Flexible GnRH antagonist versus flare-up GnRH agonist protocol in poor responders treated by IVF: a randomized controlled trial

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BACKGROUND: Although initial studies in poor responders using GnRH antagonists have reported encouraging results, they are limited in number, only a few of them are prospective, while the majority is characterized by limited power to detect a clinically important difference. METHODS: A randomized controlled trial was performed in patients with one or more previous failed IVF cycles in which five or less oocytes were retrieved, using ≥300 IU of gonadotrophins/day. Patients were randomized by computer-generated list and treated by either the flare-up GnRH agonist protocol (n = 90) or a flexible GnRH antagonist protocol (n = 180). RESULTS: Ongoing pregnancy rate, the primary outcome measure, was significantly higher in the antagonist group compared with the agonist group (12.2 versus 4.4%, P < 0.048; difference 7.8%, 95% CI: 0.2 to 14.0). Estradiol levels on the day of hCG administration were lower in the antagonist protocol [median (interquartile range): 572 (325–839) versus 727 (439–1029) pg/ml, P = 0.018]. Clinical and biochemical pregnancy rates, fertilization and implantation rates, as well as the number of oocytes retrieved, the number of mature oocytes present, the stimulation period and the gonadotrophin dosage were not significantly different between the two groups compared. CONCLUSIONS: The flexible GnRH antagonist protocol is associated with significantly higher ongoing pregnancy rates compared with the flare-up GnRH agonist protocol in poor responders (www.clinicaltrials.gov; NCT00417066).

Keywords: poor responders; GnRH antagonist; GnRH agonist

Introduction

Despite the improvements in ovarian stimulation protocols during the last two decades, there are still women who fail to respond adequately to gonadotrophin stimulation (Tarlatzis et al., 2003). These women are classified under the broad term ‘poor responders’ and are characterized by a diminished ovarian reserve (Pellicer et al., 1998) and lower pregnancy rates compared with ‘normal responders’. A universal definition of what constitutes a poor ovarian response is still lacking and this might explain its variable prevalence, reported to range from 9 to 26% (Pellicer et al., 1987; Van Rysselberge et al., 1989; Jenkins et al., 1991; Manzi et al., 1994; Stadmauer et al., 1994; Land et al., 1996).

Several stimulation protocols have been proposed for the management of poor responders (Tarlatzis et al., 2003; Ubaldi et al., 2005). Regarding the type of analog used to suppress the premature luteinizing hormone (LH) surge, the introduction of GnRH antagonists has renewed the interest in the treatment of these patients (Kolibianakis et al., 2004). Antagonists prevent premature LH surges without suppressing early follicular development, which might be important in the presence of poor ovarian response (Akman et al., 2001). Although limited, a number of randomized studies compare the long agonist and the antagonists protocol, and it appears that the latter results in significantly more cumulus oocyte complexes (COCs) (Griesinger et al., 2006). On the other hand, no major differences appear to exist between the short agonist and the antagonist protocols, although a limited number of patients has been so far analyzed (n = 449) (Griesinger et al., 2006).

The hypothesis tested in the current study was that the use of a flexible GnRH antagonist protocol as compared with the use of a flare-up GnRH agonist protocol increases pregnancy rates in poor responders undergoing IVF.

Materials and Methods

Study design

This is a single center randomized controlled trial. Random allocation was performed by a study nurse at consultation, using a computer-
generated randomization list, in a 1:2 ratio. Patients were treated either by a flare-up GnRH agonist protocol (n = 90, agonist group) or by a flexible GnRH antagonist protocol (n = 180, antagonist group) (Fig. 1). Neither patients nor doctors were blinded to the treatment assigned. The study was approved by our institutional ethics review board. An informed consent was obtained from all patients included in this study.

**Patient population**
A total of 270 poor responders patient undergoing IVF/ICSI treatment at Eugonia—Iatriki Erevna IVF unit from June 2002 to July 2006 were included in the study. Patients could enter the study only once. All patients included should have a regular menstrual cycle (duration 21–35 days) and one or more failed IVF cycles, in which five or fewer oocytes were retrieved using a high gonadotrophin dose (≥300 IU/day) (Crosignani et al., 1989; Hofmann et al., 1989).

