Outcomes of High Initial Daily Doses of Gonadotropin in Patients With Poor Ovarian Reserve

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SUMMARY

Objective: To evaluate the *in vitro* fertilization and intracytoplasmic sperm injection outcomes after high initial doses of follicle-stimulating hormone (FSH) in patients with poor ovarian reserve.

Materials and Methods: For *in vitro* fertilization/intracytoplasmic sperm injection patients younger than 40 years of age, 345 cycles were examined from April 2003 to April 2007. As a control, 218 cycles received gonadotropin-releasing hormone agonist and regular initial doses of FSH from day 3 of the treated cycle. The remaining 127 cycles were treated with high initial doses of FSH with an antagonist or low doses of gonadotropin-releasing hormone because of poor ovarian reserve.

Results: When higher initial doses of FSH were used, lower estradiol levels on the day of human chorionic gonadotropin injection and less mature oocytes were retrieved from the group with poor ovarian reserve. Clinical pregnancy rates per embryo transfer were similar (45.7% *vs.* 48.2%, $p = 0.686$). There was a trend of lower ongoing pregnancy rate per cycle (28.3% *vs.* 38.5%, $p = 0.05$) in the study compared with the control group. In the subgroups with high doses of FSH, neither protocol was superior in terms of clinical (45.5% *vs.* 46.2%, $p = 0.952$) or ongoing pregnancy rates per embryo transfer (37.9% *vs.* 42.3%, $p = 0.695$).

Conclusion: There was no significant difference in clinical pregnancy rate of the two groups when good embryos were obtained. The group with poor ovarian reserve had lower ongoing pregnancy rates per cycle. For patients with expected poor ovarian response, treatment with high doses of FSH initially is an option. [*Taiwan J Obstet Gynecol* 2010;49(4):442–448]

Key Words: gonadotropin, poor ovarian reserve, poor responder, pregnancy

Introduction

Poor response to ovarian stimulation for assisted reproductive treatment is a therapeutic challenge. Patients expected to be poor responders are generally of advanced age, have a high basal follicle-stimulating hormone (FSH) concentration, low basal inhibin B or anti-müllerian hormone concentration, and lower antral follicle counts. The estimated prevalence of poor responders among patients undergoing *in vitro* fertilization (IVF) treatment is 9–24% [1]. Over the years, different protocols have been suggested to tackle this problem, but most of these interventions have met with only limited success and the optimum stimulation protocol for poor responders is still unknown.

Most IVF programmers use long gonadotropin-releasing hormone (GnRH) agonist protocols with gonadotropins for ovarian stimulation. When the regular dose of gonadotropins (150–300 IU) fails to induce a proper multifollicular growth, the obvious clinical approach is to increase the dose of gonadotropins. A high dose of gonadotropins has been used by the vast majority of authors in poor responder patients [2].

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Several randomized control trails have demonstrated that higher starting doses of gonadotropins do not lead to improved pregnancy rates, despite lower cancellation rates or whether the patients were treated with a GnRH agonist [3,4] or antagonist protocol [5, 6]. However, patients in these trials were all expected normal responders. In a retrospective study by Karande [7], despite an increase in FSH doses, the numbers of oocytes retrieved and the pregnancy rates were low. In another retrospective study by Land et al [8], the number of oocytes retrieved increased after doubling the human menopausal gonadotropin (hMG) doses in low responders, but the pregnancy rates remained low. In a prospective randomized study by Van Hooff et al [9], increasing the starting dose up to 450 IU was ineffective in increasing ovarian response and/or in increasing pregnancy rate. In another randomized controlled study by Klinkert et al [10], doubling the starting doses of gonadotropins in expected poor responders on the basis of antral follicle counts did not lead to an improvement of the response during IVF treatment. They enrolled 52 patients with the average age above 40 years. The stimulation protocol was a long suppression protocol with starting doses of 150 IU recombinant FSH in Group I and 300 IU recombinant FSH in Group II.

Different protocols have also been proposed for the management of low ovarian response in IVF. Feldberg et al [11] compared different doses of GnRH agonist administered over a long protocol in women with previous poor response to gonadotropins. They found a higher estrogen concentration, more oocytes were collected and fertilized, more embryos transferred, and a lower cancellation rate with lower doses of GnRH. In another randomized study [12], a lower amount of gonadotropins were required and shorter duration for stimulation were also found in the group with lower doses of GnRH agonist for pituitary suppression.

