Effects of profound suppression of luteinizing hormone during ovarian stimulation on follicular activity, oocyte and embryo function in cycles stimulated with purified follicle stimulating hormone

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The effects of profound suppression of circulating luteinizing hormone (LH) during the follicular phase of in-vitro fertilization cycles were explored in normal women during treatment with a gonadotrophin-releasing hormone analogue and exogenous purified follicle stimulating hormone. Ovarian responses to treatment and the capacity of supernumerary embryos to undergo blastocyst formation were examined in groups of patients defined by the concentration of plasma LH in the mid-follicular phase. Concentrations \( \leq 0.5 \text{ IU/l} \) diagnosed the group with profoundly suppressed LH (\( \leq \text{LH}, n = 20 \)), which was compared with the remaining patients (\( \text{LH}, n = 41 \)). The \( \leq \text{LH} \) group showed lower oestradiol concentrations at human chorionic gonadotrophin administration, while the total follicular development estimated by the total follicular diameters was similar in both groups. The oestradiol secreted per follicle, estimated by the circulating concentration per mm total follicular diameter, was significantly lower in the \( \leq \text{LH} \) group. The combined effects of a trend to lower yield of oocytes (not significant) and a lower fertilization rate (not significant) resulted in a significantly reduced quantity of embryos available for cryopreservation after the fresh transfer. Supernumerary embryos were cultured for 7 days to determine blastocyst development rates, and the degree of LH suppression made no difference to embryo developmental competence (\( \text{nLH}, 23\% \); \( \leq \text{LH}, 27\% \)), or the rates of blastocyst formation. The group of patients with profoundly suppressed mid-follicular phase LH showed a reduced yield of oocytes and embryos which resulted in significantly fewer embryos available for cryopreservation. However, the developmental potential of those embryos, represented by the ability to form blastocysts in vitro, was unaffected. Key words: blastocyst formation/follicular growth/IVF/luteinizing hormone

Introduction

Normal follicular growth in the human is controlled by pituitary secretions of both follicle stimulating hormone (FSH) and luteinizing hormone (LH), and both gonadotrophins are required for oestradiol secretion through well-defined roles described in the two-cell two-gonadotrophin hypothesis (Falck, 1959; Sasano et al., 1989). The circulating LH acts on the theca cells to produce androgen substrate, which undergoes aromatization to oestradiol within the granulos cell compartment under the influence of FSH. When women with hypogonadotrophic hypogonadism undergo ovulation induction with exogenous gonadotrophins, FSH alone can be seen to induce follicular growth, confirmed by ultrasound observations, but normal concentrations of oestradiol secretion are absent. The oestradiol secretion requires an LH component, as when treated with human menopausal gonadotrophin (HMG; Couzinet et al., 1988).

Ovarian stimulation with gonadotrophin-releasing hormone analogue (GnRHa) suppression of endogenous gonadotrophin activity and with FSH monotherapy is commonplace in in-vitro fertilization (IVF) programmes. Most previous studies (Bentick et al., 1988) have shown no difference in the wide range of ovarian responses to stimulation whether FSH is used alone or in combination with LH activity, in the form of HMG. Recently, purified FSH, with high specific FSH activity and negligible LH content, has been employed in cycles of assisted reproduction technology, and it is likely that in the immediate future recombinant FSH with no LH activity will be used more widely.

Apart from the role of LH in oestradiol biosynthesis and secretion, it is possible that the differences in follicular environment resulting from LH absence will have an effect upon the oocyte and its maturation and developmental performance. Oocytes obtained from a woman with hypogonadotrophism in an IVF cycle treated with FSH showed that the LH-depleted environment yielded oocytes with a reduced fertilization rate compared with the same patient treated with combined FSH and LH (Balasch et al., 1995). Further evidence for a demand for LH during the follicular phase comes from studies in macaques (Weston et al., 1996; Zelinski-Wooten et al., 1996) in which the LH was suppressed using a GnRH antagonist. Similar degrees of follicular growth and oocyte yields were recorded for recombinant FSH (rFSH) treatment with or without rLH, but there was a higher fertilization rate when rFSH was used alone. However, LH deficiency appeared to have a detrimental effect upon embryo development and implantation. Implantation after cryopreservation and thawing was also improved after combined FSH and LH therapy (Weston et al., 1996). The inhibition of oestradiol biosynthesis in sheep at the early stages of oocyte maturation reduced the developmental capacity of retrieved oocytes (Moor, 1996), and, as an absence of LH leads to blockade of oestradiol
biosynthesis, it is possible that embryo developmental potential may be compromised through the steroid hormone pathway.

