Cessation of Low-Dose Gonadotropin Releasing Hormone Agonist Therapy Followed by High-Dose Gonadotropin Stimulation Yields a Favorable Ovarian Response in Poor Responders

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Submitted January 20, 2000; accepted August 27, 2001

Purpose: This study is a prospective nonrandomized study to determine the effect of a new protocol of controlled ovarian hyperstimulation (COH) using low doses and a half-period of gonadotropin releasing hormone agonist (GnRHa) followed by high doses of gonadotropin in patients who were supposed to be poor responders to standard long protocols of GnRHa administration.

Methods: From Dec 1996 to Nov 1998, 50 patients who were classified as "poor responders" were scheduled for 52 cycles of a modified controlled ovarian hyperstimulation protocol. They were categorized into 3 groups: a group of poor responders to COH in the previous IVF or IUI cycles, a group with elevated Day 3 FSH levels, and a group over the age of 40 years. All patients received GnRH agonist from the midluteal phase of the previous cycle to the onset of menstruation in the next cycle. Then high doses of gonadotropins (HMG/FSH) were given. The patients then had standard courses of in vitro fertilization and embryo transfer (IVF-ET) or transfallopian embryo transfer (TET).

Results: Six of the 52 cycles of the modified protocols were cancelled because of poor ovarian response. One premature ovulation was noted before ovum retrieval was performed. In the other 45 cycles, an average of 6.3 mature oocytes were retrieved. The total pregnancy rate and implantation rate were 20.5 and 11.5%, respectively.

Conclusions: The low dose and half duration of GnRHa therapy lessened the suppression of the response of the ovaries to COH compared with the regular long protocol of GnRHa down regulation therapy. This resulted in a low cancellation rate (11.8%), a favorable embryo implantation rate (11.5%), and an acceptable clinical pregnancy rate (20.5%).

KEY WORDS: Low doses of GnRH agonist; ovarian stimulation; poor responders.

INTRODUCTION

The advances in knowledge and techniques of in vitro fertilization and embryo transfer (IVF-ET) and trans-

fallopian embryo transfer (TET) have benefited many patients suffering from various causes of infertility. However, some patients still do not benefit from these procedures because of poor ovarian reserve with no oocytes or only a few poor-quality oocytes after controlled ovarian hyperstimulation (COH). The cause of poor ovarian response may be individualized and the response to COH is usually hard to predict. Age and baseline follicle stimulating hormone (FSH) level are considered two of the most important

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factors determining ovarian reserve and the response to COH. Elevated FSH level on Cycle Day 3 has proved to be correlated with poor ovarian response to COH (1). Toner and colleagues revealed that basal FSH level and age were independent predictors of IVF performance (2).

The best protocol for poor responders has still not been developed. The long protocols of GnRHa administration show benefits in follicular synchronization and prevention of premature LH surge, but have the disadvantage of inhibiting the ovarian response to gonadotropins, especially in patients with poor ovarian reserves. Tasdemir *et al.* discovered that short GnRHa protocols resulted in significantly better ovarian response in 102 patients showing poor response to previous long GnRHa protocols (3). The study of Padilla *et al.* showed the same result and strongly suggested that flare-up protocols are the method of choice in COH for supposed poor responders (4). However, not only the number but also the synchronization of the retrieved oocytes can determine the pregnancy outcome. Short protocols with flare-up effect do resolve the problems of premature LH surge but there is no follicular synchronization. Karande and colleagues used short protocols with GnRHa administered starting on Menstruation Day 2 and found it helpful in enhancing the ovarian response. However, the problems of high cancellation rate (23.8%), low pregnancy rate (11.5%), and low implantation rate (3.5%) remain unresolved (5). Can we find a protocol of GnRHa that minimizes the ovarian suppression effect of GnRHa but still preserves follicular synchronization? We tried a protocol prospectively in which the daily doses of GnRHa were decreased and were stopped at the onset of menstruation before ovarian stimulation with gonadotropins. We present the results and discuss the outcome of this modified protocol.

MATERIALS AND METHODS

From Dec 1996 to Nov 1998, 50 patients who were classified as poor responders were included in this study which resulted in 52 cycles of COH. The patients were categorized into 3 groups:

Group 1: Patients who had received COH for IUI or IVF, but with follicles ≥ 18 mm in diameter and less than 2 oocytes per cycle.

Group 2: Patients whose serum FSH level on the third day of the previous menstruation cycle was higher than 15 mIU/mL.

Group 3: Patients over the age of 40 years.

