Beneficial effect of luteal-phase GnRH agonist administration on embryo implantation after ICSI in both GnRH agonist- and antagonist-treated ovarian stimulation cycles

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BACKGROUND: GnRH agonist was recently suggested as a novel luteal-phase support that may act at different levels, including the pituitary gonadotrophs, the endometrium and the embryo itself. This prospective randomized study evaluates the effect of GnRH agonist administered in the luteal phase on ICSI outcomes in both GnRH agonist- and GnRH antagonist-treated ovarian stimulation protocols. METHODS: Six hundred women about to undergo ovarian stimulation for ICSI (300 using a long GnRH agonist protocol and 300 using a GnRH antagonist protocol) were enrolled in this study. Patients treated with each of these two protocols were randomly assigned to receive a single injection of GnRH agonist or placebo 6 days after ICSI. Implantation and live birth rates were the primary outcomes. RESULTS: Administration of 0.1 mg of GnRH agonist triptorelin on day 6 after ICSI led to a significant improvement of implantation and live birth rates after ICSI as compared with placebo. In GnRH antagonist-treated ovarian stimulation cycles, luteal-phase GnRH agonist also increased ongoing pregnancy rate. Moreover, luteal-phase GnRH agonist administration increased luteal-phase serum HCG, estradiol and progesterone concentrations in both ovarian stimulation regimens. CONCLUSIONS: Luteal-phase GnRH agonist administration enhances ICSI clinical outcomes after GnRH agonist- and GnRH antagonist-treated ovarian stimulation cycles, possibly by a combination of effects on the embryo and the corpus luteum.

Key words: corpus luteum function/embryo developmental potential/GnRH agonist/GnRH antagonist/luteal-phase support

Introduction

Luteal-phase deficiency is a common problem in current assisted reproduction techniques (ARTs) and has been described in cycles using pituitary down-regulation with a GnRH agonist as well as in those using GnRH antagonists (Macklon and Fauser, 2000; Pritts and Atwood, 2002; Beckers et al., 2003; Kolibianakis et al., 2003). To cope with this problem, different regimens of luteal-phase support were suggested (reviewed in Pritts and Atwood, 2002). Most of these regimens involve different application forms and doses of three therapeutic agents, HCG, vaginal or intramuscular progesterone and oral estradiol (E2) (Pritts and Atwood, 2002). Some recent data, however, have suggested a beneficial effect of GnRH agonist administered in the luteal phase on ART outcomes (Tesarik et al., 2004; Pirard et al., 2005).

The mechanism of the presumed beneficial effect of luteal-phase GnRH agonist administration is not clear and may be due to the drug action at multiple levels. It was hypothesized that GnRH agonist may support the corpus luteum by stimulating the secretion of LH by pituitary gonadotroph cells or by acting directly on the endometrium through the locally expressed GnRH receptors (Pirard et al., 2005). However, the administration of a single dose of GnRH agonist in the luteal phase was also shown to increase pregnancy, implantation, delivery and birth rates in recipients of donated oocytes in whom ovulation was suppressed and the corpus luteum was thus absent, suggesting a direct effect of GnRH agonist on the embryo (Tesarik et al., 2004).

To help clarify the mechanism of GnRH agonist action in the luteal phase, this prospective randomized study evaluates the effect of luteal-phase GnRH agonist administration on ICSI outcomes in the two most currently used ovarian stimulation protocols using, respectively, GnRH agonist and GnRH antagonist for the prevention of a premature LH surge. Implantation
and live birth rates were the main outcome measures. Clinical pregnancy and ongoing pregnancy rates and luteal-phase serum concentrations of HCG (in conception cycles), LH and progesterone were secondary outcome measures.