Immunoreactive LH is rarely fully suppressed during GnRHa treatment although a negative feedback effect of oestradiol upon LH can be observed during combined GnRHa and exogenous oestradiol treatment (Rademaker, 1991), and in the majority of such women treated with GnRHa for assisted reproduction it is possible that there is sufficient endogenous LH activity to allow normal follicular growth and oocyte development. However, a lower oestradiol profile during ovariian stimulation was reported by Fried et al. (1996) after comparing purified FSH with HMG, suggesting that the low LH activity in these cycles may influence follicular steroid biosynthesis, and furthermore it was shown that approximately one-third of women undergoing IVF using the GnRHa buserelin (150 µg intranasal; t.b.d.) with purified FSH demonstrate sufficient suppression of immunoassayable LH during the treatment cycle follicular phase, to reduce the oestradiol secretion in the peripheral circulation and the follicular fluids (Fleming et al., 1996). The effect of this degree of LH suppression upon oocyte fertilization, embryo development and implantation has not yet been previously reported in humans. A retrospective study by Loumaye et al. (1997) explored the roles of LH and the concept of a ratio of oestradiol per oocyte, to try to determine the impact of altered steroid biosynthesis upon IVF outcome. They showed that a reduced ratio of oestradiol per oocyte was associated with a low success rate upon IVF outcome.

Materials and methods

Patients

Sequential patients with a history of normal menstrual rhythm (α = 0.05) who were <37 years of age, with a body mass index of <29 kg/m², and who were undergoing their first IVF treatment cycle, or who had been treated previously without indication of abnormal ovarian function or abnormal semen parameters were recruited into the study. All partners showed normal semen analyses at initial assessment and also in the study IVF cycle. Sixty-one of these patients underwent oocyte retrieval and their data were collated for further analyses.

Treatment

All patients were treated with long course GnRHa (buserelin, Suprefact; Hoechst, Uxbridge, UK Ltd; 200 µg intranasally four times daily).

Follicular stimulation was effected with purified FSH (Metrodin-HP, Serono, Welwyn Garden City, UK Ltd), administered at a starting dose of 225 IU/day. Luteinization was stimulated by administration of HCG when more than three follicles of >16 mm diameter were identified. All patients were scanned 7–9 days after starting stimulation (S9–S12), and also within 24 h of HCG administration.

Embryology

Cumulus–oocyte complexes were assessed for maturity on the basis of degree of cumulus oophorus expansion, and fertilization was checked 16–21 h after insemination. After transfer of cleavage stage embryos on day 2, the supernumerary embryos were cultured in Dulbecco’s modified Eagles’s medium:Ham’s F12 supplemented with 2% Ultraser (Gibco, Paisley, UK) and graded every 22–24 h, up to day 7. Data referring to blastocyst formation on days 5 and 7 included both unexpanded and expanded blastocysts, and by days 6 and 7 also included hatched and hatching blastocysts.

Blood samples and assays

Blood samples were taken at the start of FSH treatment (S1), in the mid-follicular phase (S7, 8 or 9) and on the day of human chorionic gonadotrophin (HCG). They were separated and stored as plasma at –20°C prior to assay for LH and oestradiol using fluorimunnoassays (Delfia, Wallac Oy, Turku, Finland).

Discrimination by LH concentrations

The distributions of circulating LH concentrations amongst the patients during the mid-follicular phase showed that a number of the patients demonstrated values of <0.5 IU/l. Below this concentration the assay reliability parameters (inter- and intra-assay variation and recovery rates) deteriorated, so evaluation of the effects of profound LH suppression were estimated using this cut-off concentration value, which was recorded in approximately one-third of the patients. It was presumed that any effect of low LH would be restricted to a small proportion of cases, but discrimination below the value of 0.5 IU/l would be deemed unreliable.

