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Luteinzing Hormone Activity in Menotropins Optimizes Folliculogenesis and Treatment in Controlled Ovarian Stimulation

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ABSTRACT

Although the role that LH plays in folliculogenesis is still controversial, recent evidence points toward facilitatory actions of LH activity in ovulation induction. Thus, we compared the response to either highly purified FSH (75 IU FSH/ampoule; group A, 25 subjects) or human menopausal gonadotropin (75 IU FSH and 75 IU LH/ampoule; group B, 25 subjects) in normoovulatory GnRH agonist-suppressed women, candidates for intrauterine insemination. A fixed regimen of 2 daily ampoules of highly purified FSH or human menopausal gonadotropin was administered in the initial 14 days of treatment; menotropin dose adjustments were allowed thereafter. Treatment was monitored with daily blood samples for the measurement of LH, FSH, 17β-estradiol (E_2), progesterone, testosterone, hCG, inhibin A, and inhibin B, and transvaginal pelvic ultrasound was performed at 2-day intervals. Although preovulatory E_2 levels were similar, both the duration of treatment (16.1 $\pm$ 0.8 *vs.* 12.6 $\pm$ 0.5 days; $P < 0.005$) and the per cycle menotropin dose (33.6 $\pm$ 2.4 *vs.* 23.6 $\pm$ 1.1 ampoules; $P < 0.005$) were lower in group B. In the initial 14 treatment days the area under the curve of FSH, progesterone, testosterone, inhibin A, and inhibin B did not differ between the 2 groups, whereas LH, hCG, and E_2 areas under the curve were higher in group B. The occurrence of small follicles (<10 mm) and the inhibin B/A ratio in the late follicular phase were significantly reduced in group B. A nonsignificant trend toward a higher multiple gestation rate was present in group A (60% *vs.* 17%). We conclude that ovulation induction with LH activity-containing menotropins is associated with 1) shorter treatment duration, 2) lower menotropin consumption, and 3) reduced development of small ovarian follicles. These features can be exploited to develop regimens that optimize treatment outcome, lower costs, and reduce occurrence of complications such as multiple gestation and ovarian hyperstimulation. (*J Clin Endocrinol Metab* **86:** 337–343, 2001)

GONADOTROPIN TREATMENT is widely used in infertility to correct anovulation and in controlled ovarian stimulation (COS) to induce multiple follicular development in assisted reproduction technology (ART) procedures. Although a wide variety of gonadotropin types have been introduced in the last 3.5 decades, none has completely prevailed to date, as many physicians still employ traditional human menopausal gonadotropin (hMG) along with recently developed recombinant human (r-h) FSH. All FSH-containing gonadotropin preparations are highly effective in stimulating ovarian follicle development; although some studies suggest that certain gonadotropin preparations could be preferable (1), no agreement exists in this area (2). Furthermore, all of these compounds share the potential for causing severe complications such as multiple gestations and the ovarian hyperstimulation syndrome (OHSS).

One of the most interesting controversies in this area relates to the role that LH may play in folliculogenesis and whether LH activity should be added to the course of gonadotropin ovulation induction (3). We recently showed that LH activity supplementation in the form of low dose hCG during highly purified (HP) FSH treatment can accelerate follicle development, shorten ovulation induction, and reduce HP FSH dose requirements (4). Nevertheless, LH activity in ART ovulation induction is usually provided by more traditional gonadotropin preparations such as hMG. Thus, we elected to apply two commonly employed medications with similar FSH content and different LH activity; our goal was to carefully assess endocrine features, folliculogenesis, and clinical outcome in patients treated with a fixed regimen of either HP FSH or hMG to verify and expand our previous observation on the role of LH activity in gonadotropin ovulation induction (4).

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Materials and Methods

Patient population

A total of 50 patients diagnosed as having unexplained or mild male-related infertility were studied. All subjects had regular menstrual cycles of 26- to 34-day duration, a normal body mass index of 20–25 kg/m^2, a pelvic ultrasound showing uterus and ovaries of normal size and structure, a hysterosalpingogram and/or laparoscopy demonstrating tubal patency, normal plasma and urinary chemistry and hematological values, and thyroid and reproductive hormones within the normal range. Although ovulation induction had been previously performed in some of the subjects, no patient had received any hormone therapy (including gonadotropins) for at least 3 months preceding the study.

