Enhancement of embryo developmental potential by a single administration of GnRH agonist at the time of implantation

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BACKGROUND: Several reports have shown that inadvertent administration of a GnRH agonist in the luteal phase does not compromise pregnancy. Moreover, some studies suggested that, unexpectedly, the embryo developmental potential is improved in these conditions. This prospective controlled study was designed to test this hypothesis. METHODS: In an oocyte donation programme, oocytes from each donor (n = 138) were shared by two recipients, one of whom was given a single dose of a GnRH agonist (0.1 mg triptorelin) 6 days after ICSI, and the other received placebo at the same time. RESULTS: Oocyte recipients treated with GnRH agonist 6 days after ICSI had higher implantation (36.9 versus 25.1%), twin pregnancy (16.7 versus 3.6%), twin delivery (13.8 versus 2.2%) and birth (31.1 versus 21.5%) rates and similar miscarriage and abortion rates as compared with the placebo group. CONCLUSIONS: GnRH agonist administration at the time of implantation enhances embryo developmental potential, probably by a direct effect on the embryo.

Key words: embryo developmental potential/GnRH agonist/implantation/oocyte donation

Introduction

Based on the evidence that high doses of GnRH or GnRH agonist interfere with embryo implantation in rats and baboon monkeys (Hsueh and Jones, 1981; Kang et al., 1989), the administration of GnRH agonist in the luteal phase was originally tested as a means of contraception (Skarin et al., 1982). However, the failure to induce early abortion by luteal administration of huge doses of GnRH agonist in women (Skarin et al., 1982) was subsequently corroborated by a number of reports showing that inadvertent GnRH agonist administration in the luteal phase does not compromise the continuation of pregnancy resulting from assisted reproduction attempts but rather seems to support implantation (Golan et al., 1990; Isherwood et al., 1990; Ron-El et al., 1990; Smits et al., 1991; Jackson et al., 1992; Balasch et al., 1993; Elefant et al., 1993; Har-Toov et al., 1993; Weissman and Shohan, 1993; Wilshire et al., 1993; Young et al., 1993; Gartner et al., 1997) although an impairment of corpus luteum function was reported in one study (Herman et al., 1992). A question then arises whether luteal administration of a GnRH agonist can be envisaged as a voluntary therapeutic action aimed at enhancing implantation.

In spite of the existing circumstantial evidence for a beneficial effect of luteal GnRH agonist on implantation, this issue has not yet been addressed by a prospective controlled study. Here we use a model based on sharing sibling oocytes originating from one donor between two recipients receiving luteal GnRH agonist support and placebo respectively. A single injection of a short-acting preparation of GnRH agonist was administered 6 days after oocyte recovery (3 days after embryo transfer), at the time at which implantation normally occurs. The effects of GnRH agonist on pregnancy, implantation, multiple gestation, delivery and birth rates were examined.

Materials and methods

Clinical setting and design

This study involved 276 assisted reproduction attempts using donor oocytes. Oocyte donors were young women (19–27 years) with good ovarian reserve, as assessed by basal (cycle day 2) serum concentrations of FSH (<7 IU/l) and inhibin B (>100 pg/ml) and by the visualization of >10 small antral follicles in both ovaries on cycle days 1–3. General health status of each candidate for oocyte donation was assessed by the examinations defined by the Spanish Law of Assisted Reproduction, including karyotyping and tests for human immunodeficiency virus, hepatitis B and C, cytomegalovirus, herpes virus, rubella, chlamydia, toxoplasmosis and syphilis. Candidates for oocyte donation suffering from endometriosis and those presenting features predisposing them to the development of severe ovarian hyperstimulation syndrome were excluded from the programme. The latter applied to women whose fasting glucose:insulin ratio was <4.5 (Legro et al.,...
Oocytes from each donor were equally shared by two recipients who were chosen so as to match as close as possible the physical characteristics of the donor. For each donor, one of the oocyte-sharing recipients was allocated to the GnRH agonist agonist treatment group and the other to the placebo group. The decision of which of the two recipients would pass to either group was taken at random. The randomization was based on the alphabetical order of the patient’s surname or, if the surnames of both recipients began with the same letter, on the alphabetical order of the patient’s first name.