A GnRH antagonist has also become available in recent years. Because of its distinct pharmacological mode of action, GnRH antagonist may allow for maximum response of ovaries to the stimulation of gonadotropins without early follicular suppression. Preliminary comparisons between the GnRH agonist long protocol and GnRH antagonist protocol applied in poor responders have shown a lower consumption of gonadotropins, a shorter duration of stimulation [13], a non-significant trend for a greater number of oocytes retrieved and a lower cycle cancellation rate [14] in the GnRH antagonist group. However, a meta-analysis including five studies of fixed GnRH antagonist protocols compared with long agonist protocols, indicated 5% less clinical pregnancies in the antagonist groups [15].

The aim of our study was to evaluate the IVF/intra cytoplasmic sperm injection (ICSI) outcomes of high initial doses of FSH (at least 450 IU daily) in patients with poor ovarian reserve compared with the standard dose of FSH in IVF patients. Different methods for pituitary suppression in the group with poor ovarian reserve were also compared.

Materials and Methods

This study was a retrospective study of IVF/ICSI outcome based on the medical records of patients in the fertility unit of MacKay Memorial Hospital in Taipei, Taiwan from April 2003 to April 2007. The study protocol was approved by the Institutional Review Board of the Mackay Memorial Hospital. Patients diagnosed with systemic disease, endocrine abnormality, hydrosalpinx or cycles for oocyte donation were excluded from the study to minimize confounding factors. A total of 345 treated cycles were included in this study. A total of 218 cycles received the long protocol of GnRH agonist and regular initial doses of FSH (no more than 300 IU daily) from day 3 of the treatment cycle. The other 127 cycles were treated with high initial doses of FSH (at least 450 IU daily) from day 3 with antagonist, or by the long protocol with a low dose of GnRH agonist because of poor ovarian reserve.

Ovarian stimulation protocol

Patients treated with high initial doses of FSH were included in the study group because of cancellation of the previous assisted reproductive technology due to poor response and/or a total of four or less antral follicles on day 3 of the menstrual cycle. The threshold of four antral follicles of 2–5 mm was based on the study by Tomas et al [16] in which patients with less than five follicles measuring 2–5 mm were classified as having inactive ovaries. Bancsi et al [17] also found the threshold of no more than four antral follicles had the lowest error rate to predict poor response.

In the study group, 95 cycles were treated by the GnRH antagonist protocol and the other 32 cycles were treated by the long protocol with lower dose of leuprolide acetate (0.25 mg or 0.15 mg). In the GnRH antagonist treated cycles, recombinant FSH (Gonal-F; Serono Laboratories, Aubonne, Switzerland) and hMG (Menopur; Ferring Pharmaceuticals, Denmark) were administrated daily from the third day of the menstrual cycle. The doses were adjusted according to the patient's individual ovarian response. Cetrorelix (Cetrotide; Serono, Baxter Oncology GmbH, Halle, Germany) was administered subcutaneously at a dose of 0.25 mg

daily when the dominant follicle reached 14 mm in mean diameter, until the day of human chorionic gonadotropin (hCG) administration. In the other 32 cycles, leuprolide acetate (Takeda Pharma GmbH, Stolberg, Germany) was given at a daily dose of 0.25 mg or 0.15 mg, starting on day 21 of the previous cycle. Once serum levels of estradiol (E2) lower than 40 pg/mL were achieved on day 3 of the stimulating cycle, recombinant FSH and hMG were given until the day of hCG administration. The doses were also adjusted according to the patient's ovarian response. In the study group, the initial daily doses of FSH were at least 450 IU.

In the control group, leuprolide acetate was given at a daily dose of 0.5 mg, starting on day 21 of the previous cycle. Once serum levels of E2 were less than 40 pg/mL were achieved on day 3 of the stimulating cycle, recombinant FSH and hMG were given until the day of hCG administration. The doses were also adjusted according to the patient's ovarian response. The initial daily doses of FSH were all no more than 300 IU.

In both groups, the ovarian response was monitored by serial transvaginal ultrasound scanning and by measuring the serum E2 concentration. A dose of hCG (10,000 IU; Pregnyl, Organon, Greece) was intramuscularly given for final maturation when the leading follicles reached 20 mm together with at least two following follicles that reached diameters of 18 mm as detected on the ultrasound scan.

Oocyte retrieval was performed 34–36 hours later. Cycles in which less than two dominant follicles developed and E2 was less than 500 pg/mL on the day of hCG administration, or in which the ovaries failed to respond after stimulation, were either cancelled or converted to intrauterine insemination in patients with patent tubes.

