Article

Single-dose GnRH agonist administration in the luteal phase of GnRH antagonist cycles: a prospective randomized study

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Abstract

This study was designed to evaluate the effect of luteal-phase administration of single-dose gonadotrophin-releasing hormone (GnRH) agonist on pregnancy, implantation and live birth rates in patients who received GnRH antagonist for pituitary suppression. The study population consisted of 164 patients who underwent intracytoplasmic sperm injection (ICSI) after ovulation induction by gonadotrophins and GnRH antagonist for the prevention of a premature LH surge. For luteal-phase support, all the cases received intravaginal 600 mg micronized progesterone. In this prospective study, patients were randomly assigned to two groups. In one group, patients received an additional single dose of GnRH agonist (0.5 mg leuprolide acetate) subcutaneously on day 6 after ICSI, whereas the patients in the other group did not. Although the number of embryos transferred and the grade of the embryos were similar in the two groups, the patients in the luteal-phase agonist group had significantly higher rates of implantation and clinical pregnancy rates ($P < 0.05$). When the two groups were compared, there were also statistically significant differences in multiple pregnancy and live birth rates ($P < 0.05$). Administration of single-dose GnRH agonist as a luteal-phase support in ovarian stimulation-GnRH antagonist cycles in addition to standard luteal support seems to be effective in all cycle outcome parameters.

Keywords: *luteal phase, GnRH agonist, GnRH antagonist*

Introduction

The establishment and maintenance of pregnancy is related to adequate production of progesterone (Csapo *et al.*, 1972). A problem of defective corpus luteum function, whose major product is progesterone, is luteal-phase inadequacy. There has been an ongoing concern about the luteal-phase deficiency, particularly in IVF-stimulated cycles. In assisted reproduction techniques, in cycles with gonadotrophin-releasing hormone (GnRH) agonist, used for pituitary down-regulation, and in cycles with GnRH antagonist as well, luteal-phase deficiency is a current problem (Macklon and Fauser, 2000; Pritts and Atwood, 2002; Beckers *et al.*, 2003; Kolibianakis *et al.*, 2003).

The current data in the literature shows that, after ovulation induction for IVF with GnRH antagonist treatment, luteal support cannot be abandoned and support of corpus luteum function remains mandatory. The ovarian steroidal production during the luteal phase of IVF cycles with GnRH antagonists showed that non-supplemented luteal phase was insufficient (Beckers *et al.*, 2003). There are different theories behind the low LH concentrations in the luteal phase after ovarian stimulation for IVF in antagonist cycles. In antagonist cycles, despite the supraphysiological

steroid concentrations during the early luteal phase, luteolysis starts prematurely, which might be due to a negative effect of these high concentrations of hormones on pituitary gland resulting in suppressed pituitary LH release (Beckers *et al.*, 2003).

There are different regimens of luteal-phase support including different treatment substances like HCG, progesterone and oestradiol administered in various forms and doses (Pritts and Atwood, 2002). The most commonly used progesterone or HCG has been widely studied and the outcome of the cycles of these two regimens was similar (Daya and Gunby, 2004). Recently, Tesarik *et al.* (2004) have reported the successful use of GnRH agonist in luteal-phase support and several other investigators have made trials on the potential use of GnRH agonists for the luteal-phase support (Pirard *et al.*, 2005, 2006; Hugues *et al.*, 2006; Tesarik *et al.*, 2006). This study was designed to evaluate the effect of luteal-phase administration of single-dose GnRH agonist on pregnancy, implantation and live birth rates in patients who received GnRH antagonist for pituitary suppression.

Materials and methods

Study design and power calculation

This prospectively randomized study was conducted between January 2005 and September 2005. The study population consisted of 164 patients who underwent intracytoplasmic sperm injection (ICSI) after ovulation induction by gonadotrophins and GnRH antagonist for the prevention of a premature LH surge. Patients to be treated with antagonist protocol were randomly assigned to two groups. There were 82 patients in each group. A computer-generated random table was used for randomization and performed on the day of embryo transfer by a nurse to assign participants to their groups. The clinicians and the laboratory staff were blinded to groups. In one group, patients received an additional single-dose GnRH agonist (0.5 mg leuprolide acetate, Lucrin Daily; Abbott, Istanbul, Turkey) subcutaneously on day 6 after ICSI, whereas the patients in the other group did not. All the patients signed an informed consent.