Recipients allocated to the GnRH agonist group were given a single s.c. injection of 0.1 mg triptorelin (Decapeptyl 0.1 mg; Ipsen Pharma, Spain) 3 days after embryo transfer (6 days after fertilization). Recipients allocated to the placebo group received solvent only at the same time. The study was approved by our ethical committee.

Ovarian stimulation and oocyte recovery
Ovarian stimulation of oocyte donors was performed with the use of a combination of recombinant and urinary human gonadotrophins after pituitary down-regulation started in the mid-luteal phase as described (Tesarik and Mendoza, 2002). Briefly, a single injection of a long-acting GnRH agonist (Decapeptyl 3.75 mg; Ipsen Pharma) was administered on day 24–26 of the cycle while the donor was taking oral norethisterone (Primolut Nor; Shering, Spain), 10 mg daily, beginning 2 days before and ending 2 days after the injection to reduce the risk of ovarian cyst formation in response to GnRH agonist. Recombinant human FSH (Puregon; Organon, The Netherlands) was used as the main source of FSH for ovarian stimulation. If serum LH concentration was low (<0.7 IU/l) before the beginning of stimulation, LH activity was supplemented by adding 1 vial of hMG (Menogon; Langley, UK), containing 75 IU of FSH and 75 IU of LH, on days 1–6 of stimulation. FSH dose was adapted according to the concentration of serum estradiol and dynamics of ovarian follicular growth, assessed on day 5 of stimulation and then every other day until the final decision about the day of ovulation induction. Ovulation was induced with 10 000 IU of hCG (Profasi; Serono, Italy) when there were at least five follicles >18 mm in diameter in both ovaries. If serum estradiol concentration was >3000 pg/ml on that day, the administration of hCG was withheld until serum estradiol dropped below this level. This period did not exceed 2 days in any of the donors involved in this study.

Oocytes were recovered by ultrasound-guided transvaginal ovarian follicle aspiration, performed 36 h after hCG administration.

Oocyte preparation
Endometrial growth and secretory transformation were stimulated by progressively increasing daily doses of oral estradiol valerate (Progynova; Schering, Spain) followed by vaginal micronized progesterone (Utrogestan; Laboratoires Besins-Isovesco, France) as described (Tesarik et al., 2002). In patients who still showed ovulatory activity pituitary down-regulation was performed in the same way as described for oocyte donors but for the use of a short-acting form of the GnRH agonist preparation (Decapeptyl 0.1 mg) administered daily from day 24–26 of the cycle. After confirmation of pituitary down-regulation (bleeding and serum estradiol concentration of <45 pg/ml), the patients started the oral estradiol valerate treatment and the treatment with GnRH agonist was discontinued.

Patients who did not ovulate and who were on a hormone substitution regimen to maintain menstrual cycle were not treated for pituitary down-regulation, and the treatment with estradiol valerate was started after menstrual bleeding following withdrawal of the hormone substitution treatment. Non-ovulating patients who did not take hormone substitution were given one cycle of oral contraceptive (Tri-Minulet; Wyeth Lederle, Spain) to induce menstrual bleeding, and the treatment with estradiol valerate was started after this bleeding.

Laboratory techniques
Oocytes were fertilized by ICSI in all cases. ICSI was performed 3–6 h after oocyte recovery using previously described techniques and instrumentation (Tesarik and Mendoza, 2002). Basic sperm parameters (sperm count, motility and morphology) were evaluated on the day of ICSI according to the World Health Organization (1999) criteria.

Fertilization was assessed 14–16 h after ICSI, and two-pronucleate zygotes scoring as good-morphology zygotes according to the criteria based on the assessment of the number and distribution of nucleolar precursor bodies (Tesarik and Greco, 1999) in its simplified version (Tesarik et al., 2000) were selected. Embryos were evaluated again on days 2 and 3 after ICSI, and their cleavage speed and morphology were noted. Only embryos developing from good-morphology zygotes were selected for transfer on day 3 after ICSI. Embryo quality was expressed by a cumulative score taking into account cleavage speed, blastomere symmetry, extent of fragmentation, and the presence or absence of multinucleated blastomeres (Rienzi et al., 2002). Two or three embryos were transferred according to the couple’s decision. Endometrial thickness on the day of embryo transfer was measured by vaginal ultrasound as described (Tesarik et al., 2003).