Materials and methods

Study design and power calculation
This prospectively randomized study was conducted between September 2003 and September 2005. It involves 600 infertile couples treated by ICSI and given two different protocols of ovarian stimulation, one using GnRH agonist started in the luteal phase of the preceding cycle for pituitary down-regulation (300 couples) and the other using a GnRH antagonist for the prevention of a premature LH surge (300 couples). Patients to be treated by each of the two protocols were randomly assigned to two groups. In one group, women were given a single dose of GnRH agonist 6 days after ICSI (3 days after embryo transfer). Women in the other group received placebo at the same time after ICSI. The decision of which couples would be treated by each of the two different ovarian stimulation protocols was subjective, and the study is thus not randomized as to comparison of ICSI outcomes with ovarian stimulation protocols using GnRH agonist and GnRH antagonist, respectively, and the results can only serve to evaluate the usefulness of luteal-phase GnRH agonist administration in each of the two protocols.

Implantation and live birth rates were chosen as the main outcome measures. On the basis of previously published data (Tesarak et al., 2004) showing that the administration of GnRH agonist 6 days after ICSI to recipients of donated oocytes increases implantation rate from 24.7% to 36.2%, it was hypothesized that a similar increase can be achieved with this method in ICSI patients undergoing ovarian stimulation. Assuming a significance level of 0.05 and a power of 0.80, it was calculated that some 100 treatment cycles were needed in each branch to detect this increase. It was decided to increase the number of cycles enrolled in each group to 150 to obtain a better chance to detect significant differences in some of the secondary outcome measures of this study; these were pregnancy and delivery rates, and luteal-phase serum concentrations of HCG (in conception cycles), LH and progesterone.

The study protocol was approved by a competent institutional review board, and all participants signed an informed consent.

Randomization protocol
Couples selected for an ICSI attempt using each of the two ovarian stimulation protocols were randomized between a luteal-phase GnRH agonist group and a placebo group. Randomization was done with the use of a computer-generated randomization list. Sealed envelopes with treatment allocation instructions were opened on the day of embryo transfer by a nurse who assigned participants to their groups. The doctor and the biological team performing the ART were blinded to group assignment.

Participants
Six hundred infertile couples were enrolled in this study. Exclusion criteria were female age of >40 years and non-obstructive azoospermia requiring testicular sperm retrieval. Three couples were assigned to an ovarian stimulation protocol using long GnRH agonist and 300 to a protocol using GnRH antagonist. The decision between each of these two protocols was subjective and depended on the clinical context. Usually, the GnRH antagonist protocol was chosen in women presenting symptoms of polycystic ovaries and in those with a history of ovarian hyperstimulation syndrome (OHSS), but also in some women with expected good ovarian response without these features with the aim to reduce treatment cost and patient discomfort. The GnRH agonist protocol was mostly, but not exclusively, used in women with expected normal or low ovarian response.

Ovarian stimulation
Long GnRH agonist protocol
Pituitary down-regulation was achieved with the use of daily injections of 0.1 mg of triptorelin (Decapeptyl 0.1 mg; Ipsen Pharma, Barcelona, Spain) starting in the luteal phase of the cycle preceding ovarian stimulation and reduced to 0.05 mg daily dose from the first day of vaginal bleeding. The gonadotropin therapy was started 2–7 days after the beginning of the bleeding. This therapy was carried out with the use of recombinant human FSH (Puregon; Organon, Oss, The Netherlands or Gonal F; Serono, Rome, Italy) and HMG (Menopur; Ferring Pharmaceuticals, Langley, Berkshire, UK). The doses of FSH and HMG were continuously adapted according to serum concentrations of E2 and LH and to the dynamics of ovarian follicular growth as described (Tesarak and Mendoza, 2002) with the aim to maintain serum LH concentration in the range of 0.5–1.5 IU 1\(^{-1}\) throughout the stimulation period. Ovulation was induced with 250 μg of human recombinant HCG (Ovitrelle, Serono) when at least three follicles reached a mean diameter of 18 mm. The treatment with triptorelin was stopped on the day of HCG administration (last triptorelin injection on the day preceding HCG administration).

