Effect of disruption versus continuation of gonadotrophin-releasing agonist after human chorionic gonadotrophin administration on corpus luteum function in patients undergoing ovulation induction for in-vitro fertilization

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Previous studies have described the luteolytic effect of gonadotrophin-releasing hormone agonist (GnRHa) administered in the early luteal phase. The present work was undertaken to compare in a prospective and randomized design the effect of disruption versus continuation of daily GnRHa after human chorionic gonadotrophin (HCG) administration on corpus luteum function in patients undergoing ovulation induction for in-vitro fertilization (IVF). Two different studies were designed and a total of 38 ovum donors, aged 23–30 years, were included. In the first study, the effect of GnRHa on the early luteal phase of IVF-stimulated cycles was investigated (n = 27); the patients were divided into two groups, according to whether they stopped (n = 13) or continued with daily GnRHa injections (n = 14) for an additional period of 15 days after HCG administration. Blood was drawn from luteal phase days 2 to 6 (day 0 = day of HCG administration) and oestradiol and progesterone concentrations were analysed. The second study focused on the effects of continuation versus disruption of GnRHa administration in the mid-late luteal phase. A similar design was employed including six patients who stopped GnRH on day 0 and five other women who continued GnRHa for 15 days after HCG administration. In this second study, blood was drawn from days 5 to 11 and oestradiol, progesterone and luteinizing hormone (LH) concentrations were analysed. IVF parameters were similar in both groups. The results indicate that continuous GnRHa administration, after HCG injection, does not produce changes in oestradiol, progesterone and LH concentrations in the early, mid- and late luteal phases compared to those patients in whom GnRHa is discontinued at the day of HCG administration. The present work demonstrates that, when ovulation induction is performed, the corpus luteum is driven primarily by the HCG, regardless of the administration or disruption of GnRHa in the luteal phase. This suggests that the lack of differences between continuation versus disruption of GnRHa may be due to the accumulation of the product over the previous 2–3 weeks of treatment.

Key words: GnRHa/luteal phase/lutealysis

Introduction

The incorporation of gonadotrophin-releasing hormone agonist (GnRHa) into an ovulation induction protocol for assisted reproduction has produced a number of benefits. The reduction of cancellation rates was due to the abolition of premature luteinizing hormone (LH) surge (Fleming et al., 1985), and the increase in the number of oocytes harvested and embryos transferred led to the overall improvement in pregnancy rates (Hughes et al., 1992). However, this new pharmacological approach produces higher concentrations of serum oestradiol compared to conventional treatments. Indeed, an increased incidence of severe ovarian hyperstimulation syndrome (OHSS), ranging from 1.9 to 4.2% with the use of GnRHa in assisted reproductive techniques, has been reported (Golan et al., 1988; Forman et al., 1990). Interestingly, GnRHa administered for triggering ovulation has been suggested as an alternative to prevent OHSS in those patients at risk (Lewitt et al., 1996).

In addition to the luteolytic effect produced by inhibition of LH secretion, the presence of specific receptors for GnRHa in human preovulatory granulosa (Latouche et al., 1989) and luteal cells (Popkin et al., 1983; Bramley et al., 1986) sustains the concept that GnRHa may have a local effect on the ovary in humans. In vitro, this effect is mainly the inhibition of progesterone production in cultured granulosa luteal cells (Clayton et al., 1979; Tureck et al., 1982; Pellicer et al., 1992).

It has been reported that single-dose GnRHa administration in the luteal phase produces a luteolytic effect in humans (Koyama et al., 1978; Casper and Yen, 1979; Sheehan et al., 1982; Lemay et al., 1992) and monkeys (Hutchison and Zeleznick, 1985). Interestingly, this effect occurred if GnRHa was administered not later than 8 days after the LH peak.

In patients undergoing in-vitro fertilization (IVF), routine GnRHa administration induces a drop in progesterone 8 days after HCG administration, suggesting a cumulative drug effect or merely an alteration of corpus luteum development. However, a mid-luteal drop in progesterone also occurs when ovulation induction for IVF is performed in the absence of GnRHa (Hutchinson-Williams et al., 1989).

