Effect of reduced dose of triptorelin at the start of ovarian stimulation on the outcome of IVF: a randomized study

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BACKGROUND: Partial pituitary desensitization using gonadotrophin-releasing hormone (GnRH) agonists may be sufficient in women undergoing controlled ovarian hyperstimulation for assisted reproduction. However, the minimal effective agonist dose remains to be determined. The aim of the study was to investigate the effect of a reduced daily dose of triptorelin, administered at the start of ovarian stimulation, on the results of IVF and intracytoplasmic sperm injection. METHODS: A total of 132 patients was randomized in two groups. Pituitary desensitization was obtained in group 1 (66 patients) with a single 3.75 mg injection (i.m.) of triptorelin. In group 2, 66 patients received 100 µg triptorelin daily, which was then reduced to 50 µg at the start of follicle-stimulating hormone (FSH) stimulation. RESULTS: No significant differences were found in terms of pregnancy rate per transfer (38% in group 1 versus 34.9% in group 2), implantation rate (20.2 versus 18%) and abortion rate (8.3 versus 9.1%). The number of FSH ampoules used, as well as the number of days stimulation required, was significantly reduced in group 2 (41 ± 26 versus 46.6 ± 25.3, P < 0.03 and 11 ± 1.3 versus 11.8 ± 1.5, P < 0.002 respectively). No significant differences were seen in oestradiol concentrations and in follicle number, in the quantity of oocytes collected and fertilized, or in the number of embryos obtained or transferred. CONCLUSION: A reduced dose of triptorelin is enough for pituitary suppression during ovarian stimulation but provides no significant improvement in IVF cycle outcome when compared with depot formulation. The possibility of a shorter treatment protocol requiring lower amounts of gonadotrophins should be considered in view of its economic advantage.

Key words: GnRH-agonist/IVF/ovarian stimulation/reduced dose/triptorelin

Introduction

Over the last ten years, pre-treatment with gonadotrophin-releasing hormone (GnRH) agonists in women undergoing controlled ovarian hyperstimulation (COH) for assisted reproduction has become very common in almost all IVF centres. The suppression of pituitary gonadotrophin secretion before and during induction of multiple follicular growth has some benefits, including: (i) prevention of premature luteinizing hormone (LH) surge and luteinization, resulting in a lower cancellation rate; (ii) improvement in follicular recruitment with a larger number of oocytes recovered both in normal and poor responders; (iii) improvement in routine organization of assisted reproduction (Zorn et al., 1987). The widespread use of long-acting GnRH agonist formulations has increased patients’ and clinicians’ convenience. However, it has been suggested that complete pituitary desensitization achieved by long-term use of GnRH agonist has the disadvantages of inducing slower and lower ovarian response (especially in low responders) compared with gonadotrophins alone, requires a higher number of gonadotrophin ampoules (Ben-Rafael et al., 1991), and has a long lasting, potentially detrimental, effect in the luteal phase. What is more, GnRH agonist has been shown to have a direct effect on ovarian steroidogenesis (Casper and Yen, 1979; Tureck et al., 1982; Parinaud et al., 1988) and specific GnRH receptors have been found in murine and human granulosa cells (Bramley et al., 1985; Latouche et al., 1989).

Several authors have suggested that partial pituitary desensitization in an assisted reproduction procedure might be sufficient (Balasch et al., 1992a; Simon et al., 1994), but the minimal effective dose of GnRH agonists needed to avoid premature LH surge during ovarian stimulation has not yet been clearly determined. Some authors (Broekmans et al., 1996; Janssens et al., 1998) showed, with the use of GnRH challenge tests, that comparable degrees of suppression of the pituitary can be achieved with either 100 or 50 µg/day triptorelin. Lower doses such as 25 µg/day (Broekmans et al., 1996) or 15 µg/day (Janssens et al., 1998) still give noteworthy pituitary suppression, but the clinical usefulness of such low doses in avoiding LH surge in ovarian stimulation has yet to be demonstrated. A recent report (Janssens et al., 2000) showed...
no improvement in pregnancy rate using 15 µg triptorelin daily versus 50 or 100 µg daily.

It has been demonstrated that once the pituitary is suppressed, the dose of GnRH agonist needed to prevent an LH surge decreases with the length of treatment (Sadow and Donnez, 1990). Lowering the dose of triptorelin at the start of stimulation from 0.5 mg per day to 0.1 mg per day seems to be as effective as continuing with 0.5 mg per day (Balasch et al., 1992b; Simon et al., 1994).