Outcome measures, power calculation and statistics
Pregnancy was first assessed 12–14 days after embryo transfer by determining serum β-hCG concentration, and it was confirmed 3 weeks later by ultrasound examination. Only clinical pregnancies, characterized by the presence of at least one intrauterine gestational sac with detectable heartbeat, are taken into account. Pregnancy and delivery rates were calculated as the number of patients showing a clinical pregnancy and those having delivered at least one living child, respectively, divided by the number of embryo transfer procedures. Implantation and birth rates were calculated by dividing the number of gestational sacs with heartbeat and the number of babies born, respectively, by the number of embryos transferred.

Assuming that pregnancy rate may be only marginally influenced by an enhancement of embryo developmental potential in a clinical setting in which 2–3 embryos are transferred per attempt, implantation rate was chosen as the main outcome measure. It was calculated that some 330 embryos were needed to be transferred in both the treatment and control groups to detect a difference of 10% (from 25 to 35%) with 80% power and 5% significance level (Campbell et al., 1995).

Differences between groups were assessed by two-tailed χ2-test with Yates’ correction or Fisher’s exact test for categorical variables, and by Mann–Whitney U-test for continuous variables.

Results
Oocytes from 138 donors were shared by 276 recipients, half of whom were allocated to the GnRH agonist group and the other half formed the control group. Baseline hormonal profiles of
oocyte recipients (serum concentrations of FSH, LH, prolactin, estradiol, progesterone and testosterone determined on days 2–3 of the cycle) were not different between the GnRH agonist and control groups. The same applied to the representation of ovulating and non-ovulating recipients, female age, endometrial thickness on the day of embryo transfer and basic sperm parameters (Table I). Moreover, the number and quality of embryos transferred and luteal phase concentrations of estradiol and progesterone were similar in both groups (Table II). The two groups can thus be considered as homogeneous with regard to the main factors that can influence assisted reproduction outcomes.

In spite of the similar number and quality of embryos transferred in the GnRH agonist and control groups, pregnancy rate tended to be higher and implantation rate was significantly higher ($P < 0.05$) in the GnRH agonist group (Table III). Recipients allocated to the GnRH agonist group also developed higher numbers of twin pregnancies, had a higher number of twin deliveries and showed a higher birth rate as compared with the control group (Table IV). The number of biochemical

### Table I. Basic clinical features of treatment attempts with and without the use of GnRH agonist at the time of implantation

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of recipients</th>
<th>Ovulating</th>
<th>Non-ovulating</th>
<th>Female agea</th>
<th>Endometrial thicknessa,b</th>
<th>Basic sperm parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GnRH agonist</td>
<td>72</td>
<td>66</td>
<td>40.5 ± 4.3</td>
<td>11.6 ± 1.1</td>
<td>45 ± 7 ± 6</td>
<td>42 ± 8</td>
</tr>
<tr>
<td>Control</td>
<td>75</td>
<td>63</td>
<td>41.3 ± 4.4</td>
<td>11.5 ± 1.2</td>
<td>48 ± 7 ± 7</td>
<td>45 ± 9</td>
</tr>
</tbody>
</table>

aData are mean ± SD.

### Table II. Number and quality of embryos transferred and luteal hormone profiles of oocyte recipients randomized between the GnRH agonist and control groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Embryos transferred</th>
<th>Hormonal profiles 12 days after embryo transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Scorea</td>
</tr>
<tr>
<td>GnRH agonist</td>
<td>2.4 ± 0.5b</td>
<td>1.12 ± 0.10</td>
</tr>
<tr>
<td>Control</td>
<td>2.4 ± 0.5c</td>
<td>1.14 ± 0.11</td>
</tr>
</tbody>
</table>

Data are mean ± SD. Differences between the GnRH agonist and the control group are not significant ($P > 0.05$).

### Table III. Effects of GnRH agonist administration at the time of implantation on pregnancy outcomes

<table>
<thead>
<tr>
<th>Group</th>
<th>Pregnanciesa</th>
<th>Deliveriesa</th>
<th>Delivery rateb</th>
<th>Birth ratec</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Singleton</td>
<td>Twin</td>
<td>Triplet</td>
<td>Singleton</td>
</tr>
<tr>
<td>GnRH agonist</td>
<td>67 (48.6)</td>
<td>23 (16.7)d</td>
<td>3 (2.2)</td>
<td>64 (46.4)</td>
</tr>
<tr>
<td>Control</td>
<td>68 (49.3)</td>
<td>5 (3.6)</td>
<td>2 (1.4)</td>
<td>63 (45.7)</td>
</tr>
</tbody>
</table>

aData are percentages in parentheses calculated from the total number of embryo transfer procedures.

