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High-Dose Follicle-Stimulating Hormone (FSH) Ovarian Stimulation in Low-Responder Patients for in Vitro Fertilization[1]

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Follicle-stimulating hormone (FSH) was used in high doses (6 ampoules/day:6FSH) for ovarian hyperstimulation for in vitro fertilization in women with a previous poor response to stimulation with the equivalent of "4FSH." Luteinizing hormone levels did not differ between stimulations, but both FSH and estradiol levels were higher in the 6FSH compared to the 4FSH cycle. There were fewer cancellations in the 6FSH cycle, but similar numbers of preovulatory oocytes were retrieved, fertilized, and transferred. The pregnancy rates per attempt and retrieval were higher in the 6FSH cycle. We conclude that raising and maintaining FSH levels during stimulation in low responders reduced cancellations and may improve in vitro fertilization outcome.

KEY WORDS: in vitro fertilization (IVF); low responders; follicle-stimulating hormone (FSH); gonadotropin stimulation.

INTRODUCTION

The number of couples seeking treatment for infertility has increased dramatically over recent years. As a result, various techniques for assisted reproduction have emerged. However, certain groups of infertility patients are resistant to these therapies when conventionally applied. One such group, termed the low responders, responds poorly to the usual gonadotropin stimulation for in vitro fertilization and embryo transfer (IVF-ET). The low response, first described by Garcia *et al.* (1), was defined as a peak estradiol (E_2) of <300 pg/ml following a standard stimulation with human menopausal gonadotropin (hMG), a poor follicular response manifested by fewer oocytes retrieved, fertilized, and transferred, and a lower ongoing pregnancy rate compared to normal or high responders (1,2). Several ovarian hyperstimulation protocols for these low-responding patients have been attempted but have proved ineffective. These include the use of a pulsatile gonadotropin-releasing hormone (GnRH) pump (3) and the use of GnRH agonists prior to ovarian stimulation with gonadotropins (4–6).

Jones *et al.* (3) identified the "perimenopausal" IVF patient as a subset of the poor responder group who have elevated basal (cycle day 3) follicle-stimulating hormone (FSH) levels but normal luteinizing hormone (LH) levels. Recently, responses to gonadotropin stimulation for IVF-ET were categorized into seven groups by basal FSH and LH levels (7). Women with elevated basal FSH ($\geq$15 mIU/ml), regardless of LH level, had a poor E_2 response during ovarian stimulation with FSH and hMG (8–10), with fewer oocytes retrieved, fertilized, and transferred and no ongoing pregnancies compared to the women with normal FSH levels. It was this subset of low-responding patients, many of whom had elevated basal FSH and normal LH levels, at whom this study was directed. Because elevated FSH levels are associated with regular ovu-

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latory cycles in women in the perimenopausal period, we hypothesized that ovarian hyperstimulation in these low-responding perimenopausal patients may best be accomplished with very high doses of FSH. This report details our results.

MATERIALS AND METHODS

Twenty-three patients undergoing ovarian hyperstimulation for IVF-ET from Norfolk series 26–32 (January 1987 to June 1988) participated. All women were ±10% of ideal body weight. Each woman had at least one stimulation with a "4FSH" protocol (300 IU FSH ± 150 IU LH), consisting of four ampoules of FSH (Metrodin, Serono Laboratories, Inc., Randolf, MA) containing 75 IU of FSH and <1 IU of LH per ampoule, or a combination protocol of two ampoules of FSH plus two ampoules of hMG (Pergonal, Serono) in a step-down fashion (Fig. 1). All patients had a poor response to this "4FSH" protocol, defined as a peak E_2 of <400 pg/ml (8,9) (20/23 patients) or a poor follicular response (≤3 follicles per attempt) (19/23 patients). Fifteen of 23 (65%) of the patients had more than one 4FSH stimulation (six cycles, 2 patients; five cycles, 1 patient; four cycles, 3 patients; three cycles, 4 patients; two cycles, 5 patients). The most recent stimulation was used for this study. In a subsequent cycle, each patient received stimulation with 6 ampoules of FSH (6FSH protocol: 450 IU FSH) daily, also in a step-down fashion (Fig. 1). In both protocols, the first step-down occurred when a dominant follicle (≥12-mm diameter; all measurements were maximal diameters) was detected. Each patient had blood drawn each morning from day 3 of the menstrual cycle until the day of oocyte retrieval. The serum was separated by centrifugation and stored frozen until assayed. Transvaginal ultrasonography was performed daily from day 6 to monitor follicular development (5-mHz transducer, RT3600, General Electric, Parsippany, NJ). Human chorionic gonadotropin (hCG) (10,000 IU) was administered using the same criteria (8–10) for both the 4FSH and the 6FSH conditions. Transvaginal oocyte retrieval was performed 34 hr later. Cycle cancellation was recommended to the patient for a poor E_2 response (<200 pg/ml) or a poor follicular response (≤2 follicles), but some patients elected to go to retrieval despite meeting these cancellation criteria. Oocytes recovered were classified by the criteria of Veeck *et al.* (11), with insemination, culture, and transfer techniques as previously described (11,12). Serum was analyzed by radioimmunoassay (RIA) for FSH, LH (FSH-Quant, LH-Quant, Leeco Diagnostics, Inc., Southfield, MI), and E_2 (Pantex, Santa Monica, CA). All samples were run with kits from the same batch. The intraassay and interassay coefficients of variation for FSH, LH, and E_2 were all <7%.

