Initiation of GnRH antagonist on Day 1 of stimulation as compared to the long agonist protocol in PCOS patients. A randomized controlled trial: effect on hormonal levels and follicular development

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BACKGROUND: The optimal time for GnRH antagonist initiation is still debatable. The purpose of the current randomized controlled trial is to provide endocrine and follicular data during ovarian stimulation for IVF in patients with polycystic ovarian syndrome (PCOS) treated either with a long GnRH agonist scheme or a fixed day-1 GnRH antagonist protocol. METHODS: Randomized patients in both groups (antagonist: $n = 26$; long agonist: $n = 52$) received oral contraceptive pill treatment for three weeks and a starting dose of 150 IU of follitropin β. The primary outcome was E_2 level on Day 5 of stimulation, while secondary outcomes were follicular development, LH during ovarian stimulation and progesterone levels. RESULTS: Significantly more follicles on days 5, 7 and 8 of stimulation, significantly higher estradiol (E_2) levels on days 1, 3, 5, 7 and 8 and significantly higher progesterone levels on days 1, 5 and 8 of stimulation were observed in the antagonist when compared with the agonist group. E_2 was approximately twice as high in the antagonist when compared with the agonist group on day 5 of stimulation (432 versus 204 pg ml^{-1}, $P < 0.001$). These differences were accompanied by significantly lower LH levels on days 3 and 5 and significantly higher LH levels on days 1, 7 and 8 of stimulation in the antagonist when compared with the agonist group. CONCLUSIONS: In PCOS patients undergoing IVF, initiation of GnRH antagonist concomitantly with recombinant FSH is associated with an earlier follicular growth and a different hormonal environment during the follicular phase when compared with the long agonist protocol.

Keywords: antagonist luteinizing hormone/estradiol/follicular development/GnRH agonist/PCOS

Introduction

For several years the pharmaceutical industry sustained an interest in developing more patient-friendly analogues with immediate initiation of action. In the early 2000s, the introduction of GnRH antagonists in IVF was accomplished by five large randomized controlled trials (RCTs) which compared GnRH antagonists with the long GnRH agonist protocol. The meta-analysis of these trials showed that a lower requirement for gonadotrophins, a reduced length of treatment and a dramatic reduction in the duration of analogue treatment was present in the GnRH antagonist group (Al-Inany and Aboulghar, 2002). Moreover, a tendency for a lower occurrence of ovarian hyperstimulation syndrome (OHSS) in the antagonist group has suggested that this form of treatment is worth evaluating in patients at high risk for OHSS, such as

those with polycystic ovarian syndrome (PCOS). However, a significantly lower (5%) clinical pregnancy rate compared with the agonist group has resulted in a lower acceptance of GnRH antagonists in ovarian stimulation for IVF (Fauser and Devroey 2005; Griesinger *et al.*, 2005) and has stimulated research for optimizing the existing GnRH antagonist protocols (Kolibianakis *et al.*, 2005).

One of the currently debatable issues regarding the use of GnRH antagonists refers to the timing of GnRH antagonist initiation. A fixed protocol starting antagonist arbitrarily on Day 6 of stimulation has been used in all introductory comparative trials employing a daily antagonist administration (Al-Inany and Aboulghar, 2002). Following these trials, a flexible antagonist initiation by a follicle of 14–15 mm has been evaluated and a meta-analysis of relevant RCTs suggested

that flexible antagonist administration after Day 5 of stimulation does not appear to improve pregnancy rates (Al-Inany *et al.*, 2005).

Research for alternative GnRH antagonist protocols regarding the timing of its initiation has shifted towards earlier antagonist initiation than Day 6 of stimulation (Elkind-Hirsch *et al.*, 2003; Kolibianakis *et al.*, 2003a; Hwang *et al.*, 2004; Lainas *et al.*, 2005). Support for earlier antagonist initiation was further provided by observations that the exposure to LH and E_2 in the early follicular phase of a GnRH antagonist cycle was negatively associated with the probability of pregnancy (Kolibianakis *et al.*, 2003b).

Currently, initiation of antagonist in the early follicular phase in PCOS patients has been performed in a non-comparative intrauterine insemination trial (Elkind-Hirsch *et al.*, 2003), in which it was started concomitantly with gonadotrophin stimulation and in a comparative IVF study in which the antagonist was started one day before administration of gonadotrophins using as a control the long agonist protocol (Hwang *et al.*, 2004). In the latter study, no detailed hormonal and ultrasound data were available.

