PHYSIOLOGY

Exogenous Luteinizing Hormone (LH) Increases Estradiol Response Patterns in Poor Responders with Low Serum LH Concentrations

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Purpose: Our purpose was to investigate whether the addition of exogenous leuteinizing hormone (LH) increases estradiol secretion in LH-depleted women undergoing controlled ovarian hyperstimulation (COH) with purified follicle stimulating hormone (FSH).

Methods: We carried out case series and retrospective analysis of midfollicular serum LH concentrations and estradiol response patterns in COH cycles. All patients initially received gonadotropins containing purified FSH. Human menopausal gonadotropin containing LH was added to poor responders with low midfollicular LH concentrations.

Results: The addition of exogenous LH to the COH regimen significantly increased estradiol secretion in poor responders with low midfollicular endogenous LH concentrations. This was confirmed statistically by an average change in the slope of the estradiol patterns from 27.54 to 85.49 after the addition of exogenous LH. Furthermore, patients with midfollicular serum LH concentrations <3.0 mIU/ml had significantly lower midfollicular and peak estradiol (E_2) concentrations compared to patients with LH concentrations ≥ 3.0 mIU/ml (352.3 and 2094.3 vs 855.6 and 3757.1 pg/ml, respectively).

Conclusions: The addition of exogenous LH increases E_2 response patterns in poor responders with low midfollicular serum LH concentrations. Low midfollicular serum LH con-

centrations are associated with significantly lower midfollicular and peak E_2 concentrations.

KEY WORDS: estradiol; gonadotropins; luteinizing hormone; ovulation induction.

INTRODUCTION

The recent emergence of highly purified urinary and recombinant gonadotropins along with recent data supporting follicle stimulating hormone (FSH)-only ovulation induction has brought into question the necessity of using luteinizing hormone (LH) during controlled ovarian hyperstimulation (COH). Gonadotropin regimens consisting solely of purified FSH are reported to improve estradiol secretion patterns and are associated with a significantly higher pregnancy rate compared to regimens containing LH (1). It is thought that patients maintain sufficient levels of residual endogenous LH to support follicular development and ovarian steroidogenesis despite down regulation with gonadotropin releasing hormone (GnRH) agonist.

Further, the importance of LH during follicular development is questionable since patients with very low levels of circulating endogenous LH are reported to respond to purified FSH-containing regimens as well as those with higher levels of endogenous LH (2). Finally, inappropriately elevated levels of LH adversely affect follicular development, fertilization, embryo quality, and pregnancy rates (3–6).

In opposition to these data, there are several reports demonstrating that patients with hypogonadotropic

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hypogonadism treated with FSH alone show reduced estradiol (E_2) secretion in the follicular phase compared with those treated with regimens containing both LH and FSH (7–9). Furthermore, no pregnancies have been reported in hypogonadotropic hypogonadal patients treated with FSH alone (10). Shoham *et al.* suggest that only minute doses of exogenous LH are necessary in these patients (11). These data support the two-cell, two-gonadotropin theory of follicular steroidogenesis in which the synergistic action of both FSH and LH facilitates E_2 biosynthesis.

The role of exogenous LH in ovulation induction regimens needs to be clarified further because in a short time highly purified recombinant LH will be available. This will allow us to better individualize gonadotropin regimens for ovulation induction. The purposes of this study are (a) to examine whether E_2 secretion increases with the addition of exogenous LH to poor responders with low midfollicular serum LH concentrations and (b) to determine the relationship between midfollicular endogenous LH concentrations and E_2 patterns.

