983) first describe a poor responder as the patient with peak estradiol (E_2) levels < 300 pg/mL after a standard stimulation with hMG. Since then, other quantitative parameters have been brought into clinical use to define and categorize poor responders without consensus. Poor responders have been defined on the basis of mature oocytes (Lashen et al., 1999), elevated early follicular phase of FSH peak (Esposito et al., 2002), number of basal antral follicle (Loverro et al., 2003), number of follicle at the end of ovarian stimulation (Ulug et al.,

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2003), plasma oestradiol concentration (Surrey et al., 1998) or number of oocytes retrieved (Kumbak et al., 2009).

Several factors could be associated with reduced ovarian response to controlled ovarian stimulation (COS) in either older or younger patients with early ovarian ageing (Nikolaou et al., 2002). Young assisted reproduction patients with diminished ovarian reserve is a disappointing issue in reproductive medicine. Those patients are usually stimulated with high doses of gonadotrophins and a small number of follicles develop (Tarlatzis et al., 2003). The course of treatment often reaches a point where the dilemma is whether to carry on or cancel a cycle (Ulug et al., 2003; van der Gaast et al., 2006).

Some investigators have proposed that oocyte quality is established during fetal life, and oocytes that are less susceptible to non-disjunction are ovulated first, leaving poor quality oocytes to be ovulated later in life (Gougeon, 1996). For this reason, poor prognosis for IVF would be related to decline in ovarian follicle number than to age. On the other hand, experimental data in women demonstrated that an increased frequency of meiotic non-disjunction occur at female ovary as time goes by. This is the mechanism responsible for the majority of aneuploidies in early embryos suggesting that ovarian reserve is a better predictor of oocyte production capacity than oocyte quality, whereas age affects oocyte quality (Hanoch et al., 1998; Eldar-Geva et al., 2003).

Therefore, this study summarizes the results of assisted reproduction treatment in poor responder patients younger than 35 years compared with those age-matched normoresponder (NR). The purpose is to evaluate whether the poor response to COS is due to a reduced ovarian reserve (reflected by doses of gonadotrophins used, oestradiol concentration, number of eggs collected and cycle cancellation rate) rather than poor oocyte quality (reflected by ICSI outcomes).

Materials and methods

Patients

Data of intracytoplasmic sperm injection (ICSI) cycles performed in 416 patients younger than 35 years old were included in this retrospective cohort study. All cases of surgically retrieved sperm were excluded from the study. Patients who produced four or less MII oocytes (poor-responder, PR group, n = 52) after COS were age matched with NR patients in which five or more oocytes were retrieved (NR group, n = 364). A written informed consent was obtained, in which patients agreed to share the outcomes of their own cycles for research purposes, and the study was approved by local institutional review board.

Ovarian stimulation and oocyte retrieval

COS was achieved by long pituitary down regulation using a gonadotropin-releasing hormone agonist (GnRHa, Lupron Kit™, Abbott S.A Societé Française des Laboratoires, Paris, France) followed by ovarian stimulation with recombinant-FSH (Gonal-F®, Serono, Geneve, Switzerland). The follicular dynamic was followed by ultrasound starting on day 4 of gonadotropin administration. When adequate follicular growth and serum estradiol levels were observed, recombinant human chorionic gonadotrophin (r-hCG, Ovidrel™, Serono, Geneve, Switzerland) was administered to trigger final follicular maturation. Oocytes were collected 35 hours after hCG administration by transvaginal ultrasound ovum pick-up.

Oocytes were stored in human tubal cultured medium (HTF, Irvine Scientific, Santa Ana, USA) supplemented with 10% Human Synthetic Albumin (HSA, Irvine Scientific, Santa Ana, USA) covered with oil (Ovoil™, Vitrolife, Kungsbacka, Sweden) at 37°C in 6% CO_2 for 5 h, before cumulus cell removal. Cumulus cells were removed from the oocytes by placement HEPES buffered-medium containing hyaluronidade (80IU/mL Irvine Scientific, Santa Ana, USA). The remaining cumulus cells surrounding were then removed by gently pipetting with a hand draw Pasteur pipette (Humagen Fertility Diagnostics, Charlottesville, USA).

ICSI sperm injection procedure

Intracytoplasmatic sperm injection was performed in MII oocytes according to the technique described by Palermo et al. (1992). Oocytes were transferred into the micro-injection dish, prepared with drops of HEPES-buffered HTF (Irvine Scientific, Santa Ana, USA) covered under oil and placed on a heated stage of an inverted microscope. Approximately 16 h after ICSI, fertilization was confirmed by the presence of two pronuclei and the extrusion of the second polar body. Embryos were kept in a 50 μL drop of HTF medium supplemented with 10% HAS under oil, in a humidified atmosphere of 5% CO_2 in air, at 37°C, until transfer.

Embryo transfer was performed on the second or third day of development. For each couple, one to four embryos were transferred.