GnRH antagonist protocol
Recombinant human FSH and HMG administration was started on day 2 of menstrual bleeding following withdrawal of a contraceptive pill (Tri-Minulet; Wyeth Lederle, Madrid, Spain) administered during the cycle preceding ovarian stimulation. The doses of FSH and HMG were continuously adapted according to serum concentrations of E2 and LH and to the dynamics of ovarian follicular growth in the same way as in the long GnRH agonist protocol (discussed above). GnRH antagonist (Orgalutran; Organon or Cetrotide; Serono) was started on day 5 of gonadotropin therapy and continued, at a daily dose of 0.25 mg, until at least three follicles reached a diameter of ≥18 mm when ovulation was induced with 250 μg of human recombinant HCG (Ovitrelle).

ARTs
Mature (metaphase II) oocytes were incubated at 37°C in G-FERT medium (Vitrolife, Göteborg, Sweden) equilibrated with 6% CO\(_2\) in air for 3–6 h. They were then freed from the cumulus oophorus and corona radiata cells, subjected to ICSI as described (Tesarak and Mendoza, 2002) and incubated in G1 medium (Vitrolife) under the same gas phase. Fertilization was assessed 16–18 h after ICSI. Zygotes were scored as having good morphology or poor morphology (see section ‘Embryo selection for transfer’). After a medium change, embryos were cultured further in G1 medium until day 3 after ICSI with a medium change every day. At the time of medium change on day 2, embryo quality was assessed again (see section ‘Embryo selection for transfer’). Embryo transfer was performed 3 days after ICSI with the use of K-JETS-7019-SIVF embryo transfer set (Cook, Queensland, Australia) and under transabdominal ultrasound guidance. One to three embryos were transferred in each cycle. If suitable, supernumerary embryos were frozen for later transfer. This study, however, only deals with fresh embryo transfers.

Embryo selection for transfer
Zygotes were evaluated according to criteria based on the assessment of the number and distribution of nuclear precursor bodies in the pronuclei as described previously (Tesarak and Greco, 1999). The
scoring system was simplified by grouping together all abnormal patterns (Tesak et al., 2000), so as all zygotes were allocated to one of two groups—good-morphology zygotes and poor-morphology zygotes.

Cleaving embryos were evaluated twice, first at the time of medium change 2 days after ICSI and second 10–30 min before transfer to the uterus 3 days after ICSI as described (Tesak and Greco, 1999). Briefly, embryos that had more than three cells 2 days after ICSI and more than 6 cells 3 days after ICSI, that showed <10% of their space occupied by cell fragments and that developed from good-morphology zygotes were considered to have good morphology and were selected for transfer where available.

Luteal-phase supplementation

Irrespective of whether GnRH agonist was used as luteal-phase support or not, all women were given 4 mg of E2 valerate (Progynova; Schering, Madrid, Spain) daily and 400 mg of vaginal micronized progesterone (Utrogestan; Laboratoires Besins-Iscovesco, Paris, France) daily from the day of oocyte recovery for 17 days. In addition, all women received an injection of 250 μg of human recombinant HCG (Ovitrelle) on the day of embryo transfer.

Hormone assays

Serum concentrations of 17β-E2, progesterone and β-HCG were determined with the use of commercial enzyme immunoassay kits (Diagnostic Product Corporation, Los Angeles, CA, USA). The intra- and inter-assay coefficients of variation (CVs) were, respectively, 3.8 and 5.6% for E2, 3.7 and 4.0% for progesterone, and 3.5 and 4.1% for β-HCG. All measurements were taken according to the manufacturer’s instructions.

Statistics

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 or Welch t-test for continuous variables. The level of significance was set to P < 0.05. Efficacy analyses were performed on an intention-to-treat (ITT) basis as appropriate, taking into account all randomized patients. Multidimensional statistics were performed using multivariate logistic regression analysis. All analyses were performed using the Statistica 5.0 package (Statsoft Version 5.1, Hamburg, Germany).

