

What is the optimum maximal gonadotropin dosage used in microdose flare-up cycles in poor responders?

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Objective: To find out the optimum maximal dosage of recombinant follicle stimulating hormone (rFSH) in microdose gonadotropin-releasing hormone analog (GnRH-a) flare cycles in poor responders.

Design: Prospective randomized study.

Setting: Private infertility clinic.

Patient(s): A total of 119 women were taken into the study.

Intervention(s): The study group underwent a microdose protocol with a GnRH-agonist followed by rFSH administration. On the third day of GnRH-a administration, 119 patients were randomized in three groups to receive daily fixed doses of 300 IU of rFSH (group A, n = 38), or 450 IU of rFSH (group B, n = 39), or 600 IU of rFSH (group C, n = 42).

Main Outcome Measure(s): Peak E₂ levels, days of stimulation with rFSH, total rFSH dosage, total number of oocytes retrieved, M2 oocytes retrieved, total number of embryos, number of embryos transferred, number of Grade-1 embryos transferred, clinical pregnancy rate (positive fetal cardiac activity), and cancellation rates of stimulation and embryo transfer.

Result(s): Clinical pregnancy rates were 13.1%, 15.3%, and 16.1% for group A, group B, and group C, respectively. There were no significant differences in the age, peak serum E₂ concentration, days of stimulation with rFSH, total number of M2 oocytes retrieved, number of embryos transferred, clinical pregnancy rates, and cancellation rates of stimulation and embryo transfer between the three groups except for total rFSH dosage.

Conclusion(s): There is no need to use doses above 300 IU of rFSH to increase the pregnancy rate in microdose cycles. In addition, because the duration of stimulation does not differ between the groups, the usage of 300 IU rFSH in microdose cycles results in less total amount of rFSH consumed in a cycle compared with higher dosages, and this would obviously cost less money to the patients. (Fertil Steril® 2010;94:662–5. ©2010 by American Society for Reproductive Medicine.)

Key Words: Recombinant FSH, microdose cycle, pregnancy rate, poor responders

Diminished ovarian reserve is a condition occurring in women at any adult age, although it is more frequent, in women in their 30s. There are several tests to diagnose this problem. These include basal tests for FSH (1, 2), LH (2), estradiol (3), inhibin B (4), anti-Mullerian hormone (5) or dynamic tests, such as the clomiphene citrate challenge test (6), and gonadotrophin analogue stimulating test (7). In recent years great attention has been given to direct tests such as the antral follicle count (8). In a recent study, it was shown that good responders have a mean number of 13 antral follicles (9). Women with diminished ovarian reserve are candidates for poor responding. Poor responding patients are those women in controlled ovarian hyperstimulation (COH) for assisted reproductive technologies (ART) show poor follicle recruitment in terms of number (generally three or four follicles)

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or size, and low levels of peak serum E₂, despite the high dose of gonadotropins administered.

On the other hand, an ideal approach to patients who respond poorly to traditional COH regimens in preparation for ART has not been well established. Some investigators have sought to take the advantage of the initial endogenous gonadotropin “flare” induced by gonadotropin-releasing hormone analogue (GnRH-a) by microdoses of an agonist in the early follicular phase to enhance the effect of exogenous gonadotropins (10–12). Since 2002, in our clinic we have preferred to use a microdose flare protocol in women who have diminished ovarian reserve determined by having <12 antral follicles on baseline ultrasound.

In addition, the role of exogenous LH supplementation during COH is also a matter of debate. Previously we have showed that there is no need for the addition of exogenous LH activity of either rLH or low-dose rHCG in microdose cycles in women with diminished ovarian reserve (13). In microdose flare cycles, there is an initial rise in endogenous gonadotropins, and the protocol is associated with markedly elevated follicular phase LH concentrations, actually higher

than in subjects treated with hMG in a long GnRH agonist regimen (14).

There are also several debates about dosages of gonadotropins in poor responders besides type of COH regimens. For the first time, we aimed to find out the optimum maximal dosage of recombinant follicle stimulating hormone (rFSH) in microdose GnRH-a flare cycles in poor responders.

MATERIALS AND METHODS

Patients and Protocols

The study protocol was approved by the institutional review board. All patients gave informed consent. The study group included 119 women with <12 antral follicles undergoing a microdose protocol with a GnRH-a followed by rFSH administration. Exclusion criteria included women >42, women with only one ovary, and women with a basal FSH concentration of >12 IU/L. Only the first intracytoplasmic sperm injection (ICSI) cycle was analyzed to minimize selection bias.