Statistics

Ovarian responses displayed non-normal distributions and were compared using Mann–Whitney non-parametric test.

Log transformation of the data effectively normalized the distributions, and the groups were compared using independent sample t-tests which revealed similar results to those of the non-parametric tests. Proportions were compared using contingency table analyses, and Pearson’s correlation coefficient was used to analyse the hormone concentrations. Differences were considered significant at $P < 0.05$.

Results

LH profiles

Figure 1 shows the individual LH profiles at the critical points during the treatment cycle. The concentrations recorded show a wide range of values, but all were seen to be in the low/normal range. The values (mean ± SD) seen at mid-follicular phase (1.0 ± 0.8 IU/l) were significantly lower ($P < 0.01$) than those seen at S1 (1.4 ± 0.9 IU/l), but similar to those recorded at the time of HCG administration. The correlation between the LH concentration on day S1 and the mid-follicular phase value was $r = 0.6$ ($P < 0.01$), indicating that the S1 value showed only moderate predictive potential for that seen in the mid-follicular phase. The situation was similar with
Profiles of luteinizing hormone (LH) seen during long protocol treatment with gonadotrophin releasing hormone and purified follicle stimulating hormone. The mean values seen at S1 (start of stimulation treatment) were significantly higher than those seen during the mid-follicular phase and at human chorionic gonadotrophin (HCG) administration.

Table I. Ovarian responses to combined long course treatment with gonadotrophin-releasing hormone agonist and purified follicle stimulating hormone in the patients with profoundly suppressed luteinizing hormone (LH) during the mid-follicular phase (<LH) and in those with relatively normal LH (nLH)

<table>
<thead>
<tr>
<th></th>
<th>nLH</th>
<th>&lt;LH</th>
<th>P value</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Oestradiol at HCG (pg/ml)</td>
<td>Progesterone at HCG (ng/ml)</td>
</tr>
<tr>
<td>n</td>
<td>41</td>
<td>20</td>
<td>NS</td>
<td>2923 (2213)</td>
<td>1.35 (0.7)</td>
</tr>
<tr>
<td>LH</td>
<td>1778 (1762)</td>
<td>1.91 (1.2)</td>
<td>238 (98)</td>
<td>185</td>
<td>10.28</td>
</tr>
</tbody>
</table>

The hormone data are mean (SD) values.
FD = follicular diameter; HCG = human chorionic gonadotrophin; NS = not significant.

Ovarian responses

In order to analyse the responses with respect to the circulating LH concentrations, the distributions of the data were examined with respect to a cut-off concentration in the sample taken at mid-follicular phase. Table I shows the distributions and ovarian responses when the patients were divided into two groups based upon a cut-off value of 0.5 IU/l. The nLH group (LH >0.51; n = 41) constituted 67% of the population, with the remainder constituting the <LH group (LH <0.5 IU/l, n = 20). The two groups showed similar proportions of indications for treatment, and similar ages (<LH group mean of 33.1 years, and nLH group mean of 32.4 years).

The <LH group showed an apparent trend to reduced circulating oestradiol concentrations at the time of HCG compared with the nLH group (not significant, P = 0.06). The degree of follicular development represented by the total follicular diameter within 24 h of HCG were similar in the two groups, and there was an apparent trend to reduced oocyte yields per case (10.28 compared with 13.1: P = 0.08; not significant) in the <LH group compared with the nLH group.