ICSI was performed only in cases with male factor infertility, which was defined by the presence of any of the following parameters: sperm concentration less than 10×10^6/mL; motility less than 50%, normal morphology less than 4%; or previous fertilization failure. Upon completion of oocyte collection and IVF/ICSI, embryos were graded morphologically on day 3 and according to the system modified from Veeck. Embryos classified as grade 1 or 2 were denoted as good embryos. Embryo transfer was performed 72 hours after oocyte retrieval. When good embryos were available to transfer, this cycle was designated as the "cycle with embryo transfer".

The luteal phase was supported by vaginal supplementation with 200 mg micronized natural progesterone (Progeffik; Effik, Paris, France) three times a day or Crinone 8% vaginal gel and daily injection of 50 mg progesterone or hCG (2,500 IU) given every 3 days for three doses commencing 2 days after oocyte retrieval.

To assess treatment outcome, serum hCG was measured 14 days after oocyte retrieval. If hCG had been given as luteal phase support, a pregnancy test with urinary hCG test would be done 18 days after oocyte retrieval. Implantation was defined by the observation of a gestational sac in the uterus by transvaginal ultrasonography. An ongoing pregnancy was defined by observation of a fetal heartbeat by ultrasonography after 7 weeks of gestation. Abortion was defined as fetal loss after the gestational sac was detected.

Statistical analysis

Statistical analysis was performed using the Statistical Packages for Social Sciences version 12.0 (SPSS Inc., Chicago, IL, USA). The differences of means between two variables were calculated using the Mann-Whitney *U* test. The differences of fertilization rate, rate of embryo transfer per cycle, implantation rate, clinical pregnancy rate, ongoing pregnancy rate and abortion rate were analyzed by the χ^2 test or Fisher's exact tests as appropriate. A p value smaller than 0.05 was considered statistically significant.

Results

In total 345 cycles were analyzed, with cycle characteristics and pregnancy outcomes between the two groups compared in Table 1. Significant differences were observed between the study and control groups with respect to patient age (35.1 ± 3.0 years *vs.* 32.2 ± 3.9 years, $p < 0.01$), the starting doses of FSH (475.2 ± 39.1 IU *vs.* 234.9 ± 40.3 IU, $p < 0.01$), total dose of FSH (4,566.9 ± 1,078 IU *vs.* 2,314.3 ± 627.5 IU, $p < 0.01$), cancellation rate (14.1% *vs.* 3.2%, $p < 0.01$), serum E2 level on hCG injection day (1,191.9 ± 695.5 pg/mL *vs.* 2,714.8 ± 1,452.6 pg/mL, $p < 0.01$), mature follicles (4.7 ± 1.9 *vs.* 9.7 ± 3.9, $p < 0.01$) and oocytes obtained (6.8 ± 3.6 *vs.* 11.1 ± 5.9, $p < 0.01$). However, there was no significant difference between the control and study groups in terms of total days of stimulation (10.8 ± 2.2 *vs.* 10.7 ± 1.6, $p < 0.01$). All 18 cancelled cycles (14.1%) in the study group were the result of insufficient ovarian response, while only six (3.2%) cycles in the control group were cancelled for the same reason. The remaining one cycle cancelled in the control group was due to ovarian hyperstimulation syndrome.

Although we saw a similar fertilization rate in both groups (77.3% *vs.* 74.5%, $p = 0.43$), there were significant differences between the groups in the number of total embryos (4.4 ± 2.9 *vs.* 7.2 ± 4.2, $p < 0.01$) and good embryos obtained (2.3 ± 1.9 *vs.* 4.6 ± 3.8, $p < 0.01$), good embryos to transfer (2.3 ± 1.1 *vs.* 2.6 ± 1.0, $p < 0.05$),

Table 1. Basic characteristics and outcomes of the study and control groups*

	Study group ($n=127$)	Control group ($n=218$)	p
Age (yr)	35.1 ± 3.0	32.2 ± 3.9	< 0.01
Starting daily dose of FSH (IU)	475.2 ± 39.1	234.9 ± 40.3	< 0.01
Cancel rate (%)	14.1	3.2	< 0.01
Days of stimulation	10.8 ± 2.2	10.7 ± 1.6	NS
Total FSH dose (IU)	4,566.9 ± 1,078.0	2,314.3 ± 627.5	< 0.01
Estradiol on day of hCG injection (pg/mL)	1,191.9 ± 695.5	2,714.8 ± 1,452.6	< 0.01
No. of follicles > 14 mm	4.7 ± 1.9	9.7 ± 3.9	< 0.01
No. of oocytes	6.8 ± 3.6	11.1 ± 5.9	< 0.01
No. of mature oocytes	5.8 ± 3.3	9.9 ± 5.4	< 0.01
Fertilization rate (%)	77.3	74.5	NS
IVF (%)	80.5	76.7	NS
ICSI (%)	68.3	72.0	NS
No. of total embryos	4.4 ± 2.9	7.2 ± 4.2	< 0.01
No. of good embryos	2.3 ± 1.9	4.6 ± 3.8	< 0.01
Rate of embryo transfer per cycle (%)	72.4	89.4	< 0.01
No. of good embryo to transfer	2.3 ± 1.1	2.6 ± 1.0	< 0.05
Clinical pregnancy rate per embryo transfer (%)	45.7	48.2	NS
Implantation rate (%)	31.0	27.0	NS
Abortion rate (%)	14.3	10.6	NS
Ongoing pregnancy rate per embryo transfer, *n* (%)	36/92 (39.1)	84/195 (43.1)	NS
Ongoing pregnancy rate per cycle, *n* (%)	36/127 (28.3)	84/218 (38.5)	0.05

