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Urinary Follicle-Stimulating Hormone for Normogonadotropic Clomiphene-Resistant Anovulatory Infertility: Prospective, Randomized Comparison between Low Dose Step-Up and Step-Down Dose Regimens*

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ABSTRACT

A low dose step-up and step-down regimen for induction of ovulation using urinary FSH was compared in a prospective randomized fashion in 37 normogonadotropic clomiphene-resistant oligo- or amenorrheic infertile women. The objectives was to assess potential differences in duration of treatment, ovarian stimulation (serum FSH levels), and response [serum estradiol (E_2) levels and number and size of follicles]. Monitoring (blood sampling and transvaginal sonography) took place on the day of initiation of treatment, the first day of ovarian response as assessed by ultrasound (*i.e.* the first day a follicle ≥10 mm could be recognized), the day of hCG administration to induce ovulation, and 3 days thereafter.

The median duration of treatment in the low dose step-up group was 18 (range, 7–41) days compared to 9 (range, 4–16) days in the step-down group ($P = 0.003$), and the total numbers of ampules administered were 20 (range, 7–69) and 14 (range, 7–33), respectively (P = NS). Serum FSH levels from the first day of sonographic ovarian response until the administration of hCG were constant (median increase, 2%/day) in patients receiving the low dose step-up protocol, but showed a decrease (median, 5%/day) in step-down cycles ($P < 0.001$). Monofollicular growth, defined as not more than one follicle 16 mm or larger on the day of hCG administration, was observed in 56% of low dose step-up and 88% of step-down cycles ($P = 0.04$). The percentage of patients with normal range periovulatory E_2 serum levels (500–1500 pmol/L) was 33% in the low dose step-up group *vs.* 71% in the step-down group ($P = 0.03$).

We conclude that a step-down protocol for gonadotropin induction of ovulation exhibits a more physiological, late follicular phase FSH serum profile than a low dose step-up protocol. This results in a shorter duration of treatment, a greater number of monofollicular cycles, and more cycles with periovulatory E_2 levels within the normal range in the step-down protocol. (*J Clin Endocrinol Metab* **82:** 3597–3602, 1997)

SINCE THE early 1960s, infertile patients with anovulation have been treated with human menopausal gonadotropin and hCG. This treatment modality has proven to be effective (1), although associated complications (principally multiple pregnancies and ovarian hyperstimulation) remain an issue of major concern (2–5). Based on Brown's theory (6), conventional step-up regimens have been modified to a low dose step-up protocol (7, 8). A lower initial dose of a half-ampule or one ampule per day is used, and the dose is increased by small increments (half-ampule per day) at 1- to 2-week intervals in an attempt to slowly and prudently surpass the individual FSH threshold. In both treatment regimens the administered dose of gonadotropins is kept constant from the day of sufficient ovarian response on ultrasound (4, 7, 8) until the day of hCG administration to induce ovulation. The risk of multiple follicle development and subsequent complications is reduced with low dose step-up as compared to conventional step-up regimens (7, 8). However, low dose step-up regimens may also lead to multiple follicle development (9) and appear to be more time consuming (10). Due to the long half-life of FSH (11, 12), fixed daily doses of gonadotropin preparations may result in accumulation and, therefore, increased FSH serum levels in the late follicular phase. It has been reported that the magnitude of FSH accumulation determines ovarian hyperresponse in patients treated with *in vitro* fertilization (13).

In contrast, during the normal menstrual cycle the dominant follicle continues to grow despite decreasing FSH serum levels (14–16). Diminishing concentrations of FSH during the follicular phase have been shown to be essential for monofollicle development in the monkey model (17) as well as in the human (18). It is possible that elevated late follicular phase FSH levels during step-up regimens unintentionally interfere with single dominant follicle selection (19). Our previous studies have shown that a step-down gonadotropin dose regimen more closely resembles decreasing FSH levels found in spontaneous cycles (16, 20, 21) and can serve as a safe and successful treatment alternative for women suffering from clomiphene-resistant anovulation (22). This study is the first prospective randomized comparison between a low dose step-up and a step-down protocol for gonadotropin induction of ovulation. The objective was to assess potential

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differences in treatment duration and dose, ovarian stimulation, and response.