MATERIALS AND METHODS

Case Series

Exogenous LH was added to the ovulation induction regimen of six poor responders who had cycle day 9 serum LH concentrations <3.0 mIU/ml. Among these six patients, four were diagnosed with endometriosis and two with tubal-factor infertility. Each of these women were undergoing ovulation induction for in vitro fertilization (IVF) using the flare protocol, in which both GnRH agonist and gonadotropins are initiated during the follicular phase of the menstrual cycle, as described by Garcia *et al.* (12). The flare cycles were initiated with leuprolide acetate (TAP, Deerfield, IL) beginning on menstrual day 2, followed by gonadotropins consisting solely of purified FSH (Fertinex: Serono Laboratories Inc., Randolph, MA) on menstrual day 5. The leuprolide acetate dose was based on the patient's weight. Patients weighing <150 lb received 0.75 mg, while patients weighing ≥ 150 lb received 1.0 mg of leuprolide acetate. Purified FSH dosages were individualized based upon the patient age, ovulation history, and prior response to COH. Gonadotropins containing LH were added on cycle day 9, which was the fifth day of gonadotropin stimulation. The gonadotropins added on cycle day 9 consisted of 150 U of FSH and 150 U of LH [Humegon (Organon, West

Orange, NJ) or Repronex, (Ferring Pharmaceuticals Inc., Doylestown PA)]. The gonadotropin regimens were adjusted in each case with the addition of exogenous LH so that the total units of FSH remained the same. Poor responders were defined as those patients with follicles less than 10 mm on transvaginal sonogram on cycle day 9 who had less than a 10-fold increase in serum E_2 concentration between cycle day 5 and cycle day 9. The cutoff value of 3.0 mIU/ml for day 9 serum LH concentrations was chosen arbitrarily. A cutoff value of 2.0 mIU/ml or less could also have been chosen but was not, because in our patient population, concentrations this low occur infrequently.

Slopes of E_2 response patterns, before and after the addition of LH, were determined and compared by a generalized estimating equation to fit linear regression as described by Liang and Zeger (13).

Retrospective Analysis

Based on the findings in the case series, circulating concentrations of LH and E_2 response patterns were further analyzed, using the LH value of 3.0 mIU/ml as a point of reference. All charts for women undergoing IVF utilizing the flare protocol between January and May 1998 were reviewed. Inclusion criteria were defined as (a) initiation of leuprolide acetate on day 2 followed by purified FSH (Fertinex) on day 5 of the menstrual cycle (b) availability of baseline and repeat serum LH concentrations on cycle day 9, (c) availability of sonogram results, (d) serial E_2 results, and (e) pregnancy outcome data. Patients were excluded from the study if (a) hMG containing LH was initiated prior to obtaining a serum LH concentration on cycle day 9, (b) the dosage of FSH increased during ovulation induction, (c) embryo transfer was not performed in the same cycle as transvaginal oocyte retrieval, or (d) gonadotropins were not given on a continuous basis, once ovulation induction was initiated.

Baseline sonograms and E_2 , FSH, and LH concentrations were obtained on menstrual day 2. Patients were routinely prohibited from proceeding with ovulation induction if their baseline E_2 was >50 pg/ml, FSH was >13 mIU/ml, or there was a follicle greater than 10 mm by transvaginal sonogram. Patients undergoing ovulation induction for IVF routinely received 0.75–1.0 mg leuprolide acetate beginning on menstrual day 2, followed by initiation of gonadotropins consisting solely of purified FSH (Fertinex) on menstrual day 5. The leuprolide acetate dose was based on the patient's weight. Patients <150 lb received 0.75 mg, while patients ≥ 150 lb received 1.0 mg of leuprolide acetate.

Purified FSH dosages were individualized based upon the patient age, ovulation history, and prior response to COH. Follow-up transvaginal sonograms and E₂ and LH concentrations were obtained on cycle day 9.

Subsequent E₂ concentrations and sonograms were obtained individualized to each patient's stimulation response. Criteria for the administration of human chorionic gonadotropin (hCG; Profasi; Serono Laboratories Inc.) were the presence of at least three ovarian follicles ≥ 18 mm in diameter as determined by transvaginal sonogram and a continuous rise in the serum E₂ level. Transvaginal sonogram-guided oocyte retrieval was performed 34–36 hr following hCG administration. Embryos were transferred 2–3 days after oocyte retrieval. Patients routinely received intravaginal micronized progesterone beginning on the day of embryo transfer until determination of clinical pregnancy. Patients who were pregnant were instructed to continue progesterone for 6 weeks.