Stimulation Protocol

Following 3 weeks of oral contraceptive use (Myralon, Organon, Kenilworth, NJ), 40 μ g of leuprolide acetate (Lucrin, Abbott, Abbott Park, IL) two times daily was started on day 1 of withdrawal bleeding, and 600 IU of rFSH (Gonal F, Serono, Geneva, Switzerland; Puregon, Organon) was started on day 3. One hundred nineteen patients were then randomized by a computer-generated list in three groups to receive daily fixed doses of 300 IU of rFSH (group A, $n = 38$), or 450 IU of rFSH (group B, $n = 39$), or 600 IU of rFSH (group C, $n = 42$). Stimulation protocol was cancelled if the follicles failed to grow. When at least two follicles were >17 mm, 10,000 IU hCG (Pregnyl, Organon) was administered. Oocyte retrieval was performed 35 hours later.

Following ICSI, embryo transfers were routinely performed on day 2. The luteal phase was supported using 90 mg twice daily of intravaginal progesterone gel (Crinone gel 8%, Serono). Twelve days after the embryo transfer serum was taken for β -hCG. An ultrasound scan to confirm the number of sacs and fetal viability was performed at 6 weeks gestation.

Ultrasound and Laboratory Assays

All ultrasound measurements were performed using a 6.5-MHz vaginal probe (Siemens, Sonoline Sienna, Köhn, Germany). Antral follicles were counted on cycle day 3 prior to initiation of stimulation protocol. On day 3, serum FSH was also measured using a chemiluminescent immunoassay (Immulite, Euro/DPC, Gwynedd, UK). Stimulation response was monitored with serial measurements of serum estradiol and transvaginal ultrasonic evaluation of follicle number and size. Serum estradiol was measured using a chemiluminescent immunoassay (Immulite, Euro/DPC).

Statistical Analysis

Age, percentages of patients over 37 years of age, antral follicle count, basal (day 3) FSH, peak E_2 levels, days of stimulation with rFSH, total rFSH dosage, M2 oocytes retrieved, clinical

pregnancy rates (positive fetal cardiac activity) per transfer and program started, and cancellation rates of COH and embryo transfer were compared between the groups. Analysis of variance and chi-square tests were used for statistical comparisons. A value of $P < .05$ was considered statistically significant. Statistical calculations were performed using Sigmasat for Windows, version 3.0 (Jardel Scientific Corporation, San Rafael, CA).

RESULTS

There was no statistically significant difference in age, percentages of patients over 37 years of age, total number of antral follicles, basal FSH, peak E_2 level, days of stimulation with rFSH, total number of M2 oocytes retrieved, and number of embryos transferred between group A, group B, and group C (Table 1). But, there is a significant decrease in dosage of rFSH used in group A and group B than group C and ($P < .001$).

In addition, there was no difference in the pregnancy rate, and the cancellation rates of COH and embryo transfer between group A, group B, and group C (Table 2). Cancellation rates of stimulation were 10.5%, 15.3%, and 14.2% for group A, group B and group C, respectively. Cancellation rates of embryo transfer were 8.0%, 21.2%, and 13.8% for group A, group B, and group C, respectively. Clinical pregnancy rates were 13.1%, 15.3%, and 16.1% for group A, group B, and group C, respectively. Implantation rates were 7.0%, 5.5%, and 9.1% for group A, group B, and group C, respectively.

DISCUSSION

The ideal approach to patients who respond poorly to traditional COH regimens in preparation for ART has not been well established. There are several debates about type of COH regimens and dosages of gonadotropins. Various researchers have analyzed the effect of increasing gonadotropin dosage as a means of enhancing ovarian response (15, 16). Microdose GnRH-a flare cycles have been developed, and have shown that this type of regimen is efficacious in women with diminished ovarian reserve or poor response to luteal phase protocols (17).

In clinical practice, in an attempt to overcome the decline in ovarian response the dose of FSH is usually adjusted upward. There is evidence that exceeding the dose over 250 IU may not alter response (18, 19). Before considering egg donation, an important clinical question is to what upper limit can the dose of the FSH be raised to overcome ovarian resistance. In many clinics, the upper limit of FSH/day is 300 or 450 IU.

The only systematic review to identify evidence-based medical reasons for the highest FSH regimen to use is that of Tarlatzis et al. (20). The investigators concluded that studies of high doses of gonadotrophins used for ovarian stimulation in poor responders have inconsistent conclusions and that the few prospective randomized studies have shown either minimal or no benefit at all.

