The use of long- and short-acting forms of gonadotrophin-releasing hormone analogues in women undergoing oocyte donation

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Evidence accumulated in in-vitro fertilization (IVF) cycles suggests that the use of long-acting forms of gonadotrophin-releasing hormone analogues (GnRHa) for pituitary desensitization may impair the outcome of IVF as compared to classical short-acting formulations. Whether the negative effects are directed against the corpus luteum, the endometrium, or both is unknown. However, the presence of high affinity binding sites for gonadotrophin-releasing hormone (GnRH) in the human endometrium suggests a possible role of these analogues on this target organ, affecting embryo implantation. In the present study, we tested direct effects of two different forms of GnRHa on implantation using the ovum donation model. Patients were prospectively allocated to one of the three study groups: the short-acting form of the analogue leuprolide acetate (group I; n = 64), the long-acting form of the same analogue (group II; n = 58), and the long-acting preparation of the analogue triptorelin (group III; n = 61). A total of 68 cycles of embryo transfer was carried out in group I, whereas 67 were performed in group II and 65 in group III. Cancellation rates were respectively 18.1, 17.3 and 18.8% because of bleeding while being on the waiting list for anonymous oocyte donation. The number of oocytes donated, fertilization rates and embryos replaced in each group were similar. As a result, pregnancy rate per transfer was 38.2, 49.3 and 44.6% respectively. Implantation rates per embryo replaced were respectively 13.4, 19.1 and 17.0%. These data suggest that the use of a long-acting form of GnRHa provides success rates similar to the short-acting preparations, resulting in more convenient medication for patients with ovarian function included in ovum donation programmes.

Key words: endometrium/gonadotrophin-releasing hormone analogues/implantation/IVF/oocyte donation

Introduction

The combination of gonadotrophin-releasing hormone analogues (GnRHa) and gonadotrophins is widely employed in routine in-vitro fertilization (IVF). For this reason, clinicians have tried to find more convenient forms of GnRHa administration whilst maintaining the main advantage of this medication, i.e. pituitary desensitization and subsequent ovarian quiescence. The existence of long-acting GnRHa preparations has encouraged several groups to employ this approach, reducing administration of the drug to a single daily subcutaneous injection. The results published so far have shown reduced implantation (Devreker et al., 1996) and pregnancy rates, as well as increased miscarriages (Gonen et al., 1991; Devreker et al., 1996), when long-acting GnRHa have been compared to short-acting preparations in routine IVF. As the implantation rate is related to both embryo quality and appropriate endometrium environment, it is assumed that long-acting forms may alter the oocyte and the resulting embryo, the endocrine milieu of the endometrium, or both (Gonen et al., 1991; Devreker et al., 1996).

It has been suggested that the more pronounced inhibition of ovarian function during the follicular phase due to the administration of long-acting forms may affect both oocyte quality and the function of the corpus luteum (Devreker et al., 1996). However, there is also evidence that the inadvertent administration of GnRHa prior to an IVF cycle is not detrimental for the fetus and may potentially enhance corpus luteum function, resulting in an unexpected treatment for infertility (Smits et al., 1991; Balasch et al., 1993).

None of the authors mentioned above has mentioned the effects of GnRHa on the endometrium. However, high-affinity binding sites for the GnRH receptor have been detected in proliferative human endometria by reverse transcription–polymerase chain reaction using oligonucleotide primers synthesized according to the receptor’s sequence (Imai et al., 1994). Thus, there is also a possible role for GnRHa on the endometrium itself. In previous studies, we tested the hypothesis of whether the use of GnRHa may affect endometrial receptivity employing the oocyte donation model (Remohi et al., 1994). In this in-vivo situation, the quality of the embryos is determined by the source of the oocytes, while the endocrine environment can be modulated with exogenous steroids. We learned that short-acting GnRHa were not detrimental for implantation (Remohi et al., 1994). Current information concerning IVF cycles, however, suggests that long-acting preparations may be detrimental for implantation (Gonen et al., 1991; Devreker et al., 1996). Thus, a prospective study was carried out employing two long- and one short-acting analogues, which are all in current clinical use, in order to address the issue of a potential direct effect of the GnRHa on the endometrium and its impact on implantation employing the ovum donation model. Two long-acting forms were used
to show that long-acting analogues are not harmful, regardless of the clinical preparation employed. To this end, recipients were desensitized with both treatment modalities before steroid replacement and embryo transfer.