Estradiol, LH, and FSH concentrations were determined from venous blood. Estradiol concentrations were measured by chemiluminescent enzyme immuno-metric assay (Diagnostic Products Cooperation, Los Angeles, CA). The intraassay coefficient of variation (CV) for mean E₂ concentrations of 46, 116, 266, 422, and 480 pg/ml was 15.0, 9.5, 8.6, 7.1, and 6.3%, respectively. The interassay CV for mean E₂ concentrations of 56, 151, and 482 pg/ml was 16, 9.3, and 6.4%, respectively. Luteinizing hormone concentrations were measured by chemiluminescent enzyme immunometric assay. The intraassay CV for a mean LH value of 28.8, 53.9, and 164.0 mIU/ml was 4.8, 5.9, and 6.5%, respectively. The interassay CV for mean LH concentrations of 6.2, 30, and 60 mIU/ml was 10.6, 8.1, and 10.0%, respectively. Follicle stimulating hormone concentrations were measured by chemiluminescent enzyme immuno-metric assay. The intraassay CV for mean FSH concentrations of 7.8, 19.7, and 42.5 mIU/ml was 5.4, 6.1, and 7.7%, respectively. The interassay CV for mean FSH concentrations of 8.3, 20.8, and 42.9% was 8.1, 6.5, and 7.9%.

Pregnancy was defined as a positive β -hCG obtained 14 days after embryo transfer. Outcome variables included percentage of poor responders, percentage of cycles that progressed to transvaginal oocyte retrieval, number of oocytes obtained per retrieval, fertilization rates, embryos available for transfer, and pregnancy rates.

In addition to the case series, slopes of E₂ response patterns were also determined for women with cycle day 9 LH concentrations < 3.0 mIU/ml who did not meet our criteria for addition of exogenous LH and for women with cycle day 9 LH concentrations ≥ 3.0 mIU/ml.

Statistical significance for cause of infertility, percentage of poor responders, pregnancy rates, and percentage of cycles eventually progressing to transvaginal oocyte retrieval was determined by chi-square analysis. Statistical significance for age, mean number of oocytes retrieved, fertilization rates, mean units of gonadotropins used by the time of oocyte retrieval, embryos available for transferred, and embryos transferred per cycle was determined by Student's *t* test. A *P* value of < 0.05 was defined as statistically significant.

Institutional review board approval was not deemed necessary, as all treatments and monitoring practices and criteria were in established use before and during the study.

RESULTS

In our case series, the addition of exogenous LH to the COH regimen increased the E₂ secretion pattern in each patient (Fig. 1). This was confirmed statistically by an average change in the slope of the estradiol patterns from 27.54 to 85.49 after the addition of exogenous LH (*P* = 0.0035) (Fig. 2). Despite the increase in E₂ secretion, none of the cycles resulted in pregnancy. Patients with serum LH concentrations ≥ 3.0 mIU/ml on cycle day 9 maintained an overall steady change in their E₂ secretion over time without a significant change in slope. The pregnancy rate in this group was 33.3% (6/18). Patients with serum LH concentrations < 3.0 mIU/ml on cycle day 9 who did not meet our criteria for the addition of exogenous LH also maintained an overall steady change in their E₂ secretion over time. Exogenous LH was not added to this group of patients with cycle day 9 LH concentrations < 3.0 mIU/ml because either their serum E₂ concentrations increased by a factor of greater than 10 between cycle day 5 and cycle day 9 or there were follicles 10 mm or more in diameter on transvaginal sonogram on cycle day 9. The pregnancy rate in this group was also 33.3% (2/6).