In contrast, there are some interesting prospective trials showing that a satisfactory ovarian response and pregnancy rate were achieved, when the stimulatory regime exceeded

TABLE 1

Demographic features.				
Groups	Group A (300 IU rFSH) (n = 38)	Group B (450 IU rFSH) (n = 39)	Group C (600 IU rFSH) (n = 42)	P value
Age	35.6 ± 0.6	35.3 ± 0.6	34.1 ± 0.6	.25
Patients > 37 y (%)	31.5	28.2	30.9	.94
No. antral follicles	6.5 ± 0.5	7.0 ± 0.5	6.5 ± 0.5	.72
Day 3 FSH (IU/L)	6.9 ± 1.7	7.3 ± 1.7	7.1 ± 1.9	.98
Peak E2 levels	1,282 ± 243	1,707 ± 265	1,842 ± 386	.41
Days of stimulation	7.5 ± 0.3	8.3 ± 0.3	7.7 ± 0.3	.15
rFSH dosage (IU)	2,211.4 ± 96.1	3,749.2 ± 162.5	4,575.0 ± 203.6	< .001
Total oocytes retrieved	5.2 ± 0.4	6.3 ± 0.7	6.6 ± 0.6	.21
M2 oocytes retrieved	3.7 ± 0.4	4.9 ± 0.6	4.8 ± 0.6	.24
Total embryo number	2.5 ± 0.3	2.5 ± 0.3	2.6 ± 0.3	.96
Embryos transferred	2.3 ± 0.2	2.3 ± 0.3	2.4 ± 0.2	.94
Grade-1 embryo number	1.4 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	.27

Note: Values are mean ± SEM.

Berkkanoglu. Gonadotropin dosage in microdose cycles. Fertil Steril 2010.

the 300 IU dosage, being combined with various types of down-regulation or adjuvant strategies (21–24). There are also some prospective studies using a classic flare-up GnRH agonist regimen with 450 to 600 IU/day of hMG (11, 25, 26). But all these studies compared flare-up leuprolide acetate (LA) protocol with luteal LA protocol. They did not compare different dosages in a microdose flare-up cycle.

On the other hand, most of the retrospective studies showed that the increase of the dose was useless, as it resulted in no improvement in outcomes including number of follicles and/or oocyte retrieval rate and/or pregnancy rate (27–31).

There are also five reviews (32–36), and despite the inconsistency in results of the studies already mentioned, they found the same conclusions as the systematic review cited above.

In our study, three different dosage regimens were compared for the first time in a microdose flare-up protocol in

a prospective randomized manner. In addition, the dosage of rFSH used in each protocol was not increased or decreased throughout the stimulation protocol.

This study showed that there was no significant difference in total oocytes retrieved and total embryos developed between the three different dosage regimes. But, there was a significant difference in total rFSH dosage used between the three groups. There was nearly 2,300 IU of rFSH dosage difference in mean total rFSH dosage used between 300 IU of rFSH dosage protocol and 600 IU of rFSH dosage protocol. This would obviously result in differences in costs of money. Furthermore, the study showed that there was no difference in clinical pregnancy rate per transfer and program started between the three groups.

On the other hand, although the study is a prospective and randomized study, the sample size is small. To reach a desired power of 0.800 and an alpha value of 0.05 for comparing

TABLE 2

Clinical outcomes.				
Groups	Group A (300 IU rFSH) (n = 38)	Group B (450 IU rFSH) (n = 39)	Group C (600 IU rFSH) (n = 42)	P value
Cancellation rate of COH (%)	10.5	15.3	14.2	.85
Cancellation rate of ET (%)	8.8	21.2	13.8	.36
Clinical pregnancy rate per transfer (%)	13.1	15.3	16.1	.95
Clinical pregnancy rate per program (%)	10.5	10.2	11.9	.97
Implantation rate (%)	7.0	5.5	9.1	.66
Live birth rate (%) per transfer	13.1	11.5	12.9	.90
Live birth rate (%) per program	10.5	7.7	9.5	.81

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pregnancy rates between the three groups, a sample size of 2,826 patients is needed. A further multicentric study with a larger population would clarify this issue. But, until further multicentric studies or systematic reviews are available, it would be prudent to use 300 IU of rFSH as a maximal dosage in a microdose flare-up protocol in poor responders.

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