Another interesting issue is the administration of GnRHa inadvertently in the late luteal phase of patients undergoing IVF being associated with spontaneous pregnancies. The overall results, although conflicting, suggest that pregnancy outcome is not adversely affected by GnRHa administration in the luteal phase of the conception cycle, and some authors even suggest a beneficial effect of GnRHa administration (for review, see Balasch et al., 1993; Cahill et al., 1994).

We have therefore undertaken a prospective, randomized
study to analyse the effect of continuous GnRHα administration in the luteal phase on corpus luteum function in patients undergoing ovulation induction for IVF. To this end, two studies were performed to determine the effect of continuous administration of GnRHα in the early and the mid-late luteal phase.

Materials and methods

Study design
A total of 38 ovum donors, aged 23–30 years, volunteered to participate in this project. Two different studies were designed: in the first, the effect of GnRHα on the early luteal phase of IVF-stimulated cycles was investigated in 27 donors. They were divided into two groups, according to whether they stopped (n = 13) or continued with daily GnRHα injections (n = 14) for a further period of 15 days after human chorionic gonadotrophin (HCG) administration. Blood was drawn on luteal phase days 2–6 (day 0 = day of HCG administration) and oestradiol and progesterone concentrations were analysed.

The second study centred on the effects of continuous GnRHα administration in the mid-late luteal phase. A similar design was employed which included six patients who stopped GnRHα on day 0 and five other women who continued GnRHα for 15 days after administration of HCG. In this second study, blood was drawn from days 5 to 11 (day 0 = day of HCG administration). Similarly, oestradiol, progesterone and LH concentrations were analysed. LH was included because it has been shown that LH concentrations remain undetectable for the 10 days following the stoppage of administration of GnRHα (Smitz et al., 1988).

Ethical considerations
The protocol was approved by the Committee on Ethics of Research involving Human Subjects of the Instituto Valenciano de Infertilidad. Patients gave signed informed consent before entering the study.

Stimulation protocol
The ovarian stimulation protocol undergone by all patients, using GnRHα and gonadotrophins, has been described previously (Pellicer et al., 1989); a long protocol was used for pituitary desensitization with leuprolide acetate (Procrin; Abbott Scientific SA, Madrid, Spain), 1 mg/day s.c., commencing in the luteal phase of the previous cycle. Serum oestradiol concentrations <60 pg/ml and negative vaginal sonographic scans were used to define ovarian quiescence. On days 1 and 2 of ovarian stimulation, two ampoules/day of human menopausal gonadotrophin (HMG; Pergonal; Serono Laboratories, Madrid, Spain) together with two ampoules of follicle stimulating hormone (FSH; Fertinorm; Serono Laboratories) were administered. On days 3, 4 and 5 of ovarian stimulation, the dose of both FSH and HMG was reduced to one ampoule/day. Commencing on day 6, HMG and FSH were administered on an individual basis according to serum oestradiol concentration and transvaginal ovarian ultrasound scans. The criteria for HCG (Profasi; Serono) administration of 10 000 IU were the presence of two or more follicles >19 mm in diameter and a serum oestradiol concentration >800 pg/ml (2.94 nmol/l). Leuprolide acetate, FSH and HMG were discontinued on the day of HCG administration. GnRHα was continued in some patients for 15 days as indicated in the study design. Oocyte retrieval was scheduled 36–38 h following HCG administration.

Since the patients involved in this study were ovum donors, they did not receive the embryos back and therefore luteal support was not administered.

Steroid hormone assays
Serum oestradiol, progesterone and LH were measured by hormone assays and analysed using commercially available radioimmunoassay kits (BioMérieux, Charbonnières les Bains, France). The inter- and intra-assay variabilities for oestradiol at a concentration of <40 pg/ml (<147 pmol/l) were 2.8 and 4.3% respectively; for progesterone, at a concentration of 0.6 ng/ml (2 nmol/l), they were 9 and <10% respectively; and for LH, at a concentration of 2 IU/m, 7 and 6% respectively.

Statistical evaluation
Data are expressed as mean ± SEM. Student’s t-test and χ² were used to discriminate between groups. Significance was defined as P < 0.05. The statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA).