For the retrospective analysis, there were 68 IVF cycles between January and May 1998 in which ovulation induction was initiated solely with Fertinex using the flare protocol. Thirty cycles met the inclusion criteria. Twenty-five cycles were excluded because they did not have cycle day 9 serum LH determinations. Seven cycles were excluded because the dosage of FSH increased during ovulation induction. Four cycles with ovarian hyperstimulation were excluded because all the embryos were cryopreserved during the stimulation cycle. One cycle was excluded because a serum

Fig. 1. Improved E₂ response with the addition of exogenous LH on cycle day 9.

E₂ concentration was not obtained on cycle day 9. One cycle was excluded because the patient did not receive gonadotropins on a continuous basis.

The mean serum E₂ concentration on cycle day 9 for women with serum LH concentrations <3.0 mIU/ml was significantly lower than that for women with LH concentrations ≥3 mIU/ml (352.3 vs 855.6 pg/ml, respectively; $P = 0.0296$). The mean peak serum E₂ concentration prior to hCG injection for women with

serum LH concentrations <3.0 mIU/ml was also significantly lower than that compared to women with LH concentrations ≥3 mIU/ml (2094.3 vs 3757.1 pg/ml; $P = 0.0285$). Furthermore, cycles with serum LH concentrations <3.0 mIU/ml were prescribed significantly more units of gonadotropins by the time of oocyte retrieval (3293.8 vs 2450.0; $P = 0.0412$). The pregnancy rate for women with serum LH concentrations <3.0 mIU/ml tended to be lower than that for

Fig. 2. Change in E₂ levels over time: poor responders with serum LH concentrations <3 mIU/ml (Group 1); patients with serum LH concentrations <3 mIU/ml who were not poor responders (Group 2); all patients with serum LH concentrations ≥3 mIU/ml (Group 3).

Table I. Patient Age and Outcome Data

Factor	LH value ≥ 3.0 mIU/ml (n = 18)	LH value < 3.0 mIU/ml (n = 12) ^a	P value
Mean age (years $\pm$ SD)	34.6 $\pm$ 6.2	36.4 $\pm$ 6.2	NS ^b
Cycle day 9 E ₂ concentration	855.6 $\pm$ 715.3	352.3 $\pm$ 304.4	0.0296
Peak E ₂ concentration prior to hCG injection	3757.1 $\pm$ 2250.3	2094.3 $\pm$ 1292.1	0.0285
Pregnancy rate (%)	33.3 (6/18)	16.7 (2/12)	NS
Poor responders (%)	11.1 (2/18)	41.7 (5/12)	NS
Percentage of cycles that underwent transvaginal oocyte retrieval	94.4 (17/18)	75.0 (9/12)	NS
Pregnancy rate of cycles that underwent transvaginal oocyte retrieval (%)	35.3 (6/17)	22.2 (2/9)	NS
Mean ($\pm$ SD) No. of oocytes retrieved per cycle	14.9 $\pm$ 7.0	11.7 $\pm$ 5.7	NS
Mean percentage ($\pm$ SD) of oocytes fertilized per cycle	60.7 $\pm$ 20.0	63.0 $\pm$ 14.1	NS
Mean ($\pm$ SD) No. of embryos available for transfer per cycle	7.8 $\pm$ 3.9	6.7 $\pm$ 3.7	NS
Mean ($\pm$ SD) No. of embryos transferred per cycle	3.7 $\pm$ 1.7	4.0 $\pm$ 1.3	NS
Percentage of poor responders	11% (2/18)	33% (4/12)	NS
Mean ($\pm$ SD) U of gonadotropins used by the time of oocyte retrieval	2450.0 $\pm$ 992.7	3293.8 $\pm$ 1151.5	0.0412

^a LH value on cycle day 9.^b Not significant.

women with LH concentrations ≥ 3 mIU/ml (16.7 vs 33.3%; $P = 0.312$). Also, the percentage of poor responders was higher for women with serum LH concentrations < 3.0 mIU/ml than for women with LH concentrations ≥ 3 mIU/ml (41.7 vs 11.1%; $P = 0.053$). However, these differences were not statistically significant, most likely due to our limited sample size (Table I).