The patients received 0.5 mg of leuprolide acetate (*Lupron*, manufactured for TAP Pharmaceuticals, Deerfield, by Abbott Laboratories, North Chicago) by subcutaneous injection daily starting at the mid-luteal phase of the previous cycle. A complete basal body temperature record confirmed the phase of the cycle. The leuprolide acetate injection was stopped on Day 2 of menstruation. High dose gonadotropin was then given starting on Day 3 of the menstruation cycle. Every patient initially received 4 ~ 6 ampoules of a combination of *Metrodin* (FSH 75 IU, I.F. SERONO S.P.A. 125, Rome, Italy) and *Pergonal* (FSH 75 IU and LH 75 IU in each ampoule, I.F. SERONO S.P.A. 125 via Rome, Italy) by intramuscular injection daily. The dosages of gonadotropins were adjusted clinically according to the age of the patients, the follicular response to previous COH cycles, the number and size of follicles, and the serum E_2 levels. In most patients the daily gonadotropin doses were reduced to two ampoules per day in the last 2–3 days before human chorionic gonadotropin (hCG) administration.

The patients were monitored with transvaginal ultrasonography every 1–2 days starting on Day 7 of the menstruation cycle. The numbers and sizes of follicles were recorded by ultrasonography. The serum E_2 level was measured on Menstruation Day 2, then followed every 2–4 days after gonadotropin administration, and also on the day of hCG injection. The gonadotropins were stopped and hCG 10,000 IU was injected when at least two follicles larger than 18 mm in mean diameter were found. Transvaginal ovum retrieval was performed between 34 and 36 h after the hCG injection. IVF was done thereafter as a routine procedure. The embryos were transferred to the uterine cavity from the cervical canal (ET) or to the tube with a laparoscope (TET), 48–54 h after the oocytes were retrieved. The number of embryos transferred depended on the quality of the embryos, the outcome of previous IVF-ET or TET procedures, and the age of the patient. Usually three to four embryos, five embryos maximally, were transferred. Progesterone was given by vaginal suppositories and hCG 2500 IU by intramuscular injection every 3 days for a total of three doses after the embryos were transferred.

The parameters including the total days and ampoules of gonadotropin administered, peak E_2 level,

Table I. The Day 3 FSH Levels of Group 2 Patients

Case	Day 3 FSH level (mIU/mL)
1	30.5
2	28.0 ^a
3	27.1
4	36.2 ^a
5	19.4
6	24.0
7	17.8
8	16.2
9	15.1
10	20.6 ^b
11	16.0
12	19.9
Mean $\pm$ SD	22.5 $\pm$ 6.1

^a The cycles were finally cancelled because of poor ovarian response.

^b Triplet pregnancy with cesarean delivery.

numbers of follicles and retrieved oocytes, numbers of mature oocytes, and numbers of embryos in this modified COH protocol as well as the pregnancy outcomes were analyzed. Clinical pregnancy was defined when a gestational sac was detected by ultrasonography. The implantation rate was the total number of gestational sacs seen by ultrasonography divided by the total number of transferred embryos.

RESULTS

There were 12 patients in Group 2. The FSH level of each patient is shown in Table I. The mean FSH level and standard deviation were 22.5 and 6.1 mIU/mL, respectively. The cycles of Case 2 (basal FSH: 28 mIU/mL) and Case 4 (basal FSH: 36 mIU/mL) were cancelled because poor ovarian response. The other 10 patients underwent the complete course of COH.

Among the total 52 cycles receiving the new protocol of COH, six cycles were cancelled because fewer

than two follicles had a mean diameter ≥ 18 mm and persistent serum E₂ level < 500 pg/mL was noted. One patient had premature ovulation before ovum retrieval. The other 45 patients received full courses of COH and ovum retrieval.

The numbers and parameters of the three groups of patients are shown in Table II. Gonadotropin was injected for an average of 9.2 ± 2.0 days per cycle. An average of 41.9 ± 11.5 ampoules of gonadotropin was administered per cycle. There was an average of 3.6 ± 2.0 follicles > 14 mm in mean diameter per cycle. Retrieved oocytes averaged 7.5 ± 4.1 , with 6.3 ± 3.7 mature oocytes per cycle. An average of 2.9 ± 1.4 embryos was transferred in each ET or TET procedure.

