Gonadotrophin-releasing hormone agonist dose-dependency of pituitary desensitization during controlled ovarian hyperstimulation in IVF

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The aim of this study was to find the minimal effective daily s.c. dose of the gonadotrophin-releasing hormone (GnRH) agonist, triptorelin acetate, that suppresses the GnRH-induced release of luteinizing hormone (LH) at time of human chorionic gonadotrophin (HCG) injection and thereby prevents spontaneous LH surges during in-vitro fertilization (IVF) stimulation cycles. Therefore, a double-blind, prospective and randomized titration study was performed. A total of 48 IVF patients were divided into four groups of 12 patients. Each group received a different dose of triptorelin acetate, namely 5, 15, 50 or 100 µg s.c. daily. Standard ovarian stimulation was carried out using urinary follicle stimulating hormone (FSH) preparations. A 500 µg HCG test was performed 90 min before the HCG injection in order to measure the degree of pituitary desensitization. Spontaneous LH surges were not detected in any of the groups, although three patients in the 5 µg group had ovulated at the time of ovum retrieval. The pituitary LH response to the GnRH test at time of HCG, expressed as area under the curve (AUC), appeared to be dose-dependent. Thus, a daily s.c. dose of 100 µg triptorelin acetate appears to be too high, since adequate desensitization of the pituitary (i.e. no spontaneous LH surge) can be achieved with doses as low as 15 and 50 µg.

Key words: GnRH agonist/IVF/pituitary/triptorelin

Introduction

The use of long-acting gonadotrophin-releasing hormone (GnRH) agonists has been successfully introduced into the daily practice of in-vitro fertilization (IVF), in order to prevent undesirable spontaneous luteinizing hormone (LH) surges and so avoid premature oocyte maturation, luteinization and ovulation (Neveu et al., 1987; Ron-El et al., 1991). As reviewed by Hughes et al. (1992), a higher pregnancy rate per started cycle is found in IVF stimulation protocols that use GnRH agonists. Routinely, GnRH agonist doses, derived from treatment schedules in disseminated prostate cancer, are used which aim at complete gonadal suppression under all circumstances. It has been proven that in healthy volunteers lower doses of GnRH agonist give a dose-dependent pituitary desensitization (Broekmans et al., 1996) and that with a suppressed pituitary gland the dose needed to maintain suppression gradually decreases with the length of treatment (Sandow and Donnez, 1990). However, GnRH agonist dose-dependency of the pituitary response under peri-ovulatory IVF conditions has not been tested yet. Several authors have demonstrated the effect of GnRH agonist on ovarian function and steroidogenesis (Hsueh and Erickson, 1979; Hsueh and Jones, 1981; Sheehan et al., 1982; Brus et al., 1997). Nevertheless, data on the presence of GnRH receptors in primate ovarian tissue are conflicting (Clayton et al., 1982; Popkin et al., 1983; Bramley et al., 1985; Latouche et al., 1989). In our opinion, the lowest possible dose of GnH agonist should be used. In the present study we aimed to determine the minimal effective daily s.c. dose of triptorelin acetate which, used in a so-called long protocol in regular IVF stimulation, prevents the release of LH after a GnRH challenge test of 500 µg GnRH at the time of human chorionic gonadotrophin (HCG) injection, to such an extent that spontaneous LH surges could not be expected.

Materials and methods

Patients

From September 1993 to September 1994, 48 patients aged 23–38 years who were eligible for IVF, entered the study. Their infertility was caused by a tubal, idiopathic or male factor. Couples who had another cause of infertility and women with an elevated follicle stimulating hormone (FSH) concentration on day 3 of the menstrual cycle (FSH >10 IU/l) were excluded from the study. Patients who had undergone a previous IVF attempt with the so-called short protocol and had exhibited a stimulation phase of <7 days, were also excluded.

The protocol was approved by the Committee on Ethics of Research involving Human Subjects of the Free University Hospital, Amsterdam, The Netherlands. Informed consent was signed by all the couples participating in the study.

Treatment protocol

Four groups of 12 patients each, were treated by IVF preceded by pituitary desensitization with triptorelin acetate administered by a daily s.c. injection started in the midluteal phase of the preceding cycle (see Figure 1).