The purpose of this RCT is to provide endocrine and follicular data during ovarian stimulation for IVF in patients with PCOS treated either with a long-GnRH agonist scheme or a fixed GnRH antagonist protocol in which GnRH antagonist is initiated on Day 1 of stimulation.

Materials and Methods

Study design

This was a single-center RCT. Random allocation was performed by a study nurse on the basis of a computer-generated randomization list in a 1:2 ratio. The responsible physicians (investigators) were not involved in the randomization process. Neither patients nor doctors were blinded to the treatment assigned. More patients were randomized in the agonist group, as the antagonist used so far, has been associated with a significantly lower probability of clinical pregnancy (Al-Inany and Aboulghar, 2002). For this purpose it was deemed appropriate to introduce it more gradually in clinical practice in our centre. Patients were treated either by GnRH antagonist starting from the first day of stimulation, ($n = 26$, antagonist group), or by a long GnRH agonist protocol ($n = 52$, agonist group). 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 78 patients with PCOS undergoing IVF/ICSI treatment at the Eugonia-Iatriki Erevna IVF unit from January 2003 to January 2005 were included in the study. Patients could enter the study only once and were diagnosed as PCOS [presence of oligoovulation/anovulation (Ehrmann *et al.*, 2006) and polycystic ovaries]. Additional inclusion criteria were: age 18–39 years, less than three previous IVF/ICSI attempts, no endometriotic cyst present as assessed by transvaginal ultrasound examination and basal hormonal levels of FSH in the early follicular phase of ≤ 10 IU l^{-1}. Patients with known previous poor ovarian response (Kolibianakis *et al.*, 2004) were excluded.

Ovarian stimulation

All patients received oral contraceptive pill (OCP) starting on Day 2 of spontaneous menses of the cycle prior to the treatment cycle, after

blood test confirmed the presence of a baseline hormone profile. All patients had spontaneous menses and no one received progesterone for induction of withdrawal bleeding. The OCP contained 0.03 mg ethinyl E_2 and 0.075 mg gestodene (Minulet, Wyeth, Greece). OCPs were taken daily for 21 days.

In the GnRH antagonist protocol (antagonist group), patients started daily recombinant FSH (rFSH) treatment with s.c. injections of follitropin β (Puregon, Organon, The Netherlands) concomitantly with a daily s.c. dose of 0.25 mg ganirelix (Orgalutran, Organon, The Netherlands) on Day 2 of menses (Day 1 of stimulation) that followed the discontinuation of the OCP. Treatment with rFSH and GnRH antagonist continued daily thereafter, until and including the day of β-hCG administration.

Patients in the agonist group were administered s.c. GnRH agonist 0.1 mg triptorelin (Arvekap, Ipsen, France) daily. The agonist was started 3 days before discontinuation of the oral contraceptive. All patients had blood loss after discontinuation of the OCP. When desensitization was achieved ($\sim$10–15 days after the initiation of GnRH agonists), as evidenced by plasma E_2 levels of ≤ 50 pg ml^{-1}, the absence of ovarian follicles and the absence of a thick endometrium (endometrial thickness ≤ 6 mm) on transvaginal ultrasound examination (Barash *et al.*, 1998), daily s.c. injection of rFSH (Puregon) was commenced. The dose of GnRH agonist was decreased on that day to 0.05 mg day^{-1} and continued until and including the day of β-hCG administration.

The starting dose of rFSH was 150 IU day^{-1} for all patients in both groups. This dose was adjusted after Day 5 of stimulation, depending on the ovarian response, as assessed by E_2 levels and ultrasound. A step-up protocol was used, if necessary. As soon as three follicles reached a mean diameter of ≥ 17 mm, 10 000 IU of β-hCG (Pregnyl, Organon, The Netherlands) was administered intramuscularly. ICSI and IVF procedures were performed as described previously (Devroey and Van Steirteghem, 2004; Van Landuyt *et al.*, 2005). Conventional IVF was used for normal semen samples after assessment according to the WHO criteria (WHO, 1999). ICSI was performed in cases of severe male factor (Devroey and Van Steirteghem, 2004), or when antisperm antibodies were present (Devroey and Van Steirteghem, 2004) and also following low or failed fertilization using conventional IVF in a previous attempt (Vicdan and Isik, 1999). In cases of borderline semen both IVF and ICSI were performed (van der Westerlaken *et al.*, 2006). Up to three embryos were transferred on Day 2 or Day 3 of *in vitro* culture.

