Treatment-Associated Serum FSH Levels in Very Poor Responders to Ovarian Stimulation

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Purpose: To compare treatment-associated follicle-stimulating hormone (FSH) response in patients undergoing controlled ovarian hyperstimulation with either microdose flare (MDF) leuprolide acetate or clomiphene citrate and human menopausal gonadotropin (CC/hMG).

Methods: Thirteen patients who were deemed poor responders underwent stimulation with one of two poor responder stimulation protocols (MDF group: $n = 8$; CC/hMG group: $n = 5$). Serum FSH, estrone (E_1), estrone sulfate (E_1S), and estradiol (E_2) levels were measured at baseline, day 5 of medication, and on day of hCG administration. Ovarian and uterine responses were evaluated by ultrasound.

Results: Treatment-associated FSH levels were consistently higher in the group that took CC/hMG. However, serum E_1 , E_1S , and E_2 values were similar in both groups as were the number of oocytes retrieved and the endometrial echo complex. There were no differences between the two groups with regards to the quality of the oocytes obtained, fertilization rate, or the quality of the embryos.

Conclusion: Clomiphene citrate, when administered in conjunction with exogenous hMG, is a more potent stimulator of FSH production than MDF leuprolide acetate among poor responders to ovarian stimulation. However, the number of oocytes is not increased.

KEY WORDS: Clomiphene/hMG; in vitro fertilization; microdose flare; poor responders.

INTRODUCTION

It has long been appreciated that in the clinical practice of assisted reproductive technologies, a certain portion of patients respond to controlled ovarian stimulation by producing a less than average number of follicles. Such individuals are typically labeled “poor responders.” Previous publications have defined “poor responders” on the basis of response to clomiphene challenge test (1), advanced maternal age

(2), poor prior in vitro fertilization (IVF) cycle outcomes (3), or elevated basal follicle-stimulating hormone (FSH) levels (4–6). Poor responders experience diminished pregnancy potential as a result of diminished embryo production, but may be treated with poor responder protocols and achieve satisfactory outcomes (7–9).

The basic principle behind various poor responder protocols is the concept of maximizing FSH stimulation of the ovary. The simplest approach involves increasing the dose of human menopausal gonadotropin (hMG) administration (10). Another strategy combines hMG administration with stimulation of pituitary FSH secretion. In the latter category, clomiphene citrate and gonadotropin-releasing hormone (GnRH) agonists have been utilized for pituitary stimulation. Our clinic as well as others have utilized the

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clomiphene citrate (CC) plus hMG (CC/hMG) combination (11–13) in addition to the microdose flare (MDF) (14) with similar results. However, the relative potency of CC and MDF leuprolide acetate in stimulating pituitary FSH production has not been evaluated. Additionally, the ovarian response to these two administration schemes has not been compared.

Therefore, the purpose of this study was to evaluate the treatment-associated serum FSH levels as well as ovarian follicle and steroid production in response to CC/hMG and MDF poor responder regimens.

MATERIALS AND METHODS

This retrospective analysis focused on patients undergoing one of two poor responder protocols between 1992 and 2001. We identified 13 premenopausal women undergoing IVF cycles. All patients had normal FSH values (<20 mIU/mL) prior to initiation of therapy and were deemed poor responders on the basis of poor response to stimulation during their prior IVF cycles. Patient characteristics are summarized in Table I. The average age of the patients, as well as the body mass index, was similar in both groups. Patients were classified as “poor responders” on the basis of a poor response to stimulation during a prior cycle as follows: 1) the development of only one or two dominant follicles, or 2) slow E_2 rise (<100 pg/mL by day 7 of stimulation).

All patients were pretreated with oral contraceptives (OCs) for 10–20 days. Following cessation of OCs, stimulation was begun on the 4th day, regardless of when withdrawal bleeding was observed. Prior to 7/2000, poor responders were treated with a combination of CC and hMG. In this group ($n = 5$), patients received 100 mg of CC on cycle start days 1–5 along with eight ampules (600 IU) of hMG daily given concurrently and continued until the day of human chorionic gonadotropin (hCG) administration. All medications, including CC, were self-administered by the patients between 4 and 8 p.m. All serum samples were obtained from the patients between 8 and 10 a.m.

