BACKGROUND: Down-regulation with GnRH agonist has been suggested to result in a profound suppression of LH bioactivity, reduced estradiol synthesis, and thus impaired IVF and pregnancy outcome. The aims of this study were: (i) to assess the usefulness of serum LH measurement on stimulation day 1 as a predictor of ovarian response, conception and pregnancy outcome in patients treated with long-term down-regulation with GnRH agonist and recombinant FSH, and (ii) to define the best threshold LH value, if any, to discriminate between women with different outcomes of IVF. METHODS: Records of 2625 cycles in 1652 infertile women undergoing IVF \((n = 1856)\) and/or ICSI \((n = 769)\) treatment were reviewed. RESULTS: The range of LH concentrations on stimulation day 1 overlapped among non-conception cycles, conception cycles, ongoing pregnancies and early pregnancy losses. Receiver operating characteristic (ROC) analysis showed that serum LH concentrations on stimulation day 1 were unable to discriminate between conception and non-conception cycles \((AUC_{ROC} = 0.51; 95\% \text{ CI: } 0.49–0.54)\) or ongoing pregnancies versus early pregnancy loss groups \((AUC_{ROC} = 0.52; 95\% \text{ CI: } 0.47–0.57)\). Stratification for various low serum levels of LH did not reveal significant differences with respect to conception or pregnancy outcome among different LH levels on stimulation day 1. CONCLUSIONS: Serum LH concentration on stimulation day 1 cannot predict ovarian response, conception and pregnancy outcome in women receiving long-term down-regulation during assisted reproduction treatment.

Key words: assisted reproduction/early pregnancy loss/FSH/LH/ovarian stimulation

Introduction

Folliculogenesis and steroidogenesis are driven by FSH and LH both in a spontaneous menstrual cycle and during ovulation induction. The specific action of each gonadotrophin in different clinical settings, however, is still incompletely understood. The current understanding of human ovarian steroid synthesis is based on the ‘two cell, two gonadotrophin’ theory. This theory states that LH stimulates theca cells to synthesize and secrete androgens as substrates for the aromatase complex in the granulosa cells which convert androgens to estrogen under the influence of FSH (Fevold, 1941; Ryan et al., 1968). The extensive experience with GnRH agonist-induced LH suppression in ovarian stimulation for IVF has shown that even a minute amount of LH is sufficient for synthesis and secretion of estradiol \((E_2)\) necessary for follicular maturation (Flemming and Coutts, 1986; Ben-Chetrit et al., 1996). Furthermore, FSH alone is sufficient to stimulate follicular growth in patients with hypogonadotropic hypogonadism, without detectable increase in serum \(E_2\) and androgen concentrations (Schoot et al., 1992). However, rising \(E_2\) level during spontaneous cycles and ovulation induction in patients with hypogonadotrophic hypogonadism is necessary for induction of endometrial progesterone receptors and thereby the transformation to a receptive, secretory endometrium by exposure to progesterone during the luteal phase.

It has been suggested that down-regulation with GnRH agonist in some normogonadotropic women may result in a profound suppression of LH concentrations, which in turn impairs adequate estradiol synthesis during FSH stimulation for IVF/ICSI (Westergaard et al., 1996; Fleming et al., 1998, 2000; Janssens et al., 2000), fertilization rates (Westergaard et al., 1996; Humaidan et al., 2002), the number of clinical pregnancies (Humaidan et al., 2002), and pregnancy outcome (Westergaard et al., 2000). Indeed, inferior IVF outcome as a result of the use of GnRH agonist and recombinant \((r)\)FSH have also been reported by others (Flemming et al., 1996; Esposito et al., 1998). However, other studies do suggest that the so-called resting levels of LH, as seen in women undergoing down-regulation with GnRH antagonist and stimulation with purified or rFSH preparations, are sufficient to support development and maturation of follicles and oocytes in
normogonadotrophic women (Chappel and Howles, 1991; Daya et al., 1995; Loumaye et al., 1997; Balasch et al., 2001).