Protocol

Our institutional review board approved the protocol, and all patients provided informed consent. Patients underwent early follicular phase reproductive hormone determinations and were then randomly as-

signed to two age- and weight-matched groups. The incidence of patients who had previously undergone gonadotropin ovulation induction as well as cause of infertility studies were similar in both groups. Patients were not blinded to treatment, which was started in the midluteal phase of a spontaneous menstrual cycle with the administration of a single injection of 3.75 mg depot triptorelin (Decapeptyl 3.75, IPSEN S.p.A., Milan, Italy). Ovulation induction began 14 days thereafter. Patients in group A received two ampoules im (150 IU) daily of HP FSH (Metrodin HP, Serono Pharma S.p.A., Rome, Italy), and patients in group B received two ampoules im daily of hMG (150 IU FSH and 150 IU LH; Menogon, Ferring S.p.A., Milan, Italy). In all patients gonadotropins were administered at 1400–1600 h, and the menotropin dose was not changed for 14 days or until at least four ovarian follicles of more than 14-mm diameter and 17β-estradiol (E_2) levels of 800-1500 pg/mL were detected (final maturation parameters). If these parameters were not achieved by the 14th day of treatment, increments in the HP FSH and hMG doses were allowed with the following schedule: three daily ampoules of HP FSH (225 IU) or hMG (225 IU FSH and 225 IU LH) on days 15–17, and four daily ampoules of HP FSH (300 IU) or hMG (300 IU FSH and 300 IU LH) thereafter. At obtainment of the final maturation parameters, 10,000 IU hCG were administered to trigger ovulation, and intrauterine insemination with a sperm swim-up procedure was performed 36 h thereafter. The luteal phase was supported with 90 mg daily intravaginal progesterone (P) gel (Crinone, Wyeth Lederle S.p.A., Aprilia, Italy) administered from days 3–14 after the preovulatory hCG dose.

Monitoring

Treatment monitoring was conducted throughout menotropin administration. Each day one blood sample was drawn between 0800–0900 h in a standard manner, and two serum aliquots were obtained. E_2 was measured daily in one of the serum aliquots for clinical monitoring, and the second aliquot was stored at −20 C for later measurements of LH, FSH, E_2, P, testosterone (T), hCG, inhibin A, and inhibin B. Transvaginal pelvic ultrasound was performed on menotropin treatment days 0 and 6 and on alternate days thereafter until preovulatory hCG administration. The physician performing the pelvic ultrasound was blinded as to which arm of the protocol each patient belonged.

Hormone assays

LH, FSH, E_2, P, T, and hCG were measured with chemiluminescence assays (ACS 180, Chiron Corp., Milan, Italy). Inhibin A and inhibin B were measured with ultrasensitive enzyme-linked immunosorbent assays (Serotec Ltd., Oxford, UK). The minimal detectable dose (MDD) of LH was 0.1 IU/L; the interassay coefficient of variation (CV) was 5.1%. The *in vitro* addition of up to 200,000 IU/L hCG did not affect LH determinations in this assay, as assessed at multiple levels of the standard curve. The MDD of FSH was 0.3 IU/L; the interassay CV was 6.1%. The MDD of E_2 was 10 pg/mL; the interassay CV was 6.6%. The MDD of P was 0.1 ng/mL; the interassay CV was 7.0%. The MDD of T was 0.1 ng/mL; the interassay CV was 6.9%. The MDD of hCG in this β-specific assay was 0.1 IU/L.; the interassay CV was 5.9%. The *in vitro* addition of up to 200 IU/L LH did not affect hCG determinations in this assay, as assessed at multiple levels of the standard curve. The MDD of inhibin A was 3.9 pg/mL; the interassay CVs at low (23 pg/mL) and high (247 pg/mL) concentrations were 13.0% and 7.0%, respectively. The MDD of inhibin B was 15.6 pg/mL; the interassay CV at low (111 pg/mL) and intermediate (464 pg/mL) concentrations were 6.0% and 6.0%, respectively.

