High doses of gonadotrophins combined with stop versus non-stop protocol of GnRH analogue administration in low responder IVF patients: a prospective, randomized, controlled trial

Juan A. García-Velasco1,4, Verónica Isaza1, Antonio Requena2, Fco. Javier Martínez-Salazar1, Adriana Landázabal1, José Remohí2,3, Antonio Pellicer2,3 and Carlos Simón2,3

1IVI-Madrid, Madrid, 2Instituto Valenciano de Infertilidad (IVI) and 3Department of Paediatrics, Obstetrics and Gynaecology, Valencia University School of Medicine, Valencia, Spain
4To whom correspondence should be addressed: IVI-Madrid, C/Santiago de Compostela 88, 28035 Madrid, Spain. E-mail: ivimadrid@ivi.es

Recent evidence suggests that early cessation of gonadotrophin releasing hormone analogue (GnRHa) administration may offer some benefit to low responder patients undergoing IVF. A prospective, randomized controlled trial was designed to evaluate whether early cessation of GnRHa in an ovarian stimulation regimen is more beneficial than just increasing the doses of gonadotrophins. Seventy low responder patients (less than three mature follicles in a previous cycle) with normal basal follicle stimulating hormone concentrations and a previous cancelled IVF cycle were randomly allocated into two protocols: (i) non-stop protocol: long GnRHa suppression with high doses of gonadotrophins, and (ii) stop protocol, in which GnRHa administration is stopped with the onset of menses, while gonadotrophin doses remained similar to the non-stop protocol. A significantly higher number of mature oocytes were obtained in the study group (stop protocol) compared to the control group (non-stop protocol) (8.7 ± 0.9 versus 6.2 ± 0.7, \(P = 0.027\)). The stop protocol reduced the number of ampoules of gonadotrophins required (56.6 ± 2.7 versus 68.0 ± 3.5, \(P = 0.013\)). Both protocols resulted in a similar cancellation rate (2.7 versus 5.8%) (with no cycles cancelled due to ovulation), pregnancy rate (14.3 versus 18.7%), and implantation rate (12.1 versus 8.8%). The early cessation of GnRHa combined with high doses of gonadotrophins permitted the retrieval of a significantly higher number of oocytes.

Key words: gonadotrophin/GnRH analogue/IVF/low responders

Introduction

Low ovarian response to stimulation in IVF-embryo transfer patients occurs in 9–18% of cycles (Pellicer et al., 1987; Scott, 1996), and in most cases results in a cancelled cycle. This is a very frustrating, difficult and disappointing issue of infertility treatment. Inadequate ovarian response carries a low oestradiol peak (Garcia et al., 1983) and a small number of oocytes are retrieved. Most programmes consider that less than four dominant follicles on the day of human chorionic gonadotrophin (HCG) administration is a low response to ovarian stimulation (Karande and Gleicher, 1999), although there is no universally accepted definition for low responders. Poor IVF outcome has been related to low ovarian response, so there is a general agreement that this group of patients has a lower pregnancy rate than age-matched patients (Karande and Gleicher, 1999).

Low response to ovarian stimulation is often age-related, but this situation also occurs in young patients. Elevated basal or clomiphene-induced FSH concentrations may characterize the age-related decline in reproductive performance. However, some young patients with normal FSH concentrations present repeated low responses to aggressive stimulation protocols. In this particular group of patients, several hypothesis have been raised to explain the low ovarian response, but none has been proved. There is no doubt that whatever the underlying mechanism is, these patients have a diminished ovarian reserve, as has been recently demonstrated by three-dimensional ultrasound (Pellicer et al., 1998).

Clinicians have approached low responders in many different ways, using different dosages of gonadotrophin releasing hormone agonist (GnRHa), increasing the dose of gonadotrophins, co-treatment with oestrogens, growth hormone, clomiphene citrate or contraceptive pills, and even natural cycles (Scott, 1996; Karande and Gleicher, 1999). The optimal approach for poor responders to ovarian stimulation is still controversial. Recently, a new protocol in which early cessation of GnRHa administration was combined with high doses of gonadotrophins yielded favourable results in low responder patients (Faber et al., 1998). This retrospective report was followed by a prospective, randomized trial in which this approach offered no further advantage to these patients (Dirnfield et al., 1999). Recent evidence confirms that early follicular GnRHa cessation is still effective in the prevention of a premature rise in LH (Valbuena et al., 1997; Beckers et al., 2000). The so-called stop protocol may improve ovarian responsiveness based on a hypothetical effect on the ovary, directly via GnRH receptor or indirectly regulating the vascular network within the ovary.