In cases of excessive ovarian response that could lead to life-threatening OHSS (Navot *et al.*, 1992), elective cryopreservation was performed. Excessive ovarian response was defined by the following criteria: high E_2 levels (>4000 pg ml^{-1}) and more than 35 follicles on the day of hCG (Navot *et al.*, 1992), hematocrite >45, white blood cell count $>15 000$, ovarian size >12 cm 3 days after oocyte retrieval (Navot *et al.*, 1992; Brinsden *et al.*, 1995). For the purposes of the current study a modified system of OHSS classification previously described was adopted (Rizk and Aboulghar, 1993).

Ultrasound and laboratory assays

Ovarian stimulation was monitored by transvaginal ultrasound measurement of follicular growth, typically on days 1, 3, 5, 7 and 8 of stimulation and on the day of HCG administration. Additional evaluations were performed as required. All ultrasound measurements were performed using a 7.5, 6 or 5 MHz vaginal probe (Sonoline, Adara, Siemens).

The follow-up also included assessment of E_2, LH and progesterone concentrations on the same days. Blood samples were taken early in the morning, prior to medication administration, just before ultrasound assessment, and all biochemical tests were performed immediately.

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FSH, LH, E$_2$ and progesterone levels were measured on an Immulite analyser using the corresponding commercially available kits (DPC, Los Angeles, CA). Analytical sensitivity was 0.1 mIU ml^{-1} for FSH, 0.1 mIU ml^{-1} for LH, 15 pg ml^{-1} for E$_2$ and 0.2 ng ml^{-1} 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 E$_2$ and 7.9 and 10% for progesterone. Baseline hormonal measurements included E$_2$, progesterone, FSH and LH, in both groups, on the day of the OCP initiation (Day 2 of menses).

Outcome measures

The primary outcome measure was E$_2$ levels on Day 5 of stimulation, while secondary outcome measures evaluated were follicular development, LH and progesterone levels.

Statistical analysis

It was calculated that group sample sizes of 26 and 52 would achieve 81% power to detect a difference in E$_2$ levels on Day 5 of stimulation of 225 pg ml^{-1} between the null hypothesis that both group means are 450 pg ml^{-1} of E$_2$ and the alternative hypothesis that the mean of E$_2$ in the agonist group is 225 pg ml^{-1} with a significance level (alpha) of 0.05 using a two-sided Mann–Whitney U test (NCSS and PASS, Kaysville Utah, www.ncss.com). Continuous variables were analysed by the Mann–Whitney U test while nominal variables were analysed in the form of frequency table by the use of the exact χ-square test for trend or the Fisher's exact test (SPSS Inc., Chicago, IL, USA). Values

are expressed as median (interquartile range) unless stated otherwise. Exposure to LH, E$_2$ and progesterone during the follicular phase was calculated using the area under the curve and values are expressed as median (95% CI of the median).

Results

Baseline characteristics and hormonal profile of the patients analysed are listed in Table 1. No significant differences were observed between the antagonist and the agonist group. All patients in this study reach the HCG criteria and all patients underwent oocyte retrieval.

Ovarian stimulation characteristics and hormonal data on the day of hCG in the antagonist and the agonist group are shown in Table 1. The antagonist group was characterized by a significantly shorter stimulation period (10.0 versus 12.0 days respectively; $P < 0.001$).