Implantation and live birth rates were the main outcome measures. Previously, Tesarik *et al.* (2004) showed that in donor oocyte cycles GnRH administration to recipients during the luteal phase (on day 6 after ICSI) increases the implantation rate from 24.7% to 36.2%. On the basis of this previously published data, power analysis was performed assuming a significance level of 0.05 and a power of 0.80 and it was found that 100 cycles were needed in each group to detect this difference.

Study population

One hundred and sixty-four patients were enrolled in this study. Exclusion criteria were donor and freeze–thaw cycles and patients requiring surgical sperm extraction. Patients of all ages were included in the study. The female partners of the couples that were assigned to an ovarian stimulation using a GnRH antagonist protocol were randomized. The decision of the protocol chosen was subjective and depended on clinical context. Usually cases of advanced age and women with expected low ovarian response were chosen to receive GnRH antagonist protocol.

Ovarian stimulation

Administration of gonadotrophins either recombinant (Puregon; Organon, The Netherlands; or Gonal-F; Serono, Italy) or human-derived FSH (Menogon; Ferring, Germany) were initiated on day 2 of menstrual bleeding and doses were tailored according to the patients ovarian response. When the leading follicle reached 14 mm in diameter, GnRH antagonist 0.25 mg/day (Orgalutron; Organon; or Cetrotide; Serono) subcutaneously was started. When at least three follicles reached 17 mm or more in diameter, ovulation was triggered by intramuscular injection of 10,000 IU of HCG (Pregnyl; Organon) or 250 µg of recombinant HCG (Ovitrelle; Serono).

Assisted reproduction technologies

Oocytes were retrieved 35 h after HCG injection. After transvaginal oocyte retrieval, hyaluronidase was applied to retrieved oocytes and the metaphase II oocytes were subjected to ICSI. A Narishige micromanipulator-equipped Olympus IX 71 inverted microscope was used for the ICSI procedure. All fertilized oocytes were cultured in 25 µl microdroplets of sequential medium under mineral oil and each of them was tracked separately. Fertilization was assessed 16–18 h after ICSI. All embryos were finally graded on day 3 according to the classification by Veeck (1999). Embryo transfer was performed 3 days after ICSI using a Wallace embryo-transfer catheter (Promedicom, Turkey) under transabdominal ultrasound guidance. One to five embryos were transferred in each cycle. If suitable, supernumerary embryos were frozen for future trails. However, only the fresh cycles were included in this study.

Luteal-phase management

All patients received intravaginal 600 mg micronized progesterone (Progestan; Kocak, Istanbul) three times daily starting on the day of oocyte recovery for 17 days. In addition, all cases received a single-dose (1500 IU) HCG (Pregnyl) on day 8 after ICSI. In the GnRH agonist group, patients also received an additional single 0.5 mg dose of GnRH agonist (leuprolide acetate, Lucrin daily injection; Abbott) subcutaneously on day 6 after ICSI. βHCG testing was performed 15 days after retrieval. Clinical pregnancies were identified by the visualization of fetal heartbeat on ultrasound examination.

Statistics

Chi-squared analysis and Student's *t*-test were used and $P < 0.05$ was considered statistically significant. All analyses were performed using Epi-Info program (Centers for Disease Control and Prevention).

Results

During the study period, 179 patients were eligible in both the analogue group and the control group. Among the patients assessed for the study, five did not meet the inclusion criteria and 10 refused to participate in the study. In patients assigned to receive luteal-phase agonist supplementation ($n = 82$), six did not receive the allocated intervention. Among the 76 patients who received the allocated intervention in this group, two discontinued because of incorrect dose administration. In this group, 74 completed the study and were analysed (**Figure 1**). In the control group, all the cases who received the allocated intervention completed the study and the results of these 80 cases were analysed (**Figure 1**).