In each group a different daily dose of triptorelin acetate (Ferring BV, Hoofddorp, The Netherlands) was used, i.e. 5, 15, 50 or 100 µg in a randomized double-blind fashion. Randomization was achieved by drawing a sealed numbered envelope in which the dose of GnRH agonist to be used was noted according to a computer-generated random list in 12 permuted blocks of four patients. Patients started...
with a basal body temperature (BBT) chart on the second day of a period (menses 1). Daily triptoreline-acetate administration was started 7 days following the temperature rise and continued until and including the day of HCG administration (Profasi 10 000 IU; Serono Benelux, Den Haag, The Netherlands) preceding oocyte retrieval. All patients came to the hospital on the second day of the next bleeding (menses 2) for a vaginal ultrasound to exclude ovarian cysts and for a blood sample to determine serum concentrations of HCG, FSH, LH and 17β-oestradiol. In four patients an ovarian cyst >3 cm was observed. In these cases a transvaginal ultrasound guided puncture was performed four days later when the period was over and while triptorelin acetate was continued.

Ovarian stimulation with FSH (Metrodin 75 IU; Serono Benelux, Den Haag, The Netherlands) was started on cycle day 3, or 2 days after cyst puncture, with a daily dose of two ampoules i.m., when the patient was aged <35 years, or three ampoules i.m. in patients who were ≥35 years. Patients were monitored routinely from day 7 of the stimulated cycle onwards. Morning urinary concentrations of LH (uLH) were measured daily, starting on day 7 until the leading follicle(s) reached a diameter of 14 mm. From then on, uLH was monitored three times a day (at 07:00, 15:00, and 23:00) (Ramsewak et al., 1990) until the morning of the HCG injection. The average of the uLH concentration from day 7 till the day the leading follicle reached 14 mm was defined as the baseline uLH value. A spontaneous uLH surge was defined as two successive increasing uLH values of which the first value was more than twice that of the baseline uLH concentration. The second elevated value needed to be equal to or higher than the first elevated uLH value. According to the individual response, the dose of FSH was increased with one or two ampoules daily and monitoring was continued until the criteria for HCG were met or until an uLH surge occurred. The criteria for HCG injection were the presence of at least three follicles with a diameter >16 mm of which one was >18 mm, and serum 17β-oestradiol concentrations of >1500 pmol/l. At 1.5 h prior to the HCG injection, a GnRH challenge test was performed with 500 µg GnRH (HRF; Hoechst, Hoewelaken, The Netherlands) i.v., to test the grade of pituitary desensitization. Blood samples were taken for LH and FSH determination at 0, 30, 60 and 90 min after GnRH administration. Oocyte retrieval was carried out 35 h after HCG injection. In cases of fertilization and cleavage, the embryo transfer was performed 48 h after oocyte retrieval. Routinely, two embryos were transferred unless the patient was ≥35 years of age. In those patients a maximum of three embryos was transferred. Remaining embryos were deep frozen at −196°C. Luteal support was accomplished by vaginal administration of 200 mg micronized progesterone (Progestan; Organon, Oss, The Netherlands), three times a day.

Ultrasound
All ultrasound examinations were performed transvaginally by one of the authors (R.J.) using Toshiba ultrasound equipment (Tosbee, Model SSA-240A, probe 5 MHz model PVE-582V). To determine the diameter of the follicle, the mean of measurements in two perpendicular directions was taken. Ultrasound guidance was used in all cases for follicle aspiration and oocyte recovery.

Serum assays
FSH and LH were measured by using immunometric assays, commercially available kits (Amerlite, Amersham, UK). For measuring concentrations of 17β-oestradiol and progesterone we used a commercially available competitive immunoassay (Amerlite). Intra- and inter-assay coefficients of variation were 6–9% for FSH, 5–10% for LH, 9–11% for 17β-oestradiol, and 11–17% for progesterone.

Urinary LH assay
Urinary LH was measured by a modified immunometric assay (Amerlite). The intra- and inter-assay coefficients of variation were 6–10%.
The primary endpoint of the study was the LH response of the pituitary to the GnRH challenge test. Secondary endpoints were serum LH, FSH, progesterone and 17β-oestradiol concentrations on the day of HCG injection, number of oocytes retrieved and fertilized, and ongoing pregnancies.