In the present report we examine the impact of serum LH concentrations on the first day of ovarian stimulation (stimulation day 1) on the outcome of IVF and ICSI. The specific aims are: (i) to assess the usefulness of serum LH concentration on stimulation day 1 as a predictor of ovarian response, pregnancy, and the outcome of pregnancy; and (ii) by the use of receiver operating characteristic (ROC) curve analysis, to define the best threshold value, if any, to discriminate between women with ‘low’ and ‘normal’ LH concentration.

Materials and methods
The subjects of this retrospective study were 1652 infertile couples who received 2625 cycles of IVF or ICSI between 1996 and 2002 in our department. The couples had all undergone a diagnostic work-up consisting of one or more semen analyses evaluated according to WHO criteria, measurement of luteal phase progesterone concentrations, vaginal ultrasonography, and laparoscopy or laparotomy for evaluation of tubal patency and/or endometriosis. In women with ovulatory dysfunction, serum concentrations of FSH, LH, estradiol (E₂), prolactin, thyroid-stimulating hormone (TSH), free thyroxine, androstenedione, testosterone, and sex hormone-binding globulin (SHBG) were measured. Upper age limit of the women was 40 years; in selected cases, however, older patients were also accepted provided that they had a normal clomiphene citrate challenge test (Tanbo et al., 1992). Patients with body mass index (BMI) >35 kg/m² were excluded. In couples with severe male factor infertility, a chromosome analysis was performed in both spouses before treatment with ICSI.

All patients received mid-luteal phase down-regulation regimen. Pituitary function was suppressed with the GnRH agonist buserelin (Suprecur, Hoechst, Germany) by four doses daily as nasal spray (600 μg/day) or nafarelin (Synarel, Pfizer, USA) by three doses daily nasal spray (600 μg/g/day) given for a minimum of 14 days until a withdrawal bleeding and suppressed E₂ concentrations (<0.2 nmol/l) were achieved. Concomitant stimulation with human rFSH (Gonal F; Serono, Switzerland; or Puregon; Organon, The Netherlands) was then started. The starting daily FSH dose was 75–300 IU according to age, experience from previous cycles, baseline FSH concentration, infertility diagnosis, and BMI. Response to stimulation was evaluated by serial vaginal ultrasound examinations and serum E₂ concentrations, and the dosage of FSH was adjusted accordingly.

On stimulation day 1, before the first injection of FSH, blood samples were drawn for analysis of E₂, LH and FSH. The methods used for hormone analyses have been described in previous studies (Dale et al., 1998). In brief, serum concentrations of LH and FSH were measured using DELFIA (dissociation-enhanced Lanthamide fluoroimmunoassay) kits obtained from LKB Wallac (SF-220101; Turku, Finland). Inter- assay coefficients of variation for the individual analysis were: LH 5–8% and FSH 8%.

Ovulation was induced with 10 000IU hCG (Profasi, Serono or Pregnyl, Organon) when at least one follicle had a diameter of ≤18 mm. Oocytes were collected after 34–38 h under vaginal ultrasound guidance. Handling of gametes, fertilization, and embryo culture were according to standard IVF procedures. Briefly, Universal IVF medium (Medi-Cult, Copenhagen, Denmark) was used throughout oocyte insemination and embryo culture. In ICSI cycles only ejaculated sperm were microinjected. Embryos were scored from grade 1 (high quality) to grade 4 (poor quality) according to the size and shape of blastomeres and degree of fragmentation. Embryos were transferred on day 3, except in cases with few embryos (≤2), which were transferred on day 2. As a rule, and when available, two embryos were transferred. Luteal phase support up to 14 days after oocyte retrieval consisted of daily i.m. injection of 25 mg progesterone in oil or by vaginal administration of micronized progesterone 300 mg × 2 (Progestan; Organon A/S, Oss, The Netherlands).

Serum concentration of β-hCG was measured on day 14 after oocyte retrieval, and in case of concentrations >20 IU/l, which indicated conception, ultrasound scans were performed at 7–8 weeks gestation to verify the viability of pregnancy. Early pregnancy loss was defined as a biochemical pregnancy that failed to progress beyond 12 weeks gestation. Ongoing pregnancy was defined as a pregnancy that progressed beyond 12 weeks.