Results

Patient flow through the trial

The trial was performed according to CONSORT guidelines. Infertile couples continued to be assessed for eligibility study until 150 patients were eligible in both the treatment and the placebo arm in each of the two ovarian stimulation protocols. Among the patients in whom the long GnRH agonist ovarian stimulation protocol was indicated, 13 did not meet the inclusion criteria and eight refused to participate in the study (Figure 1A). Among the patients in whom the GnRH antagonist ovarian stimulation protocol was indicated, 11 did not meet the inclusion criteria and seven refused to participate in the study (Figure 1B).

Among the patients treated with the long GnRH agonist stimulation protocol and assigned to the luteal-phase GnRH agonist group, two discontinued the ovarian stimulation and three had no embryos for transfer. Of the remaining 145 patients who received allocated intervention, one was lost to follow-up, three were excluded from analysis because of failure to follow correctly the protocol of luteal-phase examinations, and 141 terminated the study and were analysed (Figure 1A).

Among the patients treated with the long GnRH agonist stimulation protocol and assigned to the luteal-phase GnRH agonist group, four had no embryos for transfer. Of the remaining 146 patients who received allocated intervention, one was excluded from analysis because of failure to follow correctly the protocol of luteal-phase examinations, and 145 terminated the study and were analysed (Figure 1B). Among the patients treated with the GnRH antagonist stimulation protocol and assigned to the placebo group, one was cancelled because of inadequate response to ovarian stimulation and four had no embryos for transfer. Of the remaining 145 patients who received allocated intervention, one was excluded from analysis because of failure to follow correctly the protocol of luteal-phase examinations, and 144 terminated the study and were analysed (Figure 1B).

Effects of luteal-phase GnRH agonist administration in the long GnRH agonist ovarian stimulation protocol

The patients in the luteal-phase GnRH agonist and placebo groups did not differ in their basic demographical characteristics, and the features of their ovulation stimulation cycles were also comparable (Table I). The concentrations of E2, determined on day 7 after ICSI in both groups, did not differ significantly (Table II), although there was a strong trend towards higher values in the GnRH agonist group (P = 0.053). However, serum concentration of E2, determined on day 15 after ICSI, and that of progesterone, determined on both days 7 and 15 after ICSI, were higher in the luteal-phase GnRH agonist group as compared with the placebo (Table II). Moreover, the patients given GnRH agonist 6 days after ICSI, who subsequently developed a clinical pregnancy, had a higher serum β-HCG concentration on day 15 after ICSI as compared with pregnant patients in the placebo group, and this difference was also evident when only those patients in whom a single gestational sac was subsequently detected were taken into account (Table II).

With a similar number and morphology of embryos transferred, patients in the luteal-phase GnRH agonist group had higher implantation and live birth rates than those in the placebo group, whereas the differences in the clinical pregnancy and ongoing pregnancy rates were not significant (Table III). There were 22 multiple births (21 twin and 1 triplet) in the GnRH agonist group as compared with only five multiple births (4 twin and 1 triplet) in the placebo group. Using multivariate analysis, we observed that the female age and the number of oocytes recovered were independent predictors of
Figure 1. Patient flow through the randomized trial. (A) Patients with ovarian stimulation protocol using GnRH agonist. (B) Patients with ovarian stimulation protocol using GnRH antagonist.
Table I. Basic demographic and ovarian stimulation cycle characteristics of patients treated with the long GnRH agonist ovarian stimulation protocol

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patient group*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Luteal-phase</td>
</tr>
<tr>
<td></td>
<td>GnRH agonist</td>
</tr>
<tr>
<td>Age (years)</td>
<td>34.8 ± 1.6</td>
</tr>
<tr>
<td>BMI (kg m(^{-2}))</td>
<td>23.9 ± 2.0</td>
</tr>
<tr>
<td>Duration of FSH therapy (days)</td>
<td>11.8 ± 0.6</td>
</tr>
<tr>
<td>Total amount of FSH given (IU)</td>
<td>2488 ± 181</td>
</tr>
<tr>
<td>Total amount of HMG given (IU)</td>
<td>477 ± 36</td>
</tr>
<tr>
<td>Peak serum E(_2) (pg ml(^{-1}))</td>
<td>1745 ± 201</td>
</tr>
<tr>
<td>Number of oocytes recovered(^b)</td>
<td>12.4 ± 4.6</td>
</tr>
</tbody>
</table>

Values are mean ± SD. The differences between the two groups are not significant (P > 0.05).