### Statistical analysis
Statistical analysis on differences between data in the four groups was carried out by applying the Kruskal–Wallis test followed by a Bonferroni’s significance test. Two tailed *P* values ≤ 0.05 were considered to be significant. All data are given as median with the range shown in parentheses. For statistical analysis these latter data were coverted to the logarithmic scale. Analysis of variance (ANOVA) between the groups was applied, followed by Bonferroni’s significance test, *P* < 0.05 was considered to be statistically significant. These data are presented as mean ± SD.

### Results

#### Baseline characteristics
Table I summarizes the baseline characteristics of the four groups in terms of age, body mass index (BMI), cycle length, number of smokers and infertility cause. No significant differences were noted between the four groups.

#### Clinical data
A total of 48 patients (12 in each dosage group) entered the study. Six patients discontinued treatment before oocyte retrieval because of excessive (*n* = 3) or insufficient (*n* = 3) ovarian response. These patients were equally divided over the four groups (one in dosage group 5, two in dosage group 15, two in dosage group 50 and one in dosage group 100). The remaining 42 patients completed the study. The results of the GnRH test from one patient in the 50 µg group were lost.

#### Desensitization phase
The interval between discontinuation of GnRH agonist treatment and start of FSH stimulation was defined as the desensitization phase. The time lapse between the start of the triptorelin acetate and the onset of the subsequent menses was 9.1 ± 2.2 days for the four groups together. In this respect no differences between the separate dosage groups were observed. Serum concentrations of LH, FSH and 17β-oestradiol were measured on the second day of menses 2, immediately prior to FSH stimulation. Median and range of LH concentrations (IU/l) were 1.8 (1.1–21.0), 1.5 (0.5–6.4), 1.8 (1.1–7.5) and 1.5 (0.8–4.5) for the 5, 15, 50 and 100 µg groups respectively. Median and range concentrations of FSH (IU/l) were 3.8 (2.5–7.8), 3.3 (2.0–4.8), 3.6 (2.0–6.2) and 3.0 (2.0–4.8) and median and range of 17β-oestradiol values (pmol/l) were 104 (<90–172), 102 (<90–139), 100 (<90–262) and 89 (<90–261) for the four dosage groups respectively. There was no significant difference found between the four dosage groups.

### Desensitization/FSH stimulation phase
The interval between the start of FSH in combination with GnRH agonist until HCG injection was defined as the desensitization/FSH stimulation phase.

The duration of this phase differed significantly between the groups (*P* = 0.0346). The difference between the 15 and 100 µg group was highly significant [7.5 (7.0–11.0) and 10.5 (7.0–18.0) days, *P* = 0.0147]. The number of FSH ampoules used did not differ significantly between the four dosage groups (Table II).

#### Luteinizing hormone
The LH response to the GnRH challenge test, expressed as AUC–LH, was significantly different between the 5 and 15 µg (*P* < 0.05), 50 µg (*P* < 0.01) and 100 µg groups (*P* < 0.01), as well as between the 15 µg and 50 µg (*P* < 0.05) and 15 µg and 100 µg groups (*P* < 0.01). No significant difference was found between the 50 and 100 µg groups (*P* > 0.05) (see Figure 2).

No spontaneous LH surge was detected during the FSH stimulation phase. Nevertheless, three patients appeared to have had ovulated between the GnRH challenge test and the time of ovum retrieval as demonstrated by the disappearance of follicles. These three patients had received the 5 µg triptorelin dose.

Baseline serum LH values measured on the morning of HCG administration (LH–HCG), were significantly lower in the 100 µg group, in comparison with the other three groups.

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### Table I. Baseline patient characteristics. Values shown are median with range in parentheses

<table>
<thead>
<tr>
<th>Dosage group (µg triptorelin acetate)</th>
<th>5</th>
<th>15</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.1 (22.5–37.9)</td>
<td>35.1 (31.2–37.4)</td>
<td>33.9 (26.5–37.6)</td>
<td>31.7 (25.1–37.2)</td>
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<tr>
<td>Body mass index</td>
<td>21.7 (17.2–25.2)</td>
<td>22.7 (16.1–32.0)</td>
<td>21.7 (17.5–29.3)</td>
<td>20.5 (18.1–26.9)</td>
</tr>
<tr>
<td>Cycle length (days)</td>
<td>27.0 (26.0–30.0)</td>
<td>28.0 (28.0–30.0)</td>
<td>28.0 (26.0–32.0)</td>
<td>28.0 (26.0–30.0)</td>
</tr>
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<td>No. of smokers</td>
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<td>8</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Cause of infertility</td>
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<td></td>
<td></td>
</tr>
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<td>8</td>
<td>10</td>
<td>8</td>
</tr>
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<td>male</td>
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<td>idiopathic</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
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</table>