**Materials and methods**

**Donors**

Ninety-three cycles of oocyte donation were performed in fertile women who voluntarily decided to donate oocytes, and 90 cycles in women undergoing IVF/intracytoplasmic sperm injection (ICSI) who had a high response to gonadotrophins and voluntarily donated some of their oocytes to our anonymous oocyte donation programme. Indications for IVF/ICSI in this population were (Table I): male infertility (n = 56), unexplained infertility (n = 21) and tubal infertility (n = 13). Informed consent was obtained from all donors before donation; donors were <35 years old, were screened for major sexually transmitted diseases, and had no personal or familial history of congenital malformation or hereditary disease. The ovarian stimulation protocol and general IVF procedure have been previously described (Pellicer et al., 1989). Briefly, it began with pituitary desensitization by daily subcutaneous administration of 1 mg leuprolide acetate (Procrin, Abbott S.A., Madrid, Spain) starting in the luteal phase of the menstrual cycle. Serum oestradiol levels <60 pg/ml and negative vaginal ultrasonographic scans were used to define ovarian quiescence. Days 1 and 2 of ovarian stimulation, 1 ampoule/day human menopausal gonadotrophin (HMG) (Pergonal, Serono Laboratorios, Madrid, Spain) was administered together with three ampoules of high purity follicle stimulating hormone (FSH) (Neo-Fertinorm; Serono). On days 3, 4 and 5 of ovarian stimulation, 1 ampoule FSH and 1 ampoule HMG were administered daily to each patient. Beginning on day 6, FSH and HMG were administered on an individual basis according to serum oestradiol concentrations and transvaginal ovarian ultrasound scans. The criteria for human chorionic gonadotrophin (HCG) administration (10 000 IU; Profasi, Serono) were the presence of two or more follicles >2 cm at greatest diameter, and serum oestradiol concentrations >800 pg/ml. Leuprolide acetate and FSH/HMG injections were discontinued the day of HCG administration. Oocyte retrieval was scheduled 36–38 h after HCG injection.

**Recipients**

Recipients were 183 women undergoing oocyte donation for various reasons (Table II); failed IVF (n = 24), low response to gonadotrophin stimulation (n = 143), heritable genetic disorders (n = 8) and endometriosis (n = 8). Therefore, all patients had ovarian function and were allocated to one of the treatment groups according to the time they attended the infertility clinic in the secretory phase of the menstrual cycle. Women attending the clinic in the morning were desensitized with a long-acting GnRHa, either leuprolide acetate (Ginecrin, Abbott Laboratories; group II, n = 58) or tryptorelin (Decapeptyl 3.75 mg; Laboratorios Lasa, Barcelona, Spain; group III, n = 61), while patients visiting in the afternoon were desensitized with leuprolide acetate (group I, n = 64) as described for the donors. Odd numbers were included in group II and even numbers in group III. Some patients repeated the cycle after a first failure, but they were always allocated in the same treatment group in subsequent attempts. Hormonal replacement started on day 1 of the cycle with administration of oestradiol valerate (Progynova; Schering España S.A., Madrid, Spain) 2 mg/day on days 1–8; 4 mg/day from days 9 to 11; and 8 mg/day from day 12 onwards. After 13 days of oestradiol valerate administration, recipients were ready to receive the donation, waiting until such became available. In the case of spotting during oestradiol valerate administration, the patient was cycled with 10 mg medroxyprogesterone acetate for 10 days, and a new cycle begun. On the day of recovery of donated oocytes, a daily dose of 800 mg natural micronized progesterone (Progeflik; Laboratorios Effik, Madrid, Spain) was vaginally administered. Embryo transfer was performed 48 h after oocyte recovery using the vaginal route. After transfer, the regimen of 6 mg/day oestradiol valerate and 800 mg/day progesterone was maintained for 15 days. Thereafter, a urinary β-HCG analysis was performed. In the case of positive results, oestradiol valerate was increased to 8 mg/day and progesterone was maintained at the same dosage until day 80 of pregnancy. Only ultrasound-documented pregnancies were considered for the purposes of the study.

**Statistical analysis**

Data are expressed as means ± SEM. For statistical comparison among groups, analysis of variance (ANOVA) and chi-square test were applied. A P value < 0.05 was considered statistically significant. The analysis was carried out using the statistical package for social sciences (SPSS Inc., Chicago, IL, USA). The effective size measure for this study was 0.012 (η²), and the power 0.25. The type II error was 0.74. For a power of 0.80, it was calculated that 805 patients would be needed. However, because the effective size was so small (η = 0.012), we decided that more power was not needed in our study to reject the null hypothesis. Nevertheless, mindful of the failure to reach the desired n, we determined to be cautious in our calculations.