Statistical evaluation

Data were expressed as the mean ± SE. Serum hormone levels during the initial 14 days of treatment were calculated in each cycle as the area under the curve (AUC). Between-group differences in continuous variables were assessed with Student's *t* test or the Mann-Whitney rank sum test, as appropriate. Between-group differences in noncontinuous variables were assessed by the χ^2 method with the Yates correction if needed.

Results

The patient characteristics of the two treatment groups are shown in Table 1. No significant between-group differences existed in age, height, weight, body mass index, menstrual cycle duration, ovarian volume, or baseline hormone levels. All patients completed the treatment schedule and appeared to ovulate at pelvic ultrasound. Eleven conceptions were achieved, five in group A and six in group B (pregnancy rates per cycle of 20% and 24% in groups A and B, respectively; P = NS). One spontaneous early miscarriage occurred in each group. There were four twin gestations, three in group A (60% multiple gestation rate) and one in group B (17%; P = NS). No moderate or severe OHSS cases were reported.

TABLE 1. Baseline characteristics and clinical outcome of treatment of the two groups of patients participating in the study

	Group A (HP FSH)	Group B (hMG)	*P*
Baseline parameters			
Age (yr)	32 ± 1	33 ± 1	NS
Ht (cm)	166 ± 1	164 ± 1	NS
Wt (kg)	60 ± 1	58 ± 1	NS
BMI (kg/m^2)	21.8 ± 0.3	21.7 ± 0.3	NS
Menstrual cycle duration (days)	28.2 ± 0.4	28.4 ± 0.3	NS
Mean ovarian vol (mL)	6.3 ± 0.2	6.1 ± 0.2	NS
LH (IU/L)	4.5 ± 0.3	4.0 ± 0.4	NS
FSH (IU/L)	6.9 ± 0.3	6.3 ± 0.3	NS
PRL (ng/mL)	15 ± 1	14 ± 1	NS
E_2 (pg/mL)	74 ± 7	70 ± 5	NS
P (ng/mL)	0.52 ± 0.03	0.43 ± 0.04	NS
T (ng/mL)	0.45 ± 0.04	0.41 ± 0.03	NS
hCG (IU/L)	<0.1	<0.1	NS
Inhibin A (pg/mL)	<3.9	<3.9	NS
Inhibin B (pg/mL)	<15.6	<15.6	NS
Results of gonadotropin administration			
Days of gonadotropin administration (range)	16.1 ± 0.8 (8–23)	12.6 ± 0.5 (6–17)	<0.005
Menotropin dose (ampoules)	33.6 ± 2.4	23.6 ± 1.1	<0.005
Preovulatory E_2 (pg/mL)	1133 ± 81	1080 ± 53	NS
Preovulatory follicles			
<10 mm	3.0 ± 0.3	0.8 ± 0.3	<0.0001
10–14 mm	3.7 ± 0.4	3.2 ± 0.7	NS
>14 mm	8.4 ± 0.8	6.3 ± 0.5	=0.05

The clinical results of treatment are shown in Table 1. Both the duration of gonadotropin administration and the menotropin dose employed were significantly increased in group A (Table 1); these parameters were 28% and 42% higher in group A than in group B, respectively. Preovulatory E_2 levels and the number of medium size (10–14 mm) ovarian follicles were comparable in groups A and B; however, the number of preovulatory small (<10 mm) and large (>14 mm) follicles was significantly reduced in group B (the difference in the number of large follicles was borderline significant, $P = 0.05$).