Table II. Derived assessments of ovarian function after long course gonadotrophin-releasing hormone agonist treatment combined with purified follicle stimulating hormone in the patients with profoundly suppressed luteinizing hormone (LH) during the mid-follicular phase (<LH) and in those with relatively normal LH (nLH). The oestradiol concentrations in the peripheral circulation were related to yields of oocytes and the total follicular development (total follicular diameter) observed

<table>
<thead>
<tr>
<th></th>
<th>Oestradiol per oocyte</th>
<th>Oestradiol per mm FD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>at HCG (pg/ml)</td>
<td>at HCG (pg/mm)</td>
</tr>
<tr>
<td>nLH</td>
<td>239 (139)</td>
<td>10.4 (4.3)</td>
</tr>
<tr>
<td>&lt;LH</td>
<td>169 (97)</td>
<td>7.2 (3.6)</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td>0.03</td>
</tr>
</tbody>
</table>

The hormone data are mean values (± SD).
FD = follicular diameter; HCG = human chorionic gonadotrophin; NS = not significant.

Table II shows the derived assessments of ovarian function in the two groups: the oestradiol secretion in relation to oocyte yield and total follicular diameter. It is interesting to record that despite the <LH group showing fewer oocytes and slightly lower total follicular development, the ratios of circulating oestradiol divided by these parameters were both lower in the <LH group, showing borderline significance (P = 0.07) for oestradiol per oocyte and significance for oestradiol per mm follicle diameter (P = 0.03).
LH suppression during ovarian stimulation

Table III. Embryological assessments after long course gonadotrophin-releasing hormone agonist agonist treatment combined with purified follicle stimulating hormone in the patients with profoundly suppressed luteinizing hormone (LH) during the mid-follicular phase (<LH) and in those with relatively normal LH (nLH)

<table>
<thead>
<tr>
<th></th>
<th>Oocytes (n)</th>
<th>Immature complexes n (%)</th>
<th>Total fertilized n (%)</th>
<th>Normally fertilized n (%)</th>
<th>Embryos transferred per case</th>
<th>Supernumerary embryos remaining after transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>nLH</td>
<td>536</td>
<td>31 (6)</td>
<td>450 (84.2)</td>
<td>408 (76.1)</td>
<td>2.20 (0.46)</td>
<td>321</td>
</tr>
<tr>
<td>&lt;LH</td>
<td>185</td>
<td>16 (8)</td>
<td>140 (75.7)</td>
<td>130 (70.3)</td>
<td>2.05 (0.51)</td>
<td>102</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td>&lt;0.02</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean (SD).

Table IV. Embryo developmental potential assessed by blastocyst formation in culture in the patients with profoundly suppressed luteinizing hormone (LH) during the mid-follicular phase (<LH) and in those with relatively normal LH (nLH). The embryos cultured refer to all normally fertilized embryos remaining after the fresh in-vitro fertilization transfer

<table>
<thead>
<tr>
<th></th>
<th>Supernumerary embryos per case</th>
<th>Embryos cultured (n)</th>
<th>Blastocysts (n)</th>
<th>Blastocyst formation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>nLH</td>
<td>7.8 (5.4)</td>
<td>315</td>
<td>73</td>
<td>23.2</td>
</tr>
<tr>
<td>&lt;LH</td>
<td>5.1 (4.4)</td>
<td>100</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>P value</td>
<td>0.05</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean (SD).

Embryology

Table III shows that there was no influence of the circulating LH concentration upon the incidence of immature cumulus–oocyte complexes at oocyte retrieval. However, the group with suppressed LH (<LH) demonstrated lower total fertilization (P < 0.02) and an apparent trend to reduced normal fertilization (two pronuclei) (not significant, P = 0.07) compared with the group with normal LH. The net effect of this, in combination with the reduced oocyte yield, was a reduced cohort of embryos and also fewer supernumerary embryos (remaining after the fresh embryo transfer) which would normally be available for cryopreservation (Table IV). There was no difference between the groups in the numbers of embryos transferred.

Table IV shows that although there were fewer supernumerary embryos in the <LH group of patients, the degree of suppression of follicular phase circulating LH concentrations appeared to have no impact upon the developmental capacity of the fertilized embryos to undergo cleavage and expanded blastocyst formation by day 7 of culture. The rate of blastocyst formation in the two groups, estimated as a percentage of the total formed by each day, also showed no differences between the groups (Table V). The rates of embryo hatching observed during culture were also similar in both groups.

