Comparison of LH concentrations in the early and mid-luteal phase in IVF cycles after treatment with HMG alone or in association with the GnRH antagonist Cetrorelix

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Luteinizing hormone (LH) is mandatory for the maintenance of the corpus luteum. Ovarian stimulation for IVF has been associated with a defective luteal phase. The luteal phases of two groups of patients with normal menstrual cycles and no endocrinological cause of infertility were retrospectively analysed in IVF cycles. Thirty-one infertile patients stimulated with human menopausal gonadotrophins (HMG) for IVF to whom the gonadotrophin-releasing hormone (GnRH) antagonist Cetrorelix 0.25 mg was also administered to prevent the LH surge (group I) were compared with 31 infertile patients stimulated with HMG alone (group II). Despite differences in the stimulation outcome, luteal LH serum concentrations were similar in the two groups. LH values dropped from 2.3 ± 0.6 IU/l on the day of human chorionic gonadotrophin (HCG) administration to 1.1 ± 0.7 IU/l on day HCG 2 in group I (P < 0.0001) and from 5.1 ± 3 to 1.2 ± 1.7 IU/l (P < 0.0001) in group II. In the mid-luteal phase, LH concentrations were low in both groups. Our results suggest that suppressed LH concentrations in the early and mid-luteal phase may not be attributed solely to the GnRH-antagonist administration. Pituitary LH secretion may be inhibited by supraphysiological steroid serum concentrations via long-loop feedback and/or by the central action of the exogenously administered HCG via a short-loop mechanism.

Key words: GnRH antagonist/IVF/LH/luteal phase/ovarian stimulation

Introduction

The administration of gonadotrophin-releasing hormone antagonist (GnRH) Cetrorelix has been shown to be effective in blocking the luteinizing hormone (LH) surge in ovarian stimulation cycles for IVF (Diedrich et al., 1994; Albano et al., 1996; Felberbaum et al., 1996; Albano et al., 1997). In contradiction to GnRH-agonist cycles, where pituitary function remains impaired for the entire length of the luteal phase after the arrest of the agonist (Smitz et al., 1992), adenohypophysis maintains its responsiveness to endogenous GnRH stimulus after antagonist treatment (Felberbaum et al., 1995) and it was suggested that antagonist cycles may not be in need of luteal phase support (Albano et al., 1997). Nevertheless, an impaired luteal phase in terms of duration and/or serum progesterone concentrations was observed in four out of six patients stimulated with the association of human menopausal gonadotrophin (HMG) and Cetrorelix 0.5 mg cycles with no luteal phase supplementation (Albano et al., 1998). A further analysis of the luteal phases in Cetrorelix 0.25 or 0.5 mg/HMG cycles showed that LH serum concentrations were reduced to almost undetectable levels two days after the human chorionic gonadotrophin (HCG) injection and for the whole length of the luteal phase (Albano et al., 1999).

As LH is mandatory for the maintenance and normal steroidogenic activity of the human corpus luteum (Casper and Yen, 1979; Schriock et al., 1985; Mais et al., 1986), abnormal LH secretion may account for a defective luteal phase. The aim of this study is to investigate further the possible causes of the observed decrease in serum LH concentrations after GnRH-antagonist treatment, by comparing patients stimulated with the association of HMG and the antagonist Cetrorelix 0.25 mg with patients stimulated with HMG only, for IVF.

Materials and methods

Two groups of patients were compared in this retrospective analysis. In both groups, the detection of infertility caused by endocrinopathies or polycystic ovarian syndrome or the appearance of polycystic ovaries in the ultrasound scan were main exclusion criteria. Patients with premature LH rise (e.g. two consecutive measurements of LH >10 IU/l) (Devroey et al., 1995) were also excluded from the analysis.

