GnRH agonist versus GnRH antagonist in oocyte donation cycles: a prospective randomized study

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BACKGROUND: The specific role of LH in folliculogenesis and oocyte maturation is unclear. GnRH antagonists, when administered in the late follicular phase, induce a sharp decrease in serum LH which may be detrimental for IVF outcome. This study was performed to evaluate whether the replacement of GnRH agonist (triptorelin) by a GnRH antagonist (ganirelix; NV Organon) in oocyte donation cycles has any impact on pregnancy and implantation rates.

METHODS: A total of 148 donor IVF cycles was randomly assigned to use either a GnRH antagonist daily administered from the 8th day of stimulation (group I) or a GnRH agonist long protocol (group II) for the ovarian stimulation of their donors. The primary endpoints were the pregnancy and the implantation rates.

RESULTS: The clinical pregnancy rate per transfer (39.72%, 29/73 versus 41.33%, 31/75) based on transvaginal scan findings at 7 weeks of gestation, the implantation rate (23.9 versus 25.4%) and the first trimester abortion rate (10.34 versus 12.90%) were similar in the two groups.

CONCLUSION: In oocyte donation cycles the replacement of GnRH agonist by a GnRH antagonist appears to have no impact on the pregnancy and implantation rates when its administration starts on day 8 of stimulation.

Key words: GnRH agonist/GnRH antagonist/IVF/oocyte donor/ovarian stimulation

Introduction

In oocyte donation cycles, synchronization of the donor and recipient is required (Prapas et al., 1998a). In order to allow synchronization between donor and recipient and to prevent the donor’s premature LH rise, GnRH agonists have been used to down-regulate the donor’s pituitary (Sauer et al., 1996). Recently, GnRH agonists have been replaced by GnRH antagonists in oocyte donation cycles (Lindheim and Morales, 2003; Ricciarelli et al., 2003).

In contrast with GnRH agonists, the GnRH antagonists suppress gonadotrophins within a few hours by dose-dependent effect and through competitive inhibition of the pituitary GnRH receptors (Albano et al., 1996). The use of GnRH antagonists provides several advantages for the donor, including a shorter duration of stimulation, no increased risk of cyst formation, and no increase in problems with hormonal withdrawal symptoms. (Felberbaum et al., 1999). However, it has been reported that both granulosa and endometrial cells and the embryo harbour GnRH receptors, and hence, a direct effect from the GnRH antagonist on these cells may be a possible cause for implantation failure (Hernandez, 2000).

Oocyte donation provides a unique model to eliminate confounding variables that typically occurs when comparing groups of patients undergoing IVF. It has been reported that the replacement of a GnRH agonist by a GnRH antagonist administered from day 6 of ovarian stimulation onwards, might adversely affect success rates in oocyte donation cycles (Lindheim and Morales, 2003; Ricciarelli et al., 2003). The reduced pregnancy rate has been attributed to a decrease in serum estradiol levels before hCG injection, when antagonist was administered for >3 days (Lindheim and Morales, 2003). It has also been reported that in IVF cycles low LH concentration on day 8 of stimulation has a detrimental effect on pregnancy outcome in women down-regulated with low doses of the GnRH agonist buserelin and that circulating LH concentrations above a certain critical level are required for optimal oocyte maturation (Westergaard et al., 2000).

In order to minimize the duration of GnRH antagonist administration and avoid high LH suppression on day 8 of stimulation, we decided to postpone its conventional starting day by 2 days (8th day of stimulation). In this regard, a prospective randomized study was designed to compare the pregnancy and implantation rate in oocyte donor cycles using a GnRH antagonist, daily administered from the 8th day of controlled ovarian stimulation, or a GnRH agonist (long protocol).
Materials and methods

A prospective, randomized, single blind study was performed. Oocyte donation cycles were randomized into group I (GnRH antagonist was used during the donor stimulation) or group II (GnRH agonist was used during the donor stimulation) by an allocation sequence generated from a computerized random number table. The study was approved by the Institutional Review Board and informed consent was obtained from all women.