The dynamics of ovarian follicle development during treatment are shown in Fig. 1. Although the number of medium and large ovarian follicles measured at 2-day intervals did not differ throughout treatment between groups A and B, the number of small follicles declined more rapidly across the follicular phase in patients treated with hMG ($P < 0.001$ on days 10 and 12; $P < 0.05$ on day 14). Duration of treatment ranged between 8–23 days and 6–17 days in groups A and B, respectively. Table 2 shows hormone concentrations measured during the initial 14 days of gonadotropin treatment (fixed dose period), calculated as the area under the curve (AUC). Serum FSH, P, T, and inhibin A and B did not differ between the treatment groups, whereas LH, hCG, and E_2 were higher in group B. The follicular phase secretory dynamics of daily gonadotropin and gonadal steroid levels are shown in Fig. 2.

Daily levels of inhibin A and inhibin B and the inhibin B/A ratio are shown in Fig. 3. Although inhibin A and inhibin B

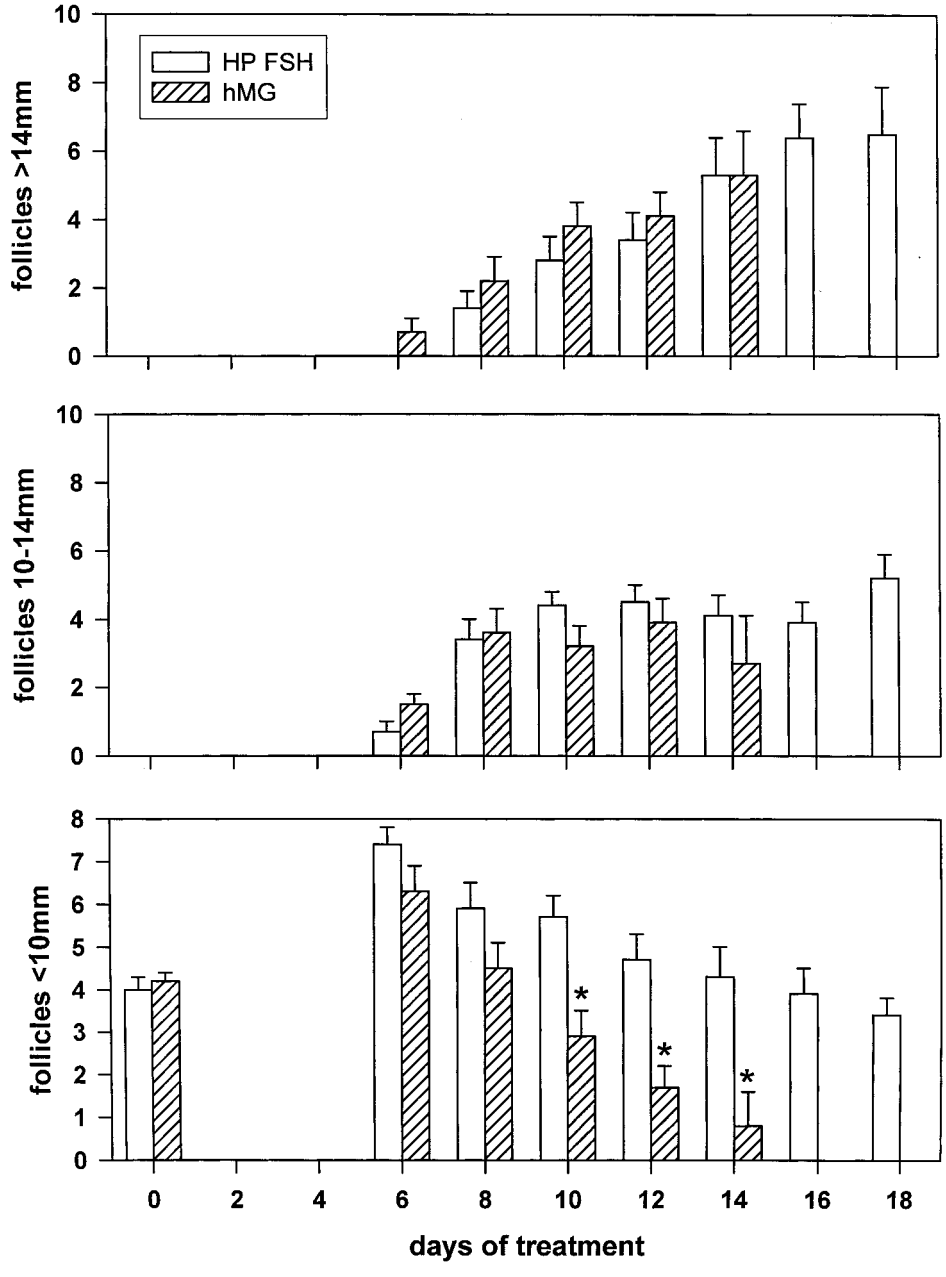

FIG. 1. Number (mean ± SE) of small (<10-mm diameter), medium (10–14 mm), and large ovarian follicles (>14 mm) measured with transvaginal pelvic ultrasound during menotropin treatment at 2-day intervals. On treatment day 0 only small follicles were detected, whereas pelvic ultrasound was not performed on days 1–5. *, $P < 0.05$ or less. Results of group B (hMG) are not reported on days 16 and 18, as only two patients or less continued treatment beyond day 15 in this group.

TABLE 2. Serum hormone levels during the initial 14 days of constant dose menotropin administration (two ampoules per day) in the two treatment groups

	Group A (HP FSH)	Group B (hMG)	*P*
LH (IU/L·day)	11.8 ± 1.0	18.7 ± 1.8	<0.005
FSH (IU/L·day)	130 ± 5	122 ± 7	NS
E_2(pg/mL·day)	2661 ± 387	3840 ± 254	<0.005
P (ng/mL·day)	5.4 ± 0.5	4.5 ± 0.5	NS
T (ng/ml·day)	3.1 ± 0.4	4.3 ± 0.5	NS
hCG (IU/L·day)	<0.1	3.8 ± 0.6	<0.0001
Inhibin A (pg/mL·day)	376 ± 66	281 ± 33	NS
Inhibin B (pg/mL·day)	6037 ± 789	5595 ± 584	NS

Values are the area under the curve.

