Timing ovulation for intrauterine insemination with a GnRH antagonist

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BACKGROUND: We aimed to assess the efficacy of a GnRH antagonist in intrauterine insemination (IUI) cycles to increase number of mature ovulatory follicles and pregnancy rates. METHODS: Prospective randomized study. Women (18–38 years old) with primary/secondary infertility were included. Eighty-two patients were randomly assigned to controlled ovarian stimulation (COS) consisting of rFSH + GnRH antagonist or rFSH alone.

RESULTS: A non-significant increase in the total amount of rFSH was seen in the GnRH antagonist group (707 ± 240 IU) with respect to the control group (657 ± 194 IU). The number of mature follicles (≥16 mm) was significantly higher in the GnRH antagonist group than in the control group (2.4 ± 1.4 versus 1.7 ± 1.2, P < 0.05). Pregnancy rates were significantly increased in the group of patients receiving the GnRH antagonist (38%) compared to the control group (14%). The only non-single pregnancy (triplets) occurred in the antagonist group.

CONCLUSIONS: In this preliminary study, adding the GnRH antagonist to the COS protocol for IUI cycles significantly increased pregnancy rates. Nevertheless, these results may not be associated directly with the antagonist itself but with the fact that more mature ovulatory follicles are present by the day of the hCG. Finally, the risk for multiple gestations needs to be carefully evaluated.

Key words: GnRH antagonist/intrauterine insemination/pregnancy rates

Introduction

Controlled ovarian stimulation (COS) is a standard procedure in intrauterine insemination (IUI) that has resulted in a significant increase in pregnancy rates with respect to rates in non-stimulated IUI cycles (Nuojua-Huttunen et al., 1999; Dickey et al., 2002; Duran et al., 2002; Houmard et al., 2002; Kaplan et al., 2002). Nevertheless, multifollicular recruitment during COS can bring about a sudden increase in estradiol (E2) serum levels that is enough to induce an LH surge while follicular growth is still in progress. Moreover, it has been calculated that 24% of IUI cycles suffer undesired premature luteinization (Ragni et al., 2004) and this can result in IUI procedure cancellation. Obviously this represents economic and psychological stress for the patients. Thus, to avoid the risk of unexpected premature follicular luteinization, standard procedure induces ovulation as soon as the leading follicle has reached the 18 mm boundary, independently of the number and developmental status of the other recruited follicles (Frydman et al., 1991; Duran et al., 2002). This will necessarily change the IUI cycle from multifollicular to monofollicular and, consequently, decrease the chances of gestation, since at least two mature follicles (≥16 mm) are needed to attain an acceptable pregnancy rate in IUI (Nuojua-Huttunen et al., 1999; Dickey et al., 2002; Duran et al., 2002; Houmard et al., 2002; Kaplan et al., 2002).

The inclusion of the GnRH antagonist in assisted reproduction techniques allows ovulation to be postponed since the antagonist will suppress gonadotrophin release, block the possibility of premature LH surges, and, consequently, premature luteinization of the follicles (Frydman et al., 1991; Diedrich et al., 1994; European and Middle East Orgalutran Study Group, 2001; Fluker et al., 2001). Furthermore, this control of untimely LH release has been extensively confirmed and demonstrated to successfully protect follicular development against unexpected luteinization in IVF (Diedrich et al., 1994; European and Middle East Orgalutran Study Group, 2001; Fluker et al., 2001; Ricciarelli et al., 2003; Acevedo-Martin et al., 2004).

With this in mind, we thought that if pregnancy rates in IUI were related to the number of mature follicles present on the day hCG was indicated (Nuojua-Huttunen et al., 1999; Dickey et al., 2002; Duran et al., 2002; Houmard et al., 2002; Kaplan et al., 2002), and if the limiting factor for follicular development was premature luteinization, why not control unexpected LH surges during the COS–IUI cycles?
with an antagonist of the GnRH? This would: (i) maintain steady growth up to $>20\text{ mm}$ in diameter among the recruited follicles; (ii) allow us to time ovulation in a synchronized manner with more than one mature follicle and, hopefully, increase pregnancy rates.

### Materials and methods

#### Study design

This study was a multicentre, prospective, randomized study performed from January to June, 2003, in 82 infertile couples to assess the efficacy of GnRH antagonist in COS combined with homologous IUI.

The GnRH antagonist is already routinely used for IVF in our centres, and the patients were recruited following the guidelines set by the Spanish Committee of Assisted Reproductive Techniques, in accordance with the Helsinki Declaration of 1975 on human research.