Ninety-eight oocyte donors were evaluated at the Iakentro IVF centre from January 2003 to December 2003. A detailed medical history was taken. The oocyte donors were ≥32 years old, had body mass index <30 kg/m², regular menstrual cycles of 25–35 days, two normal ovaries based on transvaginal scan findings, no polycystic ovaries, no endometriosis, no gynaecological or medical disorders and agreed to donate their oocytes for treatment anonymously and altruistically. Blood sample was collected for karyotype and screening for previous viral infection (hepatitis B and C, human immunodeficiency virus, cytomegalovirus and syphilis).

A total of 148 recipient cycles was included in the study (some women were used twice as donors). All recipients were ≥51 years old and had treatment for ovarian failure or failed assisted conception treatments due to poor response to ovarian stimulation. The recipients and their partners underwent blood screening similar to the donors, while a hysterosalpingogram and a diagnostic hysteroscopy had eliminated cases presenting hydrosalpinx or intrauterine pathology. The recipients had a mock transfer in a cycle previous to IVF and if difficulty was encountered a cervical dilatation was performed (Prapas et al., 2004).

All donors prior to cycle stimulation received 1–2 months treatment with oral contraceptives (Gynofen; Shering Hellas) for cycle synchronization. In the agonist group the stimulation protocol for controlled ovarian stimulation consisted of GnRH agonist (triptorelin, s.c. 0.1 mg/day) beginning in the afternoon of the 21st day of the previous cycle. Triptorelin s.c. 0.05 mg/day and recombinant (r)FSH (Puregon; NV Organon) 300 IU per day were begun on day 2 of menstruation or later when estradiol was ≥50 pg/ml. In the antagonist group rFSH (Puregon; NV Organon) 300 IU per day was begun on day 2 of the cycle and a GnRH antagonist (Orgalutran 0.25 mg; NV Organon) was added in the afternoon of the 8th day of stimulation. Cycles were monitored using vaginal ultrasound scanning and serum estradiol levels beginning the second day of menstruation and repeated after 3 days of stimulation. The daily dose of recombinant FSH was adjusted according to the donor’s ovarian response based on serum estradiol levels and the number and size of ovarian follicles as measured by transvaginal ultrasonography. hCG 10 000 IU was administered when three or more follicles ≥17 mm mean diameter were present on ultrasound and serum estradiol level ≥1500 pg/ml. Neither agonist nor antagonist was given the day of hCG administration.

For all recipients who were still cycling, down-regulation was carried out using a GnRH agonist (triptorelin, s.c. 0.1 mg/day) beginning on the 21st day of the previous cycle. The day that the donor announced the onset of her period, the recipient was informed to start triptorelin s.c. 0.05 mg/day and estradiol valerate 2 mg per day for the first 4 days, 4 mg per day for days 5–8 and 6 mg per day until the pregnancy test. Twelve hours before the oocyte retrieval, 200 mg of progesterone (Utrogestan) was given to the recipient intravaginally and continued with 200 mg, three times a day, until fetal heart beat was observed by ultrasound (Prapas et al., 1998b). When progesterone started, triptorelin administration was stopped. The recipients without menstruation followed the same protocol without triptorelin. Endometrial development was evaluated by ultrasound scan and it was considered mature when the endometrial thickness was ≥9 mm.

Transvaginal, ultrasound guided, oocyte retrieval was performed under i.v. sedation and local anaesthesia, 34–36 h following the administration of hCG. Donors with mild hyperstimulation (Golan et al., 1989) received a GnRH antagonist (Orgalutran 0.25 mg; NV Organon) for 5 days starting just after the oocyte retrieval. ICSI was used to fertilize all oocytes 4–6 h after retrieval and three good quality embryos at maximum were transferred 72 h later, under ultrasound control as described previously (Prapas et al., 1995). All cases included in the study had at least one good quality embryo. Classification of embryos was based on either the number of blastomeres or the developmental stage as described elsewhere (Prapas et al., 2001). A pregnancy test was performed 15 days after embryo transfer, and, if positive, an ultrasound scan was scheduled 2 weeks later to determine the number and status of implanted embryos. The concurrency of a positive β-hCG test and a fetal heart beat (seen by ultrasound) was defined as a clinical pregnancy.