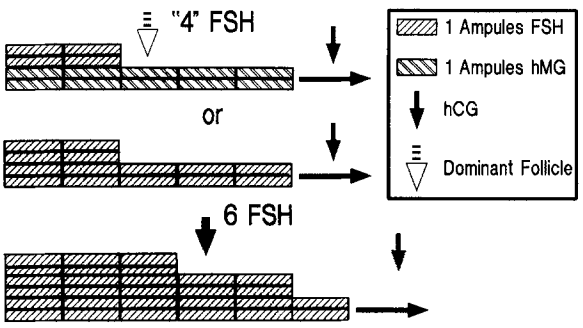


Fig. 1. Schematic representation of the step-down protocols used in the "4FSH" and 6FSH stimulations. The large downward arrowhead for the 6FSH cycle represents the day a dominant follicle >12 mm in diameter was noted on transvaginal ultrasound.

Each woman received both stimulations in a paired design; thus, data were analyzed with paired methods when appropriate. Fisher's exact test (two-tailed) was used to evaluate binary data. Analysis of variance and *t* tests (two-tailed) were applied to parametric data. Significance was defined as $P < 0.05$. All data are given as mean ± SD unless otherwise noted.

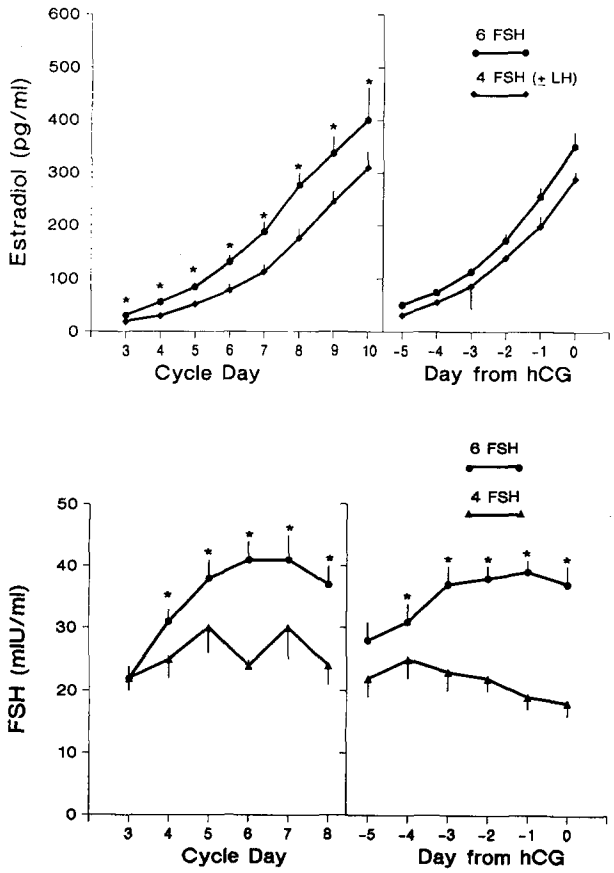
RESULTS

There were no differences in the age of the women (36.1 ± 3.3 and 36.3 ± 2.9 years), basal FSH levels (22 ± 10 and 22 ± 10 mIU/ml), LH levels (17.0 ± 6.6 and 16.0 ± 4.8 mIU/ml), or the day of hCG administration (8.5 ± 1.3 and 8.6 ± 1.0 days) between "4FSH" and 6FSH cycles, respectively. The 6FSH stimulations required significantly more ampoules of gonadotropin (28 ± 6) before hCG was given than the 4FSH stimulation (19 ± 5) ($P < 0.0001$). There were significantly ($P < 0.05$) fewer cancellations in 6FSH cycles (2/23; 9%) versus 4FSH cycles (8/23; 35%) (Fisher's exact test, $P = 0.03$). Some patients requested retrieval despite meeting criteria for cancellation. There was no bias for not cancelling the patients with a poor response (6FSH, 6/21 (14%); 4FSH, 4/15 (27%); Fisher's ex-