#### Inclusion criteria

The main inclusion criteria in women were age between 18 and 38 years, regular menstrual cycle, primary or secondary infertility lasting for $\geq 12$ months, body mass index between 19 and 25 kg/m$^2$, normal prolactin levels, normal thyroid function, normal uterine cavity and bilateral tubal patency assessed by hysterosalpingography and/or laparoscopy.

Women with hormone values outside the reference range by day 3–4 of their menstrual period (FSH levels $>10\text{ mIU/l}$) and with polycystic ovarian syndrome were excluded from this study.

Semen analysis was performed at least twice and IUI was carried out if the total motile spermatozoid swim-up count was $>10 \times 10^9$/ml.

#### Hormonal treatment

COS was performed in both groups with recombinant FSH (rFSH, Puregon; Organon Inc., Spain). Patients included in this study underwent an ultrasound scan between the third and fourth day of their menstrual cycle and were subsequently randomized by a computer-generated random listing into two groups. The study was performed in two centres. Patients were randomly assigned by a computer-generated list in the order of their enrolment.

#### GnRH antagonist group

Forty patients (30 patients from the first centre and 10 from the second centre) were subjected to the same COS protocol previously described except that the GnRH antagonist (Ganirelix) was not used and hCG (5000IU) was administered once the leading follicle reached the 18–20 mm boundary, on ultrasound examination. As in the other group, IUI was cancelled if more than four follicles ($>16–20\text{ mm}$) were present. One cycle was cancelled in order to avoid a multiple pregnancy since ultrasound had revealed seven follicles $\geq 16\text{ mm}$.

#### Control group

Forty-two patients (31 from the first centre and 11 from the second centre) were subjected to the same COS protocol previously described except that the GnRH antagonist (Ganirelix) was not used and hCG (5000IU) was administered once the leading follicle reached the 18–20 mm boundary, on ultrasound examination. As in the other group, IUI was cancelled if more than four follicles ($>16–20\text{ mm}$) were present. One cycle was cancelled in order to avoid a multiple pregnancy since ultrasound had revealed seven follicles $\geq 16\text{ mm}$.

#### Semen preparation

Semen specimens were obtained by masturbation into a sterile jar after 2–3 days of abstinence and a few hours before the scheduled insemination. After liquefaction for $\sim 20–30$ min, the specimen was mixed and diluted with 2 ml of Ham’s F-10 medium (Gibco, UK). The mixture was centrifuged at 600 for $5–10$ min, and the supernatant discarded; 0.3 ml of IVF Universal medium (Medicult, Denmark) was added and the mixture incubated for 30–45 min at room temperature. After the swim-up, the most active motile sperm were isolated by aspiration and used for insemination.

#### IUI procedure and luteal phase support

A single insemination was performed, 36–38 h post-hCG, in both groups using a Lee catheter (Vygon, France) inserted through the cervix. A limited insemination volume (0.3 ml) was delivered into the uterine cavity and bed rest was maintained for 10 min after IUI.

The luteal phase was routinely supplemented (in all patients in both groups) with 300 mg/day/vaginally of natural micronized progesterone (Utrogestan; Seid, Spain) starting after the IUI procedure on the same day.

Sixteen days after the insemination, an hCG assay (HCG; BioMerieux, France) was performed. If the assay was positive, transvaginal ultrasonography was scheduled for 2 weeks later. The concurrence of a positive hCG and embryo(s) with a positive heart beat (seen by ultrasound) was defined as a clinical pregnancy—otherwise the positive hCG test was considered to be a biochemical pregnancy. Progesterone was maintained until the 12th week of pregnancy if a clinical pregnancy was evident.

#### Hormonal determination

FSH, LH, E$_2$ and hCG serum levels were determined with a commercial enzyme immunoassay kit (Vidas; Biorémerieux, Spain). Blood samples were collected on the third or fourth day of the cycle (E$_2$, LH and FSH) and before given hCG (E$_2$).

#### Statistical analysis

Data were expressed as the mean $\pm$ SD. Continuous variables were compared with Student’s $t$-test. The $\chi^2$-test and Fisher test were used to compare clinical outcome between the two groups. The analysis was carried out using the statistical package for social sciences (SPSS Inc., USA). $P < 0.05$ was considered significant.