**Controlled ovarian stimulation**
In the flare-up GnRH agonist protocol (agonist group), patients started daily 0.05 mg/day triptorelin (Averkap, Ipsen France) from Day 2 of the cycle. GnRH agonist was administered subcutaneously and continued daily until and including the day of human chorionic gonadotrophin (hCG) administration. In the flexible antagonist protocol (antagonist group) daily administration of ganirelix 0.25 mg (Orgalutran, Organon, The Netherlands) was initiated in the presence of a follicle with a mean diameter of 14 mm at ultrasound and/or serum LH levels >10 IU/l, as previously described (Lainas et al., 2005). Treatment with GnRH antagonist continued daily thereafter until and including the day of hCG administration.

In both groups, patients were administered 400 IU of recombinant follicle-stimulating hormone (rFSH) (follitropin beta, Puregon, Organon, The Netherlands) on Day 2 of cycle. Recombinant FSH dose was adjusted in both groups during ovarian stimulation, depending on the ovarian response, as assessed by estradiol (E2) levels and ultrasound. Treatment with rFSH continued until and including the day of hCG administration.

Before initiation of treatment, a vaginal ultrasound and a basal hormonal profile, including FSH, LH, E2 and progesterone were performed in all patients to confirm the absence of any ovarian activity. When 1–2 follicles reached a mean diameter of >17 mm, 10 000 IU of hCG (Pregnyl, Organon, The Netherlands) were administrated.

**Oocyte retrieval, embryo transfer and luteal support**
Oocyte retrieval was performed 35–36 h after the hCG injection by transvaginal ultrasound-guided double lumen needle aspiration. ICSI was performed only in cases with severe male factor or previous fertilization failure. Ultrasound guidance was used for all embryo transfers, which were performed two or three days post oocyte retrieval. Luteal phase support with 600 mg of micronized progesterone (Utrogestan Laboratoires Besins-International S.A., France) was initiated two days post oocyte retrieval.

**Ultrasound and laboratory assays**
All ultrasound measurements were performed using a 7.5, 6 or 5 MHz vaginal probe (Sonoline Adara, Siemens). FSH, LH, E2 and progesterone levels were measured using an Immulite analyzer and commercially available kits (DPC, Los Angeles, CA). Analytical sensitivity were 0.1 mIU/ml for FSH, 0.1 mIU/ml for LH, 15 pg/ml for E2 and 0.2 ng/ml for progesterone. Intra- and inter-assay precision at the concentrations of most relevance to the current study (expressed as coefficients of variation) were 2.6 and 5.8% for FSH, 5.9 and 8.1% for LH, 6.3 and 6.4% for E2 and 7.9 and 10% for progesterone.

**Outcome measures**
The primary outcome measure was ongoing pregnancy rate per patient randomized. Ongoing pregnancy was defined as the presence of gestational sac with fetal heartbeat detection at 12 weeks of gestation; clinical pregnancy was defined as the presence of a gestational sac with positive heartbeat at 6 weeks of gestation.

Secondary outcome measures were duration of stimulation, total dose of rFSH, E2 concentration on hCG day, cycle cancellation rate, number of COCs retrieved, number of metaphase II (MII) oocytes, number of 2 pronuclei (2PN) oocytes, fertilization rates and ongoing implantation rates (at 12 weeks of gestation).

**Statistical analysis**
Proportions were compared with the Fisher’s exact test or the chi-square test, where appropriate. Continuous variables [age, body mass index (BMI)] were compared with the t-test for independent samples or the Mann–Whitney depending on the normality of their distribution. Statistical significance was accepted when \( P \leq 0.05 \).

It was calculated that a sample size of 88 patients in the agonist group and 176 in the antagonist group would be able to detect a difference of 10% in ongoing pregnancy rates assuming a baseline pregnancy rate of 5%, an \( \alpha \) level of 0.05 and a \( \beta \) of 0.2. To compensate for drop outs following randomization, 90 and 180 patients were randomized in each group.