Subjects and Methods

Subjects

Inclusion criteria were infertility (seeking pregnancy ≥1 yr), cycle abnormalities [oligmenorrhea (interval between menstrual bleedings >35 days) or amenorrhea (no bleeding for at least 6 months)], FSH serum concentration within the normal range (1–10 IU/L), clomiphene resistance (defined as failure to ovulate or conceive after clomiphene treatment up to a daily dose of 150 mg from cycle days 3–7 during at least three consecutive cycles), age 20–40 yr, normal PRL (<15 nmol/L) and TSH (0.2 mIU/L < TSH < 4.2 mIU/L) serum levels, absence of evidence of bilateral tubal occlusion as assessed by hysterosalpingogram, and absence of severe oligospermia (sperm count $<10 \times 10^6$ spermatozoa/mL).

Thirty-seven patients attending our Fertility Clinic fulfilling the above criteria were included in this study. Patients included (see Table 1) had a median age of 29 yr (range, 20–40 yr), a median body mass index (weight divided by height squared) of 25 kg/m^2 (range, 18–41 kg/m^2), and a median duration of infertility of 3 yr (range, 2–7 yr). The protocol was approved by the local human subject committee, and informed consent was obtained from all participants.

Study design

This was a prospective, randomized, single center study comparing a low dose step-up with a step-down dose regimen for induction of ovulation with urinary FSH. Initial screening was performed within 2 months before inclusion. Initial screening included random blood withdrawal and transvaginal sonography (TVS). Sonographic monitoring was performed by a single observer (E.J.P.v.S.), using an ultrasound machine (model EUB-415, Hitachi Medical Corp., Tokyo, Japan) with a 6.5-MHz transvaginal transducer. The ovaries were localized in relation to the iliac vessels. Follicles appeared as round or ovoid translucent structures 2 mm or more in diameter. Follicle number was established by scanning each ovary from the inner to the outer margin in longitudinal cross-section. The ovarian volume was estimated according to the following formula: ½ ($A \times B \times C$), where A is the longitudinal diameter, B is the antero-posterior diameter, and C is the transverse diameter of the ovary. Mean follicle number and mean ovarian volume were calculated from the sum of the left and right values divided by 2. Ovarian stroma echogenicity was scored as 1 (normal), 2 (moderately increased), and 3 (markedly increased) as described by Pache *et al.* (23). Total stroma count was the combined stroma scores of left and right ovaries. For each patient, blood samples for initial screening were randomly taken through venipuncture, centrifuged within 2 h after withdrawal, and stored at −20 C until assayed. Before entering the protocol, serum from each patient was assayed for immunoreactive FSH, LH (normal range, 1.3–6.9 IU/L) (24), estradiol (E_2), testosterone, androstenedione, sex hormone-binding globulin, and progesterone as previously described (25). After inclusion, patients were randomized by receiving a study number corresponding to a sealed envelope containing the protocol to be followed.

Gonadotropin treatment was started 3–5 days after the initiation of spontaneous or progestagen-induced withdrawal bleeding. Patients received daily im injections of purified urinary FSH (Follegon, Organon, Oss, The Netherlands) from a single batch (no. 74029003). Monitoring of ovarian response by TVS was performed every 2 or 3 days until hCG (Pregnyl, Organon) was administered. hCG was injected im as a single dose of 10,000 IU on the day upon which at least one follicle 18 mm or larger was observed by TVS examination. If more than three follicles 16 mm or larger were present, stimulation was canceled, and the patient was advised to use barrier contraceptives. During the study, blood samples were obtained on the day treatment was initiated, the day of sufficient ovarian response, the day of hCG injection, and 3 and 7 days thereafter. Sufficient ovarian response was defined as visualization of a follicle 10 mm or larger by TVS, which coincides with the appearance of the dominant follicle in the normal menstrual cycle (16, 23). FSH injections were administered between 0800–1200 h. Blood withdrawal was performed just before gonadotropin injection, *i.e.* 24 h after the previous injection. During treatment, the ovarian response was monitored by TVS only.

