A relative reduction in mid-follicular LH concentrations during GnRH agonist IVF/ICSI cycles leads to lower live birth rates

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BACKGROUND: The effect of early- and mid-follicular LH concentrations on the ovarian response and pregnancy outcomes was evaluated in women receiving pituitary down-regulation with a GnRH agonist and ovarian stimulation with recombinant FSH (rFSH) during IVF/ICSI treatment. METHODS: Blood samples were collected prospectively from 701 cycles (560 patients) of assisted reproduction and analysed retrospectively. On the basis of LH concentrations on stimulation day 7/8, the patients were divided into two groups: LH < 1.2 IU/l (n = 179) and LH ≥ 1.2 IU/l (n = 522). Cycle outcomes were also compared on the basis of a ratio of mid- to early-follicular LH concentrations (≤0.5, n = 210; >0.5, n = 491). RESULTS: Patients with low LH concentrations were found to have a significant reduction in the late-follicular estradiol concentrations (P < 0.001), the number of oocytes retrieved (P < 0.01) and the number of usable embryos (P < 0.01), and they required significantly more rFSH (430 IU difference, P < 0.01). These differences did not translate into a significant change in live birth rates. Conversely, a ratio of ≤0.5 mid- to early-follicular LH concentrations (a reduction of ≥50%) was associated with a significant reduction in live birth rates per embryo transfer and per cycle started (27.3 versus 19.0%, P < 0.05 and 22.2 versus 15.8%, P < 0.05, respectively). CONCLUSIONS: Low mid-follicular levels of LH have a significant impact on ovarian response but not on live birth rates. A fall in LH level of ≥50% from the early- to mid-follicular phase resulted in a lower live birth rate.

Key words: GnRH agonist/IVF/live birth rate/LH/pituitary suppression

Introduction

Recently, with the introduction of recombinant LH (rLH), the supplementation of LH in down-regulated IVF/ICSI cycles using a GnRH analogue and recombinant FSH (rFSH) has become more common. An unpublished survey performed by the authors in 2005 revealed that in Sydney (Australia) 82.4% of clinicians occasionally used LH supplementation (either rLH or low-dose HCG) and that 64.7% routinely monitored LH concentrations during controlled ovarian hyperstimulation (COH) for IVF.

Several studies have looked at the use of rLH in women undergoing GnRH analogue/rFSH therapy and IVF and have found this treatment to be of variable benefit (Marrs et al., 2004; Humaidan et al., 2004; De Placido et al., 2005; Lisi et al., 2005; Tarlatzis et al., 2006). Not all patients undergoing such treatment will benefit from LH supplementation. Hence, in this study, the question whether low LH concentrations result in adverse outcomes has been once again addressed in a large observational trial.

A recent systematic review of studies investigating an association between endogenous LH levels during ovarian stimulation using GnRH agonists failed to detect a significant negative effect of low endogenous LH levels on ongoing pregnancies past 12 weeks (Kolibianakis et al., 2006). The same review suggested that the high levels of endogenous LH were associated with a decreased probability of ongoing pregnancies beyond 12 weeks. From the experience of GnRH antagonist cycles, it has now been suggested that the dynamic changes in LH levels may well be a cause for poorer pregnancy outcomes in IVF cycles (Kol, 2005).

Using IVF/ICSI treatment cycles utilizing GnRH analogue down-regulation and COH with rFSH, we sought to explore possible associations between (i) low mid-follicular LH values and (ii) changes between mid- to early-follicular LH concentrations, and their relationship with ovarian response and pregnancy rates.

Materials and methods

A total of 701 consecutive IVF or ICSI treatment cycles from January to December 2003 were included in this study. This involved 560 patients within the age range of 20–45 years. One patient who conceived was lost to follow-up and was included in all analyses except
for the live birth data. Patients undergoing a long down-regulation protocol using nafarelin acetate nasal spray were included in the trial. The following groups were excluded: (i) patients undergoing donor oocyte treatment, (ii) women diagnosed with hypogonadotrophic hypogonadism, (iii) patients using other GnRH analogues or GnRH antagonists and (iv) patients presenting from affiliated clinics for PGD.

The standard treatment protocol was as follows: Pituitary down-regulation was achieved with GnRH agonist (Nafarelin, Synarel, Searle Australia, Australia) from the mid-luteal phase in anovulatory patients and after using medroxyprogesterone acetate (Provera, Pharmacia Australia, Australia) 10 mg daily for 5–7 days in anovulatory patients. Twelve days later, a hormonal assessment was performed. A serum estradiol (E₂) level of <200 nmol/l was accepted as adequate suppression of ovarian activity (as per IVF-Australia protocol and Tarlatzis et al., 2006). Once this was achieved, ovarian stimulation was initiated with rFSH (Gonal F®, Serono Australia or Puregon®, Organon, Australia) using an individualized dose according to age, previous response and BMI. Ultrasound examinations and serum E₂ concentrations commencing on day 7 of stimulation monitored the ovarian response. The dose of FSH administered was adjusted if necessary. When at least two follicles had reached a diameter of >18 mm, 10 000 IU of HCG (Profasi®, Serono Australia or Pregnyl, Organon) was administered to initiate final follicular maturation.