\(^*\)Intention-to-treat group (n = 300).
\(^b\)Per stimulated cycle.

Table II. Luteal-phase characteristics of patients treated with the long GnRH agonist ovarian stimulation protocol

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patient group*</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Luteal-phase</td>
</tr>
<tr>
<td></td>
<td>GnRH agonist</td>
</tr>
<tr>
<td>Serum estradiol (pg ml(^{-1}))</td>
<td></td>
</tr>
<tr>
<td>Day 7 after ICSI</td>
<td>432 ± 63</td>
</tr>
<tr>
<td>Day 15 after ICSI</td>
<td>480 ± 74(^a)</td>
</tr>
<tr>
<td>Serum progesterone (ng ml(^{-1}))</td>
<td></td>
</tr>
<tr>
<td>Day 7 after ICSI</td>
<td>44 ± 5(^a)</td>
</tr>
<tr>
<td>Day 15 after ICSI</td>
<td>47 ± 7(^a)</td>
</tr>
<tr>
<td>Serum HCG (IU (^{-1}))</td>
<td></td>
</tr>
<tr>
<td>Day 15 after ICSI</td>
<td></td>
</tr>
<tr>
<td>In all conception cycles(^b)</td>
<td>66 ± 8(^b)</td>
</tr>
<tr>
<td>In singleton pregnancies</td>
<td>53 ± 6(^c)</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

\(^*\)Group of patients having terminated the study (n = 283).
\(^b\)Only cycles that resulted in a clinical pregnancy are included.
\(^c\)Significantly different from the placebo group (P < 0.05).

Table III. Clinical outcomes of patients treated with the long GnRH agonist ovarian stimulation protocol

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Patient group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Luteal-phase</td>
</tr>
<tr>
<td>Intention to treat</td>
<td>150</td>
</tr>
<tr>
<td>Transfer procedures</td>
<td>141</td>
</tr>
<tr>
<td>Embryos transferred</td>
<td>325</td>
</tr>
<tr>
<td>Embryos per transfer(^a)</td>
<td>2.3 ± 0.5 (2.0)</td>
</tr>
<tr>
<td>Good-morphology embryos per transfer(^a)</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>Clinical pregnancy rate</td>
<td></td>
</tr>
<tr>
<td>Per embryo transfer(^a)</td>
<td>51.1% (72/141)</td>
</tr>
<tr>
<td>Per intention to treat</td>
<td>48.0% (72/150)</td>
</tr>
<tr>
<td>Clinical implantation rate</td>
<td>29.8% (97/325)(^b)</td>
</tr>
<tr>
<td>Ongoing pregnancy rate</td>
<td></td>
</tr>
<tr>
<td>Per embryo transfer</td>
<td>46.8% (66/141)</td>
</tr>
<tr>
<td>Per intention to treat</td>
<td>44.0% (66/150)</td>
</tr>
<tr>
<td>Live birth rate</td>
<td>27.4% (89/325)(^b)</td>
</tr>
</tbody>
</table>

\(^a\)Mean ± SD (median).
\(^b\)Significantly different from the placebo group (P < 0.05).

Implantation and ongoing pregnancy in both the GnRH agonist and the placebo groups (data not shown).

Effects of luteal-phase GnRH agonist administration in the GnRH antagonist ovarian stimulation protocol

The patients in the luteal-phase GnRH agonist and placebo groups did not differ in their basic demographical characteristics, and the features of their ovulation stimulation cycles were also comparable (Table IV). However, the concentrations of E\(_2\) and progesterone were higher in the luteal-phase GnRH agonist group than in the placebo group, both on days 7 and 15 after ICSI (Table V). Moreover, the patients given GnRH agonist 6 days after ICSI, who subsequently developed a clinical pregnancy, had a higher serum β-HCG concentration on day 15 after ICSI as compared with pregnant patients in the placebo group, and this difference was also evident when only those patients in whom a single gestational sac was subsequently detected were taken into account (Table V).