TABLE 1. Initial patient characteristics (median and range) of 37 normogonadotropic clomiphene-resistant oligo- or amenorrheic infertile women treated with urinary FSH for ovulation induction

	Low dose step-up	Step-down
n	19	18
Age (yr)	28 (20–35)	30 (23–40)
BMI (kg/m^2)	26 (19–40)	25 (18–41)
Infertility duration (yr)	3 (2–5)	3 (2–7)
Amenorrhea (%)	26	33
Primary infertility (%)[a]	63	61
Serum hormone concentrations[b]		
LH (IU/L)	5.6 (1.5–15.4)	5.8 (2.7–13.8)
FSH (IU/L)	4.9 (2.7–9.0)	4.5 (2.2–6.8)
E_2 (pmol/L)	238 (97–378)	250 (75–864)
T (nmol/L)	2.1 (1.3–4.6)	2.2 (1.4–4.3)
AD (nmol/L)	15.4 (8.5–30.4)	13.3 (9.3–32.8)
SHBG (nmol/L)	32 (10–128)	47 (20–164)
Transvaginal ultrasonography		
Mean ovarian vol (mL)	8.5 (4.0–14.9)	11.3 (5.7–18.4)
Mean follicle no./ovary	12.5 (1.5–15.0)	14.3 (5.5–15.0)
Total stroma count[c]	4 (2–6)	4 (2–5)

Patients were randomized for low dose step-up or step-down dose regimens.

[a] Absence of previous pregnancies in the present relationship.

[b] Blood samples were taken at random within 1 month preceding the study cycle.

[c] Scored on a scale from 1 (normal) to 3 (severely increased density) and added for both ovaries.

Low dose step-up protocol

The starting dose of FSH was one ampule (75 IU) per day. The first increase in dose by a half-ampule per day was based on absence of a follicle 10 mm or larger after 14 days. The dose was increased by a half-ampule per day every 7 days if an ovarian response was lacking. If a sufficient ovarian response was observed, the dose was kept constant until the administration of hCG. No luteal support was provided.

Step-down protocol

The starting dose of FSH was two ampules (150 IU) per day. The first decrease in dose by a half-ampule per day was based on visualization of at least one follicle 10 mm or larger. The initial dose was increased by a half-ampule per day if the ovarian response remained absent after 5 days. If follicular growth remained absent over the following 10 days (two incremental steps of a half-ampule per day), further medication was withheld, and the cycle was canceled. A further dose decrease, each time by a half-ampule per day, was performed every 3 days to a minimum dose of one ampule per day if follicular growth continued. This dose was sustained until the day hCG could be administered. No luteal support was provided.

Data analysis

Before initiation of the study, power calculations were performed to determine the required number of patients for the detection of differences in serum FSH and E_2 levels and the number of dominant follicles on the day of hCG administration, comparing both dose regimens. Based on the literature and our previous studies (20, 21), differences were estimated to be 30%. This difference was calculated to be apparent with at least 12 patients in each group.

Values given are the mean ± SD unless stated otherwise. The P values given are two-sided, and 0.05 was considered the limit of statistical significance. Differences between patient groups were tested using the Mann-Whitney test, Student's t test, or Fisher's exact test. The distribution of ovarian follicles was arbitrarily classified in categories according to size (10–12, 12–16, and ≥16 mm), as described previously (22). Ovulation after hCG administration was determined by the collapse of

the dominant follicle on TVS and midluteal P levels. Pregnancy diagnosis was confirmed by a positive urinary pregnancy test (hCG, >25 IU/L), and ongoing pregnancy was confirmed by sonographic evidence of an intrauterine gestational sac and fetal heart beat. Patients attending our clinic within 2 weeks after the last administration of gonadotropins with serious abdominal discomfort, sonographic evidence of grossly enlarged ovaries, and increased quantities of free abdominal fluid were reported to be suffering from ovarian hyperstimulation syndrome.

Results

Thirty-seven normogonadotropic patients with clomiphene-resistant anovulation entered the study protocol. After randomization, 19 (51%) patients received exogenous FSH treatment according to a low dose step-up regimen, and 18 patients received a step-down dose regimen. Patient characteristics of both groups are shown in Table 1. The groups did not differ in any of the characteristics shown.

In both groups one patient dropped out early due to extensive vaginal bleeding during gonadotropin treatment. Cancellation due to ovarian hyperresponse (more than three follicles ≥16 mm on the day of hCG administration) did occur once in the low dose step-up regimen. Seventeen patients in both groups received hCG. One patient in each group presented with low luteal progesterone levels (<10 nmol/L), suggesting the absence of ovulation.