Feldberg et al. (1994) compared three doses of triptorelin administered in a long luteal protocol in women with previous poor response to gonadotrophins: 3.75 mg in a single i.m. dose; 0.5 mg s.c. per day decreased to 0.1 mg s.c. per day; 0.1 mg s.c. per day decreased to 0.05 mg s.c. per day (Feldberg et al., 1994). In the lowest dose group they found higher oestradiol concentrations, larger numbers of oocytes collected/fertilized and more embryos transferred, as well as a lower cancellation rate and a higher, although not significant, pregnancy rate. A lower number of human menopausal gonadotrophin (HMG) ampoules and duration of stimulation were also found in this group.

The aim of the present study was to investigate whether a reduced daily dose of triptorelin at the start of ovarian stimulation produced better results versus long-acting triptorelin, in patients undergoing IVF or intracytoplasmic sperm injection (ICSI) procedures.

Materials and methods

Protocol

From September 1998 to September 1999, 132 women undergoing COH for IVF or ICSI were enrolled in the study. All subjects were aged between 25 and 38 years with infertility caused by tubal, idiopathic or male factors. Cases with active endometriosis or only idiopathic or male factors. Cases with active endometriosis or only one ovary, or with follicle-stimulating hormone (FSH) concentrations >17 mm in diameter) on the day of desensitization was performed with a single i.m. injection of triptorelin, 3.75 mg (Decapeptyl® 3.75; Ipsen Spa, Milan, Italy), on day 21 of the cycle preceding treatment.

Group 2 patients (66 patients) received a daily s.c. injection of 100 µg triptorelin (Decapeptyl® 0.1, Ipsen Spa), starting from day 21 of the cycle preceding treatment. At the onset of menses (start time of FSH stimulation) the dose was reduced to 50 µg s.c. daily until the day of human chorionic gonadotrophin (HCG) administration.

At the start of menses, patients in both groups began ovarian stimulation with low dose triptorelin (100 µg s.c./day, decreased to 50 µg /day at the beginning of FSH stimulation) and with depot triptorelin (3.75 mg i.m. in single dose).

Table 1. Results of ovarian stimulation with low dose triptorelin (100 µg s.c./day, decreased to 50 µg /day at the beginning of FSH stimulation) and with depot triptorelin (3.75 mg i.m. in single dose)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Group 1</th>
<th>Group 2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Triptorelin</td>
<td>Triptorelin</td>
<td>P value</td>
</tr>
<tr>
<td></td>
<td>3.75 mg</td>
<td>100/50 µg</td>
<td></td>
</tr>
<tr>
<td>No. of FSH ampoules</td>
<td>46.6 ± 25.3</td>
<td>41.0 ± 26.0</td>
<td>0.03</td>
</tr>
<tr>
<td>No. of days of stimulation</td>
<td>11.8 ± 1.5</td>
<td>11.0 ± 1.3</td>
<td>0.002</td>
</tr>
<tr>
<td>No. of follicles</td>
<td>19.2 ± 7.3</td>
<td>18.3 ± 8.9</td>
<td>NS</td>
</tr>
<tr>
<td>No. of follicles &gt;17 mm</td>
<td>9.5 ± 3.3</td>
<td>8.8 ± 4.0</td>
<td>NS</td>
</tr>
<tr>
<td>No. of oocytes</td>
<td>16.5 ± 6.8</td>
<td>14.4 ± 6.0</td>
<td>NS</td>
</tr>
<tr>
<td>No. of mature oocytes</td>
<td>8.8 ± 5.1</td>
<td>6.9 ± 5.3</td>
<td>0.04</td>
</tr>
<tr>
<td>No. of inseminated oocytes</td>
<td>13.2 ± 4.4</td>
<td>11.8 ± 4.4</td>
<td>NS</td>
</tr>
<tr>
<td>No. of fertilized oocytes</td>
<td>8.7 ± 3.7</td>
<td>7.7 ± 3.8</td>
<td>NS</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>65.4%</td>
<td>65.1%</td>
<td>NS</td>
</tr>
<tr>
<td>No. of cleaved embryos</td>
<td>8.4 ± 3.7</td>
<td>7.6 ± 3.7</td>
<td>NS</td>
</tr>
<tr>
<td>No. of transferred embryos</td>
<td>2.2 ± 0.6</td>
<td>2.2 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>No. of frozen embryos</td>
<td>4.4 ± 3.2</td>
<td>4 ± 3.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD. NS = not significant.