act test, $P = 0.1$). Figure 1A demonstrates the E_2 response to both the 4FSH and the 6FSH stimulations. Estradiol levels were higher in the 6FSH cycle from day 3 onward (Fig. 2). However, when canceled cycles were eliminated, there was no longer any difference in the E_2 levels. The basal E_2 values from the two stimulation cycles, while statistically different, were both within the normal range for day 3 E_2 values in our laboratory. Figure 2 demonstrates the LH levels throughout both stimulations. There was no difference in the LH levels between the two cycles by cycle day or day of hCG. The FSH levels during stimulation are shown in Fig. 2. Note the elevated basal (day 3) levels of FSH in both conditions. The FSH levels in 6FSH cycles were significantly (ANOVA, $P < 0.002$) higher than in 4FSH cycles from day 4 onward. These differences remained significant when only the cycles in which women received their hCG were considered. In 6FSH cycles, FSH levels were maintained above baseline, with peak values of 41.6 ± 15 mIU/ml on cycle day 7. The number of preovulatory oocytes retrieved, fertilized, and transferred was slightly higher in 6FSH cycles, but the differences did not reach statistical significance (Fig. 3, left panel). The number of pregnancies per attempt (initiation of stimulation) and per transfer was significantly higher in the 6FSH cycle than in the 4FSH cycle (Fig. 3, right panel) (Fishers exact test, $P = 0.02$ and $P = 0.03$, respectively). There were seven pregnancies in the 6FSH group (one clinical, three miscarriages, and three ongoing) and one which is delivered from the 4FSH group.

DISCUSSION

Women whose ovarian function has been compromised by an inflammatory process, endometriosis, extirpative surgery, or advanced age often respond poorly to gonadotropin stimulation for assisted reproduction. Various definitions of poor responders have been developed; this variability has made objective evaluation of the merits of a proposed new therapy for this group difficult. Jones *et al.* (3) defined the poor responder as those with a low serum E_2 unresponsive to gonadotropin stimulation, poor follicular development, elevated basal



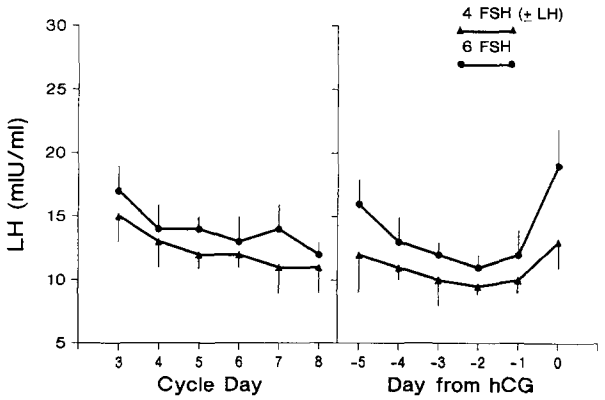


Fig. 2. E_2 levels during "4FSH" and 6FSH stimulations. The left panel shows E_2 levels by cycle day. Significant differences exist from day 3 onward (t test; $P < 0.05$). The right panel shows E_2 levels with reference to the day of hCG administration (thus canceled cycles are not represented). Significant differences between the two stimulation cycles are no longer observed. LH levels during 4FSH and 6FSH stimulations. The left panel graphs LH by cycle day. The right panel shows LH levels with reference to the day of hCG administration (canceled cycles eliminated). There were no differences in LH levels between stimulations. FSH levels during 4FSH and 6FSH stimulations. The left panel demonstrates the FSH levels by cycle day. Significant differences exist beyond day 3 (t test; $P < 0.05$). The right panel shows FSH levels with reference to the day of hCG administration (canceled cycles excluded). Significant differences exist on all days (t test; $P < 0.05$). Note the elevated basal (day 3) levels in both conditions.