Clinical treatment outcome is presented in Table 2. The median first day of ovarian response monitored by TVS in the low dose step-up group was treatment day 11 (range, 5–33) *vs.* day 5 (range, 3–13) in the step-down group ($P < 0.001$). The median day of administration of hCG in the low dose step-up group was 20 (range, 8–42) *vs.* 10 (range, 5–17) in the step-down group ($P = 0.001$). In the low dose step-up group, 7 patients (39%) acheived a preovulatory follicle using not more than 1 ampule of urinary FSH/day. In the step-down group, the initial dose of two ampules per day was sufficient to induce an ovarian response within 5 days in 14 (78%) patients.

Median serum FSH levels on the day treatment was started did not differ in the two groups and were higher on the first day of ovarian response, as determined by ultrasound, in the step-down group [6.3 IU/L (range, 4.7–10.2 IU/L) *vs.* 5.4 IU/L (range, 3.4–9.5 IU/L); $P = 0.04$; see Fig. 1]. From the day of ovarian response until the day of hCG administration, FSH levels showed an increase in 11 (61%) cycles in the low dose step-up group, whereas in the step-down group, FSH levels showed a decrease in all cycles ($P < 0.001$). The low dose step-up group exhibited a median increase in serum FSH during the late follicular phase of 0.6 IU/L·day (range, 2.0 to −4.3 IU/L·day; 2% increase/day), whereas in the step-down group, FSH levels decreased during the same period (1.3 IU/L·day; range, 0.2–5.0 IU/L·day; 5% decrease/day; see Fig. 1). Differences between the groups in changes in serum FSH during the late follicular phase were highly significant ($P < 0.001$). Patients treated according to the low dose step-up protocol with decreasing late follicular serum FSH levels exhibited monofollicle growth in five (71%) cases, whereas in the group with increasing serum FSH levels, there were five (45%) patients with monofollicular growth (P = NS.). No significant correlation was observed between absolute individual changes in serum FSH concentrations in the late follicular phase (between the day of ovarian response and the day of hCG administration) and serum E_2 levels or number of follicles (≥12 mm diameter) on the day of hCG administration in the total study group or in both subgroups separately (Fig. 2).

The distribution of follicles (arbitrarily classified in size categories of 12–16 and ≥16 mm diameter) on the day of hCG administration is shown in Fig. 3. Medium-sized follicles of 10–16 mm were absent in 22% and 29% of low dose step-up and step-down cycles, respectively (P = NS). Ovarian volumes on the day of hCG administration and 3 days thereafter showed no differences between the groups (data not shown).

The median increase in serum E_2 levels from the day of ovarian response until the day of hCG administration was 179 pmol/L·day (range, 33–742 pmol/L·day) in the low dose step-up group and 250 pmol/L·day (range, 23–1273 pmol/L·day) in the step-down group (P = NS).

In the step-up group, one pregnancy ended in an early miscarriage at 6 weeks, and one appeared to be a tubal pregnancy and had to be removed by laparoscopy. The ongoing pregnancy rate was 13% in the step-up group and 31% in the step-down group.

Discussion

Knowledge regarding the interplay between serum FSH levels and follicle growth during the normal menstrual cycle

TABLE 2. Clinical outcome of 35 normogonadotropic clomiphene-resistant oligo- or amenorrheic infertile women treated with urinary FSH for ovulation induction

	Low dose step-up	Step-down	*P*
Median duration of treatment (days)	18 (7–41)	9 (4–16)	0.003
Median no. of ampoules used per cycle	20 (7–69)	14 (7–33)	NS
Daily FSH change (IU/L) from the day of ovarian response until hCG, (%/day)	+0.6 (+2)	−1.3 (−5)	<0.001
Patients with decreasing late follicular phase FSH levels, no. (%)	6 (39)	17 (100)	<0.001
Monofollicle growth,[a] no. (%)	10 (56)	15 (88)	0.04
Patients with E_2 within normal preovulatory range (500–1500 pmol/L), no. (%)	6 (33)	12 (71)	0.03
Cycles cancelled (no.)	2	1	
Overall ovulation rate (%)	84	89	
Ongoing pregnancies (no.)	2	5	
Multiple pregnancies (no.)	0	0	
Abortion (no.)	1	0	
Tubal pregnancy (no.)	1	0	
Ovarian hyperstimulation (no.)	0	0	

Patients were randomized for low dose step-up (n = 18) or step-down (n = 17) dose regimens.
[a] Defined as 1 follicle 16 mm or larger on the day of hCG administration.