In a prospective, randomized clinical trial the beneficial effect of withholding GnRH administration upon down-regulation combined with high doses of gonadotrophins in women who previously had insufficient ovarian response to complete an IVF attempt was evaluated.
**Materials and methods**

**Study design**

This study included a total of 70 IVF cycles performed in 70 low responders treated at our institution between November 1, 1998, and February 28, 2000. The study was approved by our institution’s ethics committee, and all couples were required to sign a written informed consent after the provision of complete information.

To be included in this study, patients had to have at least one previous cancelled IVF attempt in which fewer than three follicles ≥18 mm in diameter were obtained and basal FSH concentrations were <12 IU/ml (Karande and Gleicher, 1999). There were no exclusion criteria, nor was there any age limit. The cancelled cycle was stimulated with a standard long protocol, as previously described (Simón et al., 1998). Briefly, in our standard long protocol, after pituitary desensitization with leuprolide acetate, 1 mg/day s.c. (Procrin; Abbot S.A., Madrid, Spain) on days 1 and 2 of ovarian stimulation, one ampoule of human menopausal gonadotrophin (HMG; Lepori; Farma-Lepori Laboratories S.A., Madrid, Spain) was administered together with three ampoules of highly purified FSH (Neo-Fertinorm; Serono Laboratories S.A., Madrid, Spain). On days 3, 4 and 5 of ovarian stimulation, one ampoule of HMG and one ampoule of FSH were given to each patient. Beginning on day 6, HMG and FSH were administered on an individual basis according to serum oestradiol concentrations and transvaginal ovarian ultrasound scans.

During their next cycle, the patients were informed about the possibility of being included in the study with two study groups (stop protocol versus non-stop protocol). Thirty-six patients were treated in 36 cycles with a high dose regimen, whereas 34 were treated in 34 cycles with a stop protocol and a high dose regimen. Successive ovarian stimulation was separated by a minimum of two or more months in order to avoid any potential source of error, as this is our routine clinical practice.

**Assignment and masking**

Considering low ovarian response to ovarian stimulation as less than 10 follicles ≥18 mm in diameter were obtained and basal FSH concentrations were >10 mm on vaginal ultrasound scans, were used to define ovarian quiescence. If a cyst >10 mm was observed, serum oestradiol concentrations <60 pg/ml was sufficient to confirm ovarian quiescence. If serum oestradiol concentrations were beyond this threshold value, the patient was excluded from the study.

For the high dose, non-stop protocol, a similar approach was used (long protocol) but on days 1 and 2 of ovarian stimulation, three ampoules of HMG were administered together with five ampoules of FSH. On days 3, 4 and 5 of ovarian stimulation, two ampoules of HMG and three ampoules of FSH were administered. From day 6 onward, gonadotrophin dosage was estimated according to serum oestradiol concentrations and transvaginal ovarian ultrasound scans. Patients included in the stop protocol group received the same gonadotrophin doses as in the high dose, non-stop protocol group.

In both protocols, the criteria for HCG administration (10 000 IU, Profasi; Serono Laboratories) was the same. The criteria for HCG administration included the presence of at least three follicles measuring >18 mm in diameter. Gonadotrophin administration, and leuprolide acetate in non-stop protocol, were discontinued the day of HCG administration. Oocyte retrieval was scheduled for 36–38 h after HCG injection and intravaginal micronized progesterone, 400 mg/day (Progeffik; Efikk Laboratories, Madrid, Spain), was administered as luteal support.

**IVF/intracytoplasmic sperm injection**

The standard IVF/intracytoplasmic sperm injection (ICSI) procedure has been described previously (Pellicer et al., 1989; Gil-Salom et al., 1995). Briefly, oocyte-cumulus complexes were evaluated under the dissecting microscope and classified. Oocyte-cumulus complexes were incubated at 37°C under 5% CO₂ in atmospheric air. Embryos were scored on the day of transfer according to their morphology under the dissecting microscope. Four types of embryos were established, ranging from types I to IV. Type I embryos were the best and were defined as round and well-shaped blastomeres without fragments. The policy for embryo transfer was to select as many type I and type II embryos as possible: the remaining embryos were cryopreserved with 1,2-propanediol and sucrose. Only patients with freshly transferred embryos were included in the study. The maximum number of embryos transferred was three or four, depending on previous IVF attempts and age of the patient.