Oocyte retrieval was performed 38 h later by vaginal ultrasound-guided follicle aspiration. A maximum of two embryos were transferred on day 2, 3 or 5 after retrieval. Luteal phase support was given by daily administration of vaginal progesterone pessaries (200 mg per day, Orion, Australia) or HCG (Pregnyl 1500 IU in three doses at 72 h intervals).

A clinical pregnancy was defined as a gestational sac seen on ultrasound. A miscarriage was defined as a clinical pregnancy loss before 20 weeks of gestation. The implantation rate was calculated as the number of sacs seen on ultrasound divided by the number of embryos transferred. The number of usable embryos was defined as the number of embryos that were suitable for transfer or cryopreservation. Embryos were assessed on the basis of developmental stage and the level of cell fragmentation. For example, on day 2 after insemination, embryos with four cells or more and <20% fragmentation were deemed to be suitable for fresh transfer and those with <10% suitable for freezing. Obstetric outcomes were confirmed with the patients. The multiple pregnancy rate was calculated as the number of multiple births divided by the total number of births after 20 weeks of gestation.

The study was approved by the Research and Development Committee of IVFAustralia.

Blood samples were collected as part of the routine monitoring on stimulation, day 0 (i.e. before commencing FSH), in the mid-follicular phase (day 7/8) and just before HCG administration (late-follicular phase). Sera were immediately analysed for E₂, LH and progesterone. A low LH concentration was defined as <1.2 IU/l because this is the defining concentration for the diagnosis for World Health Organization (WHO) type I anovulation (The European Recombinant Human LH Study Group, 1998).

The assay used for LH, E₂ and progesterone measurements was a commercially available ACS180 automated chemiluminescent immunoassay system (Bayer Diagnostics, Australia). LH was assessed against the WHO second international standard 80/552 and had an assay sensitivity of 0.07 IU/l. Similarly, the sensitivity values for progesterone and E₂ were 0.35 nmol/l and 36.7 pmol/l, respectively. The intra- and inter-assay coefficients of variation (CVs) for LH were <5.6 and <7.2%, for progesterone <10 and <12% and for E₂ <9.4 and <9.8%, respectively, in the range of concentrations measured in this study.

Statistical methods

Comparisons of means for normally distributed parameters were made using Student’s t-test. Similar comparisons for data requiring non-parametric analysis were tested using Mann–Whitney U-tests.

Logistic regression analyses for confounding factors were performed when data were presented in the form of a percentage or success/failure outcomes. Significance was recorded for tests with P < 0.05.

Results

Based on mid-follicular LH measurements (cycle day 7/8), treatment cycles were allocated to one of the two groups: Group 1, LH < 1.2 IU/l and Group 2, LH ≥ 1.2 IU/l. The number of cycles in Groups 1 and 2 was 179 and 522, respectively. The frequency of cycles with mid-follicular LH concentrations <1.2 IU/l was 25.5%. The median value of LH was 1.90 IU/l.

Some summary characteristics for the patients are compared in Table I. There were no statistically significant differences among the groups regarding the age and number of previous IVF/ICSI attempts and the number of embryos transferred.

Group 1 required statistically significantly more rFSH during COH than Group 2 (437 IU difference, 2423 versus 1986 IU, P < 0.001) (Table II). Furthermore, Group 1 had a 2221 pmol/l lower mean E₂ level before HCG administration (5279 versus 7500 pmol/l, P < 0.001) (Table II). The mean number of oocytes retrieved was also significantly reduced for Group 1 (9.6 versus 11.2, P < 0.01) (Table II). Finally, the total number of usable embryos was 17.4% less in Group 1 (2.96 versus 3.58, P < 0.01) (Table II).