In the first group, 31 subjects were stimulated with a combination of HMG and the antagonist Cetrorelix 0.25mg. The stimulation protocol has been previously described in detail (Albano et al., 1996, 1997). In brief, subjects were infertile women between 23 and 37 years of age, with regular menstrual cycles (24–35 days) undergoing...
IVF in five cycles (16%) or intracytoplasmic sperm injection (ICSI) in 26 cycles (84%). Controlled ovarian stimulation was carried out with three ampoules (225 IU) HMG (Humegen; Organon, Oss, The Netherlands and Menogon; Ferring, Kiel, Germany) starting on day 2 of the menstrual cycle. The dose was adjusted individually from day 6 of the treatment according to oestradiol values and ultrasonographic follicular measurements. From day 6 of the HMG injection onwards (day 7 of the menstrual cycle), 0.25 mg of Cetrorelix (ASTA Medica AG, Frankfurt Main, Germany) were also administered s.c. in the anterior abdominal wall, up to and including the last day of HMG administration. Ovulation was induced when at least three follicles were ≥17 mm in diameter, by the injection of 10.000 IU of HCG (Pregnyl; Organon Oss, The Netherlands). A maximum of three embryos was replaced 2 days after the oocyte retrieval. All the subjects received luteal-phase support by means of one injection of 1500 IU of HCG every three days starting on the day of embryo transfer.

The second group consisted of 31 infertile patients stimulated with HMG for IVF. The stimulation protocol has also been previously described in detail (Devroey et al., 1995). In brief, patients were 25–36 years of age with regular menstrual cycles. Controlled ovarian stimulation was carried out with three ampoules (225 IU) HMG (Normegon; Organon Oss, The Netherlands and Metrodin; Serono, Geneva, Switzerland) starting on day 2 or 3 of the menstrual cycle. After 4 days of treatment the dose was adjusted individually according to serum oestradiol concentrations and ultrasonic follicular measurements. Ovulation was induced by 10 000 IU of HCG (Pregnyl; Organon Oss, the Netherlands) when three follicles ≥17 mm were detected by ultrasonography. The luteal phase was supplemented with HCG 1500 IU every 3 days starting 2 days after the embryo transfer.

In both groups, intensive hormonal monitoring was performed through daily blood samples during the peri-ovulatory period for the detection of a premature LH surge until the day of embryo transfer. Serum gonadotrophins were measured by specific monoclonal immunoradiometric assays (IRMA) for follicle stimulating hormone (FSH) and LH and expressed in IU/l (conversion factor to SI unit 1.00; First International Reference preparation for LH 68/40 and Second International Reference preparation for FSH 78/549). The LH assay had a sensitivity of 0.3 IU/l and within- and between-assay coefficients of variation of 7 and 9% respectively. Steroid serum concentrations were expressed in ng/l for oestradiol and µg/l for progesterone (conversion factor to SI unit 3.671 for oestradiol and 3.180 for progesterone).

Clinical pregnancy was defined as the presence of a gestational sac in the ultrasound scan at 7 weeks. Data were analysed by means of a Wilcoxon rank sum test using MedCalc software statistical program (MedCalc Software; Mariakerke, Belgium). Values are expressed as mean ± SD. Statistical significance was defined as a P value of <0.05.

Results

Patients’ age and day 3 FSH levels were comparable in both groups. In group I (HMG/Cetrorelix 0.25 group) the mean age of the patients was 30.7 ± 4.1 years and in group II (HMG group) it was 30.3 ± 2.5 years. Although cycle characteristics were different between the two groups, the number of transferred embryos was similar (2.7 ± 0.4 in the HMG/Cetrorelix 0.25 mg and 2.5 ± 0.5 in the HMG group), resulting in eight clinical pregnancies per group (pregnancy rate/embryo transfer of 25.8%) (Table I).

Steroid serum values were different between the two protocols. In the Cetrorelix 0.25 mg group, significantly higher oestrogen concentrations were observed during the 4 days preceding HCG administration, on the day of HCG and 4 days after the HCG (Figure 1, Table II). Progesterone serum concentrations were higher in the HMG group before the ovulatory HCG, but after the HCG injection progesterone values were higher in the Cetrorelix 0.25 mg/HMG group (Figure 2, Table II). FSH serum concentrations were lower in the antagonist protocol before the HCG injection, but did not attain statistically significant differences on any of the days studied (data not shown).