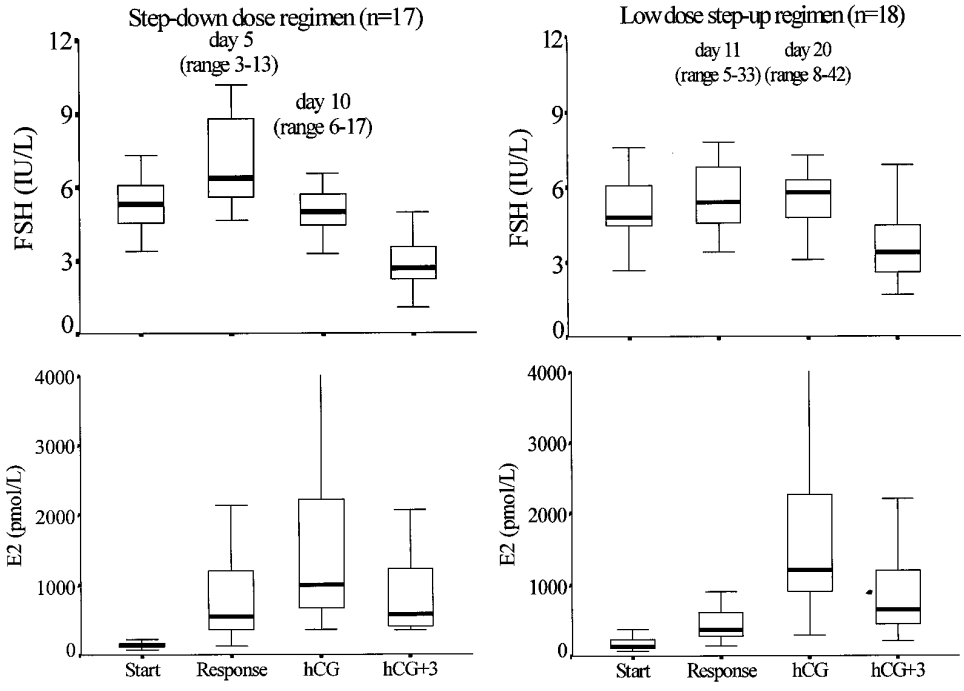

FIG. 1. Serum FSH (international units per L) and E_2 (picomoles per L) levels on the day of initiation of ovulation induction with urinary FSH (Start); the first day of ovarian response, defined as the first day a dominant follicle could be recognized using transvaginal sonography (Response); the day of hCG administration to induce ovulation (hCG); and 3 days thereafter (hCG+3) in 35 normogonadotropic clomiphene-resistant oligomenorrheic or amenorrheic infertile women. This group was randomized for a step-down dose regimen (*left panel*) or a low dose step-up regimen (*right panel*). Only cycles with ongoing follicle growth are included. Data are presented as box and whisker plots, whereas boxes encompass values between the 25th and 75th percentiles, *horizontal lines* represent median values, and "whiskers" give the 95% range of the values. See text for statistical evaluation.

has increased, and the process of selection of a single dominant follicle has been recognized to be dependent on decreasing FSH concentrations during the late follicular phase (17, 18). In contrast, increasing serum FSH levels in the late follicular phase have been described during conventional step-up regimens for induction of ovulation, even using a low dose step-up regimen (26). Step-up regimens ignore the concept that the FSH threshold should be surpassed for a limited period of time only (a narrow window) sufficient to allow a single follicle to gain dominance (6, 10). As complications of gonadotropin induction of ovulation, such as ovarian hyperstimulation and multiple pregnancy, are related to multiple follicle development (27), it may be worthwhile to focus on various dose regimens and resulting patterns in serum FSH levels. In some ovulation induction studies (26), late follicular steady state FSH levels were observed during low dose step-up regimens. Moreover, preliminary data suggest that decreasing serum FSH levels may occur due to negative estrogen feedback action in some patients (28) or iv low dose step-up FSH administration (29).