With a similar number and morphology of embryos transferred, patients in the luteal-phase GnRH agonist group had

Table IV. Basic demographic and ovarian stimulation cycle characteristics of patients treated with the GnRH antagonist ovarian stimulation protocol

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patient group*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Luteal-phase</td>
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<tr>
<td></td>
<td>GnRH agonist</td>
</tr>
<tr>
<td>Age (years)</td>
<td>31.3 ± 2.1</td>
</tr>
<tr>
<td>BMI (kg m(^{-2}))</td>
<td>23.8 ± 2.2</td>
</tr>
<tr>
<td>Duration of FSH therapy (days)</td>
<td>11.0 ± 0.5</td>
</tr>
<tr>
<td>Total amount of FSH given (IU)</td>
<td>2150 ± 164</td>
</tr>
<tr>
<td>Total amount of HMG given (IU)</td>
<td>282 ± 21</td>
</tr>
<tr>
<td>Peak serum E(_2) (pg ml(^{-1}))</td>
<td>1931 ± 208</td>
</tr>
<tr>
<td>Number of oocytes recovered(^b)</td>
<td>12.1 ± 4.3</td>
</tr>
</tbody>
</table>

Values are mean ± SD. The differences between the two groups are not significant (P > 0.05).

\(^*\)Intention-to-treat group (n = 300).
\(^b\)Per stimulated cycle.

Table V. Luteal-phase characteristics of patients treated with the GnRH antagonist ovarian stimulation protocol

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patient group*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Luteal-phase</td>
</tr>
<tr>
<td></td>
<td>GnRH agonist</td>
</tr>
<tr>
<td>Serum estradiol (pg ml(^{-1}))</td>
<td></td>
</tr>
<tr>
<td>Day 7 after ICSI</td>
<td>405 ± 52(^e)</td>
</tr>
<tr>
<td>Day 15 after ICSI</td>
<td>420 ± 56(^e)</td>
</tr>
<tr>
<td>Serum progesterone (ng ml(^{-1}))</td>
<td></td>
</tr>
<tr>
<td>Day 7 after ICSI</td>
<td>42 ± 8(^e)</td>
</tr>
<tr>
<td>Day 15 after ICSI</td>
<td>48 ± 9(^e)</td>
</tr>
<tr>
<td>Serum HCG (IU (^{-1}))</td>
<td></td>
</tr>
<tr>
<td>Day 15 after ICSI</td>
<td></td>
</tr>
<tr>
<td>In all conception cycles(^b)</td>
<td>64 ± 9(^e)</td>
</tr>
<tr>
<td>In singleton pregnancies</td>
<td>50 ± 6(^e)</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

\(^*\)Group of patients having terminated the study (n = 289).
\(^b\)Only cycle that resulted in a clinical pregnancy are included.
\(^e\)Significantly different from the placebo group (P < 0.05).
higher implantation, ongoing pregnancy and live birth rates than those in the placebo group (Table VI), and there was also a trend towards a higher clinical pregnancy rate in the GnRH agonist group ($P = 0.083$). There were 15 multiple births (all twins) in the GnRH agonist group as compared with only two multiple births (both twins) in the placebo group. Using multivariate analysis, we observed that the female age and the number of oocytes recovered were independent predictors of implantation and ongoing pregnancy in both the GnRH agonist and the placebo groups (data not shown).