In contrast, there was no significant difference between Groups 1 and 2 in the clinical pregnancy rate per embryo transfer (35.1 versus 32.9%, P = 0.62) and per cycle started (30.2 versus 26.4%, P = 0.34) (Table II). The miscarriage rate was comparable in the two groups (28.3 versus 21.7%, P = 0.60) (Table II). Neither the live birth rate per transfer (23.5 versus 25.2%, P = 0.67) nor the live birth rate per cycle (20.2 versus 20.3%, P = 0.98) was significantly different. There was, however, a greater incidence in multiple pregnancies observed for

### Table I. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (LH &lt; 1.2 IU/l; n = 179)</th>
<th>Group 2 (LH ≥ 1.2 IU/l; n = 522)</th>
<th>Statistical comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age (SD)</td>
<td>34.0 years (±5.0)</td>
<td>33.6 years (±4.7)</td>
<td>ns</td>
</tr>
<tr>
<td>Average number of IVF attempts (SD)</td>
<td>2.26 (±2.37)</td>
<td>2.20 (±2.25)</td>
<td>ns</td>
</tr>
<tr>
<td>Average number of embryos transferred (SD)</td>
<td>1.27 (±0.40)</td>
<td>1.25 (±0.50)</td>
<td>ns</td>
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patients in Group 2 (23.9 versus 7.9%, \( P < 0.022 \)). The implantation rate was 29.4% in Group 1 and 27.2% in Group 2 (\( P = 0.54 \)).

Subjects in the upper quartile (LH \( \geq 2.8 \) IU/l) were not found to have a significant difference in live birth rates per transfer when compared with patients in the lower three quartiles (LH \( < 2.8 \) IU/l) (25.3 versus 24.6%, \( P = 0.86 \)) (Table III).

The next section of this study explored associations between changing mid- to early-follicular LH concentrations (Table IV). A mid- to early-follicular LH ratio of \( \leq 0.5 \) compared with >0.5 was associated with a significant reduction in live birth rate per transfer (19.0 versus 27.3%, \( P = 0.030 \)) and per cycle started (15.8 versus 22.2%, \( P = 0.049 \)). Logistic regression analysis of live birth data incorporating the LH ratio with potential confounding factors such as female age, IVF attempt number and the number of embryos transferred showed a significant association (\( P = 0.027 \)).

A further analysis of patients with a low LH ratio (\( \leq 0.5 \)) and categorized on the basis of their day 7/8 LH concentration (1.9 IU/l, median value) showed a greater reduction in live birth rates per transfer (5.8 versus 22.1%, \( P = 0.033 \)) and per treatment cycle (4.8 versus 18.6%, \( P = 0.024 \)) for patients with higher day 7/8 LH concentrations. Only 5.9% of patients had a low LH ratio and raised day 7/8 concentrations (LH > 1.9 IU/l).

**Discussion**

COH for the purposes of IVF has certainly changed over the last 25 years. Clomiphene citrate alone was quickly replaced by its use in combination with urinary gonadotrophins. With the introduction of more purified forms of urinary FSH and then rFSH, the LH content in these medications has been gradually eradicated. The increasing use of GnRH analogues (Porter et al., 1984) for pituitary down-regulation has in many patients resulted in the depletion of endogenous LH production.

More recently among clinicians, there has been interest in supplementing LH to rFSH in COH. A recent survey performed by the authors (unpublished observation) indicated that 82.4% of clinicians in Sydney (Australia) are using supplemental LH or low-dose HCG on some occasions and that this decision is often initiated by low-serum LH concentrations. Worthy of comment is that there is no solid evidence yet to support such action.

rLH has been used in COH with varying outcomes. Some benefits have been suggested in women >35 years of age and in poor responders (Marrs et al., 2004; Humaidan et al., 2004; De Placido et al., 2005). A recent study has shown improved live birth rates when rLH was added to rFSH for COH (Lisi et al., 2005). These findings stand in contrast to a meta-analysis that suggests that rFSH preparations (Daya and Gunby, 2000)
lead to better pregnancy rates than the urinary products (which contain LH). Hence, the question still remains, which group of patients benefits from LH supplementation of benefit and what those benefits are.

The authors of this study have tried to address this question by attempting to identify negative effects of low mid-follicular LH concentrations. Recent studies have shown that low LH concentrations were associated with negative treatment outcomes (Fleming et al., 1996, 1998; Westergaard et al., 2000; Esposito et al., 2001; Humaidan et al., 2002). On the contrary, some publications have found no effect and have questioned the value of measuring LH (Fleming et al., 2000; Balasch et al., 2001; Penarrubia et al., 2003; Cabrera et al., 2005).

A recent systematic review of studies investigating an association between endogenous LH levels during ovarian stimulation using GnRH agonists failed to detect a significant negative effect of low endogenous LH levels on ongoing pregnancies past 12 weeks (Kolibianakis et al., 2006). The same review suggested that high levels of endogenous LH were associated with a decreased probability of ongoing pregnancies beyond 12 weeks. From the experience of GnRH antagonist cycles, it has now been suggested that the dynamic changes in LH levels may well be a cause for poorer pregnancy outcomes in IVF cycles (Kol, 2005).

The results of the study presented here show a significant increase in the amount of rFSH required during COH (437 IU) if the mid-follicular LH concentration is low (<1.2 IU/l). This increase is associated with increased cost. This study has also shown that a low mid-follicular level of LH is associated with reduced late-follicular E2 concentrations (42% reduction) and oocyte numbers (17% reduction). Most importantly, the number of usable embryos was 17% less in the low LH group. This may ultimately translate into a lower cumulative pregnancy rate.

