Follicular phase serum levels of luteinizing hormone do not influence delivery rates in in vitro fertilization cycles down-regulated with a gonadotropin-releasing hormone agonist and stimulated with recombinant follicle-stimulating hormone

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Objective: To assess the value of serum LH measurements in early and late follicular phase as predictors of ovarian response and IVF outcome in patients treated with recombinant FSH with GnRH agonist (GnRH-a) pituitary down-regulation.

Design: Retrospective cohort analysis.

Setting: Institutional.

Patient(s): Women undergoing 157 consecutive IVF cycles suppressed with leuprolide acetate (LA) started in the midluteal phase and stimulated with recombinant FSH. Only women <40 years of age and with a basal cycle day 3 serum FSH ≤ 9 IU/L were included.

Intervention(s): Serum LH levels were measured on cycle days 3 (D3) and 10 (D10).

Main Outcome Measure(s): Delivery rates. Other secondary outcome measures included fertilization rate, clinical pregnancy rate, and parameters of ovarian response (peak E2, number of metaphase II oocytes, and number of ampules of recombinant FSH).

Results: No significant differences were found with respect to ovarian response, fertilization rate, and outcome of pregnancy, when three threshold values of D3 and D10 serum LH (1, 1.5, and 2 mIU/mL) were analyzed. In addition, no significant differences were found between conception (n = 87) and no conception (n = 71) groups with respect to D3 or D10 LH. Receiver operator characteristic (ROC) analysis showed that neither the serum LH concentration on D3 nor on D10 was able to discriminate between conception and nonconception cycles (area under the curve [AUCROC] = 0.54, AUCROC = 0.56), or between delivered pregnancies and first trimester pregnancy loss (AUCROC = 0.53, AUCROC = 0.61).

Conclusions: The suppressed levels of early and late follicular serum LH in women <40 years of age with normal ovarian function desensitized with a GnRH-a and treated with recombinant FSH are not predictive of ovarian response, pregnancy, or delivery. These data do not support the use of exogenous LH supplementation in this clinical scenario. (Fertil Steril 2005;83:42–8. ©2005 by American Society for Reproductive Medicine.)

Key Words: GnRH agonist, IVF, pregnancy, recombinant FSH, serum LH levels

Pituitary down-regulation with a GnRH agonist (GnRH-a) is commonly used in controlled ovarian hyperstimulation (COH) protocols before the beginning of exogenous gonadotropin administration in women undergoing IVF. A switch in COH regimens to a more widespread use of FSH-only preparations, totally or partially devoid of LH activity, has been associated with an increase in overall pregnancy rate in some studies, although not in other studies (1–3). The relatively recent availability of recombinant human FSH, and differing views on the role of exogenous LH in ovarian stimulation has resulted in the virtual elimination of LH bioactivity from several protocols for IVF (4, 5). Nevertheless, the role of exogenous LH during ovarian stimulation in normogonadotropic women down-regulated or not with a GnRH-a has been a matter of considerable debate (3, 6–10).

Luteinizing hormone has essential and well-established roles in both ovarian steroid synthesis and ovulation. The “two-cell, two-gonadotropin” concept emphasizes that stimulation of both theca cells by LH and granulosa cells by FSH and LH is required for adequate synthesis of E2, but probably also for optimal follicle–oocyte maturation (4, 11). Although only FSH is required for follicular growth, some LH is necessary to achieve adequate follicular steroidogenesis, and develop the capacity of the follicle to ovulate and luteinize when exposed to the ovulatory LH surge. The amount of LH activity actually necessary for normal follicle and oocyte development is unknown, but it is likely to be very low, as

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1% of follicular LH receptors need to be occupied to allow normal steroidogenesis (11).

The need for LH supplementation is not questioned in the case of patients with hypogonadotropic hypogonadism undergoing ovulation induction. Treatment of such profoundly hypogonadotropic women with FSH-only regimens results in reduced folliculogenesis and reduced levels of serum estrogen (E). The addition of LH enhances E2 secretion, follicle development, and the chances of achieving term pregnancy (12).

In normogonadotropic women, the administration of a GnRH-a results in a variable level of suppression of pituitary gonadotropins secretion that is dependent on the type, dose, and mode of administration of the analogue (2). It is estimated that the amount of residual endogenous LH remaining during GnRH-a pituitary suppression may be sufficient in most clinical cases to achieve adequate follicular maturation during ovarian stimulation with pure FSH. Nevertheless, it is also possible that in some cases GnRH down-regulation may result in profound suppression of LH concentrations with an adverse effect on steroidogenesis/oocyte quality, therefore having an impact on IVF outcome. Such cases might benefit from preparations containing LH.