concentrations in both groups mostly overlapped during gonadotropin administration, the inhibin B/A ratio in group B (hMG) was increased during treatment days 3–6 ($P < 0.05$), but then significantly declined over group A levels across the late follicular phase (treatment days 11–15; $P < 0.05$).

Discussion

FSH induces the development of ovarian follicles of any size, as FSH receptors are present on granulosa cells (but not on thecal cells) at every stage of follicle development (5). High dose FSH stimulation is primarily responsible for the excessive multiple follicle development that can predispose ART patients to severe complications such as multiple gestation and OHSS; the presence of many antral follicles of small diameter before hCG administration can be particularly threatening, as it was associated with a high rate of these complications (6). Conversely, LH receptors are expressed by both thecal cells and granulosa cells of larger antral follicles (>10 mm diameter) (5, 7). Thus, in addition to inducing androgen synthesis by thecal cells, LH activity is capable of stimulating larger ovarian follicle growth and function in hypogonadotropic hypogonadism (8, 9) and in ART ovulation induction regimens (4, 10). The effect of LH activity on smaller follicles may be different and cause small follicle atresia, possibly through increments in intrafollicular androgen levels (11). These physiological principles have relevant clinical implications that we tried to elucidate in this study.

The issue of which gonadotropin preparation used to induce ovulation is preferable in terms of efficacy and safety is still controversial. Numerous studies, both prospective (12–14) and retrospective (1, 2), have compared different gonadotropin preparations such as hMG, purified and HP FSH, and r-hFSH; nevertheless, no widely accepted conclusion has been reached, although some recent clinical studies suggest greater efficacy of hMG- over FSH-only-containing gonadotropins in regular ART (15) and blastocyst transfer procedures (16).