Data analysis
Results are expressed as mean ± SD. Continuous data were analysed with Student’s t-test or the Mann–Whitney U-test, where appropriate. The χ²-test and χ²-test for linear trend were used for frequencies. P < 0.05 was considered statistically significant.

The discrimination attained between two compared groups (conception versus non-conception cycles and ongoing pregnancy versus early pregnancy loss) was evaluated with the ROC analysis (Hanley and McNeil, 1982; Zweig and Campbell, 1993). ROC curves are plots of all the sensitivity and specificity pairs that are possible for all levels of a particular parameter. They are constructed by plotting the false positive rate or (1 – specificity) on the x-axis and the true positive rate or sensitivity on the y-axis. The best threshold value discriminating between the two conditions is typically located at the greatest distance from the diagonal. The area under the ROC curve (AUCROC) may serve as an estimate of accuracy, i.e. the ability of a particular parameter (LH concentration on stimulation day 1 in this paper) to discriminate between two conditions (conception versus non-conception and ongoing pregnancy versus early pregnancy loss).

Results
A total of 1652 women undergoing 2625 treatment cycles of IVF (n = 1856) or ICSI (n = 769) were included in the present investigation. Of the 1652 patients, 916 were treated for one cycle, 529 for two cycles, 179 for three, 26 for four and two patients underwent five treatment cycles. Compared to non-conception cycles, conception cycles were characterized by a lower mean age of women, shorter duration of stimulation, lower total dose of FSH during stimulation, increased number of collected and fertilized oocytes, increased mean number of transferred embryos, and higher quality of transferred embryos. Hormone concentrations on first day of stimulation were not significantly different between conception and non-conception cycles (Table I). Statistically significant differences in duration and total dose of FSH stimulation, number of collected and fertilized oocytes, and hormone concentrations of stimulation day 1 were not observed between treatment cycles leading to ongoing pregnancies compared to cycles with early pregnancy loss (Table I).

To assess further the potential impact of suppressed LH concentrations on the outcome of IVF/ICSI treatment, cycles
were stratified according to serum LH concentration on stimulation day 1 (LH: <0.50; 0.51–1.00; 1.01–1.50; 1.51–2.00; 2.01–5.00; >5.00 IU/l). Significant association between LH concentration on stimulation day 1 and treatment outcome was not observed (Table II).

In a subgroup of cycles (n = 33), GnRH agonist down-regulation was given for >90 days, mostly for treatment of advanced endometriosis. These women had a lower BMI, lower serum LH concentrations on stimulation day 1, and lower pregnancy rate than the women receiving GnRH agonist treatment for <90 days (Table III).

Treatment cycles performed for polycystic ovarian syndrome (PCOS) as the main infertility diagnosis (n = 151) were characterized by significantly lower FSH concentrations on stimulation day 1 (3.1 ± 1.3 IU/l versus 3.7 ± 1.9 IU/l, P < 0.001) and higher E2 concentrations on stimulation day 1 (0.14 ± 0.04 nmol/l versus 0.12 ± 0.03 nmol/l, P < 0.001) compared to cycles performed for other reasons.

The diagnostic accuracy of serum LH concentration on stimulation day 1 to discriminate between conception versus non-conception cycles and ongoing pregnancies versus early pregnancy losses was examined with ROC analysis, which did not reveal a statistically significant diagnostic value of LH concentration (Figure 1, Table IV). In order to examine the sensitivity of ROC analysis, the analysis was repeated in the following subsets: (i) only the first cycle of each couple; (ii) all IVF cycles; (iii) all ICSI cycles; (iv) excluding cycles with GnRH agonist down-regulation for >90 days and/or presence of PCOS; (v) combination of these criteria. Serum LH concentration on stimulation day 1 did not discriminate significantly between conception versus non-conception cycles and ongoing pregnancies versus early pregnancy losses in these analyses (Table IV).