The objective of the present study was to evaluate the value of measuring serum LH levels in the early and late follicular phase in predicting pregnancy outcome and ovarian response to stimulation. To address this question we studied a group of women undergoing IVF with a normal ovarian reserve and <40 years of age (with or without intracytoplasmic sperm injection [ICSI]) treated with a single protocol using recombinant FSH under GnRH-a pituitary down-regulation (long protocol).

**MATERIALS AND METHODS**

This study evaluated a total of 157 consecutive IVF cycles in women undergoing IVF (with or without ICSI) in which ovarian stimulation was carried out with recombinant FSH under pituitary suppression with a GnRH-a between July 1999 and December 2001. The protocol was approved by the Institutional Review Board at Eastern Virginia Medical School. The inclusion criteria were: women <40 years of age and with a basal cycle day 3 serum FSH ≤9 IU/L (and an E2 <80 pg/mL), independently of the cause of infertility.

Pituitary down-regulation in all women was achieved with daily leuprolide acetate (LA) (0.5 mg SC) starting in the midluteal phase of the previous cycle, then decreased to 0.25 mg starting on day 3 of menses and continued daily until the day of hCG administration. Recombinant FSH (Gonal-F, Serono Lab, Randolph, MA) was started on day 3 of menses and administered SC. The initial dose of recombinant FSH was individualized between 150 and 300 IU/d depending on the age of the woman and screening of ovarian reserve (basal cycle day 3 measurement of serum FSH, LH, and E2) (13). The dose was adjusted in a step-down fashion according to the ovarian response as assessed by sequential transvaginal ultrasonography to assess follicular development and increase of serum E2 levels.

Human chorionic gonadotropin at a dose of 10,000 IU was given by IM injection when leading follicles reached 16 mm in diameter. Oocytes were retrieved under ultrasound guidance 36 hours after hCG administration. Metaphase II oocytes were diagnosed either by examination of cumulus oocytes complexes (for IVF) or oocyte assessment after hyaluronidase treatment (for ICSI) and following the criteria of Veek (14). Typically three embryos were transferred to the uterine cavity 3 days after insemination (range 2 to 4 embryos). Luteal support was provided with 200 mg of intravaginal progesterone (Prometrium, Solvay Pharmaceuticals, Marietta, GA) three times daily, starting 2 days after retrieval and continued until the day of pregnancy test. Clinical pregnancies were diagnosed by ultrasound confirmation of a gestational sac.

Serum LH determinations were performed using frozen serum samples collected on stimulation cycle day 3 (D3, first day of stimulation) and day 10 (D10). All frozen sera were thawed and batch analyzed for measurement of LH using the IMx LH assay (Abbott Laboratories, Abbott Park, IL), which is a Microparticle Enzyme Immunoassay (MEIA) standardized against the WHO Second International Standard 80/552 for LH. The sensitivity of the assay was 0.1 IU/L. The intra-assay and interassay coefficients of variation were 5.5% and 6.7% in the range of LH concentrations measured in this study. To evaluate the effects of profound LH suppression we used three arbitrary serum LH cutoff concentrations (1, 1.5, and 2 mIU/mL). Below the concentration of 1 IU/L the interassay variability, especially the interassay variation between different lots of reagents, deteriorates and discrimination at <1.0 IU/L was deemed unreliable.

**Statistical Analysis**

Outcome variables examined were: [1] COH response (assessed by peak serum E2, number of metaphase II oocytes retrieved, and number of ampules of recombinant FSH used per cycle), and [2] fertilization and pregnancy rates (clinical, delivery, and miscarriage rates). Results are expressed as mean ± SE. Mean values were compared using the unpaired t test. The level of significance was set at P<.05. Receiver operator characteristics (ROC) curves analysis was used to evaluate the ability of different LH concentrations (based on arbitrary selected thresholds) on D3 and D10 to discriminate between conception and no-conception cycles (reported as clinical pregnancies).