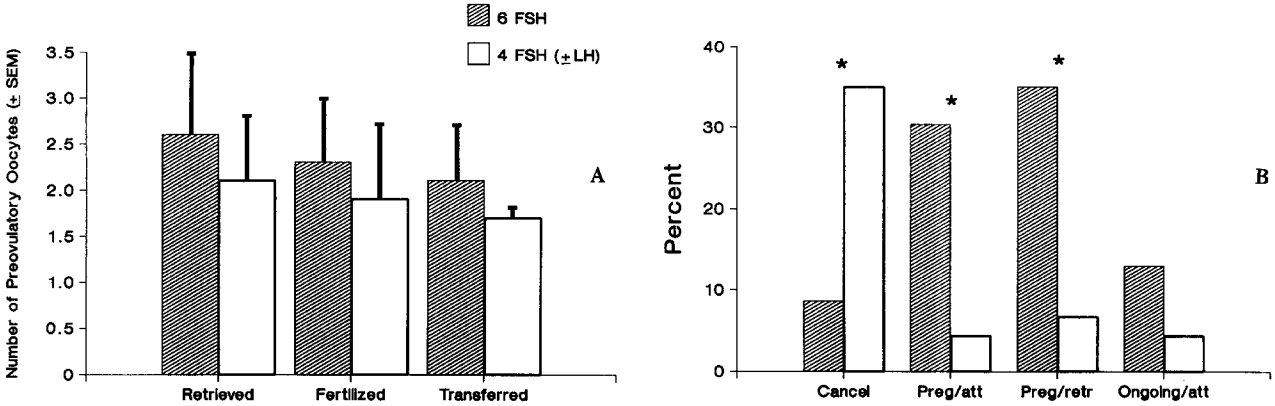


Fig. 3. (A) Average number of preovulatory oocytes retrieved, fertilized, and transferred per patient going to retrieval in the "4FSH" and 6FSH stimulations. No significant differences were found. (B) The decreased cancellation rate (Fisher's exact test; $P = 0.03$) and increased pregnancy rate per attempt (Fisher's exact test; $P = 0.03$) and increased pregnancy rate per attempt (Fisher's exact test; $P = 0.02$) and per retrieval (Fisher's exact test; $P = 0.05$) during 4FSH stimulation vs 6FSH stimulation. More ongoing pregnancies were observed in the 6FSH cycle, but this was not statistically significant.

E_2 levels with or without a cyst, or a rapidly rising E_2 followed by an LH surge in women with perimenopausal basal gonadotropins. The GnRH pump was used in these patients with no apparent benefit (3). Other studies using GnRH agonists to suppress poor-responding patients prior to gonadotropin stimulation have not shown any advantage to using this approach. In these studies, the low response was defined as (a) recruitment of a small number of asynchronous follicles (4), (b) peak E_2 <500 pg/ml, an LH surge before hCG administration, or whether a dominant follicle emerged (5), or (c) development of <3 mature follicles or a dominant follicle, follicular growth arrest, or a poor E_2 response (6).

The present study adopted a stricter definition of a poor response as a peak E_2 <400 pg/ml or a follicular response of ≤3 follicles, with most patients having elevated basal FSH levels (>15 mIU/ml) associated with a poor stimulation response (3,7). This strict definition defined a subset of the poor-responder group that would have failed treatment with the GnRH pump or GnRH agonists as outlined above. Each patient in this study was selected for the 6FSH protocol on the basis of at least one previous poor response to the "4FSH" protocol, and because patients respond in a similar fashion from one cycle to the next on a given stimulation protocol (13). Additionally, the majority of the patients (65%) had failed a 4FSH stimulation at least twice. The fact that patients from the 4FSH stimulation had done poorly was, in principle, a selection bias but one common to all studies cited on poor responders. Hence, while we report three ongoing pregnancies for the patients treated with 6FSH, the selection bias and small numbers preclude any conclusions regarding ongoing pregnancy rates.

It is generally accepted that basal FSH levels rise as women become perimenopausal (3,14) in order to maintain ovulatory competence. We chose to stimulate poor-responding patients, many with elevated basal FSH levels, by using high doses of FSH. One group has reported that this strategy of increasing the dose of gonadotropins (hMG) gave higher levels of FSH, LH, and E_2 in high responders (peak E_2 > 1000 pg/ml). However, in low responders (peak E_2 < 1000 pg/ml), increasing the dose of hMG increased the FSH and LH levels but had no effect on E_2 (15).

In the present study, women with elevated basal FSH levels who responded poorly to a 4FSH protocol were stimulated with high-dose FSH (6FSH). The results demonstrate a sustained, elevated level of FSH throughout the stimulation cycle. E_2 levels were also significantly elevated throughout 6FSH cycles. Fewer cancellations occurred in 6FSH cycles versus 4FSH cycles. The number of oocytes recovered, fertilized, and transferred in 6FSH cycles was not significantly greater than in 4FSH cycles. The pregnancy rate per attempt and per transfer was significantly higher in 6FSH cycles than in 4FSH cycles. Whether similar results could have been obtained with similar doses of hMG with the associated LH is unknown.

We conclude that in poor responders, ovarian hyperstimulation with high doses of FSH (6FSH) resulted in sustained elevated FSH levels, improved

E_2 response, and fewer cancellations; it may yield more ongoing pregnancies. We suggest that high-dose FSH may offer an alternative for these difficult patients.

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