One of the most notable developments in this field has been the progression toward gonadotropin ovulation induction protocols characterized by an increasingly LH-depleted hormone environment; this was due to the use of gonadotropin preparations virtually (HP FSH) or completely (r-hFSH) devoid of LH activity combined with the application of long GnRH agonist regimens (17). Even the use of GnRH antagonists that are started only in the midfollicular phase may not obviate this problem, as these compounds can rapidly and profoundly suppress LH activity and E_2 levels (18) and remove endogenous LH support at a critical stage of follicle maturation. Thus, we performed our study to elucidate the effect of COS conducted with menotropin preparations containing only FSH (HP FSH) or both FSH and LH activities (hMG) in normoovulatory patients with reduced endogenous gonadotropin secretion obtained with a depot GnRH agonist. We chose to administer a fixed menotropin dose for at least 14 consecutive days to provide a constant FSH input to all subjects and thus isolate the impact of LH activity supplementation from potential FSH dose-related effects.

In a previous study, also performed with an invariable 14-day gonadotropin regimen, we had supplemented HP FSH with LH activity in the form of low dose hCG (50 IU/day) and compared this regimen to HP FSH alone; we found that low dose hCG enhanced E_2 secretion and large ovarian follicle development and significantly reduced ovulation induction duration (by 38%), and HP FSH dose regimens (by 55%) (4). The results of the present study partly confirm our previous findings; compared with HP FSH, hMG administration was associated with a significant, albeit less dramatic, reduction of treatment duration (28%) and menotropin dose requirements (42%). This finding suggests that a daily dose of 50 IU hCG is markedly more potent than 150 IU LH daily to supplement FSH activity in ovulation induction. In our study we also confirmed that LH activity in hMG preparations includes low, but measurable, amounts of hCG, as this hormone was detected in the peripheral blood of most hMG-treated women; this phenomenon could be related to accidental contamination of menotropin preparations by pregnant women's urine samples or intentional hCG addition to titrate LH activity in hMG (19–21). We thus confirmed (19) that hMG administration in women causes low, but measurable, increments in daily serum hCG concentrations. Nevertheless, neither two daily ampoules of hMG nor up to 50 IU hCG daily for 14 days appeared to have excessively stimulated theca-granulosa cell function, as neither T nor P was significantly increased in hMG or hCG-treated patients (4) compared with HP FSH cycles.

We also assessed the AUC of LH, FSH, E_2, P, T, hCG, inhibin A, and inhibin B levels in daily blood samples obtained in the initial 14 days of treatment (fixed gonadotropin dose period); this is the first time that such an extensive and detailed measurement of inhibin secretion is conducted during gonadotropin ovulation induction. As expected, the AUCs of serum LH and hCG were higher in group B, as HP FSH may contain only minute amounts of these hormones. The AUC measurements of FSH, inhibin A, and inhibin B did not significantly differ in the two treatment groups, thus indirectly confirming that the FSH content in each ampoule of HP FSH and hMG is similar and that all the patients in this study received a comparable degree of FSH stimulation in the initial 14 days of treatment. Conversely, serum E_2 levels were higher in group B, suggesting that more androgen substrate was locally available in these patients and/or that