### Discussion

Several lines of evidence suggest that the luteal phase after embryo transfer is deficient in cycles stimulated with HMG or FSH, irrespectively of whether a GnRH agonist (Macklon and Fauser, 2000; Pritts and Atwood, 2002) or a GnRH antagonist (Beckers et al., 2003; Kolibianakis et al., 2003) is used for the prevention of a premature LH surge. To solve this problem, most clinics use some form of luteal-phase support after embryo transfer. This can be done by stimulating progesterone secretion by the corpus luteum with exogenous HCG administration or by a direct supplementation of exogenous progesterone (in Brocks and Atwood, 2002). In this prospective randomized study, evaluating the effects of GnRH agonist administered in a single dose 6 days after ICSI on luteal-phase characteristics and clinical outcomes, both HCG and progesterone supplementation were used in both the treatment and the placebo groups, in addition to oral E2. Consequently, any effect of luteal-phase GnRH agonist administration described in this study must be interpreted on the background of this basic luteal-phase support.

The effects of luteal-phase GnRH agonist administration were evaluated in the context of two different ovulation stimulation regimens, one using GnRH agonist and the other GnRH antagonist. The results concerning ICSI clinical outcomes, namely clinical implantation and birth rates, confirm previous findings obtained in an oocyte donation model (Tesarik et al., 2004). The observed increase in clinical implantation rate in patients treated with GnRH agonist in the luteal phase, as compared with the placebo group, indicates an increased risk of multiple pregnancies after this luteal-phase supplementation. Hence, it might be interesting to combine luteal-phase GnRH agonist administration with single embryo transfer.

In addition, unlike patients in whom ovulation stimulation had been performed with the use of a long GnRH agonist protocol, patients treated with the use of GnRH antagonist and receiving GnRH agonist in the luteal phase had also a higher delivery rate and showed a trend towards a higher clinical pregnancy rate as compared with the corresponding placebo group. The relationship between the protocol of ovarian stimulation and the subsequent effects of luteal-phase administration of GnRH agonist is not clear and remains an intriguing challenge for further research, namely in the context of the current efforts at optimization of the use of GnRH antagonists in ovarian stimulation (Fauser and Devroey, 2005).

The fact that beneficial effects of luteal-phase GnRH agonist on these variables was achieved here on the background of luteal-phase support with HCG, progesterone and E2 supports the originally formulated hypothesis that GnRH agonist exerts a direct beneficial effect on the implanting embryo (Tesarik et al., 2004). The hypothesis of a direct action of GnRH agonist on the embryo (Tesarik et al., 2004) is further corroborated by the present observation of higher levels of serum β-HCG, both in the overall group of patients who achieved a pregnancy and after the exclusion of multiple pregnancies from the analysis, after luteal-phase administration of GnRH agonist as compared with placebo. It was reported previously that GnRH increases serum HCG in pregnant women (Iwashita et al., 1993), presumably by acting on a placental GnRH receptor (Lin et al., 1995), and results in excessive binding of circulating GnRH by a yet-undefined GnRH-binding substance, possibly an auto-antibody reported in patients with repeated early pregnancy wastage (Siler-Khodr et al., 1997).

However, the arguments in favour of a direct effect of GnRH agonist on the embryo do not exclude other mechanisms of action acting in parallel. Unlike the situation in the oocyte donation model, the corpus luteum may have been another target of GnRH agonist action in the present setting. In fact, an increase in serum concentrations of both E2 and progesterone in the luteal phase of patients given GnRH agonist 6 days after ICSI as compared with patients receiving placebo was observed in this study. These observations were made in both types of ovarian stimulation regimens employed in this study.