An ROC curve is a line graph that plots the probability of a true positive result—or the sensitivity of the test—against the probability of a false-positive result for a range of different cutoff points (15, 16). When an existing diagnostic test (e.g., LH concentrations on D3 and D10) is being eval-
TABLE 1

Patients' characteristics, ovarian response, and fertilization rate in patients with low or high LH on cycle day 3 (D3) according to three different threshold values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LH (IU/L)</th>
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<tbody>
<tr>
<td></td>
<td>&gt;1 (n = 142)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>32.4 ± 0.3</td>
</tr>
<tr>
<td>Basal E2 (pg/mL)</td>
<td>49.7 ± 1.8</td>
</tr>
<tr>
<td>Basal LH (IU/L)</td>
<td>4.9 ± 0.2</td>
</tr>
<tr>
<td>Basal FSH (IU/L)</td>
<td>5.8 ± 0.1</td>
</tr>
<tr>
<td>Suppressed day 3 E2 (pg/mL)</td>
<td>44.6 ± 1.3</td>
</tr>
<tr>
<td>Day 10 LH (IU/L)</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>Units of recombinant-FSH</td>
<td>1,957.5 ± 37.5</td>
</tr>
<tr>
<td>E2 on day of hCG</td>
<td>2,785 ± 111</td>
</tr>
<tr>
<td>No. of oocytes inseminated</td>
<td>10.4 ± 0.4</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>82.4 ± 1.5</td>
</tr>
<tr>
<td>No. of embryos cryopreserved</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>No. of embryos per transfer</td>
<td>2.9 ± 0.1</td>
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</table>

Note: Values are mean ± SE.

AP=.004.


Ulated, this type of graph can be used to assess the usefulness of the test to determine the most appropriate cutoff point to discriminate between two conditions (e.g., conception vs. no-conception). A straight line that extends from the left lower corner to the right upper corner (with an area under the curve [AUC] of 0.5) corresponds to a test that gives positive and negative results by chance alone; such a test has no inherent value. The closer the line to the upper left-hand corner of the graph (with an AUC closer to 1.0), the more accurate the test is. Furthermore, the point that lies closest to this corner is usually chosen as the cutoff that maximizes both sensitivity and specificity simultaneously.

RESULTS

To evaluate the impact of suppressed circulating LH concentrations on IVF outcomes, three arbitrary serum LH threshold values were used for both the early (D3; stimulation day 1) and late follicular (D10; stimulation day 7) phase. The three threshold values were 1, 1.5, and 2 mIU/mL. No significant differences were found with respect to patients' age, ovarian response (peak E2, number of metaphase II oocytes, and number of ampules of recombinant FSH) and fertilization rate (Tables 1 and 2), or pregnancy outcomes (clinical, delivery, or miscarriage rates) (Tables 3 and 4), when comparing groups with LH levels above or under the selected cutoff levels (high and low LH groups).

When conception (n = 87) and no-conception (n = 70) groups were analyzed, again no significant differences were found in any of the variables investigated with respect to ovarian response (peak E2, number of metaphase II oocytes, and number of ampules of recombinant FSH) or fertilization rate. The same was true when delivered pregnancies (n = 74) were compared with pregnancy losses (n = 13). In addition, no significant differences were found between the conception and no-conception groups, or between delivered pregnancies and first trimester pregnancy loss groups with respect to basal cycle D3 serum gonadotropins or D10 LH (data not shown). There were no significant differences between LH groups with respect to early pregnancy loss.

The ROC analysis was used to evaluate the ability of LH concentrations on D3 and D10 to discriminate between conception and no-conception and between delivered pregnancies and first trimester pregnancy loss groups with respect to basal cycle D3 serum gonadotropins or D10 LH (data not shown). The calculation of the AUCROCC showed that neither the serum LH concentration on D3 nor on D10 were able to discriminate between conception and no-conception cycles (AUCROC = 0.54, AUCROC = 0.56), or between delivered pregnancies and first
significantly reduced E$_2$ levels at the late follicular phase and highly purified FSH or recombinant human FSH with pro-

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another study, Westergaard et al. (18) used a standard stim-

embryo yields, and a higher incidence of poor response. In

stimulation with recombinant human FSH. This study found that women with low LH concentration (<0.5 IU/L) had similar pregnancy rates but a fivefold higher risk of early pregnancy loss (45% vs. 9%).