Discussion

The GnRHa protocol used in this study produced low circulating concentrations of LH by the start of treatment, which were reduced even further during the stimulated follicular phase. The concentrations of LH appear to be slightly lower than those reported previously in cycles treated with a lower dose of GnRHa, but in which an association between low circulating LH and reduced oestradiol biosynthesis and secretion was recorded (Fleming et al., 1996). In the present study, the cycles showing profoundly suppressed mid-follicular phase LH concentrations (<0.5 IU/l) during GnRHa treatment confirmed the observation of reduced circulating oestradiol concentrations and therefore reduced oestradiol biosynthesis and secretion. Comparison of the cycles with profoundly suppressed LH with those in which LH was >0.5 IU/l in the mid-follicular phase, revealed that the total degree of follicular development in the two groups of cycles did not appear to be significantly influenced by the LH concentration, indicating that there was reduced oestradiol secretion by the individual follicles in the <LH group. This was confirmed by the significant difference observed in the oestradiol per mm follicular diameter ratio, and the lower oestradiol per oocyte value recorded. It is presumed that this effect is due to the lack of LH-stimulated thecal androgen produced, as follicular fluid testosterone concentrations have been shown to be reduced under these circumstances (Fleming et al., 1996).

There appeared to be no effect of the degree of LH suppression upon the incidence of immature cumulus–oocyte complexes. Furthermore, the circulating LH appeared to have no impact on the proportion of normally fertilized oocytes that developed to the blastocyst stage, nor upon the rate at which they did so. However, the reduced yields of oocytes, combined with the lower fertilization rate, conspired to produce a significantly reduced yield of viable embryos in the group with suppressed LH. This has important implications for embryo cryopreservation programmes.

The demonstration of an effect of the low LH concentrations upon oestradiol output while showing a negligible effect upon the degree of follicular development, concurs with the
observations in women with hypogonadotrophic hypogonadism (Couzinet et al., 1988). Although the apparent reduction in oocyte yield has not been previously documented, probably because of the small numbers of appropriate patients, the reduced fertilization rate amongst the cycles with <LH concurs with the observation in a hypogonadotrophic patient treated in an IVF programme (Balasch et al. 1995), albeit to a reduced degree.

The observation that a low ratio of oestradiol per oocyte was associated with subnormal implantation rates in IVF (Loumaye et al., 1997) may indicate that excessively suppressed LH has a more profound impact than hitherto considered, since the <LH group showed lower oestradiol/oocyte ratios than the nLH group. However, the rates of blastocyst formation did not indicate that embryo developmental potential was compromised by the degree of LH suppression, or by the oestradiol per oocyte value (data not presented). More work is required to explore this phenomenon, but the specific cases referred to probably represent a small subgroup of the <LH group.

The mechanism by which a reduced yield of oocytes may be associated with low circulating LH may centre on the degree of follicular development in a manner undetected by the ultrasound observations, or it may derive from a reduced rate of recovery of oocytes from the follicles. It is possible that the maturation of the cumulus structure may be reduced by the low LH concentrations and therefore oocyte retrieval may require a more aggressive approach. However, there was no apparent increase in the proportion of immature cumulus–oocyte complexes in those oocytes that were retrieved.

The recent introduction of recombinant FSH into IVF programmes has led to an increased yield of oocytes and embryos compared with patients treated with urinary products, implying that rFSH enjoys a higher potency than urinary FSH. This appeared to be the case despite the virtual absence of LH in the recombinant preparation (Out et al., 1996). However, there has been no investigation into the responses to recombinant FSH of the relatively small subgroup of patients with profoundly suppressed LH during their mid-follicular phase, as in this study. It would be interesting to determine if the differences observed above would be reproducible under these circumstances.

In conclusion, the excessive suppression of LH during the follicular phase of a proportion of IVF cycles in normal women treated with purified FSH leads to a reduced cohort of embryos available for cryopreservation, and a reduced ratio of oestradiol per mm of follicular development, or oestradiol per oocyte, which may have implications for implantation potential. However, the developmental competence of the ‘spare’ embryos, as assessed by blastocyst development in vitro, was unaffected by the degree of LH suppression.

References


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