Table III shows the pregnancy outcomes of these patients. Among the 45 patients receiving full courses of COH and ovum retrieval, one patient did not undergo further embryo transfer because all seven oocytes retrieved were poor in quality and failed to be fertilized. A total of 131 embryos were transferred in 44 cycles. Fifteen gestational sacs in nine patients were detected by ultrasonography 3 weeks after the embryos were transferred. The pregnancy rate was 20.5% (9/44). The implantation rate was 11.5% (15/131). There were five single, two twin, and two triplet pregnancies in the nine pregnant patients. One triplet pregnancy (Group 3) was reduced to a single fetus because two fetuses died spontaneously during the first trimester. The remaining baby was delivered uneventfully in the 40th week of gestation. The babies in the other triplet pregnancy (Group 2) were delivered in the 33rd week via cesarean section because of malpresentation. The two twin pregnancies, one from Group 1 and the other from Group 3, were delivered at term via cesarean section because of malpresentation with no complications. Of the five single pregnancies all from Group 1, one patient had a spontaneous abortion during the first trimester and the other four

Table II. The Parameters of COH in the Three Groups of Patients

Parameters (mean $\pm$ SD)	Group 1 (N = 31)	Group 2 (N = 12)	Group 3 (N = 9)	Total (N = 52)
Age	35.2 $\pm$ 3.3	36.1 $\pm$ 3.3	41 $\pm$ 1.1	36.4 $\pm$ 3.7
Ampoules of HMG/FSH	41.0 $\pm$ 11.2	44.4 $\pm$ 14.2	41.9 $\pm$ 8.4	41.9 $\pm$ 11.5
Days of HMG/FSH inj.	9.1 $\pm$ 1.7	9.5 $\pm$ 2.8	9 $\pm$ 1.4	9.2 $\pm$ 2.0
Peak E ₂ level	796 $\pm$ 371	994 $\pm$ 705	2040 $\pm$ 1310	1089 $\pm$ 875
No. of follicles > 14 mm	3.3 $\pm$ 1.9	4.0 $\pm$ 1.9	3.9 $\pm$ 2.2	3.6 $\pm$ 2.0
No. of oocytes aspirated	7.2 $\pm$ 3.6	6.8 $\pm$ 4.7	9.4 $\pm$ 4.1	7.5 $\pm$ 4.1
No. of mature oocytes	6.0 $\pm$ 3.0	5.7 $\pm$ 4.5	7.9 $\pm$ 4.4	6.3 $\pm$ 3.7
No. of embryos transferred	3.0 $\pm$ 1.1	2.0 $\pm$ 1.6	3.7 $\pm$ 1.4	2.9 $\pm$ 1.4

Note. Group 1: Patients with poor ovarian response in previous IUI and IVF cycles; Group 2: Patients with elevated Day 3 FSH, > 15 mIU/mL; Group 3: Patients over 40 years of age.

Table III. The Outcomes of the COH and IVF+ET or TET

Outcomes	Group 1 (N = 31)	Group 2 (N = 12)	Group 3 (N = 9)	Total (N = 52)
Cancelled	4	2	0	6
Premature ovulation	1	0	0	1
Ovum postmaturity	1	0	0	1
TET/ET	25	10	9	44
Pregnancy	6	1	2	9
Total implantation sacs	7	3	5	15
Pregnancy rate (per transfer)	24%	10%	22.2%	20.5%
Implantation rate	9% (7/78)	15% (3/20)	15.2% (5/33)	11.5% (15/131)
Cancellation rate	16.1% (5/31)	16.7% (2/12)	0% (0/9)	13.5% (7/52)

Note. Group 1: Patients with poor ovarian response in previous IUI and IVF cycles; Group 2: Patients with elevated Day 3 FSH, >15 mIU/mL; Group 3: Patients over the age of 40 years.

patients had smooth pregnancy and delivery courses. The delivery rate was 18.2% (8/44).

DISCUSSION

A good outcome for assisted reproductive techniques (ART) depends on many factors, and a sufficient number of oocytes retrieved is one of the most important factors in performing IVF-ET or TET. Women whose ovarian reserve is too poor to retrieve enough mature oocytes have to suffer more, pay more, and have less hope of having their own children. Poor ovarian response to gonadotropins usually results in poor results in ART. This condition becomes more common as the women grow older.

To overcome the problem of poor ovarian response some modifications of the COH protocol were developed. Ibrahim *et al.* reported that growth hormone may augment the effect of gonadotropins in poor responders when combined with the gonadotropins (6). However, Dor *et al.* found that administration of growth hormone did not improve the ovarian response in a randomized placebo-controlled study (7). Another way to increase the ovarian response is to increase the dosage of gonadotropins. In some cases there may be a correlation between the dosage of gonadotropins and the response to ovarian stimulation. However, it does not definitely increase the numbers of follicles and the pregnancy outcome. Land and colleagues reported that in a group of poor responders, high-dose HMG injection increased the numbers of follicles and retrieved oocytes. But there was no significant increase in the numbers of embryos. The pregnancy outcome was still low (8).