Statistical analysis

Based on preliminary data, power analysis calculations showed that 14 738 cycles would be needed for each group in the study in order to achieve statistical significance at a 5% level with power 80%, while statistical significance with power 95% would be achieved with 15 625 cycles in each group. However, the limited number of donor cycles performed annually in our IVF centre rendered the above numbers impossible. Consequently, a 1 year study was conducted to compare the two protocols. The χ²-test was used to compare pregnancy and implantation rates between different groups. P < 0.05 was considered statistically significant. The t-test was used where appropriate. Values are expressed as mean ± SD.

Results

Data were prospectively collected for 148 IVF cycles of the Iakentro’s oocyte donation programme from January 2003 to December 2003. A flow chart of inclusion, randomization and drop-out of oocyte donation cycles is shown in Figure 1.

A total of 148 recipients received oocytes from 98 donors. In 73 cycles the donors were stimulated using the GnRH antagonist protocol (group I) whereas 75 cycles were given the GnRH agonist protocol (group II) described in Materials and methods.

All recipients were nulliparous. The mean age of the donors and recipients was 28 ± 2.3 and 47 ± 3.2 years for the GnRH antagonist group and 27 ± 1.9 and 48 ± 2.1 years for the GnRH agonist group respectively. In the GnRH agonist group, two out of 73 cases (2.73%) had premature LH surge (LH > 10 IU/l and progesterone > 1.2 ng/ml) whereas three cases (4.1%) did not achieve embryo transfer due to donors’ poor ovarian response (two cases) and immaturity of all oocytes retrieved (one case). Oocytes were obtained from all donors who presented premature LH surge but no pregnancy was achieved. The mean duration of antagonist administration was 1.86 ± 0.73 (1–3 days). In the GnRH agonist group, two out of 75 cases (2.66%) presented an ovarian cyst on the second day of their stimulated cycle, the cyst being aspirated under ultrasound control in all cases without complications. In four cases out of 75 (5.33%) embryo transfer was not carried out due to the donors’ poor ovarian response.
In groups I and II respectively the days of stimulation (9.3 ± 1.5 versus 9.6 ± 1.4), the recombinant FSH dose used (2052.1 ± 375.05 versus 2138 ± 407.3 IU), the donors’ mean serum estradiol level (1900 ± 562 versus 2140 ± 730 pg) and the number of oocytes donated (13.8 ± 3.2 versus 14.3 ± 2.7) were similar. The fertilization rate (73 versus 79%), the mean number of embryos transferred (2.34 ± 0.77 versus 2.36 ± 0.73) and the embryo quality were similar in both groups as assessed by scoring (Veeck, 1998) (Table I). Donors presented mild hyperstimulation in two out of 73 cycles (2.73%) in group I and in three out of 75 cycles in group II (4%). None of the donors had severe hyperstimulation.

The clinical pregnancy rate per cycle started (39.72%, 29/73 versus 41.33%, 31/75) based on transvaginal scan findings at 7 weeks of gestation, the implantation rate (23.9 versus 25.4%), the first trimester abortion rate (10.34%, 3/29 versus 12.90%, 4/31) and the biochemical pregnancy rate (1.36%, 1/73 versus 2.66%, 2/75) were similar in groups I and II respectively (Table I).

Table I. Comparing data between the GnRH antagonist group (group I) and the GnRH agonist group (group II)