**Hormone measurements**

Blood was drawn with every transvaginal ovarian ultrasound scan. The samples were stored at −20°C in aliquots for subsequent oestradiol analysis. Serum oestradiol and FSH were analysed using commercially available microparticle enzyme immunoassay kits (Abbot Laboratories, Abbot Park, IL, USA). Inter- and intra-assay variability for oestradiol at a concentration of <40 pg/ml was 2.8 and 4.3%, respectively, and 4.6 and 6.1% for FSH at a concentration of 22 IU/ml.

**Statistical analysis**

Data were expressed as means ± SEM. Comparisons between different cycles of the same patient were performed with the use of the Student’s t-test, except that pregnancy and implantation rates were compared with the use of χ²-test. When homogeneity and normality (Kolmogorov-Smirnov test) of the samples were not appropriate, non-parametric statistical methods (Wilcoxon test) were used for comparison. P < 0.05 was considered significant. Statistical calculations were performed using Sigmastat for Windows, version 2.0 (Jandel Scientific Corporation, San Rafael, CA, USA).
Table I. Stimulated cycle characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-stop protocol (n = 36)</th>
<th>Stop protocol (n = 34)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age (years)</td>
<td>34.0 ± 0.5</td>
<td>34.4 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Basal FSH concentration (mIU/ml)</td>
<td>7.1 ± 0.8</td>
<td>7.3 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>No. of FSH ampoules</td>
<td>45.2 ± 2.2</td>
<td>37.2 ± 1.8</td>
<td>0.007</td>
</tr>
<tr>
<td>No. of HMG ampoules</td>
<td>22.8 ± 1.5</td>
<td>19.3 ± 1.2</td>
<td>0.078</td>
</tr>
<tr>
<td>No. of ampoules (total)</td>
<td>68.0 ± 3.5</td>
<td>56.6 ± 2.7</td>
<td>0.013</td>
</tr>
<tr>
<td>No. of days FSH/HMG administration</td>
<td>11.3 ± 0.4</td>
<td>10.1 ± 0.5</td>
<td>0.082</td>
</tr>
<tr>
<td>Peak oestradiol concentration (pg/ml)</td>
<td>708.6 ± 107.2</td>
<td>859.5 ± 132.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table II. Characteristics of IVF cycle and outcome

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-stop protocol (n = 36)</th>
<th>Stop protocol (n = 34)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of follicles &gt;18 mm</td>
<td>3.5 ± 0.6</td>
<td>4.1 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Total no. of oocytes aspirated</td>
<td>6.2 ± 0.7</td>
<td>8.7 ± 0.9</td>
<td>0.027</td>
</tr>
<tr>
<td>No. of mature oocytes aspirated</td>
<td>4.1 ± 0.6</td>
<td>6.9 ± 0.9</td>
<td>0.018</td>
</tr>
<tr>
<td>Fertilized oocytes (fertilization rate, %)</td>
<td>3.7 ± 0.5 (85.2)</td>
<td>5.2 ± 1.1 (76.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Cleaved embryos (cleavage rate, %)</td>
<td>3.1 ± 0.4 (83.8)</td>
<td>4.2 ± 0.8 (80.8)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>2.7 ± 0.4</td>
<td>2.8 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>No. of cancellations due to low response (%)</td>
<td>1/36 (2.8)</td>
<td>2/34 (5.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Pregancies/initiated cycle (%)</td>
<td>5/36 (13.9)</td>
<td>6/34 (17.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Pregancies/transfer (%)</td>
<td>5/35 (14.3)</td>
<td>6/32 (18.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Implantation (%)</td>
<td>6/68 (8.8)</td>
<td>8/66 (12.1)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Implantation rate calculated from good quality embryos only.

Results

Table I presents the data from the study cycle (high dose gonadotrophins + stop protocol) compared to the high dose, non-stop cycle, which is our routine practice in low responders with a previous cancelled cycle. Both groups were comparable in terms of age and basal FSH concentrations.