**Discussion**

We found that the clinical outcome of IVF and ICSI was not related to the serum LH concentration on stimulation day 1 in women undergoing mid-luteal phase GnRH agonist down-regulation with nasal spray and rFSH stimulation.
Table III. Patient characteristics, gonadotrophin treatment, LH stimulation day 1, ovarian response, ovum retrieval, and IVF/ICSI outcome in patients with GnRH agonist treatment for <90 days versus GnRH agonist treatment for >90 days

<table>
<thead>
<tr>
<th>Duration of GnRH agonist down-regulation</th>
<th>&gt;90 days</th>
<th>&lt;90 days</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of cycles'</td>
<td>33</td>
<td>2585</td>
<td></td>
</tr>
<tr>
<td>Female age (years)</td>
<td>31.4 ± 4.0</td>
<td>32.9 ± 3.6</td>
<td>0.06</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.9 ± 2.6</td>
<td>23.6 ± 4.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Hormone concentrations on stimulation day 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>3.8 ± 1.9</td>
<td>3.7 ± 1.3</td>
<td>0.96</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>1.2 ± 1.1</td>
<td>2.0 ± 1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Estradiol (nmol/l)</td>
<td>0.13 ± 0.03</td>
<td>0.13 ± 0.03</td>
<td>0.96</td>
</tr>
<tr>
<td>No. of days of stimulation</td>
<td>11.5 ± 4.0</td>
<td>11.4 ± 2.6</td>
<td>0.37</td>
</tr>
<tr>
<td>Total FSH dose (IU)</td>
<td>2322 ± 1317</td>
<td>2188 ± 916</td>
<td>0.90</td>
</tr>
<tr>
<td>No of oocytes retrieved</td>
<td>7.6 ± 4.5</td>
<td>9.2 ± 5.7</td>
<td>0.15</td>
</tr>
<tr>
<td>No of diplodified fertilized oocytes</td>
<td>4.4 ± 3.7</td>
<td>5.4 ± 3.7</td>
<td>0.08</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>1.9 ± 0.3</td>
<td>1.9 ± 0.3</td>
<td>0.98</td>
</tr>
<tr>
<td>No. of blastomers in the first transferred embryo</td>
<td>5.1 ± 2.6</td>
<td>5.7 ± 2.4</td>
<td>0.21</td>
</tr>
<tr>
<td>Grade of the first transferred embryo</td>
<td>2.1 ± 0.6</td>
<td>2.2 ± 0.5</td>
<td>0.67</td>
</tr>
<tr>
<td>No. of blastomers in the second transferred embryo</td>
<td>5.5 ± 2.7</td>
<td>5.8 ± 2.4</td>
<td>0.46</td>
</tr>
<tr>
<td>Grade of the second transferred embryo</td>
<td>2.1 ± 0.4</td>
<td>2.3 ± 0.5</td>
<td>0.07</td>
</tr>
<tr>
<td>No pregnancies (%)</td>
<td>3 (9.1)</td>
<td>699 (27.7)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values are mean ± SD or n (%).

*Duration of GnRH agonist down-regulation could not be established in 34 treatment cycles.

Figure 1. Receiver operating characteristics curve of LH concentration on stimulation day 1 for discriminating conception versus non-conception cycle (solid line) and ongoing pregnancies versus early pregnancy losses (dashed line).