### Table I. Several parameters and causes of infertility in the donor population

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
<th>Age (years)*</th>
<th>GnRHs (days)*</th>
<th>Duration of stimulation (days)*</th>
<th>Number of FSH/HMG (ampoules)*</th>
<th>Indication for IVF</th>
<th>Fertile donors (%)</th>
<th>Male factor (%)</th>
<th>Unexplained infertility (%)</th>
<th>Tubal infertility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>64</td>
<td>27.8 ± 4.8</td>
<td>20.5 ± 5.4</td>
<td>9.3 ± 1.5</td>
<td>25.9 ± 7.3</td>
<td>IVF</td>
<td>33 (51.5)</td>
<td>20 (31.2)</td>
<td>8 (12.5)</td>
<td>3 (4.8)</td>
</tr>
<tr>
<td>II</td>
<td>58</td>
<td>26.9 ± 4.9</td>
<td>21.1 ± 4.5</td>
<td>9.3 ± 1.1</td>
<td>26.3 ± 8.9</td>
<td>ICSI</td>
<td>28 (48.3)</td>
<td>17 (29.3)</td>
<td>7 (12.0)</td>
<td>6 (10.4)</td>
</tr>
<tr>
<td>III</td>
<td>61</td>
<td>28.5 ± 4.8</td>
<td>20.6 ± 7.0</td>
<td>9.5 ± 2.1</td>
<td>25.2 ± 8.1</td>
<td>Tubal infertility</td>
<td>32 (52.4)</td>
<td>19 (31.2)</td>
<td>6 (9.9)</td>
<td>4 (6.5)</td>
</tr>
</tbody>
</table>

*Values are means ± SE.

Group I = short-acting leuprolide acetate.

Group II = long-acting leuprolide acetate.

Group III = long-acting tryptorelin.

There were no significant differences between groups.
Table II. Characteristics of the recipients allocated in each group

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>64</td>
<td>58</td>
<td>61</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>37.7 ± 0.6</td>
<td>40.0 ± 0.6</td>
<td>38.1 ± 1.1</td>
</tr>
<tr>
<td>GnRHa (days)*</td>
<td>25.5 ± 2.6</td>
<td>24.9 ± 1.4</td>
<td>25.1 ± 0.8</td>
</tr>
</tbody>
</table>

Indication for oocyte donation

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low responder (%)</td>
<td>50 (78.2)</td>
<td>46 (79.3)</td>
<td>47 (77.0)</td>
</tr>
<tr>
<td>Failed IVF (%)</td>
<td>9 (14.0)</td>
<td>7 (12.1)</td>
<td>8 (13.1)</td>
</tr>
<tr>
<td>Endometrosis (%)</td>
<td>2 (3.1)</td>
<td>2 (3.4)</td>
<td>4 (6.6)</td>
</tr>
<tr>
<td>Genetic (%)</td>
<td>3 (4.7)</td>
<td>3 (5.2)</td>
<td>2 (3.3)</td>
</tr>
</tbody>
</table>

*Values are means ± SE. See Table I for definition of groups. There were no significant differences between groups.

Table III. In-vitro fertilization parameters in each group of recipients

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of women</td>
<td>64</td>
<td>58</td>
<td>61</td>
</tr>
<tr>
<td>Initiated cycles</td>
<td>83</td>
<td>81</td>
<td>80</td>
</tr>
<tr>
<td>Cancelled cycles (%)</td>
<td>15 (18.1)</td>
<td>14 (17.3)</td>
<td>15 (18.8)</td>
</tr>
<tr>
<td>Cycles with embryo replacement</td>
<td>68</td>
<td>67</td>
<td>65</td>
</tr>
<tr>
<td>Oocytes retrieved*</td>
<td>7.8 ± 2.1</td>
<td>7.4 ± 1.6</td>
<td>8.0 ± 2.5</td>
</tr>
<tr>
<td>Fertilization rate* (%)</td>
<td>69.5 ± 2.3</td>
<td>69.0 ± 2.6</td>
<td>68.0 ± 2.0</td>
</tr>
<tr>
<td>Transferred embryos/patient*</td>
<td>3.8 ± 0.1</td>
<td>3.8 ± 0.1</td>
<td>3.9 ± 0.7</td>
</tr>
<tr>
<td>Good-quality embryos transferred*</td>
<td>3.5 ± 0.1</td>
<td>3.6 ± 0.7</td>
<td>3.6 ± 0.8</td>
</tr>
<tr>
<td>Pregnancies (%)</td>
<td>26 (38.2)</td>
<td>33 (49.3)</td>
<td>29 (44.6)</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>13.4</td>
<td>19.1</td>
<td>17.0</td>
</tr>
<tr>
<td>Miscarriages (%)</td>
<td>4 (15.3)</td>
<td>3 (9.1)</td>
<td>5 (17.2)</td>
</tr>
<tr>
<td>Ectopic pregnancies (%)</td>
<td>0</td>
<td>1 (3.0)</td>
<td>1 (3.4)</td>
</tr>
</tbody>
</table>

*Values are means ± SE. See Table I for definition of groups. There were no significant differences between groups.