#### Results

To determine the impact that inclusion of GnRH antagonist in COS–IUI cycles may have on pregnancy rates, 82 patients were randomly divided as described in Materials and methods.
The rationale for this hypothesis was based on the capacity of the GnRH antagonist to rapidly inhibit LH release by the gonadotrophs and thereby control and avoid premature luteinization in IVF (Frydman et al., 1991; Diedrich et al., 1994; Reissmann et al., 1995; Olivennes et al., 2002). We thought that the same mechanism could be used in COS–IUI advantageously to avoid premature luteinization of the leading follicle(s) and to expand follicular development so that ovulation could be induced once follicle size was between 19 and 20 mm. Furthermore, since COS generally develops a cohort of follicles, avoiding premature luteinization with ganirelix acetate would increase the number of ovulatory follicles available for the IUI procedure and, hopefully, increase pregnancy rates (Nuojua-Huttunen et al., 1999; Dickey et al., 2002; Duran et al., 2002; Houmard et al., 2002; Kaplan et al., 2002).

Our results support this hypothesis since the inclusion of the GnRH antagonist (1.8 ± 0.7 ampoules) in the COS–IUI protocol significantly ($P < 0.05$) increased the number of mature follicles (≥16 mm) in the GnRH antagonist group with respect to the control group (2.4 ± 1.4 versus 1.7 ± 1.2 respectively). The total amount of FSH used by patients receiving GnRH antagonist (707 ± 240 total IU) was slightly greater than in the controls (657 ± 194 total units), but this difference was not significant (Table II).

Nevertheless, we were concerned by the increased cost of including the GnRH antagonist in the IUI protocols. We calculated that including this treatment would increase the cost of each cycle by a minimum of €100 and a maximum of €150. In return by using a GnRH antagonist that will significantly reduce the risk of cycle cancellation and the associated emotional stress to the patients.

We were aware of the risk that increasing the number of mature follicles could also increase multiple pregnancy rates (Dickey et al., 2001; Khalil et al., 2001; Tur et al., 2001), and, in fact, the only multiple gestation in this study was in the group treated with GnRH antagonist (Table III). For this reason, IUI was cancelled whenever a patient had more than four 16–20 mm follicles, regardless of how many follicles were <15 mm (Claman, 2004; Osuna et al., 2004). Several

**Discussion**

The purpose of this preliminary study was to determine if the inclusion of a GnRH antagonist (ganirelix acetate) in COS–IUI protocols could significantly increase the number of mature follicles and, by extension, improve pregnancy rates, since these two parameters are known to be positively correlated (Nuojua-Huttunen et al., 1999; Dickeys et al., 2002; Duran et al., 2002; Houmard et al., 2002; Kaplan et al., 2002).

As seen in Table I, no significant differences in basal serum levels (third and fourth days of their menstrual cycle) for FSH (5.5 ± 2.1 versus 6.1 ± 1.7), LH (4.7 ± 4.2 versus 6.1 ± 4.8) or E₂ (38.2 ± 15.4 versus 38.3 ± 12.7) were noted between the GnRH antagonist-treated group and the control group.

No significant differences in the total amount of rFSH (707 ± 240 versus 657 ± 194 total IU) and E₂ serum levels (428 ± 154 pg/ml versus 598 ± 167 pg/ml) were seen between the GnRH antagonist and the control groups (Table II).

Nevertheless, the number of mature follicles (≥16 mm in diameter) determined by ultrasound before beginning hCG was significantly higher in patients receiving GnRH antagonist than in the control group (2.4 ± 1.4 versus 1.7 ± 1.2, $P < 0.05$). As expected, cycle length was extended to almost 1 day more (7.6 ± 1.9 versus 6.6 ± 1.8 days) in the GnRH antagonist group than in the control group, although this difference was not statistically significant.

Pregnancy rates were significantly increased in the group of patients receiving the GnRH antagonist (38%) compared to the control group (14%). To date, except for one biochemical pregnancy in the control group, all the other pregnancies are ongoing. Furthermore, 93% of the pregnancies in the GnRH antagonist group (14/15) and 100% of the pregnancies in the control group (6/6) were single gestations. The only non-single pregnancy (triplets) occurred in the antagonist group.

**Table I.** Timing ovulation for intrauterine insemination with a GnRH antagonist

<table>
<thead>
<tr>
<th></th>
<th>GnRH antagonist</th>
<th>Control</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>40</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Age (years$^a$)</td>
<td>33.9 ± 2.6</td>
<td>32.05 ± 3.3</td>
<td>NS</td>
</tr>
<tr>
<td>Basal FSH (mIU/l)$^a$</td>
<td>5.5 ± 2.1</td>
<td>6.1 ± 1.7</td>
<td>NS</td>
</tr>
<tr>
<td>Basal LH (mIU/l)$^a$</td>
<td>4.7 ± 4.2</td>
<td>6.1 ± 4.8</td>
<td>NS</td>
</tr>
<tr>
<td>Basal estradiol (pg/ml)$^a$</td>
<td>38.2 ± 15.4</td>
<td>38.3 ± 12.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^a$Values are means ± SD. NS = not significant.