Of 26 cycles that progressed to transvaginal oocyte retrieval, the mean numbers of oocytes obtained per retrieval and embryos available for transfer were 13.8 ± 6.7 and 7.4 ± 3.8 , respectively. There was no significant difference in the mean numbers of oocytes obtained per retrieval and embryos available for transfer between women with serum LH concentrations < 3.0 mIU/ml and those with concentrations ≥ 3 mIU/ml. There was also no significant difference in the etiology of infertility between the two groups (Table II).

DISCUSSION

Over the past few years the availability, first, of highly purified urinary FSH and, then, of recombinant FSH has altered ovulation induction and challenged the notion that LH is necessary for ovarian follicular development. This is based on reports of an improved ovarian response with purified FSH products compared with older urinary products containing both FSH and LH. There is also the presumption that if LH is neces-

sary, patients probably maintain adequate levels of endogenous LH during ovarian stimulation to support follicular steroidogenesis.

In our study, as in others, the majority of women underwent successful ovulation induction using gonadotropins consisting solely of purified FSH. This suggests that, normally, exogenous LH is not necessary for folliculogenesis during ovulation induction. We agree that most women probably maintain endogenous LH concentrations that are adequate to support folliculogenesis. However, we believe that there is a subset of women with low endogenous LH concentrations that may benefit from the addition of exogenous LH to their gonadotropin regimen. This is supported by our findings that in women with low midfollicular LH concentrations, not responding well to ovulation

Table II. Etiology of Infertility

Infertility diagnosis	LH value ≥ 3.0 mIU/ml (n = 18)	LH value < 3.0 mIU/ml (n = 12) ^a
Tubal factor	5	3
Endometriosis	4	6
Male factor	6	1
Age	1	1
Tubal and endometriosis	0	1
Tubal and male factor	1	0
Endometriosis and male factor	1	0

^a LH value on cycle day 9.

induction, the addition of LH significantly improved their E₂ secretion pattern. Despite the improvement in E₂ secretion during the midfollicular phase, there were no pregnancies. This emphasizes the importance of a minimal LH threshold during the early follicular phase of ovulation induction and its critical influence on both oocyte development and pregnancy outcome. The impact of endogenous LH concentrations on folliculogenesis is further supported by our findings that women with low midfollicular LH concentrations had significantly lower serum E₂ concentrations.

The serum LH concentration on cycle day 9 of 3.0 mIU/ml was chosen arbitrarily. However, statistical analysis confirms that this is a reasonable point of comparison for our patient population. Patients with a serum LH concentration <3.0 pg/ml on cycle day 9 had significantly lower E₂ concentrations on cycle day 9 ($P = 0.0296$) as well as lower peak E₂ concentrations ($P = 0.0285$). This was not true when LH concentrations of 4.0 mIU/ml or more were used ($P > 0.5$). There were too few patients ($n = 5$) with LH values adequately <2.0 mIU/ml to evaluate this as a point of reference.

Our findings of (a) an improved E₂ response pattern by the addition of exogenous LH and (b) a blunted E₂ response pattern in patients with low endogenous LH concentrations emphasize the importance of the synergistic action of the two-cell, two-gonadotropin system. Although FSH has a dominant role in stimulating the granulosa cells to produce E₂, the presence of LH enhances steroidogenesis by providing an androgen substrate. In concordance with the two-cell theory, we suggest that patients with low endogenous LH concentrations may benefit by the inclusion of gonadotropins containing LH in their ovulation induction regimen.

Although our study is limited by the fact that it is a case series and retrospective analysis, we feel that it illustrates a very important aspect of ovulation induction that requires further investigation. Our results justify the need for a prospective, randomized clinical trial. The study should be designed so that poor responders with low midfollicular serum LH concentrations are randomized either to continue their gonadotropin regimen consisting solely of FSH or to have their regimen altered by the addition of LH.

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