Our group has focused on a method of obtaining serum FSH levels during gonadotropin induction of ovulation that more closely mimic physiology (19). These studies have shown that the late follicular phase FSH profile during a step-down dose regimen with initial doses of two ampules per day and decreasing steps of a half-ampule per day closely resembles serum FSH levels of the spontaneous cycle (20, 21), and that treatment outcome is at least comparable to that using low dose step-up protocols (22). To substantiate potential differences, we compared the step-down induction regimen with a low dose step-up protocol in a prospective randomized trial. The results obtained indicate that in the step-down group, serum FSH levels decrease in the late follicular phase in all patients (median, 5%/day), whereas in the low dose step-up group, individual serum FSH levels decrease in only 39% of patients ($P < 0.001$). The small number of patients (39%) in the low dose step-up group that present with decreasing late follicular phase FSH levels consists of patients that in the majority of cases (71%) exhibit monofollicle growth; the "good responders." Consistent with pharmacokinetic studies (11, 12), the overall median serum FSH concentration in the low dose step-up group remains fairly stable in the late follicular phase. Another study in which exogenous FSH was replaced by pulsatile administration of GnRH when dominant follicle growth was first recognized showed that multiple follicle growth was minimized, and a low multiple pregnancy rate could be obtained (30). The decrease in serum FSH during step-down ovulation induction in a protocol that combined exogenous gonadotropins with GnRH agonists showed a more pronounced median decrease of 10%/day (20) compared to the 5% daily decrease demonstrated in the present study. In a study in which patients were treated according to a low dose step-up protocol using iv gonadotropin administration with and without pituitary down-regulation by GnRH agonists (29), the effect was reversed; in the group treated with GnRH agonists, serum FSH levels were stable, whereas a minimal decrease occurred in the group treated with gonadotropins alone. This may be related to the changes in endogenous FSH production in nonsuppressed patients. All of the above-mentioned observations favor a limited role for E_2 in late follicular FSH patterns in gonadotropin-stimulated cycles.

We observed more monofollicle cycles (88% *vs.* 56%) and more preovulatory serum E_2 levels in the physiological range (71% *vs.* 33%) in the step-down group than in the low dose step-up group. This may coincide with reduced chances for multiple pregnancy and ovarian hyperstimulation (27, 31). The reported percentages of monofollicle cycles in low dose step-up studies vary substantially due to different criteria used for defining monofollicular growth and cycle cancel-

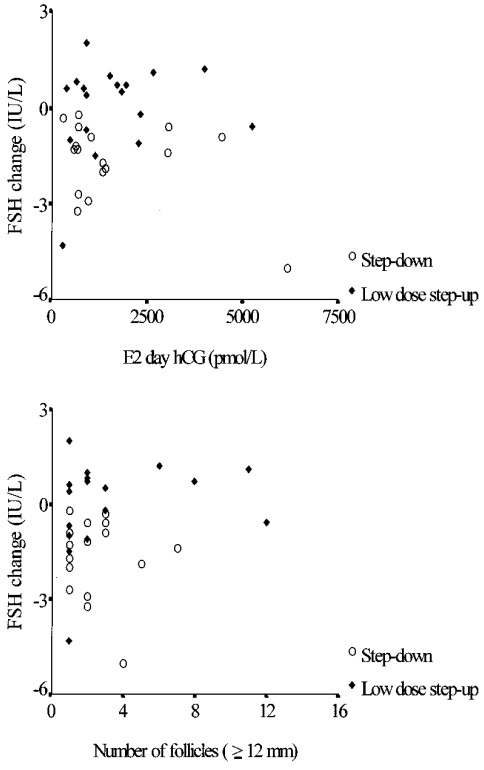

FIG. 2. Distribution of individual serum E_2 levels (*upper panel*) and number of follicles 12 mm or more in diameter (*lower panel*) on the day of hCG administration in a group of 35 normogonadotropic clomiphene-resistant oligomenorrheic or amenorrheic infertile patients treated after randomization according to a low dose step-up regimen (♦; n = 18) or a step-down dose regimen (○; n = 17) for induction of ovulation with urinary FSH. Significant correlation between these parameters and late follicular phase (between the day of ovarian response and the day of hCG administration) changes in serum FSH concentrations could not be observed in the total study group or separately in the two subgroups.