**Table II.** Timing ovulation for intrauterine insemination with a GnRH antagonist

<table>
<thead>
<tr>
<th></th>
<th>GnRH antagonist</th>
<th>Control</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of ampoules</td>
<td>1.8 ± 0.7</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>FSH (total IU)</td>
<td>707 ± 240</td>
<td>657 ± 194</td>
<td>NS</td>
</tr>
<tr>
<td>Days of stimulation</td>
<td>7.6 ± 1.9</td>
<td>6.6 ± 1.8</td>
<td>NS</td>
</tr>
<tr>
<td>Follicles ≥16 mm</td>
<td>2.4 ± 1.4</td>
<td>1.7 ± 1.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Estradiol</td>
<td>428 ± 154</td>
<td>598 ± 167</td>
<td>NS</td>
</tr>
<tr>
<td>Swim-up sperm (× 10⁹/ml)</td>
<td>23.4 ± 9.3</td>
<td>19.9 ± 18.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

All values are means ± SD. NS = not significant.

As calculated that including this treatment would increase the cost of each cycle by a minimum of €100 and a maximum of €150. In return by using a GnRH antagonist that will significantly reduce the risk of cycle cancellation and the associated emotional stress to the patients.

We were aware of the risk that increasing the number of mature follicles could also increase multiple pregnancy rates (Dickey, 2001; Khalil et al., 2001; Tur et al., 2001) and, in fact, the only multiple gestation in this study was in the group treated with GnRH antagonist (Table III). For this reason, IUI was cancelled whenever a patient had more than four 16–20 mm follicles, regardless of how many follicles were <15 mm (Claman, 2004; Osuna et al., 2004). Several
publications have indicated that the risk of multiple pregnancies rises with the number of 15 mm follicles (Tur et al., 2001; Dickey et al., 2001; Khalil et al., 2001; Ghosh et al., 2003) and some of the follicles we classified as 15 mm could actually have measured 16 mm (given the difficulties of distinguishing between a 16 or 15 mm follicle by ultrasound). However, our clinic’s historical average for multiple gestations using the criterion of ignoring the number of <15 mm follicles when deciding IUI is 20% (only twins, no triplets), so we followed the same rationale in this study and only one couple in the GnRH antagonist group had a multiple pregnancy.

As also described previously in IVF/GnRH antagonist cycles (Diedrich et al., 1994; European and Middle East Orgalutran Study Group, 2001; Fluker et al., 2001; Ricciarelli et al., 2003; Acevedo-Martin et al., 2004), E2 serum levels decreased in the GnRH antagonist–IUI group in respect to the control group (428 ± 154 versus 598 ± 167 respectively), although not significantly. This decrease did not affect oocyte differentiation (Hernández, 2000; Tur et al., 2001; Ricciarelli et al., 2003; Acevedo-Martin et al., 2004) since pregnancy rates were significantly increased in patients receiving the GnRH antagonist with respect to the controls (38 versus 14%). Furthermore, at the time of writing, all the pregnancies are ongoing in the GnRH antagonist group and there have been no miscarriages (Table III). However, it must be remembered that the increases in the pregnancy rate are probably not related to the antagonist itself but to the fact that the use of an antagonist gives the clinician more time to produce more ovulatory follicles that can give rise to a pregnancy.

Although Kolibianakis et al. (2003) report that patients receiving the GnRH antagonist during IVF cycles seem to present a dysfunctional endometrium, Ragni et al. (2001) have demonstrated that the luteal phase profile (progesterone concentration) in COS–IUI/GnRH antagonist cycles seems to be unaffected in IUI cycles treated with recombinant FSH in combination with GnRH antagonist. Nevertheless, to avoid a hypothetical effect of the antagonist on the function of the corpus luteum, we routinely supplemented the luteal phase with natural micronized progesterone in all the IUI procedures.

In conclusion, the addition of the GnRH antagonist to COS–IUI cycles significantly increased pregnancy rates in our patients. Since this increase seems to be related to the number of follicles recruited, clinicians should balance this benefit against the risk of multiple gestation in IUI.

Finally, because only a relatively small number of patients has been analysed, the results of this study are only preliminary. However, in our opinion some immediate emotional and clinical benefits (lower cancellation rates, avoidance of premature luteinization) can be obtained using the GnRH antagonist in the COS–IUI cycles.

References


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