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GnRH and pituitary desensitization

Table II. Stimulation outcome. Values shown are median with range in parentheses

<table>
<thead>
<tr>
<th>Dosage group (µg triptorelin acetate)</th>
<th>5</th>
<th>15</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>11</td>
<td>10</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Duration of stimulation (days)</td>
<td>9.0 (7.0–12.0)</td>
<td>7.5 (7.0–11.0)</td>
<td>10.0 (7.0–17.0)</td>
<td>10.5 (7.0–18.0)</td>
</tr>
<tr>
<td>No. of ampoules of FSH</td>
<td>24.0 (16.0–30.0)</td>
<td>22.5 (14.0–33.0)</td>
<td>27.0 (16.0–57.0)</td>
<td>28.5 (20.0–50.0)</td>
</tr>
</tbody>
</table>

FSH = follicle stimulating hormone.

*Values are significantly different ($P < 0.05$).

Figure 2. Area under curve (AUC) of luteinizing hormone (LH) response to an i.v. bolus of 500 µg gonadotrophin-releasing hormone (GnRH) prior to human chorionic gonadotrophin (HCG) injection during in-vitro fertilization (IVF) treatment after desensitization with four different doses of triptorelin acetate.

Table III. Hormonal profiles of the stimulation phase. Values shown are median with range in parentheses

<table>
<thead>
<tr>
<th>Dosage group (µg triptorelin acetate)</th>
<th>5</th>
<th>15</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH–S7 (IU/l)</td>
<td>3.3 (1.0–19.0)</td>
<td>1.5 (0.5–5.7)</td>
<td>1.3 (1.0–2.9)</td>
<td>0.8 (0.5–1.7)</td>
</tr>
<tr>
<td>LH–HCG (IU/l)</td>
<td>2.5 (1.0–8.0)</td>
<td>2.3 (1.3–5.6)</td>
<td>1.8 (1.1–4.5)</td>
<td>0.95 (0.4–2.7)</td>
</tr>
<tr>
<td>E2–S7 (pMol/l)</td>
<td>1460 (232–9380)</td>
<td>2385 (89–9010)</td>
<td>942 (90–21960)</td>
<td>282 (89–14000)</td>
</tr>
<tr>
<td>E2–HCG (pMol/l)</td>
<td>4640 (1790–17 540)</td>
<td>9900 (1750–18 000)</td>
<td>6470 (1690–12 280)</td>
<td>4395 (1520–38 780)</td>
</tr>
<tr>
<td>FSH–S7 (IU/l)</td>
<td>8.3 (6.6–11.0)</td>
<td>10.0 (7.3–15.0)</td>
<td>8.7 (2.2–14.0)</td>
<td>9.9 (6.2–13.0)</td>
</tr>
<tr>
<td>FSH–HCG (IU/l)</td>
<td>8.4 (6.8–11.0)</td>
<td>9.4 (8.5–12.0)</td>
<td>9.5 (6.9–13.0)</td>
<td>9.3 (6.3–12.0)</td>
</tr>
<tr>
<td>P4–HCG (nM/l)</td>
<td>4.6 (0.9–6.0)</td>
<td>3.1 (1.2–8.3)</td>
<td>2.4 (0.9–8.6)</td>
<td>1.4 (0.5–3.5)</td>
</tr>
</tbody>
</table>

LH = luteinizing hormone; E2 = 17β-oestradiol; FSH = follicle stimulating hormone; P4 = progesterone; S7 = serum concentrations on stimulation day 7; HCG = serum concentrations on morning of human chorionic gonadotrophin injection.

*Values are significantly different from the 100 µg group ($P < 0.05$).

($P = 0.0047$). A trend for this difference was already found on day 7 of FSH stimulation (LH–S7). The data are shown in Table III.