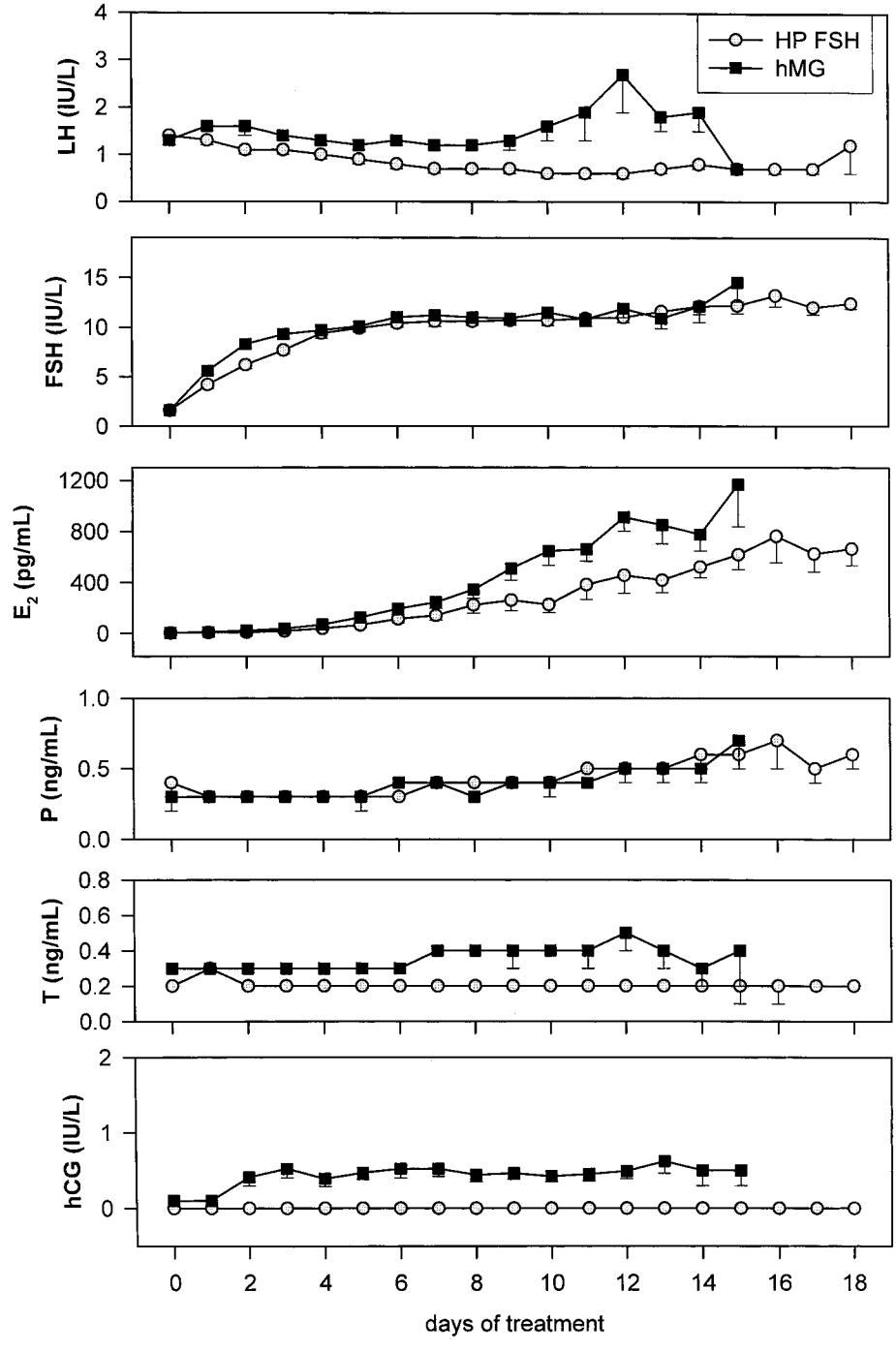

FIG. 2. Daily gonadotropin and gonadal steroid serum levels (mean ± SE) in groups A (HP FSH) and B (hMG). Results of group B (hMG) are not reported on days 16–18, as only two patients or less continued treatment beyond day 15 in this group.

increased LH activity directly stimulated the aromatase system (22) through the granulosa cell LH receptors found in larger follicles (5, 7). The likelihood of this latter mechanism of action is supported by the observation that significantly higher E_2 levels only occurred in group B after the eighth day of treatment, *i.e.* when a sufficient number of ovarian follicles more than 10 mm in diameter began to emerge.

A critical finding of this study is related to the pattern of folliculogenesis encountered in menotropin-treated patients. Although the growth of medium and large ovarian follicles progressed comparably in both groups, the occurrence of small follicles declined in a significantly more rapid manner in hMG-treated patients, so that small follicles were virtually undetectable just before preovulatory hCG administration in group B. We indirectly confirmed this phenomenon by assessing inhibin B to inhibin A ratios. Although inhibin B is produced in large amounts by preantral and small antral follicles, inhibin A prevails in larger preovulatory follicles (23–26); thus, we used the inhibin B/A ratio as a qualitative marker of follicle development. Our observation that the inhibin B/A ratio decreased more significantly in the late gonadotropin stimulation stages of group B confirmed our