**Data are presented as mean ± standard deviation, % or n (%). FSH = follicle stimulating hormone; NS = not significant; hCG = human chorionic gonadotropin.*

and the rate of embryo transfer per cycle (72.4% *vs.* 89.4%, $p<0.01$). There were no significant differences in the clinical pregnancy rate per embryo transfer (45.7% *vs.* 48.2%, $p=0.686$), implantation rate (31.0% *vs.* 27.0%, $p=0.276$), abortion rate (14.3% *vs.* 10.6%, $p=0.571$), and ongoing pregnancy rate per embryo transfer (39.1% *vs.* 43.1%, $p=0.527$). A trend of higher ongoing pregnancy rate per cycle in the control group compared with the study group was still noted (28.3% *vs.* 38.5%, $p=0.05$).

To compare the treatment outcomes of high initial doses of FSH with different protocols for pituitary suppression, the 127 cycles in the study group were further divided into the subgroups "antagonist protocol" and "long protocol with lower doses of GnRH agonist". Cycle characteristics and pregnancy outcomes between the two groups are list in Table 2. No significance difference was observed between the two groups in patient age (35.4 ± 2.9 years *vs.* 34.3 ± 3.2 years, $p=0.07$) and cancellation rate (16.8% *vs.* 3.1%, $p=0.069$). However, with more total FSH doses used (4,804.3 ± 1,104.7 IU *vs.* 3,869.5 ± 593.3 IU, $p<0.01$) and longer stimulation duration (11.1 ± 2.4 days *vs.* 9.9 ± 1.3 days, $p<0.01$) in the antagonist group, lower serum levels of E2 upon hCG injection (1,089.1 ± 688.6 pg/mL *vs.* 1,493.9 ± 634.0 pg/mL, $p<0.01$), a low number of follicles larger than 1.4 cm (4.5 ± 1.9 *vs.* 5.4 ± 1.8, $p<0.05$) and less oocytes obtained (6.3 ± 3.4 *vs.* 8.2 ± 3.8, $p<0.05$) were observed in the antagonist group. There were no significant differences in the fertilization rate (76.7% *vs.* 78.5%, $p=0.432$), numbers of total embryos (4.1 ± 2.5 *vs.* 5.2 ± 3.7, $p=0.186$) and good embryos (2.3 ± 1.9 *vs.* 2.4 ± 1.9, $p=0.693$), rate of embryo transfer per cycle (69.5% *vs.* 81.3%, $p=0.197$), numbers of good embryo to transfer (2.2 ± 1.1 *vs.* 2.5 ± 1.1, $p=0.29$), implantation rate (30.4% *vs.* 32.3%, $p=0.782$), multiple pregnancy rate (34.4% *vs.* 58.3%, $p=0.417$), clinical pregnancy rate per embryo transfer (45.5% *vs.* 46.2%, $p=0.952$), ongoing pregnancy rate per embryo transfer (37.9% *vs.* 42.3%, $p=0.695$) and ongoing pregnancy rate per cycle (26.3% *vs.* 34.4%, $p=0.382$).

Discussion

Our study focused on those expected poor responders younger than 40 years of age. A similar duration of ovarian stimulation was found in both the study and

Table 2. Comparison of basic characteristics and outcomes of Group I (antagonist protocol) and Group II (long protocol with lower doses of gonadotropin-releasing hormone agonist)*