Our study is in agreement with the study by Balasch et al. (9) that found no significant differences with respect to ovarian response, IVF/ICSI outcome, implantation, and the outcome of pregnancy between women with low or high late follicular LH as defined by three different thresholds (1, 0.5, or <0.7 IU/L). In that study pituitary desensitization was achieved by SC administration of LA started in the mid luteal phase of the previous cycle at a dose of 1 mg daily, then reduced to 0.5 mg after ovarian suppression was con-

Tesarik and Mendoza (19) reported on a prospective study in which oocyte donors were randomized to groups stimulated with FSH alone or with a combination of FSH and LH after pituitary down-regulation using 3.75 mg of triptorelin administered in the midluteal phase. They found that in donors with deep suppression of pituitary LH (serum levels <1 IU/L) before the beginning of ovarian stimulation, LH activity supplementation in the form of hMG enhanced FSH-

trimester pregnancy loss (AUC$_{ROC}$ = 0.53, AUC$_{ROC}$ = 0.61) (Figs. 1 and 2).

**DISCUSSION**

This study could not identify any significant difference in ovarian response or treatment outcome based on three different thresholds of early (D3) and late follicular (D10) LH serum levels. Neither could it identify any significant difference in any of the variables investigated when analyzing the conception vs. no-conception groups nor when the delivered pregnancies and early pregnancy loss groups were compared.

Fleming et al. (17) found that patients treated with either highly purified FSH or recombinant human FSH with pro-

TABLE 2

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</tr>
<tr>
<td>Basal FSH (IU/L)</td>
<td>5.7 ± 0.1</td>
</tr>
<tr>
<td>Suppressed day 3 E$_2$ (pg/mL)</td>
<td>45.5 ± 1.4</td>
</tr>
<tr>
<td>Suppressed day 3 LH (IU/L)</td>
<td>5.2 ± 0.2$^a$</td>
</tr>
<tr>
<td>Units of recombinant-FSH</td>
<td>1,935 ± 37.5</td>
</tr>
<tr>
<td>E$_2$ on day of hCG</td>
<td>2,800 ± 108</td>
</tr>
<tr>
<td>No. of oocytes inseminated</td>
<td>10.3 ± 0.4</td>
</tr>
<tr>
<td>Fertilization rate</td>
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**Note:** Values are mean ± SE. $^{a,b,c}P<.001$

induced folliculogenesis and increased the number of retrieved metaphase II oocytes and good-quality zygotes and embryos. In contrast, in donors in whom residual LH after pituitary down-regulation was \( \leq 1 \) IU/L, the addition of exogenous LH to the ovarian stimulation protocol resulted in a lower number of developmental competent oocytes and lower implantation rate. These investigators therefore postulated that their data support the concept of a “window” for LH with a threshold requirement and a ceiling level beyond which LH might be deleterious to ovarian stimulation. This concept of threshold and ceiling has also been presented by some researchers (20–22), although debated by others (23–25).

In our study, the peak E₂ concentration on the day of hCG administration was higher, although not statistically significant, in women having high LH as compared with low LH, irrespective of the LH threshold value considered. This result is also in agreement with Balasch et al. (9) that did not detect any significant difference in serum E₂ concentrations on D7 and on day of hCG administration in the groups of patients with lower D7 LH levels. Conversely, this result differs from those of other studies that found significant higher E₂ levels in the groups of patients with normal LH levels at stimulation D8 (18) and at both stimulation D7 and on day of hCG administration (17).

As mentioned, the administration of GnRH-a results in a variable level of suppression of pituitary gonadotropins secretion that is dependent in its type, dose, and mode of administration. It is possible that because of the less intense GnRH-a regimen used (i.e., half the dose of SC LA used by Balasch et al. [9]), the great majority of women studied in this study may not have been as depleted of endogenous LH.