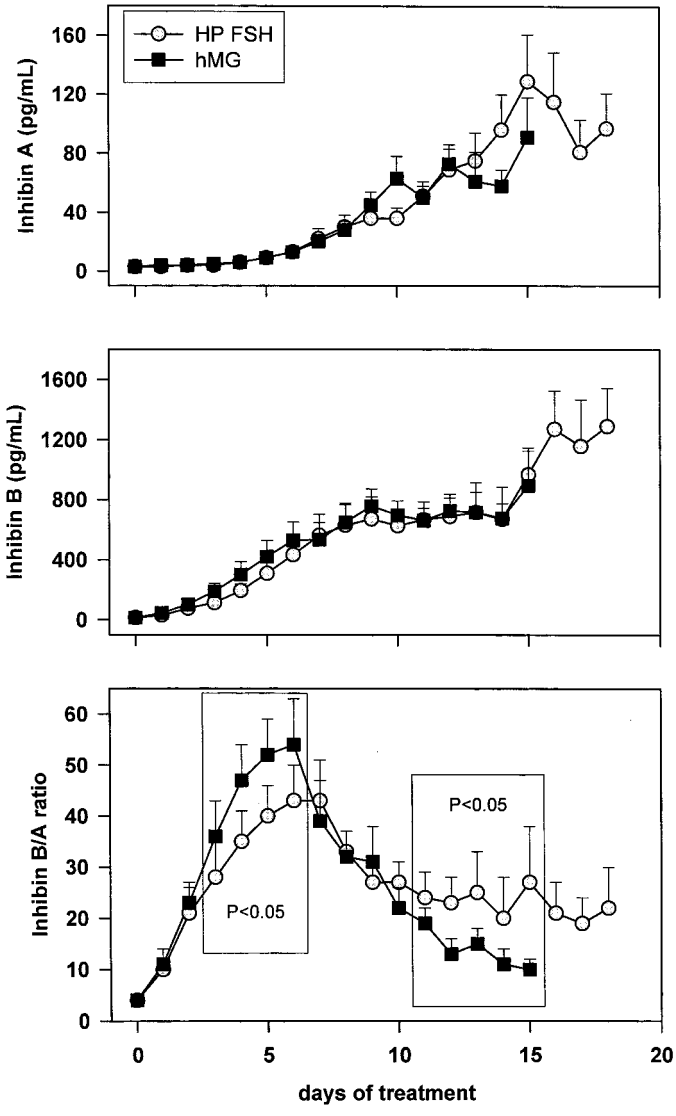

FIG. 3. Daily serum levels of inhibin A and B and daily inhibin B/A ratios (mean ± SE) in patients in group A (HP FSH) and group B (hMG). Differences in mean inhibin B/A ratios achieved statistical significance on treatment days 3–6 and 11–15. Results for group B (hMG) are not reported on days 16–18, as only two patients or less continued treatment beyond day 15 in this group.

ultrasound finding that hMG administration was associated with a greater decline in the number of small follicles across COS. This follicle pattern may also explain the nonsignificant trend toward a lower incidence of twin gestations we found in group B. Therefore, ultrasound, endocrine data, and clinical outcome of treatment point toward a less intense development of small antral follicles in hMG- *vs*. HP FSH-treated patients. The mechanism for this pattern of folliculogenesis could be dual, as LH activity may induce androgen-mediated atresia of small follicles (11) while supporting larger follicles whose granulosa cells have begun to express LH receptors (10).

In summary, when administered at similar dosages, hMG appears to be moderately, but significantly, more effective than HP FSH at inducing ovulation in GnRH agonist-suppressed women. In addition, a valuable potential characteristic of LH-containing gonadotropins appears to be their capacity to reduce the formation of small ovarian follicles while intensely stimulating large follicle growth and function. If properly exploited, this feature could allow the development of safer ovulation induction regimens aimed at reducing multiple gestation and OHSS through the optimization of ovarian follicle growth.

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