	Group I ($n=95$)	Group II ($n=32$)	p
Age (yr)	35.4 ± 2.9	34.3 ± 3.2	NS
Starting dose of FSH (IU)	481.6 ± 41.8	454.7 ± 18.4	< 0.01
Cancel rate (%)	16.8	3.1	NS
Days of stimulation (d)	11.1 ± 2.4	9.9 ± 1.3	< 0.01
Total FSH dose (IU)	4,804.3 ± 1,104.7	3,869.5 ± 593.3	< 0.01
Estradiol on day of HCG injection (pg/mL)	1,089.1 ± 688.6	1,493.9 ± 634.0	< 0.01
No. of follicles > 14 mm	4.5 ± 1.9	5.4 ± 1.8	< 0.05
No. of oocytes	6.3 ± 3.4	8.2 ± 3.8	< 0.05
No. of mature oocytes	5.5 ± 3.1	6.7 ± 3.7	NS
Fertilization rate (%)	76.7	78.5	NS
IVF (%)	80.4	80.6	NS
ICSI (%)	67.0	71.4	NS
No. of total embryo	4.1 ± 2.5	5.2 ± 3.7	NS
No. of good embryo	2.3 ± 1.9	2.4 ± 1.9	NS
Rate of embryo transfer per cycle (%)	69.5	81.3	NS
No. of good embryo to transfer	2.2 ± 1.1	2.5 ± 1.1	NS
Clinical pregnancy rate per embryo transfer (%)	45.5	46.2	NS
Implantation rate (%)	30.4	32.3	NS
Abortion rate (%)	16.7	8.3	NS
Ongoing pregnancy rate per embryo transfer (%)	37.9	42.3	NS
Ongoing pregnancy rate per cycle (%)	26.3	34.4	NS

**Data are presented as mean ± standard deviation, % or n (%). NS = not significant; GnRH = gonadotropin-releasing hormone; FSH = follicle stimulating hormone.*

standard groups. Even with high starting doses of recombinant FSH in the study group, a higher cancellation rate, lower serum E2 levels on the day of hCG injection and lower numbers of oocytes obtained were still observed in these expected poor responders. A high initial daily dose of FSH stimulation could not compensate for the poor ovarian reserve. These patients with less ovarian reserve also had less chance for embryo transfer in each cycle of IVF treatment because of the higher cancellation rate, and the lower number of oocytes and good embryos obtained.

It is noteworthy that the implantation rate and the clinical pregnancy rate per embryo transfer were similar in the study and control groups when embryos with good quality could be obtained. This may be because all patients were younger than 40 years of age in both groups, and implantation was more greatly affected by the age and quality of embryos than the expected poor ovarian response. With the similar implantation rate, both groups achieved a similar clinical pregnancy rate per embryo transfer and ongoing pregnancy rate per embryo transfer. The abortion rate in both study and control groups were also without significant differences. This supports the idea that the implantation rate may not be affected in these expected poor responders aged younger than 40.

For patients, the ongoing pregnancy rate for each cycle of IVF treatment is of greatest concern. In our study, the ongoing pregnancy rate per cycle was lower in patients with poor ovarian reserve. This could be explained by the high cancellation rate and lower embryo transfer rate in the study group. In addition to looking for an egg donor, a high starting dose of recombinant FSH with antagonist protocol/a long protocol with lower doses of GnRH agonist may be another option for patients with poor ovarian reserve.

Different methods for pituitary suppression, antagonist protocol (Group I) and long protocol with lower doses of GnRH agonist (Group II), in the group with high initial doses of FSH treatment were also compared in

our study. With similar average age of patients, patients in Group I had a less favorable result of ovarian stimulation (lower serum E2 on the day of hCG injection and less oocytes obtained). This result is comparable with previous studies [18,19] where the number of follicles and number of oocytes retrieved in a flexible antagonist protocol were less favorable than in a long GnRH agonist protocol. The rate of embryo transfer in each cycle was higher in Group II but the difference was not statistically significant.

The possible direct or indirect negative effect on the endometrium of the GnRH antagonist has been discussed [20–22]. With similar number of embryos transferred, lower implantation and pregnancy rates have been observed in woman undergoing ovarian stimulation with the GnRH antagonist protocol [23]. Conversely, another recent study compared the endometrial development in egg-donors treated with the GnRH antagonist protocol, the GnRH agonist long protocol or in natural cycles. They found more similarity in the endometrial development of donors treated with GnRH antagonist and natural cycles [24]. Our study also demonstrated similar implantation and abortion rates in Groups I and II. There were no significant differences in the clinical and ongoing pregnancy rates per embryo transfer when a similar number of good embryos were transferred.

In conclusion, this study demonstrates that the outcomes of IVF treatment in patients with expected poor response and younger age who received high initial daily doses of FSH are still not compatible to a normal responder. The similar implantation rate and an acceptable ongoing pregnancy rate per embryo transfer may be achieved in patients with poor ovarian reserve if embryos with good quality could be obtained. However, because of the limitation of retrospective studies and the imbalanced case numbers in the two groups, a further study is recommended to confirm these conclusions.

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