Transvaginal oocyte retrieval was performed under ultrasound guidance 34 h after HCG administration.

Two days after oocyte retrieval, a maximum of two embryos in women aged <35 years and a maximum of three in women aged >35 years were replaced via transcervical route. All remaining embryos presenting adequate morphology were cryopreserved for future use.

Luteal phase was sustained with natural progesterone in oil (Prontogest®; Amsa, Firenze, Italy), 50 mg i.m. daily from day 1 after oocyte retrieval.

Pregnancy was defined as the presence of one or more gestational sacs detected on ultrasound scan performed at least 4 weeks after embryo transfer. Biochemical pregnancies (a rise of β-HCG with no further evidence of gestational sac on ultrasound scan) were not considered.

Parameters evaluated

The primary endpoint was the total number of oocytes retrieved from patients who received HCG. The following secondary endpoints were also recorded: number of FSH ampoules used, number of days of stimulation, 17β-oestradiol concentration on the day of HCG administration, LH on day 7 of stimulation and on the day of HCG administration, progesterone on the day of HCG administration, number of follicles (total and >17 mm in diameter) on the day of HCG administration, number of oocytes collected, number of mature oocytes, fertilization rate, number of cleaved embryos, number of embryos transferred or frozen, number of clinical pregnancies, miscarriages and multiple pregnancies.

Sample size

A sample size of 66 patients in each group would have 90% power and a significance level of 0.05 to detect a difference in the mean number of oocytes of 2.3 (SD 6.5).

Assignment

Randomization was performed on an individual basis using sealed envelopes containing the name of one of the two treatments. The assignment took place when eligible patients agreed to participate, about 2 weeks before the day of triptorelin administration. Each envelope and allocation was sequentially numbered. The contents of the envelopes were only known to medical staff who had no involvement with the trial.
Low dose triptorelin and in-vitro fertilization

Statistical analysis
The analysis was performed with an SPSS program on an intention-to-treat basis and included all randomized patients who received HCG. Descriptive statistics have been performed for each variable, quantitative results were presented by using mean and standard deviations, qualitative results were summarized by using distribution of frequencies.

Before comparing the two groups, each variable was tested in order to check the normality distribution using Kolmogorov–Smirnov test, the comparisons of means was performed using a two-sample unpaired t-test or Mann–Whitney test. Proportions for the two groups were compared using a χ² test and a Fisher’s exact test. A value of P < 0.05 was considered to be statistically significant.

The results being presented here represent the first interim analysis of this study, which is still ongoing.

The 95% confidence interval (CI) around the point estimates of the effect of treatment were calculated for all the primary and secondary endpoints.

Results
Participant flow and follow up
A total of 132 patients was randomized into the study; 66 into depot triptorelin group (group 1) and 66 into daily triptorelin group (group 2). Patients’ mean age was comparable in the two groups: 33 ± 3.6 years in group 1 and 33.8 ± 3.1 years in group 2 (Table I). Of the 132 randomized patients, 128 had oocyte retrieval and 126 completed the study up to embryo transfer. In total four cycles were cancelled before oocyte retrieval for poor response to stimulation and were not included in efficacy analyses (two in both groups). Figure 1 shows the participant flow and follow-up.

IVF was performed in 33 patients in group 1 and in 35 patients in group 2. ICSI was performed in 31 patients in group 1 and in 29 patients in group 2. One patient in group 1 and one in group 2 did not undergo embryo transfer because of failed fertilization.

Table I shows the characteristics of the ovarian stimulation in the two groups of patients. The number of FSH ampoules used was lower in group 2 (41 ± 26 versus 46.6 ± 25.3 in group 1, P < 0.03). Days of stimulation were significantly lower in group 2 (11 ± 1.3 versus 11.8 ± 1.5, P < 0.002). No significant difference was seen in oestradiol concentrations and in the number of follicles (total and >17 mm) seen on the day of HCG administration or of total oocytes collected. The number of mature oocytes was significantly higher in the 3.75 triptorelin group (Table I), but no differences were detected in the number of fertilized oocytes or embryos obtained/transfered.

If ICSI patients only (in which an evaluation of oocyte nuclear maturity is feasible) are considered, no significant differences were found between the two groups in the number of metaphase II oocytes (12 ± 4.6 in group 1 versus 11 ± 3.5 in group 2).