Clinical follow-up

Serum β-hCG levels were assessed for the first time 12 days after replacement of the embryos. Clinical

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pregnancy was defined when a transvaginal ultrasound scan, performed 3–4 weeks after embryo transfer, revealed the presence of a gestational sac. To calculate the implantation rate, the number of gestational sacs was divided by the number of embryos transferred. Miscarriage was defined as the spontaneous abortion before 20 weeks' gestation.

Data analysis

The two groups were compared with regard to: (i) age; (ii) total gonadotrophin dose (IU); (iii) duration of gonadotrophin stimulation (days); (iv) oestradiol concentration on day of hCG (pg/mL); (v) oocyte yield (no. of retrieved oocytes / no. follicles); (vi) metaphase II oocyte rate (MII oocyte / total number of retrieved oocyte); (vii) percentage of high quality embryos on the third day of development (no. of high quality embryos / no. of fertilized MII oocytes); (viii) normal fertilization (no. of zygote showing two clearly distinct pronuclei/no. of injected oocytes); (ix) cycle cancellation (no. of embryo transfers/no. of initiated cycles); (x) pregnancy rate; (xi) implantation and (xii) miscarriage rates.

High quality embryos were defined as those showing 6–8 cells on the third day of development; less than 15% fragmentation; symmetric blastomeres; absence of multinucleation and absence of zona pellucida dysmorphism.

Statistical analysis

Results were expressed as mean ± standard deviation for numeric variables, while proportions (%) were used for categorical variables. Mean values were compared by Student's *t* parametric test or Mann–Whitney non-parametric test, accordingly Gaussian distribution. Proportions were compared by the Chi-squared or Fisher exact test, only when expected frequency was five or fewer. Results were considered to be significant at the 5% critical level ($p < 0.05$). Data analysis was carried out using GraphPad Prism version 4.0 Statistical Program.

Results

The range of maternal age was 25–35 years. Although similar duration of gonadotrophin stimulation between the two groups; significantly increased doses of gonadotrophins were used in the PR group. Oestradiol concentration on day of hCG and oocyte yield were significantly lower in PR group.

The oocyte retrieval rate was significantly lower in the PR group, however, the MII oocyte rate was found to be similar (Table I). Normal fertilization rate was found to be similar between the two groups. Although the higher cycle ending without embryo transfer and the lower number of embryos transferred (1.9 ± 1.2 *versus* 3.1 ± 1.1; $p < 0.0001$), poor responder patients presented similar percentage of high quality embryos on the third day of development.

A trend towards increased pregnancy rate per embryo transfer in NR group was also noted, but did not reach statistical significance. On the other hand, implantation rate between the two groups were not significantly different. Likewise, no difference was found between the two groups for ongoing pregnancy rate and abortion rate. The results of ICSI cycles outcomes of poor responder patients are summarized in Table II.

Discussion

Despite the reasonable percentage of women undergoing infertility treatment that respond poorly to the usual gonadotrophin stimulation protocol, there is no uniformity in the definition of the poor response. The number of developed follicles and/or number of oocytes retrieved after an ovarian stimulation protocol are, hierarchically, two of the most important criteria for defining poor ovarian response.

Previous reports have proposed different numbers of MII retrieved oocytes after ovarian stimulation to define a poor responder patient and it ranges from less than three to less than five retrieved oocytes (Rombauts et al., 1998; Surrey et al., 1998). With

Table I. Stimulation cycles characteristics in a group of poor responder patients younger than 35 years (PR group) compared with those age-matched normoresponders (NR group).

Variable	Study group		*P* value
	PR ($n = 52$)	NR ($n = 364$)	
Gonadotrophin stimulation period (days)	10.5 ± 0.4	9.3 ± 0.8	0.1358*
Total gonadotrophin dose (IU)	2510 ± 865	2253 ± 572	0.0061†
Oestradiol on day of hCG (pg/mL)	807 ± 838	2433 ± 1804	<0.0001*
No. of retrieved oocytes/No. follicles (%)	51.4	70.2	<0.0001*
MII oocyte / total number of retrieved oocyte (%)	66.8	73.3	0.2741*

Values expressed as mean ± SD, unless otherwise noted.
*Statistical Mann–Whitney test.
†Student's *t*-test.

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Table II. Intracytoplasmic sperm injection cycles outcomes in a group of poor responder patients younger than 35 years (PR group) compared with those age-matched normoresponders.

Variable	Study group PR ($n=52$)	NR ($n=364$)	P value
Normal fertilization rate	70.2	72.0	0.1190⋆
Rate of high quality embryos	50.0	45.2	0.4895⋆
Cycle cancellation rate	26.9	3.1	$<0.0001^{\dagger}$
Pregnancy rate	26.3	42.2	$0.0818^{\dagger}$
Implantation rate	14.5	19.7	0.2246⋆
Abortion rate	10.0	15.4	$1.0^{\dagger}$

Values are in percentages.
⋆Statistical Mann–Whitney test.
†Fisher exact test.

respect to our study, we choose to assign patients with less than four MII retrieved oocytes in the poor responder group.