Table I. Demographics of Patients in the Two Groups

Group	CC/hMG	Microdose flare
Age	36.8 ± 0.8	39.6 ± 1.5
Body mass index	25.4 ± 1.7	27.9 ± 2.5

Note. Results are reported as mean value $\pm$ standard error of the mean (SEM).

After 7/2000, poor responders were treated with the MDF protocol. In this group ($n = 8$), patients received 40 μ g of leuprolide acetate twice daily starting on cycle start day 1 along with eight ampules of hMG (600 IU) daily starting on day 3 as previously described (7–9).

Serum FSH, E_1 , E_1S , and E_2 values were measured at baseline, day 5 of medication, and on day of hCG administration using specific radioimmunoassays. The number and size of follicles and the endometrial echo complex (EEC) were measured by transvaginal ultrasound and recorded at baseline, day 5, and then every 1–2 days until the day of hCG administration.

Oocytes were rated as mature or immature on the day of oocyte retrieval. Fertilization was confirmed by the presence of two pronuclear stage embryo 24 h after oocyte retrieval. Embryo assessment was carried out on the day of embryo transfer (ET) (3 days after oocyte retrieval). Scoring was based on developmental stage and morphology, using previously described criteria (15,16). Grading criteria for day 3 embryos included cell number, regularity of blastomeres, fragmentation, and morphologic aspects such as granulation. When no fragmentation was evident and the developmental stage was appropriate for their age, embryos were described as high grade and given a grade of “A.” Embryos showing developmental delay and more than 50% fragmentation were described as low grade and graded as “F.” Embryos of better and worse intermediate quality were given scores of “B” or “C.”

Data were analyzed by linear regression and the unpaired Student’s *t* test.

RESULTS

Serum FSH and E_2 levels along with oocyte number and EEC are summarized in Table II. Treatment-associated FSH levels were consistently higher in the CC/hMG group than in the MDF group ($P < 0.05$). Additionally, the increase in FSH levels from baseline to day 5 and day of hCG administration was greater in the CC/hMG group ($P < 0.05$). However, serum E_2 levels were similar in both groups. The number of oocytes retrieved and the mean EEC was also similar in both groups (Table II). Serum FSH levels on day of hCG did not correlate with serum E_2 nor with the number of oocytes retrieved ($r = 0.2$, $P = \text{ns}$).

Serum levels of the three estrogens measured are depicted in Table III. As with E_2 , in spite of markedly different FSH levels in the two groups, the mean E_1

Table II. Serum Estradiol (E₂), FSH Levels, Number of Oocytes Retrieved, and Endometrial Echo Complex (EEC) in CC/hMG Versus MDF

Group	Baseline		Day 5 of medication		Day of hCG			
	E ₂ (pg/mL)	FSH (mIU/mL)	E ₂ (pg/mL)	FSH (mIU/mL)	E ₂ (pg/mL)	FSH (mIU/mL)	No. of oocytes	EEC (mm)
CC/hMG	24 ± 3.3	9.9 ± 1.2	396 ± 80	41.9 ± 5	1321 ± 275	45 ± 3.9	7.8 ± 1.0	9.6 ± 0.8
MDF	20.4 ± 5.4	9.5 ± 1.3	324 ± 86	29.7 ± 2.8	1162 ± 199	30 ± 3.3	6.4 ± 1.5	8.6 ± 0.7
P value	ns	ns	ns	0.04	ns	0.007	ns	ns

Note. Results are reported as mean value ± SEM; ns, not significant.

and E₁S levels were not different between the two groups at baseline, day 5 of hMG, or day of hCG administration.

There were no statistically significant differences between the MDF and the CC/hMG groups with regards to oocyte maturity at the time of retrieval, fertilization rate, embryo quality, or the number of 8-cell embryos on day 3 post-oocyte retrieval (Table IV). There were no pregnancies achieved in either treatment group.

DISCUSSION

In the ovary, FSH is the primary inducer of follicle growth and maturity. In general, higher levels of FSH produce enhanced stimulation, resulting in larger numbers of follicles, and consequently, greater numbers of oocytes retrieved (17–19). Additionally, enhanced follicle growth is reflected in higher serum estrogen levels, including E₂, E₁, and E₁S (20).