These findings extend the earlier observations, where ovarian response, IVF/ICSI outcome, implantation and the outcome of pregnancy were unrelated to early and mid-follicular phase serum LH concentrations, during long-term down-regulation with s.c. GnRH agonist preparations and stimulation with a protocol similar to ours (Balasch et al., 2001; Carbrera et al., 2005). Since rFSH is devoid of LH activity, LH immunoactivity detected in the serum before and during stimulation with rFSH must be the result of the endogenous LH secretion. Lack of association between LH concentration and treatment outcome suggests that such a residual LH secretion (remaining after a prolonged GnRH agonist treatment) may be sufficient to support a normal follicle maturation. Consequently, administration of exogenous LH during the long protocol stimulation is probably not justified by the argument that some women may experience a too profound suppression of serum immunoactive LH concentration that needs to be corrected. This notion, however, has several limitations. First, immunoactive LH concentrations do not necessarily correlate with LH bioactivity, which was not measured in this report. Second, LH concentrations later during stimulation may still be related to treatment outcome, although such an association was not observed in other reports (Balasch et al., 2001; Lisi et al., 2005). Finally, recent experience with rLH treatment during ovarian stimulation in normo-ovulatory women suggests that rLH can improve rates of implantation and pregnancy in these women, but curiously not among those who have a profound LH suppression (Lisi et al., 2005). It has been reported that mid-follicular phase levels of circulating E2 and LH is of significance for the outcome of assisted reproductive treatment (ART) after long GnRH agonist protocol and FSH stimulation, and that the results correlate with the regimen of desensitization [intranasal (i.n.) versus subcutaneous (s.c.) administration] and with the type of gonadotrophin used (hMG versus rFSH) (Westergaard et al., 2001). The most favourable outcome is reported to be in the i.n. hMG group indicating that administration of LH (part of hMG) after administration of i.n. GnRH agonist is likely to improve the results (Westergaard et al., 2001). In our study, only intranasal formulation of two different GnRH agonist compounds was used.

Suggestions have been made that GnRH agonist down-regulation in some normogonadotrophic women may result in profound suppression of LH concentrations, impairing adequate E2 synthesis (Westergaard et al., 1996; Flemming et al., 1998, 2000; Janssens et al., 2000), fertilization rates (Westergard et al., 1996) or the final clinical treatment outcome by increasing the risk of early pregnancy loss (Westergaard et al., 2000). It has also been suggested that in addition to a ‘threshold’ there might exist a ‘ceiling’ in LH activity (i.e. an optimal range for LH action), and that excessive LH administration might impair treatment outcome in ovulation induction and assisted reproductive treatment (Shoham, 2002). Our results, however, show that the level of LH on stimulation day 1 did not have any implication on the clinical outcome. The requirement for LH during the follicular phase is likely to be low, because <1% of pre-ovulatory follicular theca LH receptors need to be occupied to allow normal ovarian steroidogenesis (Chapell and Howles, 1991). The fair assisted reproductive treatment results obtained with the use of rFSH and i.n. GnRH agonist tend to support this notion (Westergaard et al., 2001). Furthermore, patients with intermediate levels of residual endogenous LH secretion on stimulation day 8 have been found to respond optimally,
whereas women with high or low LH concentrations had reduced success rates after assisted reproductive treatment (Humaidan et al., 2002). We could find no such correlation by stratification of the LH concentrations in our study (Table III).

Women with hypogonadotrophic hypogonadism (HH) most often need addition of gonadotrophin preparations containing LH activity (hMG or rLH) to the stimulation protocol in order to restore E₂ production and to improve the reproductive outcome (Shoham et al., 1991; Kousta et al., 1996). LH of <1.2 IU/l is suggestive of HH (European Recombinant Human LH Study Group, 1998). Accordingly, levels of LH < 1.2 IU/l on stimulation day 1 in i.n. GnRH agonist down-regulated women have been applied as an indication for supplementation of LH during the FSH stimulation phase. Even after promotion of optimal follicular development in HH by adding rLH in sufficient amounts, LH remains <1 IU/l (European Recombinant Human LH Study Group, 1998). This further confirms that, as shown in pituitary down-regulated patients, minimal circulating levels of LH are required to initiate follicle steroidogenesis. Therefore, measurements of serum immunoactive LH levels are of limited value, if any, for identifying whether a patient has enough endogenous LH activity to respond adequately to stimulation with FSH alone (Chappel and Howles, 1991; Loumaye et al., 1997). Thus there may be significant differences related to how longstanding minimal LH levels may affect the folliculogenesis in HH women, in contradiction to the very short period of time with low LH levels as a result of i.n. GnRH agonist down-regulation.