Following oocyte retrieval, embryo transfer was not performed in one patient in the antagonist group (3.85%, 95% CI: 0.68–18.89) and in six patients in the agonist group (11.54%, 95% CI: 5.40–22.97) due to excessive ovarian response. All these women developed OHSS. Overall, the incidence of moderate–severe OHSS (Rizk and Aboulghar, 1993) was significantly lower in the antagonist when compared with the agonist group (11.54 versus 38.46%, respectively; $P < 0.02$; difference −26.92%, 95% CI: −42.45 to −5.76). When considering

Table 1. Baseline characteristics, hormonal profile of patients, ovarian stimulation characteristics and hormonal data on the day of hCG in the antagonist and in the agonist group

	Antagonist group ($n = 26$)	Agonist group ($n = 52$)	P-value
Baseline characteristics			
Age (years)	32.0 (14)	30.5 (16)	0.079
BMI (kg m^{-2})	23.2 (20.9)	23.6 (18.9)	0.585
Duration of infertility (years)	4.0 (11)	3.5 (11)	0.905
Subjects with previous IVF attempts n (%)	10 (38.5)	16 (30.8)	0.497
Indication for IVF n (%, 95% CI)			
PCOS only	6 (23.1, +11.0 to +42.0)	13 (25, +15.2 to +38.2)	
PCOS + male factor	15 (57.7, +38.9 to +74.5)	22 (42.3, +29.9 to +55.8)	0.616
PCOS + tubal factor	4 (15.4, +6.1 to +33.5)	13 (25, +15.2 to +38.2)	
PCOS + endometriosis	1 (3.8, +0.7 to +18.9)	4 (7.7, +3.0 to +18.2)	
Baseline hormonal profile[a]			
FSH (IU l^{-1})	6.3 (1.7)	5.8 (2.6)	0.129
LH (IU l^{-1})	5.4 (3.6)	5.6 (4.5)	0.948
E$_2$ (pg ml^{-1})	35 (66.0)	30.5 (20.0)	0.251
Progesterone (ng ml^{-1})	0.66 (0.4)	0.61 (0.4)	0.162
Stimulation period (days)	10.0 (3)	12.0 (2)	0.001
Number of oocytes retrieved	26.0 (19.5)	29.0 (16.2)	0.166
Mature oocytes (in ICSI patients)	17.5 (12.75)	15 (16.5)	0.919
Type of fertilization n (%, 95% CI)			
IVF	10 (38.5, +22.43 to +57.47)	30 (57.7, +44.2 to +70.1)	
ICSI	11 (42.3, +25.5 to +61.0)	17 (32.7, +21.5 to +46.2)	0.229
IVF + ICSI	5 (19.2, +8.5 to +37.9)	5 (9.6, +4.2 to +20.6)	
Fertilization rate	52.8 (23.5)	58.1 (27.3)	0.869
Within IVF	56.3 (21.6)	64.7 (24.0)	0.450
Within ICSI	50.0 (30.2)	44.7 (16.2)	0.264
Within ICSI + IVF	46.2 (13.8)	44.4 (54.4)	0.841
E$_2$ concentration on hCG day (pg ml^{-1})	2333 (1505)	2858 (1449)	0.153
LH concentration on hCG day (mIU ml^{-1})	1.6 (1.8)	0.9 (0.8)	0.109
PRG concentration on hCG day (ng ml^{-1})	1.1 (0.5)	1.1 (0.7)	0.431
OHSS Moderate–severe n (%)	3/26 (11.54)	20/52 (38.46)	0.02[b]

Values are expressed as median (interquartile range) unless stated otherwise.
[a]Prior to OCP initiation.
[b]Difference, 95% CI: −26.92%, 95% CI: −42.45 to −5.76.

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only those patients who had their embryo transfer cancelled, the difference in the incidence of OHSS between the two analogue groups was not statistically significant.

No LH surges were observed in the agonist group while a transient LH surge (LH 14.8 IU L^{-1}) was observed in the antagonist group on day 8 of stimulation. LH levels returned to normal on the next day of stimulation (3 IU L^{-1}) and no progesterone rise occurred.

Table 2 shows the follicular development in the groups compared, while Table 3 and Figure 1 show the hormonal profile of patients stimulated with GnRH antagonists and GnRH agonists. The antagonist group was characterized by an earlier follicular growth than the agonist group. Already on Day 5 of stimulation, the proportion of women who had at least one follicle of ≥ 12 mm was significantly higher when compared with the agonist group (30.77 versus 5.77%; $P < 0.005$).

The same pattern was followed on days 7 and 8 of stimulation, regarding the proportion of women who had at least one follicle of ≥ 15 mm, which was significantly higher in the antagonist group (34.6 versus 9.6%; $P < 0.011$ and 46.2 versus 19.2%; $P < 0.017$, respectively). Although on Day 7 neither group had any women with follicles of greater than 18 mm, on Day 8 of stimulation significantly more ($P < 0.001$) women in the antagonist group had at least one follicle of ≥ 18 mm (34.6%) when compared with those in the agonist group (1.9%) (Table 2).