LH values were higher in the HMG group than in the Cetrorelix group 2 days prior to administration of the HCG, but this reached statistically significant differences 1 day before HCG administration (5.6 ± 2.9 versus 2.5 ± 1.2 IU/I).
Table II. Steroid serum concentrations during the peri-ovulatory phase of the Cetrorelix 0.25 mg/HMG and HMG groups

<table>
<thead>
<tr>
<th>Day</th>
<th>Progesterone (µg/l)</th>
<th>Oestradiol (ng/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HMG</td>
<td>Cetrorelix 0.25/HMG</td>
</tr>
<tr>
<td></td>
<td>mean ± (SD)</td>
<td>mean ± (SD)</td>
</tr>
<tr>
<td>−2</td>
<td>0.4 ± (0.3)</td>
<td>0.2 ± (0.1)</td>
</tr>
<tr>
<td>0</td>
<td>0.6 ± (0.4)</td>
<td>0.4 ± (0.2)</td>
</tr>
<tr>
<td>+2</td>
<td>6.1 ± (3.8)</td>
<td>11.4 ± (5.7)</td>
</tr>
<tr>
<td>+4</td>
<td>35 ± (15)</td>
<td>50.9 ± (9.8)</td>
</tr>
</tbody>
</table>

Day 0 = the day of ovulatory HCG.

Figure 2. Progesterone serum concentrations in Cetrorelix 0.25 mg/HMG and HMG cycles. Progesterone concentrations were higher in the HMG group before the ovulatory HCG injection, but higher in the Cetrorelix group in the early luteal phase. Values were similar in both groups in the mid-luteal phase. The values are plotted on a logarithmic scale. *P < 0.05 between the two groups.

Figure 3. LH serum concentrations in Cetrorelix 0.25 mg/HMG and HMG cycles. LH values are significantly higher in the HMG cycles one day before and on the day of HCG. Two days after the HCG, LH values drop significantly in both groups, reaching almost undetectable levels. *P < 0.05 between the two groups. **P < 0.05 versus day of HCG injection.

(P < 0.0001) and on the day of HCG (5.1 ± 3 versus 2.3 ± 1 IU/l) (P < 0.0001). One day after the ovulatory HCG, LH serum concentrations dropped in the HMG group and reached a plateau or even slightly increased in the antagonist group (from 2.3 ± 1 to 2.5 ± 1.9 IU/l) (Figure 3). Two days after the HCG injection, LH values decreased in both groups. In the Cetrorelix group, LH serum values dropped from 2.3 ± 1 IU/l on the day of HCG to 1.1 ± 0.7 IU/l on day HCG +2 (P < 0.0001) and in the HMG group they dropped from 5.1 ± 3 to 1.2 ± 1.7 IU/l (P < 0.0001). For the next 4 days following the HCG injection, no statistically significant differences were detected in LH serum concentrations between the two groups and on days 3, 4 and 8 after HCG these concentrations were at almost undetectable levels in both stimulation protocols. On the 8th day after the HCG, LH values were 0.7 ± 0.6 IU/l in the antagonist group and 0.5 ± 0.6 in the HMG group (Figure 3).

No statistically significant differences were detected in LH concentrations between pregnant and non-pregnant patients for any of the days studied, before and after the HCG injection, within the Cetrorelix/HMG group or within the HMG group (Figure 4).

Discussion

Cycle characteristics were different between the two stimulation protocols. In the HMG/Cetrorelix 0.25 mg group, ovarian stimulation was longer, the number of administered ampoules and the number of retrieved oocytes were significantly higher. This may be explained by the fact that as GnRH antagonists successfully prevent a premature LH surge, ovarian stimulation may be prolonged more than in cycles treated without antagonists, in order to obtain a larger number of mature oocytes.

In the pre-ovulatory phase, LH serum concentrations were lower in the antagonist group, as antagonist treatment significantly reduces LH levels (Leroy et al., 1994). One day after the ovulatory HCG, LH serum concentrations dropped in the HMG group but not in the Cetrorelix group, probably due to the arrest of the antagonist. Despite the differences in cycle characteristics between the two protocols, the luteal phase LH serum concentrations were similar from day 2 following the ovulatory HCG onwards. It has been previously reported from our group that treatment with the association of HMG and Cetrorelix 0.25 or 0.5 mg for ovarian stimulation reduces LH serum concentrations to almost undetectable levels for the whole length of the luteal phase in cycles supplemented with HCG (Albano et al., 1999). Similarly, in GnRH-agonist/HMG cycles, undetectable LH levels and low progesterone secretion
in the luteal phase have been described, accounting for a luteal-phase defect and making luteal-phase support necessary (Smitz et al., 1988). In GnRH-agonist cycles, these low LH levels may be attributed to a prolonged impairment of the pituitary gonadotrophin secretory capacity after GnRH-agonist treatment (Smitz et al., 1992). On the contrary, the pituitary remains responsive to GnRH after antagonist treatment (Gordon et al., 1990; Felberbaum et al., 1995) and normal corpus luteum function is preserved after mid-follicular antagonist administration (Mais et al., 1986). Since, in our results, LH values were strikingly similar in the early and mid-luteal phase in the two groups, it may be postulated that this observed decrease in LH serum concentrations may not be attributed solely to the GnRH antagonist administration.