Other experimental and clinical studies indicate that LH is not required for follicular growth, but exogenously administered LH probably plays a primary role in complete maturation of the follicle and oocyte competence in patients with longstanding HH (Balasch et al., 1995; Fox et al., 1997; Cortvrindt et al., 1998). Our results indicated that this might also be the case for women who have been treated with GnRH agonist for > 90 days. As there were no differences in the consumption of FSH, the ovarian response and the embryo quality regardless of whether or not i.n. GnRH agonist were used for >90 days, the lower pregnancy rate after long-term use of GnRH agonist might be due to a reduced endometrial receptivity (Table III). Another explanation could be that the dominant diagnosis within this group was extensive endometriosis, which may be associated with a reduced implantation rate (Bernhart et al., 2002; Kao et al., 2003). However, our results are in contrast to those of Surrey et al. (2002) who observed a higher ongoing pregnancy rate in endometriosis patients down-regulated for 3 months before ovarian hyperstimulation compared to those who were treated with a conventional mid-luteal down-regulation regimen. In their paper, however, the type of gonadotrophin is not stated and patients with ovarian endometriosis were excluded. Due to the limited number of patients in both studies, more studies are needed to confirm these findings.

The present study shows that even ‘profoundly’ suppressed LH serum concentrations (LH < 0.5 IU/l) failed to affect the clinical outcome. LH suppression probably had no significant effect on estradiol biosynthesis since serum estradiol concentrations on stimulation day 1 were not significantly different when groups with various LH concentrations were compared. In a study from our own group (Tanbo et al., 2001) it has been shown that during the time of rFSH stimulation serum LH concentration decreased by an average of 35%. Furthermore androgen levels increased during rFSH stimulation in down-regulated IVF patients despite a decline in immunoactive LH during the stimulation period. FSH therefore, probably via inhibin production, is by itself capable of stimulating thecal androgen biosynthesis.

The patients in this study received two types of GnRH agonist. One half received buserelin, the other half nafarelin. It has been claimed that these two GnRH agonists might have different suppressive effects. Dada et al. (1999) showed that buserelin needed one more week to obtain the same suppressive effect on estradiol as nafarelin. Simberg et al. (1998) also showed that serum LH was lower in patients after 2 weeks of administration of buserelin compared to nafarelin. Furthermore the latter report claimed that the use of buserelin resulted in more oocytes recovered, while the fertilization rate was higher with the use of nafarelin (Simberg et al., 1998). No studies, however, have shown differences in implantation, miscarriage and pregnancy rate to be dependent on whether buserelin or nafarelin was administered (Lockwood et al., 1995; Simberg et al., 1998; Dada et al., 2002).

| Table IV. Diagnostic accuracy, as measured by AUCROC, of stimulation day 1 serum LH concentrations in predicting treatment outcome in all treatment cycles and various subsets of cycles |
|---------------------------------|------------------|------------------|
| Conception versus non-conception | Ongoing pregnancy versus early pregnancy loss |
| n | AUCROC (95% CI) | n | AUCROC (95% CI) |
| All cycles | 2625 | 0.51 (0.49–0.54) | 702 | 0.52 (0.47–0.57) |
| First cycles only | 1652 | 0.52 (0.49–0.55) | 442 | 0.51 (0.45–0.57) |
| IVF cycles only | 1856 | 0.52 (0.49–0.55) | 501 | 0.53 (0.47–0.59) |
| ICSI cycles only | 769 | 0.51 (0.46–0.56) | 201 | 0.51 (0.42–0.59) |
| Cycles without endometriosis or PCOS | 2327 | 0.51 (0.49–0.54) | 631 | 0.52 (0.47–0.57) |
| First IVF cycles without endometriosis or PCOS | 1047 | 0.51 (0.47–0.55) | 288 | 0.51 (0.43–0.59) |
| First ICSI cycles without endometriosis or PCOS | 405 | 0.51 (0.44–0.57) | 105 | 0.49 (0.37–0.60) |

AUCROC = area under the curve of the receiver operator characteristics curve; PCOS = polycystic ovarian syndrome.
1999). The subjects incorporated in the first two of these studies were few and the results from the studies partly contradictory. More investigations are needed to determine whether there are any differences in outcome of IVF/ICSI after i.n. administration of buserelin or nafarelin.

In conclusion, we found that serum LH concentration on the first day of FSH stimulation is not predictive of pregnancy and the outcome of pregnancy in women who had received long-term down-regulation with an intranasal GnRH agonist.

References


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