Since poor responders exhibit diminished ovarian response to standard FSH stimulation, it is logical to attempt to enhance ovarian exposure by increasing FSH levels in the circulation. Manzi *et al.* (18) reported the use of high-dose hMG in poor responders with some success. However, other investigators (8) have also evaluated the utility of this approach and have not found it to be successful.

A second approach, the one investigated here, is to combine high-dose exogenous FSH administration with stimulation of endogenous FSH by the pituitary.

The CC/hMG protocol was described early in the history of IVF (11,12) and its potential application to poor responders was appreciated shortly thereafter (13). At the present time, a more commonly employed approach is the MDF protocol, which uses low doses of a GnRH agonist to stimulate the pituitary (21). Initially described by Scott *et al.* (17), the MDF protocol appears to maximize FSH levels and its successful use has been reported by other groups (14,21).

We sought to evaluate the relative potency of the MDF and CC/hMG protocols to produce maximal levels of FSH and then to evaluate the response of the ovaries. We found that FSH levels were substantially higher in the CC/hMG protocol as compared with MDF. Since the amount of exogenous gonadotropins used was the same in both groups, we believe that the observed difference in FSH levels reflects pituitary FSH production. In spite of a nearly twofold greater increase in levels of FSH in the CC/hMG group, as compared with the MDF group, there was no statistically significant difference in E₂, total estrogenicity, the number of oocytes retrieved, oocyte maturity, fertilization rate, or embryo quality. Additionally, in spite of the antiestrogenic effects of CC, EEC measurements on the day of hCG were similar in the two groups. We therefore speculate that elevation in FSH levels beyond a certain threshold value does not result in any additional follicular development in poor responders to ovarian stimulation. Alternatively, the antiestrogenic effects of CC may have blunted the potential added benefits of additional FSH levels in the CC/hMG group.

Table III. Serum Estradiol (E₂), Estrone (E₁), and Estrone Sulfate (E₁S) Levels in the CC/hMG Versus MDF

Group type of estrogen	Baseline			Day 5 of medication			Day of hCG		
	E ₂ (pg/mL)	E ₁ (pg/mL)	E ₁ S (ng/mL)	E ₂ (pg/mL)	E ₁ (pg/mL)	E ₁ S (ng/mL)	E ₂ (pg/mL)	E ₁ (pg/mL)	E ₁ S (ng/mL)
CC/hMG	24 ± 3.3	24 ± 3	1.9 ± 0.7	396 ± 80	129 ± 46	18.4 ± 8.1	1321 ± 275	462 ± 122	19.7 ± 6.0
MDF	20.4 ± 5.4	16 ± 4.9	6.7 ± 3.2	324 ± 86	52 ± 28	19.4 ± 7.7	1162 ± 199	370 ± 144	32.2 ± 12.5
P value	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note. Results are reported as mean value ± SEM; ns, not significant.

Table IV. Oocyte Maturation, Fertilization Rate, and Embryo Quality in the CC/hMG Versus the MDF

Group	CC/hMG	Microdose flare	p value
Percent of mature oocytes	51 ± 15	45 ± 16	ns
Percent fertilization	63 ± 22	54 ± 19	ns
Percent of grade A and B embryos	29 ± 11	24 ± 10	ns
Percent of 8-cell stage embryos on day of ET	30 ± 8	38 ± 9	ns

Note. Results are reported as percent mean value ± standard error of the mean (SEM); ns, not significant.

In an effort to evaluate the total estrogenicity of the cycle, we measured serum levels of E₁ and E₁S in addition to serum E₂ levels. The potential benefits of this approach have previously been reported by us and others (20,22,23); however, in the present study, we found no advantage in measuring total estrogenicity over measuring serum E₂ alone in this group of poor responder patients.

We also evaluated the EEC in the two groups. We found no statistically significant differences in this measurement between the CC/hMG and the MDF groups. The endometrium has been previously reported to respond poorly in the presence of CC (24,25). However, since the serum E₂ levels were similar in both groups, it does not appear that the addition of CC in the CC/hMG group had an adverse effect on the endometrium in this cohort of patients.

We conclude that CC is a more potent stimulator of FSH production than MDF leuprolide acetate when administered in conjunction with exogenous hMG in poor responders to ovarian stimulation. However, this additional FSH production does not result in additional follicular growth, suggesting that elevation in FSH levels beyond a certain threshold value does not result in any additional follicular development in poor responders to ovarian stimulation.

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