In this present study, poor responders' patients used significantly higher gonadotrophins doses when compared with age-matched NRs stimulated during similar interval of days. Ovarian stimulation protocols are employed to stimulated multifollicular growth and to allow the retrieval of multiple oocytes. In most cases, when submitted to COS protocols, patients with a low number of antral follicles often receive a higher initial dose of gonadotrophins. In addition, when the standard dose of gonadotrophins fails to induce a proper multifollicular growth, the expected clinical approach is to increase the dose (Surrey & Schoolcraft, 2000). As a result, the total dose of gonadotrophins is significantly higher in poor responder patients when compared with age-matched NRs stimulated during similar interval of days. There is, however, increasing evidence that excessive ovary stimulation may have detrimental effects on oocyte and embryo quality and endometrial receptivity (Karande et al., 1990; Simon et al., 1995; Land et al. 1996; Bourgain & Devroey, 2003; Greb et al., 2005).

Although the lower total number of oocytes retrieved, poor responder patients have similar MII oocytes ratio, fertilization, high-quality embryos and implantation rates. Even though it has been described that the quality of sperm plays a key role during fertilization (Swann et al., 2006; Saunders et al., 2007), investigation of oocytes remaining unfertilized revealed that missing or disturbed oocyte activation may be the main cause of fertilization failure after ICSI (Sousa & Tesarik, 1994). Furthermore, the expression of the embryonic genome, which is a combination of the sperm and oocyte contribution, starts between the four- and eight-cell stage of human embryo development (Tesarik et al., 1986, 1988). Therefore, the oocyte is essential for the embryos development until the third day (i.e., 72 h after ICSI), when embryonic genome activation should have occurred. Together with the above cited studies, the fertilization and high quality embryo's rates observed in our study suggest that in young patients the poor ovarian response may be rather due to a decreased ovarian reserve than a poor oocyte quality.

The reason why, in spite of similar fertilization, high-quality embryos and implantation rates, a trend toward increased pregnancy rate per embryo transfer was observed in the NR group, may be explained by the higher number of available embryos. In fact, the number of embryos transferred was significantly lower in PR group and the selection for transfer was limited. Nevertheless, implantation rate in PR group was found to be not statistically different from those of NR patients indicating that the low oocyte yield has no impact upon the biological capacity of those eggs. In addition, our results show that young poor responder patients have a significantly increased chance of the cycle ending without embryo transfer. However, the similarity in the implantation rate, among the groups, strengthens the argument against cancel the cycles of young poor responders. In addition, once the pregnancy is achieved, similar abortion rates were observed in PR patients when compared with age-matched NRs.

During follicular development, the vast majority of follicles will go to atresia either by apoptosis or necrosis at some stage of development. The criteria for determining the number and selection of follicles which are removed from the pool become less stringent with increasing age. Assuming that follicle atresia plays a role in determining the overall quality of follicles which reach the final stage of development, this implies a reduced quality control of follicles in older women (van Rooij et al., 2003). A diminished ovarian reserve in young women could be caused by a genetically regulated mechanism of higher intrinsic rate of atresia. Previous ovary surgery (Nargund & Bromhan, 1995), severe endometriosis (Wardle et al., 1985), post infectious adhesions (Keay et al., 1998), chemotherapy (Nargund & Bromhan 1995) and smoking (Sharara et al., 1994) are known factors that can also affect the ovarian reserve (De Sutter & Dhont, 2003). Therefore, oocytes of poor responders may be of good quality although they are the last oocytes available from the ovarian pool. In addition, young PR patients could be protected from some age-related deleterious effects (Hanoch et al., 1998; El-Toukhy et al., 2002).

Another possibility is that some patients have had a destructive process that left behind fewer follicles but the same proportion of good quality oocytes (Check, 1999; Check et al., 2002). In addition, besides diminished ovarian reserve, several possible

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etiologies have been associated with poor ovarian response: a decreased number of FSH receptors available in granulosa cells (Zeleznik et al., 1981), defective signal transduction after FSH receptor binding (Salgado Jacobo et al., 2003), the presence of a special FSH receptor binding inhibitor in the follicular fluid (van der Gaast et al., 2006), an inappropriate local vascular network for the distribution of gonadotrophins, the presence of autoantibodies against granulosa cells (Hanoch et al., 1998), the presence of heterophylic antibodies (Lambalk, 2003), lowered circulating gonadotrophin surge-attenuating factor (GnSAF) bioactivity (Martinez et al., 2002) and FSH receptor polymorphism could also result in an elevated value in patients with otherwise normal ovaries (Lambalk, 2003).

Although poor ovarian response to gonadotrophin stimulation could be considered one of the most challenging issues of assisted reproduction, minimal data are available regarding oocyte quality, embryo development and endometrial receptivity in poor responders who are elected to undergo oocyte retrieval. In our study, the comparable outcomes in the different groups could be related to the good quality of the produced oocytes and embryos and the potential for implantation in poor responders. Therefore, our results suggest that women younger than 35 years with low ovarian response have mainly a quantitative rather than a qualitative decline in ovarian function.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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