Progesterone modulates LH secretion during the luteal phase by influencing the LH pulse amplitude and pituitary release of LH (Soules et al., 1984). A short exposure to physiological levels of progesterone, in the range of the early luteal phase, has a stimulatory effect on LH secretion by acting directly at the pituitary level (Couzinet et al., 1992; Couzinet and Schaison, 1993). On the other hand, a longer exposure to progesterone or the combined action of oestrogen and progesterone, results in reduced frequency of LH secretion by a possible action at the hypothalamic level (Steele and Judd, 1986, 1988; Nippoldt et al., 1989). Consequently, as ovarian stimulation results in supraphysiological steroid serum concentrations as compared to natural cycles, it may be postulated that these high steroid serum concentrations may adversely effect the LH secretion by disturbing the feedback mechanisms.

The fact that ovarian stimulation reduces LH concentrations in the luteal phase and results in a luteal-phase defect has also been previously proposed (Messinis and Templeton, 1987). In cycles stimulated with FSH, lower early serum LH concentrations have been detected than in natural cycles, or in natural cycles supplemented with exogenous oestrogen (Messinis and Templeton, 1987). Similarly, in cycles stimulated with clomiphene citrate/HMG/HCG for IVF, serum LH concentrations decreased from 20 IU/l (the day after the HCG injection) to a nadir of 3–5 IU/l in the mid-luteal phase (Smitz et al., 1988).

As well as supraphysiological steroid concentrations in ovarian stimulation, there is one more possible mechanism that might further affect pituitary LH secretion. A long time ago it was suggested that a negative short-loop feedback mechanism might exist which controls LH secretion (David et al., 1966). According to this hypothesis, HCG may affect pituitary LH secretion negatively, by reducing hypothalamic GnRh due to its similarity to the LH molecule but also due to its longer half-life (Damewood et al., 1989). Although in animal studies such a negative feedback exists (Silverman et al., 1981; Patritti-Laborde et al., 1982) there is a debate in the literature about its existence in humans, with some of the studies supporting this hypothesis (Miyake et al., 1978, 1979) and others not (Kyle et al., 1989; Nader and Berkowitz, 1992).

Nevertheless, recent findings in in-vitro studies further support this idea. GT1-7 neurons which are morphologically and functionally similar to GnRH neurons were found to contain LH/HCG receptors (Lei and Rao, 1994). In addition, exogenously administered HCG was found to decrease the expression of GnRH receptor gene in GT1-7 cells (Li et al., 1996) or GnRH secretion in immortalized GnRH neurons (Mores et al., 1996).

In stimulated cycles this assumption was supported by Demoulin et al. (1991). As mid-luteal LH serum concentrations were significantly lower in stimulated cycles compared to control natural cycles, the authors postulated that this might be due a possible short negative feedback from the exogenously administered ovulatory HCG. Furthermore, in cycles stimulated with HMG/Cetrorelix 0.5 mg and receiving no luteal phase supplementation, LH concentrations started to increase 8 days after the HCG (Albano et al., 1998), which coincides with the time that exogenous HCG is cleared from the circulation (Mannaerts et al., 1998). On the contrary, LH concentrations remained low in cycles supplemented with HCG (Albano et al., 1999). As, in GnRH antagonist cycles, triggering of ovulation is possible with a GnRH-agonist (Olivennes et al., 1996), the effect that alternative methods to induce the final oocyte maturation (e.g. GnRH-agonist or recombinant LH) exert on the luteal phase, remains be investigated.

In conclusion, ovarian stimulation with virtually all the currently used stimulation protocols results in reduced LH serum concentrations in the early and mid-luteal phase. These low LH serum concentrations may contribute to the luteal phase defect observed after ovarian stimulation. Supraphysiological steroid serum concentrations may interfere with LH secretion via long-loop feedback, but, additionally, the exogenously administered HCG might amplify LH secretion arrest via a second short-loop negative feedback.

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LH concentrations during ovarian stimulation

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