LH concentrations were lower in the depot triptorelin group: on day 7 of stimulation the difference was not significant (0.91 ± 0.48 IU/l in group 1 versus 1.06 ± 0.41 IU/l in group 2: P = 0.06), but it became highly significant on the day of HCG.
In light of the above, using low doses of this kind of drug signifi-
cantly increased GnRH release in responders, with no adverse effect on the quality of ovarian response and a need for higher gonadotrophin dosage for response with low dose GnRH agonist. In our study ovarian response was reduced in poor responder patients, resulting in reduced ovarian stimulation phase and a lower number of FSH ampoules used. However, unlike the above reports, we did not see excessive pituitary suppression, especially in our depot formulation, which usually induces pituitary LH surge decreases with the length of treatment (Sandow and Donnez, 1990). Halving the dose of a daily administered GnRH agonist at the beginning of stimulation has been successfully performed both in normal (Elgendy et al., 1998) and poor (Feldberg et al., 1994; Olivennes et al., 1996) responders, with no adverse effect on the quality of ovarian response to stimulation.

Our results, in agreement with Feldberg (Feldberg et al., 1994) and Olivennes (Olivennes et al., 1996), show a shorter stimulation phase and a lower number of FSH ampoules administered in the low dose s.c. GnRH agonist group. However, unlike the above reports, we did not find better ovarian response with low dose GnRH agonist. In our study ovarian stimulation did not change with the two different GnRH agonist regimens, showing similar oestradiol concentrations at the beginning of FSH stimulation) and with depot triptorelin (3.75 mg i.m. in single dose)

Table III. Comparison of clinical results with low dose triptorelin (100 µg s.c./day, decreased to 50 µg /day at the beginning of FSH stimulation) and with depot triptorelin (3.75 mg i.m. in single dose)

<table>
<thead>
<tr>
<th></th>
<th>Group 1: Triptorelin 3.75 mg</th>
<th>Group 2: Triptorelin 100/50 µg</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IVF</td>
<td>ICSI</td>
<td>Total</td>
</tr>
<tr>
<td>No. of retrievals</td>
<td>33</td>
<td>31</td>
<td>64</td>
</tr>
<tr>
<td>No. of transfers</td>
<td>33</td>
<td>30</td>
<td>63</td>
</tr>
<tr>
<td>Pregnancy rate/transfer (%)</td>
<td>14 (42.4)</td>
<td>10 (33.3)</td>
<td>24 (38)</td>
</tr>
<tr>
<td>Miscarriages (%)</td>
<td>2 (14.3)</td>
<td>0</td>
<td>2 (8.3)</td>
</tr>
<tr>
<td>Ectopic pregnancy (%)</td>
<td>1 (7.1)</td>
<td>0</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>No gestational sacs</td>
<td>17</td>
<td>12</td>
<td>29</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>22</td>
<td>18.2</td>
<td>20.2</td>
</tr>
</tbody>
</table>

NS = not significant.

Discussion

The most widely used protocol for administration of GnRH analogues in assisted reproduction centres today involves the use of depot formulation, which usually induces pituitary suppression for a long time after oocyte retrieval. Two studies showed that recovery of pituitary function begins only 8 weeks after a single dose injection of depot triptorelin (Porcu et al., 1994) or depot leuprorelin (Porcu et al., 1995). In contrast, an almost normal response to GnRH challenge test was seen 1 week after discontinuation of a daily administered form of GnRH agonist (Porcu et al., 1994, 1995).

A key benefit of GnRH agonist lies in its inhibiting effect on the endogenous secretion of LH. However, concern has been raised about excessive pituitary suppression, especially in poor responder patients, resulting in reduced ovarian response and a need for higher gonadotrophin dosage for stimulation. Furthermore, it is now well known that GnRH analogues have extra-pituitary collateral effects, including a direct effect on ovarian steroidogenesis. An inhibitory action has been suggested by various authors with in-vitro studies (Tureck et al., 1982; Parinaud et al., 1988; Smitz et al., 1992).

In light of the above, using low doses of this kind of drug would seem appropriate (Loumaye et al., 1989), though the minimal effective dose of GnRH agonists to prevent premature LH surge during ovarian stimulation has still not been clearly defined.