Combining clomiphene citrate and gonadotropins was once considered an alternative in COH for poor responders, not only because of the possible effect on ovarian stimulation but also because of the relative

economic benefit. However, the outcome in terms of numbers of oocytes, synchronization of follicular growth, fertilization rates, and implantation rates was not better than COH with a combination of gonadotropins and GnRHa. But the combination of clomiphene citrate with gonadotropins is still considered an alternative in poor responders (9).

COH with long GnRHa protocols with daily GnRHa administration from the midluteal phase of the previous menstrual cycle through the whole course of ovarian stimulation can be roughly divided into two major aspects: down-regulation on pituitary function and ovarian hyperstimulation by exogenous gonadotropins. Pituitary down-regulation is achieved by daily doses of GnRHa and its purpose is to prevent the effects of endogenous gonadotropins released by the pituitary gland, and thus to make each follicle grow "homogeneously," that is, to achieve synchronization of follicular growth. However, it is now recognized that long-term use of GnRHa will also decrease the ovarian response to gonadotropins. In patients with poor ovarian reserve the negative effects of long-term GnRHa may cause a poor outcome with COH. This negative effect of GnRHa also increases the doses of exogenous gonadotropins needed to achieve an adequate ovarian response and therefore increases the cost of COH. Restriction of the dose of GnRHa has been considered since the mid-1990s. Feldberg discovered that minidose GnRHa will increase the E₂ level, and increase the number of oocytes retrieved, and also the pregnancy and implantation rates (10).

The flare-up effect of GnRH agonist has been established in the COH cycle since the late 1980s. It is suggested that short-term GnRHa administration in the follicular phase of menstruation stimulates the ovary by releasing gonadotropin from the pituitary gland. Many studies suggest the use of short GnRHa flare-up protocols instead of long GnRHa protocols in poor responders (3–5). Surrey concluded that the

improved effect of flare-up protocols may be due to enhanced release of early follicular phase endogenous FSH without concomitant deleterious rises in androgen levels or corpus luteum rescue (11). The flare-up protocol also minimizes the doses of GnRHa to prevent the suppression of ovarian response to gonadotropins. Many centers use the flare-up protocols as the standard protocols in poor responders. Focusing on the flare-up effect, microdoses of gonadotropin administration were also reported. Essentially about 20–40 μ g leuprolide acetate was given twice a day starting on Menstruation Day 3 to promote the flare-up effect and also to minimize the ovarian suppression. The results were encouraging although the problem of premature LH surge, asynchronous growth of follicles, and deterioration of oocyte function are still not resolved (11–13).

In choosing a new protocol for poor responders, adjusting the doses and duration of GnRHa administration was considered. Dr. Faber and colleagues used a protocol of GnRHa administration that began in the midluteal phase and ceased with the onset of menses in 224 IVF cycles. Their criteria for low responders was a Day 3 FSH ≥ 9 mIU/mL, or retrieved mature oocytes ≤ 4 in previous COH cycles. The pregnancy rate was 32%, an encouraging result compared with the results of previous studies focusing on poor responders (14). However, the criteria for “low responders” was somewhat looser than in our study. We ceased use of low dose GnRHa at the onset of the menses, and used high-dose gonadotropins in patients meeting a more strict definition of “poor responders.”

Long-term GnRHa suppresses the ovarian response but an inadequate GnRHa dose may result in insufficient pituitary down-regulation and may cause spontaneous LH surge. To reduce the GnRHa dose we considered decreasing the daily GnRHa doses. We used 0.5 mg leuprolide acetate instead of the conventional 1.0 mg daily subcutaneous injection to maintain pituitary down-regulation. In addition to the change in daily GnRHa dose the time course was also changed. Leuprolide acetate was stopped at menstruation onset to shorten the duration of GnRHa administration. Both changes, the reduced daily dose and the shortened course, lowered the total dose of GnRHa to minimize ovarian suppression. The result, 6.3 ± 3.7 mature oocytes per cycle, confirmed good ovarian response and good follicular synchronization.

We also considered changing the GnRHa course. We started the leuprolide acetate injection at the midluteal phase as in the conventional long GnRHa protocol but stopped the injection at the onset of

menses. Thus pituitary down regulation was maintained until the administration of high-dose gonadotropins. Although the reappearance of pituitary function after cessation of GnRHa may lead to the disadvantages of spontaneous LH surge and premature ovulation, in our study only one patient had premature ovulation and this cycle was cancelled.