Results

General data
Table I compares the clinical outcome of the control and luteal GnRHα-treated patients included in the early luteal phase study. The number of patients and cycles, number of ampoules administered, number of oocytes retrieved, and oestradiol concentrations on the day of HCG administration did not differ between the groups. Only the age (P < 0.001), and obviously the total number of days GnRHα was administered (P < 0.05), were significantly different.

In Table II, we compare the outcome of ovulation induction in patients included in the mid- and late luteal phase study. Only the total number of days of GnRHα administration differed between the groups, as expected. There was no statistical difference in the rest of the parameters analysed.

This first approach demonstrates that the groups analysed in both studies were comparable in terms of outcome of ovulation induction and, therefore, steroidogenic behaviour.

Effects of luteal GnRHα disruption/continuation on corpus luteum function
The circulating amounts of oestradiol and progesterone during the 5 days following the day of oocyte retrieval (from day 2 up to day 6 after HCG injection) are shown for the early luteal phase study in Figure 1. There was no difference between the two groups analysed, indicating that continuation of GnRHα does not affect oestradiol and progesterone concentrations in the early luteal phase compared to the cessation of this drug at the time of HCG administration.

In the mid-late luteal phase study (Figure 2), we compare serum concentrations of oestradiol, progesterone and LH from day 3 through day 9 following oocyte retrieval (day 5 through day 11 after HCG injection). There were no significant differences established between the groups. These results indicate that continuous GnRHα administration after the HCG injection does not produce changes in oestradiol, progesterone and LH concentrations in the mid- and late luteal phases compared to those patients in whom GnRHα has been stopped on the day of HCG administration.

Discussion
The luteolytic effect of GnRHα in humans after menstrual (Sheeham et al., 1982) or luteal (Casper and Yen 1979; Lemay
et al., 1992) administration has been reported. A window of luteolysis has been described by giving GnRHa until day 7 after ovulation (Asch et al., 1981). The scope of the present work was to compare, in a prospective and randomized design, the effect of disruption versus continuation of GnRHa after HCG administration on corpus luteum function in patients undergoing ovulation induction for IVF. Since we were investigating the luteal phase, this study had two important requirements: first, progesterone supplementation should not be administered, and second, the embryos could not be transferred back to the mother. For these reasons, we chose ovum donors as the subjects for this work.

Our results demonstrate a similar early/mid/late luteal hormonal profile in those patients in whom GnRHa was stopped after HCG administration versus those in whom GnRHa was continued. The oestradiol profile showed a decrease after HCG administration until day 3, increasing thereafter and peaking between days 5 and 7. Progesterone profile increased from day 2, peaking from days 5 to 7 and falling thereafter. These results are similar to the reported luteal profile from cycles in which GnRHa administration was disrupted following HCG administration (Smitz et al., 1987). Moreover, LH remained <1 mIU/ml throughout the luteal phase regardless of whether GnRHa was disrupted or continued after HCG administration (Figure 2). This is in agreement with the report of Smitz et al. (1988) indicating that LH remains undetectable for the 10 days following the stoppage of GnRHa administration on the day of the ovulation-inducing HCG injection. However, this previous report did not consider oestradiol and progesterone profiles in patients treated with or without GnRHa in the luteal phase. Also, this profile is similar to the non-conception cycles in which only gonadotrophins were used in ovulation induction (Hutchinson-Williams et al., 1989). Taken together, this information suggests that when ovulation induction is performed, the corpus luteum is driven primarily by the HCG, regardless of the presence or absence of GnRHa in the luteal

Table I. In-vitro fertilization parameters in patients undergoing ovulation induction with or without gonadotrophin-releasing hormone agonist (GnRHa) in the early luteal phase. Values are means ± SEM

<table>
<thead>
<tr>
<th>With GnRHa</th>
<th>Without GnRHa</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Age (years)</td>
<td>23.9 ± 1.2</td>
<td>30.4 ± 0.8</td>
</tr>
<tr>
<td>Previous cycles</td>
<td>1.8 ± 0.43</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>Total no. of days GnRHa administered</td>
<td>28.6 ± 1.4</td>
<td>18.1 ± 1.5</td>
</tr>
<tr>
<td>Total dose of FSH-HMG (no. of ampoules)</td>
<td>23.3 ± 0.9</td>
<td>30.4 ± 3.5</td>
</tr>
<tr>
<td>Oestradiol on day of HCG (pg/ml)</td>
<td>1980 ± 278</td>
<td>3110 ± 464</td>
</tr>
<tr>
<td>No. of oocytes collected</td>
<td>22.6 ± 2.9</td>
<td>20.2 ± 1.2</td>
</tr>
</tbody>
</table>

NS = not significant; FSH = follicle stimulating hormone; HMG = human menopausal gonadotrophin; HCG = human chorionic gonadotrophin.