Follicle stimulating hormone

Serum FSH concentrations on either day 7 of FSH stimulation (FSH–S7) and on the morning of the HCG administration (FSH–HCG) did not differ significantly between the four dosage groups (Table III).

17β-oestradiol

17β-oestradiol concentrations on day 7 of FSH stimulation (E2–S7) and on the morning of HCG administration (E2–HCG) did not differ between the groups (Table III).

Progesterone

Progesterone values measured on the morning of HCG administration (P4–HCG) were significantly higher in the 5 and 15 µg groups, in comparison with the 100 µg group. No significant differences were noted between the other dosage groups (Table III).

Results of the IVF treatment

The median (range) number of cumulus–oocyte complexes retrieved after follicular aspiration was 6.0 (2.0–18.0), 14 (3.0–23.0), 8.0 (2.0–18.0) and 5.5 (3.0–35.0) respectively for the 5, 15, 50 and 100 µg groups. This did not differ significantly between the four groups. The median (range)
percentage of fertilization was 50.0 (0.0–89.0) for the 5 µg group, 59.2 (28.5–71.4) for the 15 µg group, 71.4 (42.8–100.0) for the 50 µg group and 63.3 (0.0–100.0) for the 100 µg group. No significant difference was found between the different dosage groups. The number of pregnancies achieved (a total of nine pregnancies of which four are ongoing, were divided among the four groups) did not differ either.

Discussion
To our knowledge, this is the first study in which the GnRH agonist dose-dependency of the pituitary LH response to 500 µg GnRH has been evaluated under peri-ovulatory IVF conditions. In view of the possible effects of the agonist on maturing oocytes and later on the developing embryo, several authors have recognized the need to reduce the dosage of the analogue used in IVF (Loumaye et al., 1989; Balasch et al., 1992; Simón et al., 1994; Edwards et al., 1996). Monroe et al. (1986) and Ron-El et al. (1992), suggested that partial desensitization of the pituitary in an IVF treatment might be sufficient. In the present study, the LH response was significantly higher in the 5 and 15 µg groups, in comparison with the 50 and 100 µg groups, indicating a less profound state of pituitary desensitization in the patients of the two lower dose groups. Since three patients, in the 5 µg group, ovulated before the time of ovum retrieval, we concluded that 5 µg daily s.c. triptorelin is too low to be used in IVF treatment. However, the partial suppression of the pituitary obtained with 15 µg triptorelin might be sufficient.

Broekmans et al. (1996) has already shown that 50 µg triptorelin creates a state of pituitary desensitization comparable with the 100 µg dose in healthy regularly cycling volunteers. We now confirm this under regular IVF conditions. There was no statistical difference in the LH response of the pituitary to the supramaximal GnRH challenge between 50 or 100 µg triptorelin treatment. This implies that 50 µg is enough as an analogue dose during regular IVF treatment in a long protocol. This would mean a possible reduction of at least 50% of the currently used dose.

However, reducing the dose of analogue in IVF treatment is only beneficial if clinical success is maintained. Our study was not designed to investigate this. So far, there have been no differences found in the number of oocytes obtained or in the percentage fertilization between the four groups. The ongoing pregnancy rate per started cycle was 8.3%. Two of the ongoing pregnancies were found in the 5 µg group, one in the 50 µg group and one in the 100 µg group. This suggests that the lower doses of agonist used had no influence on this relatively low pregnancy rate. We cannot exclude the possibility that the high GnRH test dose may be responsible. Similar or higher dosages of GnRH or its agonist have been given in ovulation induction protocols to induce the midcycle LH surge (Gerris et al., 1995). From these studies it does not seem that the peri-ovulatory GnRH in comparison with HCG has adverse effects on pregnancy outcome. However it should be realized that, in our study, GnRH was given in addition to GnRH agonist treatment.

Based on the present study we conclude that in regular IVF, in a so-called long protocol, it is possible to adjust the dose of analogue to lower values than routinely given, while adequate desensitization of the pituitary at the time of HCG injection is maintained. In addition, earlier reports have shown improvement of ovarian responsiveness with microdoses of GnRH agonist during ovulation induction for IVF in poor responders (Feldberg et al., 1994; Scott and Novot, 1994).

On the basis of these results, further studies aimed at dose adjustments of agonist treatment in routine IVF treatment protocols are justified.

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References


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