The results of this study support the current evidence (Kolibianakis et al., 2006) that low LH concentrations (<1.2 IU/l) in the mid-follicular level bare no significant negative effect with regard to the clinical pregnancy rate per transfer (35.1 versus 32.9%), miscarriage rate (28.3 versus 21.7%) and live birth rate (23.5 versus 25.2%).

In this study, 25.5% of the women demonstrated LH concentrations <1.2 IU/l on day 7/8 of FSH stimulation. This proportion varies from other studies because of different down-regulation regimens, differing dosages and modes of administration of GnRH analogues and variations in the LH assays (Westergaard et al., 2000; Humaidan et al., 2002).

The authors sought to determine whether a reduction in serum LH concentrations from the early- (day 0) to the mid-follicular levels (day 7/8) might be associated with a less favourable outcome. For this purpose, a new measure was introduced—a ratio of mid- to early-follicular LH concentrations [day 7/8 LH/day (0) LH]. A ratio of 1:2 (0.5) indicating a 50% reduction in the LH level was established to be significant. The ratio of ≤0.5 was associated with a lower pregnancy rate and a higher miscarriage rate. Even though these results did not individually reach statistical significance, the combined effect of these results in a significant difference in live birth outcomes manifesting in an 8.3% reduction (27.3 versus 19.0%) in live birth rate per embryo transfer. With decreasing LH ratios, there appeared to be a further decrease in the live birth rates. In fact, an LH ratio of ≤0.25 resulted in a 9.5% live birth rate per cycle started, compared with 22.2% in those cases with an LH ratio of >0.5. Furthermore, the subanalysis of LH ratios identified a small group of patients with the above median levels of LH (day 7/8) and a low LH ratio who performed particularly poorly. This may suggest that not just the LH ratio but also the absolute change of LH values may be predictive of outcomes. Either way, these findings suggest that the dynamic changes in LH concentrations are of importance in predicting outcomes.

A review of patients included in more than one treatment cycle revealed a significant cycle-to-cycle correlation between LH concentrations on day 0 (r = 0.23, P < 0.05) and day 7/8 (r = 0.24, P < 0.05). This suggests a consistent effect of GnRH agonists on LH concentrations between cycles, which may lend itself to treatment with modified drug regimes.

How mid-follicular LH depletion might lead to increased pregnancy loss requires more study. According to the 2-cell two-gonadotrophin theory, both FSH and LH are required for normal follicular steroidogenesis (Falck, 1959). Women with hypogonadotrophic hypogonadism can achieve follicular development with FSH alone, but the E2-depleted environment affects the fertilization rates of retrieved oocytes (Balasch et al., 1995), and endometrial priming is poor resulting in markedly decreased implantation rates. Zeleznik and Hiller (1984) proposed that the presence of LH receptors is a key issue in the survival of the dominant follicle.

Westergaard has also reported an increase in early pregnancy loss when serum LH concentrations were low (Westergaard et al., 2000). Whether the supplementation of LH in patients with a low LH ratio improves live birth rates still needs to be established and certainly would be worth investigating further.

The strengths of this study lie in the large sample size, giving it enough power to look at live birth rates. The authors reported pregnancy outcomes per IVF cycle started to take into account any effect of cycle cancellations on the outcomes. Furthermore, a new concept—that of falling LH concentrations—was introduced (the LH ratio). Low numbers of embryos were transferred complying with modern trends in IVF. Older age groups were included. This is important because rLH appears to benefit in the older patient (Marrs et al., 2004).

The retrospective nature of the study can result in some biases. Selection bias was minimized by including all patients for 2003 who met the criteria. The IVF programme had no active protocols to deal with either low or high LH concentrations when this study sample was taken, reducing the risk of selection bias. Extended embryo culture can be a confounder. The proportion of extended embryo culture was only 4.5% of the transfers and did not significantly differ in the groups investigated. Unfortunately, the study was unable to reliably establish the diagnoses for infertility.

This study supports the clinical value of LH measurement during COH. It shows that low mid-follicular LH concentrations (<1.2 IU/l) are associated with an increase in FSH use.
Reducing LH concentrations affect pregnancy outcomes

and with a decrease in oocyte numbers and the number of usable embryos. No effect on pregnancy or live birth rates was detected despite the large sample size. Whether the lower number of usable embryos will result in a significant reduction in the cumulative pregnancy rate requires later evaluation.

Patients with a reduction of $>50\%$ (LH ratio $<0.5$) in the serum LH concentrations (from day 0 to day 7/8) were found to have significantly lower live birth rates. Whether LH supplementation in such cases can improve outcomes requires prospective interventional trials.

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