Table II. In-vitro fertilization parameters in patients undergoing ovulation induction with or without gonadotrophin-releasing hormone agonist (GnRHa) in the mid-late luteal phase. Values are means ± SEM

<table>
<thead>
<tr>
<th>With GnRHa</th>
<th>Without GnRHa</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>25.2 ± 2.1</td>
<td>25.3 ± 1.2</td>
</tr>
<tr>
<td>Previous cycles</td>
<td>1.2 ± 0.2</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>Total no. of days GnRHa administered</td>
<td>34.2 ± 0.4</td>
<td>18.3 ± 1</td>
</tr>
<tr>
<td>Total dose of FSH-HMG (no. of ampoules)</td>
<td>22.4 ± 1.7</td>
<td>24.3 ± 2</td>
</tr>
<tr>
<td>Oestradiol on day of HCG (pg/ml)</td>
<td>1986 ± 99</td>
<td>2546 ± 562</td>
</tr>
<tr>
<td>No. of oocytes collected</td>
<td>22.4 ± 4</td>
<td>24.5 ± 4.9</td>
</tr>
</tbody>
</table>

NS = not significant; FSH = follicle stimulating hormone; HMG = human menopausal gonadotrophin; HCG = human chorionic gonadotrophin.

Figure 1. Early luteal phase study, oestradiol and progesterone concentrations during the 5 days following the day of oocyte retrieval, from day 2 up to day 6 after human chorionic gonadotrophin administration. GnRHa = gonadotrophin-releasing hormone agonist.
phase. Based on this observation, we question whether luteal phase supplementation with progesterone from day 4 after HCG is necessary, or whether administration of progesterone starting 8 days after HCG would be sufficient to prevent the documented progesterone drop.

Based on the expected luteolytic effects, this study was planned considering two possible clinical implications: first, the potential effect of continuous administration of GnRHa on the prevention of OHSS, and secondly, the adverse effect of luteal administration of GnRHa in the luteal phase of the conception cycle. Since OHSS is linked to excessively high serum oestradiol concentrations, a luteolytic strategy that could prevent excessive hormonal levels within the luteal phase of stimulated cycles, without affecting the pregnancy outcome, could be of interest. Unfortunately the absence of hormonal drop-out renders this strategy useless in the prevention of OHSS if this entity can be prevented or ameliorated by reducing luteal steroid concentrations. There is probably no causal relationship between luteal LH/oestradiol/progesterone patterns and OHSS. The reason why luteal GnRHa treatment does not prevent OHSS could be because a predisposition to develop OHSS had already been acquired in the follicular phase.

For the same reason, this approach may not be hormonally deleterious in the luteal phase of the conception cycle. Indeed, trials to induce abortions with GnRHa (Casper et al., 1980; Skarin et al., 1982) failed to do so. Moreover, additional luteal support is given routinely to patients undergoing ovulation induction for assisted reproduction treatment. Nonetheless, this practice must be considered with caution, since a possible teratogenic effect of GnRHa cannot be ruled out at this time. GnRHa has been reported to cross the placenta and causes a reduction of testicular weight in rhesus monkeys (Sopelak and Hodgen, 1987). This issue remains unresolved and further clinical trials are required.

In conclusion, continuation of GnRHa administration during the luteal phase in patients undergoing ovulation induction for IVF does not impair corpus luteum function compared to patients in whom GnRHa is routinely discontinued on the day of HCG administration, suggesting that HCG is the main driving force for the development of the corpus luteum. The lack of differences between continuation and disruption of GnRHa may be due to accumulation of the product during the 18 days of treatment.

References


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