### Table IV. Effects of GnRH agonist administration at the time of implantation on pregnancy and implantation rates

<table>
<thead>
<tr>
<th>Group</th>
<th>Transfers</th>
<th>Pregnancies</th>
<th>Pregnancy rate (%)</th>
<th>Embryos transferred</th>
<th>Gestational sacs</th>
<th>Implantation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH agonist</td>
<td>138</td>
<td>93</td>
<td>67.4</td>
<td>331</td>
<td>122</td>
<td>36.9b</td>
</tr>
<tr>
<td>Control</td>
<td>138</td>
<td>75</td>
<td>54.3</td>
<td>335</td>
<td>84</td>
<td>25.1</td>
</tr>
</tbody>
</table>

aData are only gestational sacs with detectable heartbeat are taken into account.

bSignificantly different from the control group ($P < 0.05$).
pregnancies and spontaneous abortions did not differ between the two groups.

Discussion

Previous studies have reported cases in which a GnRH agonist was administered inadvertently in the early pregnancy (Golan et al., 1990; Ron-El et al., 1990; Isherwood et al., 1990; Smits et al., 1991; Jackson et al., 1992; Balasch et al., 1993; Elefant et al., 1993; Har-Toov et al., 1993; Weissman and Shohan, 1993; Wilshire et al., 1993; Young et al., 1993; Gartner et al., 1997). However, this is the first prospective controlled study in which the effects GnRH agonist administration in the luteal phase after embryo transfer on assisted reproduction outcomes are analysed. Data obtained show that mid-luteal GnRH agonist administration increases the implantation rate without affecting the miscarriage and abortion rates, thus leading to a higher birth rate. Multiple pregnancies (twin) were also more frequent after GnRH agonist administration.

The fact that a short-acting GnRH agonist was administered to oocyte recipients as a single injection 6 days after ICSI excludes any possible effect on oocyte quality. The possibility that GnRH agonist acted primarily on the recipients’ uterine receptivity is unlikely, because if this were the case, an augmentation of the pregnancy rate without a corresponding increase in the implantation rate would be a more probable consequence. A direct effect of GnRH agonist on the early embryo is thus the most probable explanation of the present observations.

Previous studies have shown that GnRH and GnRH agonists stimulate placental hCG production in vivo (Iwashita et al., 1993; Lin et al., 1995; Siler-Khodr et al., 1997) and in vitro (Barnea et al., 1991), and the stimulatory effect of GnRH and GnRH agonist on in vitro production of hCG in placental explants was reversibly inhibited by a GnRH antagonist (Barnea et al., 1991). Moreover, preimplantation mouse embryos express GnRH receptor mRNA, and their in vitro development is significantly enhanced by incubation with increasing concentrations of GnRH agonist and decreased or completely inhibited by incubation with increasing concentrations of GnRH antagonist (Raga et al., 1999). It is thus possible that the administration of GnRH agonist, which was performed in this study 6 days after ICSI, stimulated the secretion of hCG by the early-implanting embryos and thus enhanced the embryo implantation potential. Interestingly, an antibody against GnRH has been detected in maternal circulation of pregnant women with a history of miscarriages and low levels of hCG (Siler-Khodr et al., 1997). These observations corroborate the concept of a GnRH–hCG axis in the physiological control of early pregnancy.

Although the mechanism of the beneficial effect of GnRH agonist on human implantation is merely hypothetical at this stage, the present observations suggest that assisted reproduction outcomes can be improved by a single administration of a short-acting GnRH agonist preparation in the mid-luteal phase. In the present study, the mid-luteal GnRH agonist administration increased the implantation rate in an oocyte donation programme in which the success rates were high even without this additional treatment. Further study is needed to determine whether mid-luteal GnRH agonist administration can improve clinical outcomes also in cases at high risk of implantation failure or early pregnancy loss.

References


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