<table>
<thead>
<tr>
<th></th>
<th>Group I (n = 73)</th>
<th>Group II (n = 75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor’s age (years)a</td>
<td>28±2.3</td>
<td>27±1.9</td>
</tr>
<tr>
<td>Recipient age (years)a</td>
<td>47±3.2</td>
<td>48±2.1</td>
</tr>
<tr>
<td>Total recombinant FSH (IU)a</td>
<td>2052.1±375.05</td>
<td>2138±407.3</td>
</tr>
<tr>
<td>Days of stimulationa</td>
<td>9.3±1.5</td>
<td>9.6±1.4</td>
</tr>
<tr>
<td>Days of antagonist administrationa</td>
<td>1.86±0.73</td>
<td></td>
</tr>
<tr>
<td>Estradiol (pg/ml)a</td>
<td>1900±562</td>
<td>2140±730</td>
</tr>
<tr>
<td>Oocytes donateda</td>
<td>13.8±3.2</td>
<td>14.3±2.7</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>73</td>
<td>79</td>
</tr>
<tr>
<td>Embryos transferreda</td>
<td>2.34±0.77</td>
<td>2.36±0.73</td>
</tr>
<tr>
<td>Embryonic scorea</td>
<td>4.60±0.78</td>
<td>4.56±0.75</td>
</tr>
<tr>
<td>No transfer</td>
<td>3 (4.1)</td>
<td>4 (5.33)</td>
</tr>
<tr>
<td>Mild hyperstimulation</td>
<td>2 (2.73)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Clinical pregnancy/cycle started</td>
<td>29 (39.72)</td>
<td>31 (41.33)</td>
</tr>
<tr>
<td>Implantation (%)</td>
<td>23.9</td>
<td>25.4</td>
</tr>
<tr>
<td>Twins</td>
<td>3 (10.34)</td>
<td>4 (12.9)</td>
</tr>
<tr>
<td>Triplet</td>
<td>1 (3.44)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>First trimester abortion</td>
<td>3 (10.34)</td>
<td>4 (12.90)</td>
</tr>
<tr>
<td>Biochemical pregnancy</td>
<td>1 (1.66)</td>
<td>2 (2.6)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.

aMean ± SD.

No differences were statistically significant.

Discussion

This study has demonstrated that implantation rates, pregnancy rates and overall mean embryo development did not differ in oocyte donation cycles that a GnRH agonist is replaced by a GnRH antagonist daily administered from the 8th day of stimulation. In our study, a high starting dose of 300 IU rFSH for the first 3 days (Remohi et al., 1997; Garcia-Velasco et al., 2004) and ICSI procedure was used in all cases, in order to avoid unexpected poor responses, fertilization failures and statistical biases.

Two previous studies have reported adverse effects of replacing a GnRH agonist by a GnRH antagonist (Lindheim and Morales, 2003; Ricciarelli et al., 2003). Lindheim and Morales (2003) in 37 donor cycles, starting GnRH antagonist administration on day 6 of stimulation, reported that 35% of donor cycles had a decrease in serum estradiol prior to hCG administration and 93% of them showed a decrease in serum estradiol at >3 days after GnRH antagonist administration. They concluded that the use of GnRH antagonists has an unpredictable effect on estradiol production during follicular recruitment which appears to adversely affect pregnancy outcome if a decline in serum estradiol occurs. Ricciarelli et al. (2003), comparing GnRH antagonist administration starting on day 6 of donor stimulation (cetrorelix 0.25 mg per day) versus GnRH agonist (triptorelin 3.75 mg on 21st day of previous cycle) in their oocyte donation programme reported a non-significant decrease in pregnancy rates for the GnRH antagonist group (52 versus 43%), while they noted a statistically significant lower estradiol level, implantation rate and higher biochemical pregnancy rate (P < 0.05) for the antagonist group. They concluded that the small decrease in the pregnancy rates seen in the GnRH antagonist donor cycles could be of oocyte or embryonic origin, and may explain the significant decrease reported in implantation rates (15 versus 32% P < 0.05).

The findings in our donor oocyte programme are different to those found in the above studies. No significant differences were found in estradiol level on the day of hCG and the number of oocytes donated, comparing the ovarian response between donors treated with the GnRH antagonist (ganirelix 0.25 mg/day starting on the 8th day of stimulation) and those
treated with GnRH agonist (triptorelin 0.1 mg/day starting on 21st day of previous cycle). Equally, fertilization rates, embryonic score and number of embryos transferred were similar. The clinical pregnancy rates, the implantation rates, the abortion rates and the biochemical pregnancy rates were not different between the two groups. Furthermore the percentage of women failing to achieve embryo transfer was similar between the two groups while the percentage of premature LH surge (2.73%) presented in the antagonist group was similar to the 2.8% reported by the European Orgalutran Study Group (2000). Our results are in accordance with those reported by Sauer et al. (1997), who in a small study of 15 oocyte donor cycles concluded that the replacement of the GnRH agonist (Leuprolide) by a GnRH antagonist (Nal-Glu) in an oocyte donation programme did not affect the pregnancy rates.