In this study we categorized the poor responders into 3 groups. Group 1 and Group 2 were defined as patients with a previous poor response during the cycles and those with elevated basal serum FSH levels, respectively. Group 3 patients were over 40 years old but without definite data to prove ovarian “poor-response.” Thus some patients in the Group 3 may not be “true” poor responders and may have adequate ovarian reserves. It may somewhat explain why the peak serum E_2 level and deviation are higher in Group 3 (Table II).

The number of mature oocytes, 6.3 ± 3.7 oocytes per cycle, revealed a fair response and good synchronization of follicles in this study. For patients with a history of poor response or elevated basal serum FSH levels, an average of 7.5 oocytes were retrieved per cycle, the total pregnancy rate was 20.5%, and the implantation rate was 11.5%, all encouraging results. The cancellation rate was still low (11.5%). The case number in this study may not be enough to make a definite, general, and conclusive answer in choosing an appropriate protocol for poor responders. However, as a preliminary study it shows that achieving the goals of enhancing follicular number and maintaining follicular synchronization in poor responders to COH is possible.

REFERENCES

1. Bassil S, Godin PA, Gillerot S, Verougstraete JC, Donnez J: In vitro fertilization outcome according to age and follicle-stimulating hormone levels on cycle day 3. *J Assist Reprod Genet* 1999;16:236–241
2. Toner JP, Philput CB, Jones GS, Muasher SJ: Basal follicle-stimulating hormone level is a better predictor of in vitro fertilization performance than age. *Fertil Steril* 1991;55:784–791
3. Tasdemir M, Tasdemir I, Kodama H, Fukuda J, Tanaka T: Short protocol of gonadotropin releasing hormone agonist administration gave better results in long protocol poor responders in IVF-ET. *J Obs Gyn Research* 1996;22:73–77
4. Padilla SL, Dugan K, Maruschak V, Shalika S, Smith RD: Use of the flare-up protocol with high dose human follicle stimulating hormone and human menopausal gonadotropins for in vitro fertilization in poor responders. *Fertil Steril* 1996;65:796–799
5. Karande V, Morris R, Rinehart J, Miller C, Rao R, Gleicher N: Limited success using the “flare” protocol in poor responders

- in cycles with low basal follicle-stimulating hormone levels during in vitro fertilization. *Fertil Steril* 1997;67:900–903
6. Ibrahim ZH, Matson PL, Lieberman BA: The use of biosynthetic human growth hormone to augment ovulation induction with buserelin acetate/human menopausal gonadotropin in women with a poor ovarian response. *Fertil Steril* 1991;55:202–204
 7. Dor J, Seidman DS, Amudai E, Bider D, Levran D, Mashiah S: Adjuvant growth hormone therapy in poor responders to in-vitro fertilization. A prospective randomized placebo-controlled double-blind study. *Hum Reprod* 1995;10:40–43
 8. Land JA, Yarmolinskaya MI, Dumoulin JC, Evers JL: High-dose human menopausal gonadotropin stimulation in poor responders does not improve in vitro fertilization outcome. *Fertil Steril* 1996;65:961–965
 9. Benadiva CA, Davis O, Kligman I, Liu HC, Rosenwaks Z: Clomiphene citrate and hMG: An alternative stimulation protocol for selected failed in vitro fertilization patients. *J Assist Reprod Genet* 1995;12:8–12
 10. Feldberg D, Dicker D, Farhi J, Shalev J, Ashkenazi J, Ben-Rafael Z: Minidose gonadotropin-releasing hormone agonist is the treatment of choice in poor responders with high follicle-stimulating hormone levels. *Fertil Steril* 1994;62:343–346
 11. Surrey ES, Bower J, Hill DM, Ramsey J, Surrey MW: Clinical and endocrine effects of a microdose GnRH agonist flare regimen administered to poor responders who are undergoing in vitro fertilization. *Fertil Steril* 1998;69:419–424
 12. Scott R, Navot D: Enhancement of ovarian responsiveness with microdoses of gonadotropin-releasing hormone agonist during ovulation induction for in vitro fertilization. *Fertil Steril* 1994;61:880–885
 13. Schoolcraft W, Schlenker T, Gee M, Stevens J, Wagley L: Improved controlled ovarian hyperstimulation in poor responder in vitro fertilization patients with a microdose follicle-stimulating hormone flare, growth hormone protocol. *Fertil Steril* 1997;67:93–97
 14. Faber BM, Mayer J, Cox B, Jones D, Toner JP, Oehninger S, Muasher SJ: Cessation of gonadotropin-releasing hormone agonist therapy combined with high-dose gonadotropin stimulation yields favorable pregnancy results in low responders. *Fertil Steril* 1998;69:826–830