**Results**
Table I describes the baseline characteristics and hormonal profile of patients in the antagonist and the agonist group. No differences were observed in patients’ age, BMI, duration of infertility and number of previous attempts, as well as in baseline hormonal levels (FSH, LH, E2 and progesterone). The proportion of patients in whom ICSI was performed was similar in the agonist and the antagonist group (72.2 versus 69.4%, respectively, \( P = 0.6 \)).
The total units of FSH administered during stimulation as well as the number of oocytes retrieved, embryos transferred and the implantation and fertilization rate were similar between the two study groups (Table II). However, E2 levels on the day of hCG administration were significantly higher in the agonist \[727(439–1029)\] pg/ml versus the antagonist group \[572(325–839)\] pg/ml, \(P = 0.018\) (Table II). No more than five oocytes were retrieved per woman in either group.

Ongoing pregnancy rate was significantly higher in the antagonist group compared with the agonist group (12.2 vs 4.4\%, \(P = 0.048\)) (Table III). The difference between the two groups was 7.8\% (95% CI: 0.2 to 14.0).  

**Discussion**  
In the present study, a significantly higher ongoing pregnancy rate was observed with the use of the flexible antagonist compared with the short GnRH agonist protocol in poor responders patients treated by IVF.

There are currently five published randomized controlled trials (RCTs) comparing the short agonist and GnRH antagonist protocols in poor responders (Akman et al., 2001; Martinez et al., 2003; Malmusi et al., 2005; Schmidt et al., 2005; De Placido et al., 2006), and one abstract (Mollo et al., 2005), reviewed in the meta-analysis by Griesinger et al. (2006) on the use of GnRH antagonists in poor responders. All the
above studies have used a flexible antagonist protocol with the exception of the study by Martinez et al. (2003) in which a fixed protocol was used. To our knowledge this is the largest RCT so far and the first to suggest that a higher ongoing pregnancy rate is expected with the use of the flexible antagonist protocol.

Four out of the six studies in the meta-analysis by Griesinger et al. (2006) showed a higher but non-significant clinical pregnancy rate in favour of the GnRH antagonist protocol. This might be due to the inadequate power of those studies to detect an existing difference or might, however, be due to the absence of a true difference between the two protocols. Thus, there is a need for further RCTs regarding this comparison. By reducing clinical heterogeneity, a unanimous definition of poor ovarian response would allow more solid conclusions to be drawn. However, this has not been made possible so far. It is not clear what is the source of the difference, observed in the current study regarding ongoing pregnancy rates between the two groups compared. Whether or not this is related to the fact that the flexible antagonist protocol creates a hormonal environment, which resembles the natural cycle more closely, avoiding the high levels of LH in the early follicular phase, caused by the GnRH agonist flare-up in the short protocol might need further investigation. Interestingly, significantly lower E2 levels on the day of hCG were observed in the antagonist group, corroborating previous studies (Akman et al., 2001; Mohamed et al., 2005).

Currently no study has reported that ongoing pregnancy rates are significantly better with the use of a short antagonist compared with the antagonist protocol. On the contrary, there is now evidence to suggest that the probability of pregnancy might be better with the use of the antagonist protocol. It has to be noted, that the lower 95% CI of the difference observed in ongoing pregnancy rates is 0.2%, and thus its clinical significance might be small. However, a flexible antagonist protocol is a simpler and more patient friendly method of ovarian stimulation compared with the short antagonist protocol. Therefore, the GnRH antagonist protocol might represent the preferred way for stimulating poor responders for IVF.

Future studies, besides confirming the results reported here, should also focus on the comparative use of a fixed versus a flexible protocol in patients with poor ovarian response.

In conclusion, the present study suggests that the flexible GnRH antagonist protocol is associated with significantly higher ongoing pregnancy rates compared with the flare-up GnRH agonist protocol in poor responders treated for IVF.

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References
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