Results

Table I shows some characteristics of the donors and their distribution among the established groups of recipients. The data show no differences among groups, thus providing the rationale for accepting a homogeneous assignment of the oocytes obtained. Table II shows some of the recipients’ characteristics included in each group. Again, there were no differences among groups in age and/or cause of entering the ovum donation programme, or in the number of days they were under the effect of the GnRHa before starting progesterone and receiving the embryos.

In Table III, the number of initiated and finished cycles is presented. The number of cancelled cycles was not different among groups. In all cases cancellation was due to spotting during oestradiol valerate ingestion. Table III also shows the results of the ovum donation cycle. The data show no differences in the number of oocytes donated, good quality embryos obtained and replaced, and pregnancy rates. Importantly, implantation and miscarriage rates were also similar when those women desensitized with a short-acting analogue were compared with those treated with the same analogue in its long-acting form, or a different long-acting preparation.

Discussion

Several lines of investigation suggest a role for GnRH in primate and human implantation. There is clinical evidence that uterine receptivity may be enhanced by GnRHa (Testart et al., 1993). Moreover, there is evidence in monkeys that the early embryo secretes GnRH and that a lack of GnRH production is associated with impaired implantation (Seshagiri et al., 1994). Since we know that there is a close cross-talk between the embryo and the endometrium around the time of implantation which probably involves many different molecules (Simón et al., 1996), it is appropriate to speculate that GnRH may be one of the signals relevant to implantation in primates, and that the endometrium may have the availability to receive the message sent by the embryo using specific receptors or other not yet understood mechanisms. If this is true, the use of exogenous GnRHa may affect the endocrine status of the endometrium.

In addition, high-affinity binding sites for the GnRH receptor have been detected in proliferative human endometria (Imai et al., 1994). Further, the mechanism of GnRH coupling to its receptor has been recently described in detail (Imai et al., 1996). GnRHa display anti-tumour effects in postmenopausal women with recurrent endometrial cancer (Jeyarajah et al., 1996), providing the rationale for a direct action of the drug on the endometrial neoplastic tissue in women lacking ovarian function. Thus, all the above information prompted us to investigate whether the negative impact of long-acting preparations described by several authors (Gonen et al., 1991; Devreker et al., 1996) may be directed against the endometrium, although we had evidence.
that short-acting GnRH\(a\), per se, do not affect implantation (Remohi et al., 1994).

An analysis of the population of donors divided according to the three groups of recipients showed no difference among groups in the main parameters that could affect the quality of the donated oocytes, i.e. age (Tan et al., 1992), ovarian stimulation (Simón et al., 1995) and cause of infertility (Tan et al., 1992). From this analysis, it can be deduced that the distribution of the oocytes was homogeneous between both groups of recipients. Moreover, Table II shows that the distribution of the recipient uteri was also homogeneous. Age of the recipient, the only factor that we have previously found could affect the outcome of ovum donation (Cano et al., 1995; Pellicer et al., 1995), was similar among groups, as was the reason for entering the ovum donation programme. Since the number of good quality embryos replaced was similar, it can be assumed that the actual implantation rates observed in the study reflected the endometrial environment and ability to sustain a human pregnancy to term. From our study, it can be deduced that the use of long-acting preparations had no impact on the endometrium as compared with short-acting forms. It can also be indirectly inferred that the use of long-acting forms on IVF may not affect the endometrium, and that the adverse effect of the analogue observed in some studies might be directed against the ovary as the authors suggested (Gonen et al., 1991; Devreker et al., 1996).

The data presented here suggest that irrespective of the form of GnRH\(a\) administration, pregnancy and implantation rates remain similar, although a higher sample size may show a different picture in the future. The data also compared two different long-acting analogues, suggesting that the specific compound may not have additional effects and that the conclusions drawn in this study are valid for most of the analogues employed in clinical practice. Since administration of long-acting preparations is more convenient for patients, we feel confident in recommending this option for those women undergoing oocyte donation who still maintain functional ovaries. Employing long-acting preparations and a prolonged oestradiol regimen (Remohi et al., 1995), recipients can be maintained for approximately 40 days ready to receive progesterone and, subsequently, embryos, having the same chance of pregnancy no matter how long they have been receiving oestradiol valerate. Similar cancellation rates of almost 20% were found in both treatment modalities after unopposed oestradiol valerate administration. This is lower than our original report of this protocol of oestrogen replacement, wherein we found a 45% cancellation rate (Remohi et al., 1995). The reason for the discrepancy may lie in the fact that the number of donation cycles in fertile donors has increased in the last 2 years.

References