When both protocols in the present study were compared (Table I), it was found that the stop protocol required fewer ampoules of gonadotrophins compared to the non-stop protocol (P = 0.013). The mean number of days of FSH/HMG administration was lower in the stop protocol, although the difference did not reach statistical significance. A similar peak of serum oestradiol was found in both protocols.

Table II shows that in patients undergoing ovarian stimulation with the stop protocol a significantly higher number of oocytes was retrieved (8.7 versus 6.2, P = 0.027) as well as a higher number of mature oocytes (6.8 versus 4.1, P = 0.018). However, similar fertilization and cleavage rates were obtained. Also, the mean number of embryos transferred (2.8 versus 2.6) was similar. No differences were found in cancellation rate (5.8 versus 2.7%), pregnancy rate (18.7 versus 14.3%) and implantation rate between both treatment groups (12.1 versus 8.8%) (Table II).

The number of patients undergoing ICSI was similar in both groups: 90.6% (29/32) of the subjects underwent ICSI in the stop protocol and 85.7% (30/35) in the non-stop protocol. Fertilization rates were similar in both groups, regardless of IVF or ICSI: stop protocol 72.7 ± 4.7% and 79.7 ± 4.3% respectively; non-stop protocol, 74.5 ± 3.9% and 88.6 ± 4.9% respectively.

Obviously, a higher number of FSH/HMG ampoules were administered in the high dose protocol irrespective of whether the patient was included in the stop or non-stop protocol when compared to the previous cancelled cycle (62.3 versus 35.5, P < 0.001). For the same reason, the number of days of stimulation (10.7 versus 9.5) as well as the peak oestradiol concentration (783.5 versus 367.2 pg/ml) was also higher in this group.

Discussion

The results of this prospective, randomized trial show that early cessation of GnRHAs combined with high doses of gonadotrophins, although it recruited a significantly higher number of mature oocytes, resulted in similar IVF outcome than a conventional non-stop, long down-regulation protocol.

Several mechanisms may contribute to the improved ovarian response observed with the stop protocol. The pituitary gonadotrophin down-regulation induced by GnRHa decreases cancellation rates by suppressing endogenous LH surges, although this may require significantly higher requirements of gonadotrophins (Horvarth et al., 1988), probably due to a direct, partial inhibition of ovarian response, modulating ovarian steroidogenesis and oocyte maturation. A direct inhibitory
Early GnRHa discontinuation in low responders

effect of the GnRHa on the ovaries has been proposed, so reducing the dose or even stopping the administration would remove this suppression, improving ovarian responsiveness (Kowalik et al., 1998). This hypothesis is based on the presence of GnRH receptors on the ovary (Latouche et al., 1989). On the other hand, GnRHa have been proved to decrease blood flow assessed by pulsed Doppler analysis (Aleem and Predanic, 1995). Some investigators believe that follicular growth is dependent on an appropriate vascular network responsible for the distribution of circulating gonadotrophins (Zeleznik et al., 1981). In fact, ovarian blood flow velocity, after pituitary suppression is confirmed, has been shown to be predictive of ovarian responsiveness and the outcome of IVF treatment (Engmann et al., 1999). Thus, it could be postulated that GnRHa early cessation, while maintaining pituitary suppression, restores the diminished perifollicular blood flow, which correlates with the number of oocytes retrieved and IVF outcome (Bhal et al., 1999). Whether this is true or not for ovarian vessels in low responders remains to be elucidated.

GnRHa is routinely used until oocyte retrieval, but even with short-acting molecules pituitary down-regulation continues following cessation of GnRHa during ovarian stimulation for IVF (Sungurtekin and Jansen, 1995). In a recent prospective study, the follicular and luteal phase characteristics following early cessation of GnRHa during ovarian stimulation were evaluated (Beckers et al., 2000). Early follicular phase cessation of GnRHa is still effective in the prevention of a premature rise in LH or progesterone, as some pituitary recovery occurred 16–22 days after GnRHa cessation. This is in accordance with the fact that most of the cancellations in the study group in the current study as well as in others (Faber et al., 1998; Dirnfeld et al., 1999; Karande and Gleicher, 1999) were not due to a premature ovulation.