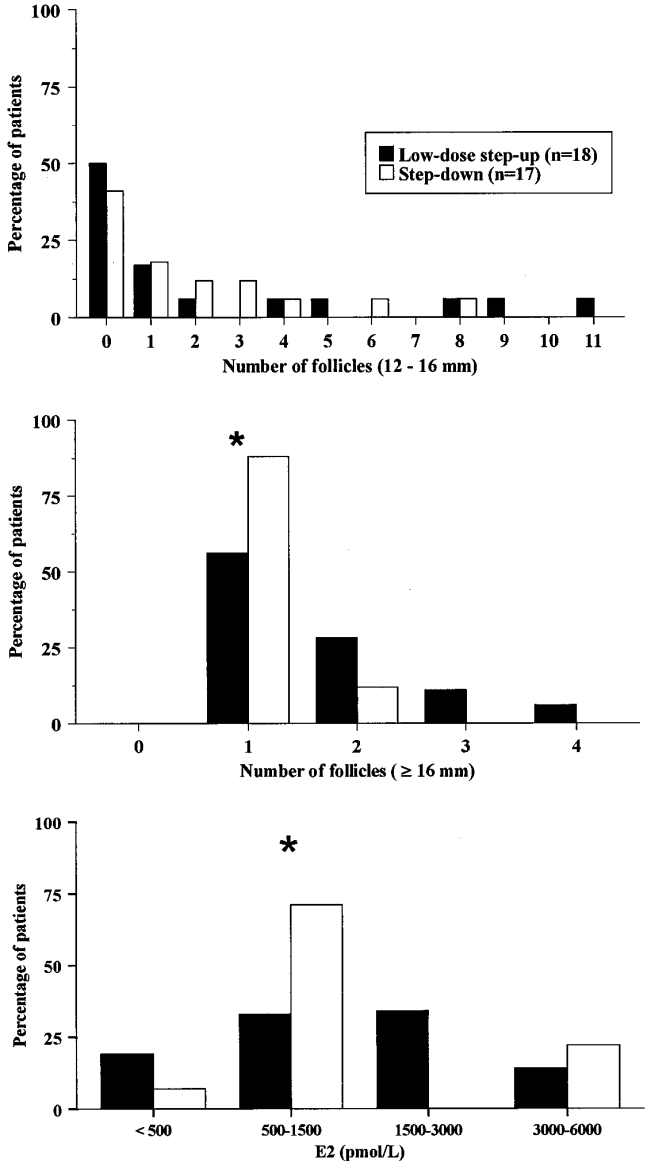

FIG. 3. Distribution of number of ovarian follicles (arbitrarily classified as 12–16 mm in diameter (*upper panel*) and ≥16 mm (*middle panel*)] and serum E_2 levels (*lower panel*) on the day of hCG administration in 35 normogonadotropic clomiphene-resistant oligomenorrheic or amenorrheic infertile women randomized for a step-down dose regimen (n = 17) or a low dose step-up regimen (n = 18) for induction of ovulation with urinary FSH. The *asterisk* indicates the significant difference ($P = 0.04$) in the percentage of patients (56% *vs.* 88%) presenting with monofollicular cycles (1 follicle ≥16 mm only), comparing both treatment schedules. The percentage of patients (33% *vs.* 71%) with normal preovulatory serum E_2 levels (500–1500 pmol/L) is also significantly ($P = 0.03$) different.

lation. The small subgroup of patients in the step-down protocol with very high late follicular phase serum E_2 levels (patients that hyperstimulate with the starting dose) may respond better to a lower starting dose of gonadotropins. Another feature observed in this study is that the step-down group ovulated after a shorter induction time (9 *vs.* 18 days). Using a low dose step-up protocol, patients may initially be exposed to serum FSH levels below the threshold for an extended period of time, resulting in a longer treatment period than strictly necessary. The treatment duration and number of ampules used in the low dose step-up regimen in our study are comparable to data reported previously (7, 8). Few other studies have reported the application of exogenous FSH in a step-down fashion for induction of ovulation (32, 33). The inclusion criteria used and a rigid step-down protocol render it difficult to draw conclusions from these studies. A large randomized study comparing conventional step-up and step-down regimens (initial dose of three ampules per day) has only appeared in abstract form (33).

In conclusion, our findings suggest that in a group of normogonadotropic clomiphene-resistant anovulatory infertile women, induction of ovulation using a step-down gonadotropin dose regimen results in comparable ovulation and pregnancy rates and a much shorter induction period than that required with a low dose step-up regimen. This may bring health economic benefits (more ovulations per given period of time). In addition, the late follicular phase serum FSH profile is more physiological and results in more monofollicular cycles and more cycles in which E_2 levels are within the normal preovulatory range. These observations may have important implications for the risk of ovarian hyperstimulation and multiple pregnancy.

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