The patients in the luteal-phase GnRH analogue-supplemented group did not differ in their basic demographic characteristics regarding the mean age of the female partner, basal FSH concentrations, duration of infertility, duration of stimulation, daily gonadotrophin dosages and total dosage of gonadotrophins (**Table 1**). Moreover, the mean numbers of oocytes retrieved, mean number of metaphase II and fertilized oocytes, mean number of embryos and grade-1 embryos transferred were also similar in two groups (**Table 2**).

Although, the number of embryos transferred and the grade of the embryos were similar in two groups, the patients in the luteal-phase agonist group had significantly higher rates of implantation and clinical pregnancy rates ($P < 0.05$). When the two groups were compared, there were also statistically significant differences in multiple pregnancy and live birth rates ($P < 0.05$) (**Table 3**).

Discussion

Administration of single-dose GnRH agonist as a luteal-phase support in ovarian stimulation-GnRH antagonist cycles, in addition to standard luteal support, seems to be effective in all cycle outcome parameters. The results concerning implantation, clinical pregnancy and live birth rates were significantly higher in agonist supplemented group. The results of this study are compatible with the findings obtained in previous studies (Pirard *et al.*, 2005, 2006; Tesarik *et al.*, 2006).

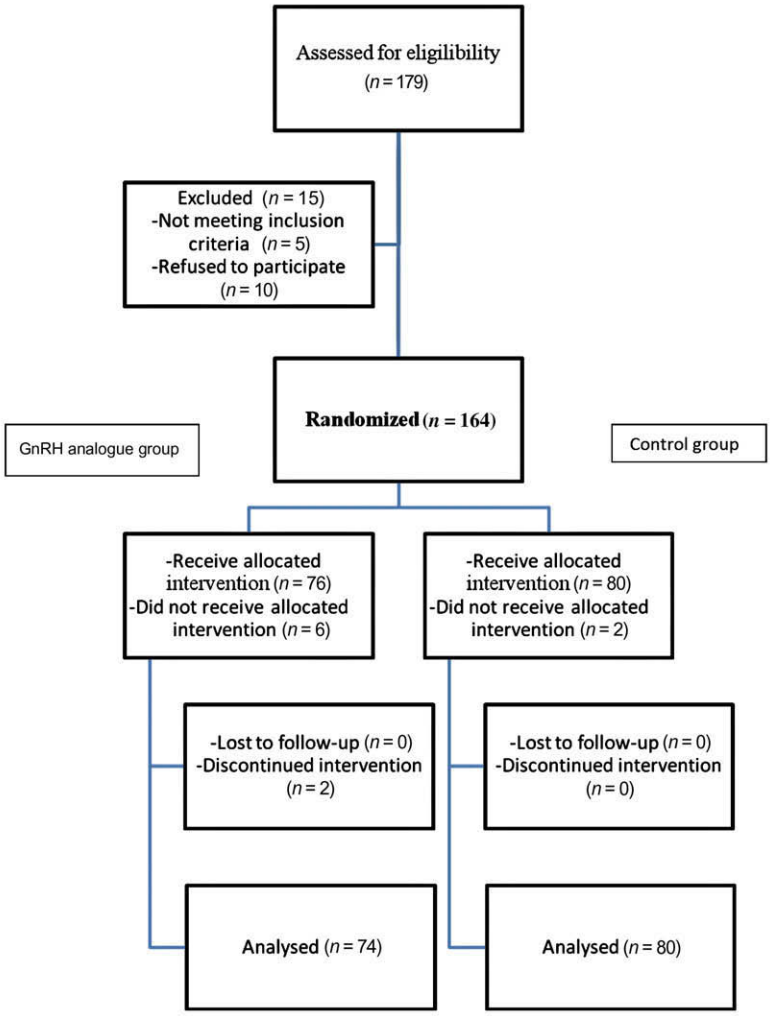

Figure 1. Patient flow through the randomized trial. GnRH = gonadotrophin-releasing hormone.