When the previous cancelled cycles with a conventional IVF protocol were compared using the study cycles (either high dose or high dose combined with early cessation of GnRHa) a significant improvement in the ovarian response was found. It is interesting to note that regardless of the protocol used, stop or non-stop, we found that a satisfactory number of oocytes was retrieved in both groups of re-stimulated patients. If it is considered that they were enrolled in this study on the basis of a previous cancelled stimulation with less than three mature follicles, the only explainable hypothesis is the use of high doses of gonadotrophins. Although this explanation appears reasonable, conflicting reports have been published. Increasing the gonadotrophin dose during the course of treatment was initially advocated by Laufer (Laufer et al., 1986), in an attempt to recruit a higher number of follicles. Several other reports including our data support this approach (Chong et al., 1986; Crosignani et al., 1989). However, other authors have not reported such a beneficial effect (Karande and Gleicher, 1990; Van-Hoof et al., 1993; Land et al., 1996). Patients show a great variability in their ovarian response to the same stimulation protocol, especially those who qualify as poor responders to IVF stimulation, probably based on the intercycle variability of basal FSH concentrations (Scott et al., 1990). Basal day 3 FSH concentrations were comparable between groups, so it is believed that the higher dose adminis-

tered during the study cycles allowed the recruitment of a higher number of follicles and enabled most women to finish their cycle and undergo oocyte retrieval. Whether the FSH threshold in these women is higher or if the intra-individual variability in the ovarian response is responsible for the outcome is something to be determined.

The primary efficacy result, i.e. a significantly higher number of mature oocytes, was supported by the fact that the number of days of treatment and the dose of FSH required to reach criteria for triggering follicle maturation were both lower for the stop protocol. Gonadotrophin ampoule consumption was lower in the stop protocol, and less GnRHa was used, so the cost of this cycle in terms of medication is significantly reduced. The reduced gonadotrophin usage together with the higher number of mature oocytes retrieved make this protocol appealing, something that would be definitive if it allowed higher pregnancy rates. Although a trend was observed (18.7 versus 14.3%), a prospective study with pregnancy rate as primary end-goal should be designed to prove this hypothesis. The stop protocol, in spite of having significantly more oocytes (8.7 ± 0.9 versus 6.2 ± 0.7), had similar oestradiol concentrations to those observed in the non-stop protocol. In fact, serum concentrations of oestradiol were higher in the stop protocol, although the values did not reach statistically significant differences (85.9 ± 132.7 versus 708.6 ± 107.2 pg/ml, P = 0.380), probably due to the limited sample size of this study.

The ‘flare effect’ derived by the GnRHa administration during the luteal phase does not seem to be relevant in the ovarian stimulation outcome in this kind of patient. San Roman (San Roman et al., 1992) did not find a significant increase in gonadotrophin concentrations in patients receiving GnRHa initiated in the luteal phase opposed to the concentrations found in patients who were initiated with GnRHa during the follicular phase. Consistent findings were described by Gelety (Gelety et al., 1995).

When clinicians observe a low response to IVF stimulation (less than three follicles), cycle cancellation is usually counselled to the patients, in the hope that a better response might be obtained in a subsequent cycle. This trend is based on the assumption that poor responders have a poor IVF outcome, as they usually have a low oocyte quality. Not all low responders are the same, as they represent a very heterogeneous group. According to their basal FSH concentrations, young low responders with high basal FSH concentrations have a poor outcome based on the low quality of the oocytes retrieved. However, young low responders with normal day 3 FSH concentrations, although their ovarian reserve may be compromised (Pelliccet et al., 1998), might benefit from alternative protocols such as the stop protocol, as their chances of achieving a pregnancy seem to be similar to those of normal responders (Lashen et al., 1999).

It is concluded that low responders with normal basal FSH concentrations may benefit from increasing the doses of gonadotrophins in order to proceed to oocyte retrieval and avoid a new cancelled cycle, with the financial and emotional implications and frustration for both the patient and physician. The stop protocol increases the number of available embryos
and decreases the quantity of gonadotrophins required. Further studies are required to determine whether early cessation of GnRHa leads to an increase in LH concentrations in the late induction phase, and subsequently to decreased implantation.

References


Received on April 10, 2000; accepted on August 2, 2000