Some authors have suggested that only partial pituitary desensitization in an assisted reproduction procedure might be sufficient and have put forward protocols using reduced GnRH agonist doses. As regards depot formulation, both the full (3.75 mg) and half (1.87 mg) dose of GnRH agonist triptorelin seem to be equally effective in pituitary desensitization, with similar duration for both desensitization and recovery time (Balasch et al., 1992a).

No difference in terms of stimulation quality and IVF outcome was reported (Porcu et al., 1994, 1995) in two different studies comparing a depot and a daily administered form of GnRH agonist (triptorelin 3.75 mg, 1 ampoule i.m. versus triptorelin 100 µg s.c. daily and leuprorelin 3.75 mg, 1 ampoule i.m. versus buserelin 0.3 mg s.c. daily respectively). The clinical results of our research confirm such studies, however, the daily dose administered during stimulation in those studies remained unchanged throughout treatment and was not reduced as in ours.

Indeed, there is some evidence that once the pituitary is suppressed, the dose of GnRH agonist needed to prevent the LH surge decreases with the length of treatment (Sandow and Donnez, 1990). Halving the dose of a daily administered GnRH agonist at the beginning of stimulation has still not been clearly defined.

The most widely used protocol for administration of GnRH analogues in assisted reproduction centres today involves the use of depot formulation, which usually induces pituitary suppression for a long time after oocyte retrieval. Two studies showed that recovery of pituitary function begins only 8 weeks after a single dose injection of depot triptorelin (Porcu et al., 1994) or depot leuprorelin (Porcu et al., 1995). In contrast, an almost normal response to GnRH challenge test was seen 1 week after discontinuation of a daily administered form of GnRH agonist (Porcu et al., 1994, 1995).
A daily dose of 50 µg triptorelin has proven sufficient to prevent the spontaneous LH surge (Broekmans et al., 1996; Janssens et al., 1998). None of our patients seemed to have ovulated before oocyte retrieval: LH or progesterone assay performed on the day of HCG administration showed no evidence of an LH surge or premature luteinization. Moreover, some oocytes were found in every retrieval and our study conformed to the report by Janssens et al. (2000), who compared a placebo with three doses of triptorelin: 100, 50 and 15 µg daily, and showed that 50 µg was equivalent to 100 µg in terms of IVF outcome (Janssens et al., 2000). A 15 µg dose was capable of preventing an LH surge, with a lower degree of pituitary desensitization, yet this lower suppression does not seem to affect the clinical results of our study, which are similar in both groups.

Our findings confirm the report by Janssens et al. (2000), who compared a placebo with three doses of triptorelin: 100, 50 and 15 µg daily, and showed that 50 µg was equivalent to 100 µg in terms of IVF outcome (Janssens et al., 2000). A 15 µg dose was capable of preventing an LH surge, with a lower degree of pituitary desensitization, yet the IVF results appeared to be worse, although not significantly so.

We may postulate that probably in young woman (in our study all were aged 38 years), with normal ovarian reserve and hormonal pattern, the degree of pituitary suppression might be almost irrelevant as regards response to stimulation and treatment outcome. It would be of interest to check in women aged >38 years, when ovarian reserve begins to decrease, whether lowering the GnRH agonist dosage may bring some improvement in the success of assisted reproduction. Such a trial is now in progress in our centre.

It is well known that GnRH analogues exert a luteolytic effect (Smits et al., 1988). In our study the implantation rate is similar in the two groups of patients. Since the same dose of exogenous progesterone was given in both groups as luteal support, no difference can be inferred as regards the effect of long- and short-acting GnRH analogues on luteal phase. This finding is in accordance with previous studies which showed no differences in hormonal profile in the luteal phase with both GnRH analogues (Porcu et al., 1994). Despite the different duration of pituitary suppression, depot formulations do not seem to impair luteal function and fetal development, more than daily-administered forms. Luteal support is necessary with both formulations, as has recently been confirmed (Beckers et al., 2000).

Our study confirms that a low dose of triptorelin is capable of inducing adequate pituitary suppression during ovarian stimulation. Although this dose does not significantly improve IVF outcome, the possibility of a shorter treatment, requiring a lower amount of gonadotrophins, should be taken into consideration on account of its economic advantage.

It may be sensible to adopt a case-by-case approach, weighing up the convenience of a single i.m. administration with the risk of higher treatment costs against the discomfort of multiple injections, albeit with lower costs.

Additional studies are needed to evaluate the benefit of further reducing the dose of GnRH agonist on the outcome of assisted reproduction.

References


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