Table 1. Patient and cycle characteristics.

Parameter	*GnRH agonist (+) (n = 74)*	*Control (n = 80)*
Age (years)	35.56 ± 4.46	35.59 ± 5.54
Basal FSH (mIU/ml)	7.73 ± 2.29	7.7 ± 2.27
Duration of infertility (years)	10.0 ± 6.6	9.9 ± 5.5
Duration of stimulation (days)	9.9 ± 2.2	9.9 ± 2.2
Daily gonadotrophin dosage (IU)	452.2 ± 102.2	411.11 ± 135.5
Total gonadotrophin dose (IU)	3882.2 ± 1598.0	3871.1 ± 1273.3

Values are means ± SD. GnRH, gonadotrophin-releasing hormone. There were no statistically significant differences between the two groups.

Table 2. Cycle characteristics.

Parameter	*GnRH agonist (+) (n = 74)*	*Control group (n = 80)*
Oocytes retrieved	10.00 ± 7.74	10.07 ± 8.87
MII oocytes	7.71 ± 5.57	7.77 ± 6.63
Fertilized oocytes	5.58 ± 4.45	5.53 ± 4.49
Embryos transferred	2.27 ± 1.15	2.23 ± 1.15
Grade-1 embryos transferred	1.14 ± 1.18	1.16 ± 1.17

Values are means ± SD. GnRH, gonadotrophin-releasing hormone; MII, metaphase II. There were no statistically significant differences between the two groups.

Table 3. Pregnancy outcome.

Parameter	*GnRH agonist (+) (n = 74)*	*Control (n = 80)*	P-*value*
βHCG positive	32 (43.2)	23 (28.8)	NS
Implantation rate	54/204 (26.5)	21/227 (9.3)	<0.0001
Clinical pregnancies	30 (40.5)	16 (20.0)	0.0055
Multiple pregnancies	17 (56.7)	3 (18.8)	0.0145
Live birth/embryo transfer	26/74 (35.1)	13/80 (16.3)	0.007

Values are number (percentage); GnRH, gonadotrophin-releasing hormone; NS, not statistically significant.

The study evaluating the luteal phases of the cycles where ovarian stimulation performed with human menopausal gonadotrophins and GnRH antagonists reported a decrease in serum LH concentrations which were detected 2 days after the pre-ovulatory HCG injection and maintained at almost undetectable concentrations throughout the entire luteal phase (Albano *et al.*, 1999). Abnormal luteal hormonal function was demonstrated in another study (Tavaniotou and Devroey, 2006) evaluating the cycle characteristics of 23 fertile donors stimulated with recombinant FSH and GnRH antagonist receiving luteal supplementation and were compared with control, natural cycles. The results of this study also showed extremely low LH concentrations in the luteal phase of the donor cycles, reaching their lowest values in the mid-luteal phase (Tavaniotou and Devroey, 2006).

As data in the literature (Ditkoff *et al.*, 1991; Fattinger *et al.*, 1996; Oberye *et al.*, 1999) showed that gonadotrophin concentrations recovered within 24 h after stopping the GnRH antagonist, the low LH concentrations observed in the luteal phase of antagonist cycles might be attributed to negative feedback from the HCG-induced increase in steroid concentrations on pituitary secretion. Despite the inhibition of gonadotrophin secretion in the luteal phase of antagonist cycles, it was demonstrated that the pituitary remained responsive to single or serial i.v. boluses of GnRH (Chillik *et al.*, 1987; Gordon *et al.*, 1990; Felberbaum *et al.*, 1995). Therefore, GnRH agonist as a luteal-phase support in antagonist cycles may be effective to support corpus luteum function at different levels. It might be speculated that in antagonist cycles the support of the corpus luteum by GnRH analogue administered in the luteal phase might cause the stimulation of the secretion of LH by pituitary gonadotroph cells. However, the preliminary report of Hugues *et al.* (2006) in a similar trial did not observe any difference in the hormonal profile of the luteal phase. On the contrary, luteal-phase GnRH agonist administration increased luteal-phase serum βHCG, oestradiol and progesterone concentrations in both agonist and antagonist ovarian stimulation regimens in the study of Tesarik *et al.* (2006). In another study by Pirard *et al.* (2006) the luteal-phase duration correlated well with the frequency of buserelin administration. In that study, ovulation was triggered with 200 µg intranasal buserelin in the patients undergoing antagonist cycles followed by buserelin 100 µg every 2 days,