There are several possible explanations for these findings. There is an obvious difference in the serum estradiol levels between the above-mentioned studies. Lindheim and Morales (2003) reported plateau or decrease in serum estradiol level in some donor cycles, 3 days after starting GnRH antagonist administration. In the study by Ricciarelli et al. (2003) the GnRH antagonist group presented a statistically significant lower serum estradiol level on the day of hCG administration in comparison to the GnRH agonist group. In our study by the day that hCG was scheduled (follicles ≥17 mm) serum estradiol levels were lower in donors treated with GnRH antagonist than in the donors treated with GnRH agonist, although this difference was not statistically significant. It is important to note that the estradiol levels in donors receiving GnRH antagonist vary from donor to donor in an unpredictable manner. Obviously in our study the estradiol results were pooled, but from all our donors none of them presented a decrease or plateau in serum estradiol level after starting GnRH antagonist administration.

In IVF cycles, the starting day of GnRH antagonist administration in the conventional daily treatment with 0.25 mg has been fixed at day 6 of rFSH stimulation (European Middle East Orgalutran Study Group, 2001). The lower pregnancy rate consistently observed in the antagonist group during Phase III comparative trials between agonists and antagonists has resulted in a tendency to modify existing GnRH antagonist protocols in order to improve the reproductive outcome (Al-Inany and Aboulghar, 2002). Ludwig et al. (2002) proposed a tailored approach to the administration of the GnRH antagonists according to follicular size. They concluded that tailoring of GnRH antagonist protocols leads to an optimization of ovarian stimulation with more oocytes retrieved with the same monitoring visits. Kolibianakis et al. (2003) reported that in patients with no follicle of ≥15 mm after 5 days of ovarian stimulation with rFSH, a significantly lower implantation rate was observed if the antagonist was delayed beyond the 6th day of stimulation as compared with fixed antagonist administration on day 6 of stimulation. This difference was not related to the occurrence of premature LH rise nor to the levels of serum estradiol, progesterone or LH on the day of hCG administration, but it was suggested that the high levels of LH and estradiol found in the flexible group before the antagonist administration was started might have adversely affected the implantation rate mainly by altering endometrial receptivity. In a preliminary study, Thong et al. (2003) starting the GnRH antagonist administration on the 8th day of cycle (4th day of stimulation) in their oocyte donation programme reported 50% pregnancy rate. Eliminating the endometrial factor by using recipients in our study we have shown that the replacement of GnRH agonist by a GnRH antagonist daily administered from day 8 of stimulation appears to have no impact on the oocyte maturation, fertilization rate, embryo development, pregnancy and implantation rates. In our donors the use of contraception for one or two cycles before the ovarian stimulation started might have lowered the LH and estradiol levels in the early follicular phase of stimulation.

The dose-finding study of ganirelix (Ganirelix dose-finding study group, 1998) reported that the extent of pituitary LH suppression in IVF cycles does not affect follicular growth. Additionally, Oberye et al. (1999) have shown that a steady state of GnRH antagonist (ganirelix) is reached within 2–3 days of treatment and that maximal suppression of endogenous LH production occurs shortly (4 h) after each injection. In donor oocyte cycles though, Lindheim and Morales (2003) reported a possible decline in serum estradiol levels due to LH over-suppression, resulting in adverse outcome when the GnRH antagonist was administered for >3 days. Recently, in another study with donor oocyte cycles it has been reported that LH supplementation, when the GnRH antagonist administration is initiated, increases pregnancy rates (Acevedo et al., 2004). Data from Westergaard et al. (2000) suggest that low LH concentrations on day 8 of stimulation have a detrimental effect on pregnancy outcome in women down-regulated with low doses of the GnRH agonist buserelin. By starting the GnRH antagonist administration on day 8 of donor stimulation in our study the days of LH suppression are decreased to ≤3 and any possible high LH suppression before the 8th day of stimulation which could adversely affect the pregnancy outcome was eliminated.

In conclusion, this study has shown that in oocyte donation cycles the replacement of GnRH agonist by a GnRH antagonist has no impact on the pregnancy and implantation rates when its administration starts on day 8 of stimulation. However, the fact that there was no statistically significant difference might be due to the sampling size. We recommend the use of GnRH antagonist in the oocyte donation cycles because it is better tolerated by the donors and simplifies the management of the donor.

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