The mechanism of action of GnRH agonist on the corpus luteum remains a controversial issue. Early studies suggested that GnRH agonist treatment in the luteal phase (one or two administrations of 500 µg of buserelin) may act as a luteolytic agent for contraceptive purposes (Lemay et al., 1982, 1983), supposedly due to GnRH receptor desensitization in gonadotroph cells. In fact, this may be true for certain doses of GnRH agonist, although the hypothesis of a contraceptive action of GnRH agonists has never been definitely confirmed, and attempts at inducing early abortion in women by huge doses of a superactive GnRH agonist resulted in a failure (Skarin et al., 1982). More recently, a number of observational clinical studies...
reported the consequences of an inadvertent administration of GnRH agonist in the luteal phase, and with only one exception (Herman et al., 1992), all agree in that luteal-phase GnRH agonist administration 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; Smitz et al., 1991; Jackson et al., 1992; Balasch et al., 1993; Elefant et al., 1993; Har-Toov et al., 1993; Weissman and Shoham, 1993; Wilshire et al., 1993; Young et al., 1993; Gartner et al., 1997). Moreover, a recent small randomized study suggested that low doses of GnRH agonist (100 μg of buserelin) exert a stimulatory effect on the corpus luteum (Pirard et al., 2005). The present data strongly support this hypothesis. It is not known whether the increase in the synthesis of E2 and progesterone in patients given GnRH agonist in the luteal phase is mediated by pituitary hormones or results from a direct action of GnRH agonist in the ovary. Our failure to detect a measurable increase in serum LH concentration 1 day after GnRH agonist administration does not support a role for the pituitary in these events, but it must be reminded that a short LH peak, sufficient to produce this effect, may not be detected with the design of this study.

If GnRH agonist stimulates placental production of HCG, which is known to promote the secretion of vascular endothelial growth factor (VEGF), the main player in the development of OHSS (McCune et al., 1994; Neulen et al., 1995; Krasnow et al., 1996), a question arises about the safety of luteal-phase GnRH agonist administration in patients at high risk of OHSS. Surprisingly, however, a recent study performed in an animal (rat) model has shown that GnRH agonist administration during the luteal phase reduces the expression of VEGF and VEGF receptors, both at mRNA and protein levels, and decreases vascular permeability in the hyperstimulated ovaries (Kitajima et al., 2004). Moreover, the continuation of GnRH agonist administration in GnRH agonist-down-regulated IVF cycles has been shown to prevent the development of the early OHSS in at-risk patients in whom embryo transfer is not performed and all embryos are cryopreserved (Endo et al., 2002). The GnRH agonist-induced increase in HCG production by the embryo at early stages of implantation is probably too low to affect significantly VEGF synthesis in extraterine locations, and the decrease in the ovarian VEGF and VEGF receptor expression operated by GnRH agonist (Kitajima et al., 2004) may be related to GnRH agonist action on the pituitary–ovarian axis, independent of its effect on the early embryo. In this study, we have not observed any case of severe OHSS requiring hospitalization. There is thus no a priori reason why GnRH agonist should be avoided as luteal-phase support in this category of patients, although caution is recommended until more complete information about the effects of luteal-phase GnRH agonist administration at different levels of the reproductive system is available.

Moreover, a GnRH receptor site was immunolocalized in murine endometrium (Murdoch, 1995), and a functional LH receptor has been detected in the human uterus (Reshef et al., 1990). A direct action of GnRH agonist or GnRH agonist-induced LH in the uterine tissues can thus also be responsible for the observed luteal-phase GnRH agonist effects.

In conclusion, the results of this prospective randomized study extend previous findings of a beneficial effect of GnRH agonist administration in the luteal phase on embryo implantation potential, obtained in an oocyte donation programme, to ovarian stimulation cycles using both GnRH agonist and GnRH antagonist for prevention of premature LH surge. This effect was particularly evident in ovarian stimulation cycles using GnRH antagonist. Moreover, these data corroborate the hypothesis of a direct action of GnRH agonist on the implanting embryo by demonstrating a stimulatory effect on β-HCG secretion, but they also suggest a direct or indirect positive effect on the corpus luteum function. These observations open the idea of a broader use of GnRH agonist as a novel luteal-phase support, possibly in combination with the conventionally employed HCG, E2, and progesterone, especially in GnRH antagonist-using ovarian stimulation regimens. However, caution is needed as to eventual late-appearing health effects of this therapy on the offspring. The present data are likely to prompt further studies into the mechanism of action of GnRH agonist in the luteal phase. Pre-clinical studies are also needed to determine the optimal dose, time and frequency of GnRH agonist administration.

References