### Table 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LH (IU/L)</th>
<th>&gt;1 (IU/L)</th>
<th>≤1 (IU/L)</th>
<th>&gt;1.5 (IU/L)</th>
<th>≤1.5 (IU/L)</th>
<th>&gt;2 (IU/L)</th>
<th>≤2 (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients (n = 157)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>143 (91%)</td>
<td>14 (9%)</td>
<td>137 (87%)</td>
<td>20 (13%)</td>
<td>129 (82%)</td>
<td>28 (18%)</td>
<td></td>
</tr>
<tr>
<td>No conception (n = 70)</td>
<td>66 (94%)</td>
<td>4 (6%)</td>
<td>64 (91%)</td>
<td>6 (9%)</td>
<td>61 (87%)</td>
<td>9 (13%)</td>
<td></td>
</tr>
<tr>
<td>Conception (n = 87)</td>
<td>77 (89%)</td>
<td>10 (11%)</td>
<td>73 (84%)</td>
<td>14 (16%)</td>
<td>68 (78%)</td>
<td>19 (22%)</td>
<td></td>
</tr>
<tr>
<td>Early pregnancy loss (n = 13)</td>
<td>11 (85%)</td>
<td>2 (15%)</td>
<td>11 (85%)</td>
<td>2 (15%)</td>
<td>9 (69%)</td>
<td>4 (31%)</td>
<td></td>
</tr>
<tr>
<td>Delivered pregnancies (n = 74)</td>
<td>66 (89%)</td>
<td>8 (11%)</td>
<td>62 (84%)</td>
<td>12 (16%)</td>
<td>59 (80%)</td>
<td>15 (20%)</td>
<td></td>
</tr>
<tr>
<td>Singleton deliveries (n = 39)</td>
<td>34 (87%)</td>
<td>5 (13%)</td>
<td>31 (79%)</td>
<td>8 (21%)</td>
<td>29 (74%)</td>
<td>10 (26%)</td>
<td></td>
</tr>
<tr>
<td>Multiple deliveries (n = 35)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32 (82%)</td>
<td>3 (8%)</td>
<td>31 (79%)</td>
<td>4 (10%)</td>
<td>30 (77%)</td>
<td>5 (13%)</td>
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</tr>
</tbody>
</table>

<sup>Note: Values are n (%).<br>aImplantation rate = 27%.<br>bTwins = 32 (43%), triplets = 3 (4%).</sup>
activity as was found by Westergaard and Fleming and their colleagues (9, 17, 18). This assumption is supported by the fact that only 10% of the patients in this study had serum LH levels <1 IU/L, as compared with 31%, 15%, and 7% with LH <1, ≥0.7, and <0.5 IU/L, respectively, in the study by Balasch et al. (9); 49% with LH <0.5 IU/L in Westergaard et al. (18); 30%–48% with LH <0.7 IU/L in Fleming et al. (17); and 68% with LH <1 IU/L in the report by Tesarik and Mendoza (19). It is conceivable that the failure to detect significant differences in both suppressed and peak serum E2 levels is a reflection of the less depleted endogenous LH activity—in agreement with the two-cell, two-gonadotropin concept of ovarian E2 biosynthesis. Therefore, the less intense GnRH regimen possibly resulted in sufficient residual endogenous LH secretion that allowed adequate synthesis of E2 and follicle–oocyte maturation.

The level of LH activity likely to influence the theca and granulosa cell activity, and thereby follicular steroid biosynthesis and secretion, has been estimated at <1.0 IU/L (11). Thus, studies of the effect of LH suppression must operate at around the limits of assay sensitivity or below.

The European Recombinant Human LH Study Group compared results of ovulation induction in 38 hypogonadotropic hypogonadal (WHO group I anovulation) patients stimulated with recombinant human FSH and different doses of supplemental recombinant human LH. Interestingly, the majority of patients receiving 75 IU/d of recombinant LH showed an adequate response, while exhibiting undetectable concentrations of serum LH. The serum LH concentration also remained below the sensitivity limit (1.0 IU/L) in 7 of 10 patients who received 225 IU/d of recombinant human LH (12). This further confirms that (as shown in pituitary

**FIGURE 1**

Receiver operating characteristic curves of LH concentration on cycle day 3 (stimulation day 1) for discriminating conception vs. no-conception cycles (A) and ongoing pregnancy vs. early pregnancy loss (B).

**FIGURE 2**

Receiver operating characteristic curves of LH concentration on cycle day 10 (stimulation day 7) for discriminating conception vs. no-conception cycles (A) and ongoing pregnancy vs. early pregnancy loss (B).
down-regulated patients) minimal circulating levels of LH are required for normal follicular function, and measurements of serum immunoactive LH levels are of limited value (if any) to identify whether a patient has enough LH secretion to respond adequately to stimulation with FSH alone.

In conclusion, our study has demonstrated that the measured suppressed serum LH levels during the early and late follicular phase in women <40 years of age with normal ovarian function desensitized with GnRH-a and treated with recombinant FSH are not predictive of ovarian response, pregnancy, or delivery. The concentrations of residual endogenous LH remaining after GnRH-a pituitary suppression with this regimen appear to be certainly sufficient to achieve adequate follicular maturation during ovarian stimulation with recombinant FSH. Therefore, it is evident that our data do not support the need for exogenous LH supplementation in this clinical scenario.

REFERENCES