100 μg every day, 100 μg twice a day or 100 μg three times a day. The results showed that buserelin once every 2 days, and even once a day, was associated with luteal-phase deficiency (luteal-phase durations under 10 days and low serum progesterone concentrations). However, Pirard *et al.* (2006) with a limited number of patients in each group detected that the patients receiving three administrations per day did not appear to differ from the group of patients in whom ovulation was triggered by HCG and luteal-phase supplementation by vaginal administration of micronized progesterone (3 × 200 mg/day). In this study, it is not possible to explain the effectiveness of GnRH agonist supplementation in the luteal phase with respect to hormonal milieu because this study lacks detailed luteal-phase endocrine data.

The hormonal characteristics of the luteal phase after ovarian stimulation for IVF using GnRH antagonists when compared with those after GnRH agonists, with members of both groups given progesterone only as luteal support, showed similar luteal hormonal profile and dynamics between the study and control group (Friedler *et al.*, 2006). Endometrial thickness was similar in both treatment groups on the day of HCG administration. Therefore, Friedler *et al.* (2006) suggested that other parameters, especially endometrial factors playing a role in implantation should be investigated to explain the clinical differences regarding the outcome of the use of GnRH antagonists. A study by Saadat *et al.* (2004), evaluated the endometrium of patients undergoing ovarian stimulation with GnRH agonists and compared the results with cycles utilizing antagonists. In that study, the authors reported no difference between the endometrial maturation in cycles utilizing GnRH agonists or antagonists. Hence, the observed differences in pregnancy rates between cycles utilizing GnRH antagonists with agonists or without agonists in the luteal phase are unlikely to be due to a difference in the endometrial maturation. On the other hand, the improved implantation rates in antagonist IVF cycles with GnRH analogue supplementation in the luteal phase might be through the specific GnRH receptors present on the ovary and embryo or by acting directly on the endometrium through the locally expressed GnRH receptors (Pirard *et al.*, 2005). Besides, the beneficial effect of administration of luteal GnRH analogue to oocyte recipients in a donation programme, in whom ovulation was suppressed and the corpus luteum was absent, suggested a direct effect of the analogue on the embryo (Tesarik *et al.*, 2004). The reason for this positive effect can also be the combination of some of the above mechanisms (Lambalk and Homburg, 2006).

Contrary to above-mentioned findings in the prospective randomized study of Hugues *et al.* (2006), the effects of GnRH agonist (0.1 mg triptorelin) administered on the day of transfer and 3 days later did not show any beneficial effect of GnRH analogue administration during the luteal phase of an antagonist cycle. On the other hand, in the study by Tesarik *et al.* (2006), 0.1 mg of GnRH agonist triptorelin was administered on day 6 after ICSI in agonist and antagonist cycles and a significant improvement in implantation and live birth rates after ICSI was found as compared with placebo. Moreover, Tesarik *et al.* (2006) reported that in GnRH antagonist-treated ovarian stimulation cycles, luteal-phase GnRH agonist also increased ongoing pregnancy rate, supporting these results.

Although administration of GnRH agonist for luteal support seems to be an attractive and effective means of luteal-phase improvement in IVF cycles, much work is necessary before the routine administration of this drug. Especially minimal effective dose and most effective timing and protocol should be established and more studies should be performed in order to identify the exact mechanism or mechanisms in the background of improved outcome.

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