E_2 rose in line with follicular growth. Patients in the antagonist group started with a significantly higher E_2 concentration when compared with the agonist group (35 versus 12 pg ml^{-1}, respectively; $P < 0.001$). E_2 was approximately twice as high in the antagonist when compared with the agonist group on Day 5 of stimulation 432 versus 204 pg ml^{-1}; $P < 0.001$). E_2 remained significantly higher up to Day 8 of stimulation (Table 3; Fig. 1) in the antagonist group. E_2 exposure, as

Table 3. Hormonal profile in the antagonist and the agonist group during ovarian stimulation up to day 8 of stimulation

	Antagonist	Agonist	P-value
FSH (IU 1^{-1})			
Day 1	6.2 (1.6)	3.6 (1.7)	0.001
LH (IU L^{-1})			
Day 1	5.2 (2.4)	1.9 (1.9)	0.001
Day 3	0.6 (0.3)	1.4 (0.5)	0.003
Day 5	0.6 (0.4)	0.9 (0.5)	0.008
Day 7	1.6 (1.6)	1.0 (0.8)	0.006
Day 8	2.3 (2.7)	0.8 (0.7)	0.002
E_2 (pg ml^{-1})			
Day 1	35 (27.2)	12 (13.7)	0.001
Day 3	121 (112)	55 (69)	0.001
Day 5	432 (425)	204 (211)	0.001
Day 7	1121 (994)	571 (558)	0.001
Day 8	1558 (1460)	954 (1118)	0.033
Progesterone (ng ml^{-1})			
Day 1	0.7 (0.3)	0.4 (0.2)	0.01
Day 3	0.5 (0.5)	0.7 (0.6)	0.195
Day 5	0.7 (0.4)	0.4 (0.4)	0.045
Day 7	0.9 (0.4)	0.7 (0.4)	0.077
Day 8	1.1 (0.6)	0.6 (0.4)	0.016

Values are expressed as median (interquartile range).

expressed by the area under the curve (median, 95% CI) for E_2 (pg ml^{-1} days) was significantly higher in the antagonist group when compared with the agonist group (3761.85, 95% CI: $+2519.83$ to $+5529.66$ versus 2010.50, 95% CI: $+1488.81$ to $+2412.82$).

In the agonist group a minor decline of plasma LH levels was observed from day 1 to Day 8 of stimulation (Table 3; Fig. 1). On the contrary, in the antagonist group there was a significant decrease of LH levels from Day 1 to Day 3 of stimulation. This resulted in significantly lower plasma LH concentrations on Day 3 (0.6 versus 1.4 IU L^{-1}; $P < 0.003$) and on Day 5 of stimulation (0.6 versus 0.9 IU L^{-1}; $P < 0.008$) in the antagonist group than the agonist group, respectively. However, from Day 5 onwards, the LH levels in the antagonist group started to rise. This resulted in significantly higher plasma LH levels on Day 7 (1.6 versus 1.0 IU L^{-1}; $P < 0.006$) and on Day 8 (2.3 versus 0.8 IU L^{-1}; $P < 0.002$) of stimulation in the antagonist when compared with the agonist group (Table 3, Fig. 1). LH exposure, as expressed by the area under the curve (median, 95% CI) for LH (IU $\times$ days) was higher in the antagonist group when compared with the agonist group but not significantly (12.96, 95% CI: $+11.61$ to $+15.37$ versus 12.17, 95% CI: $+10.31$ to $+12.98$).

Significantly higher progesterone levels were observed in the antagonist group when compared with the agonist group on Day 1 (0.7 versus 0.4 ng ml^{-1}; $P < 0.01$), Day 5 (0.7 versus 0.4 ng ml^{-1}; $P < 0.045$) and Day 8 (1.1 versus 0.6 ng ml^{-1}; $P < 0.016$) of stimulation, respectively (Table 3, Fig. 1). Progesterone exposure, as expressed by the area under the curve (median, 95% CI) (ng ml^{-1} days) was higher in the antagonist group when compared with the agonist group, but not significantly (5.04, 95% CI: $+4.19$ to $+5.98$ versus 4.65, 95% CI: $+4.44$ to $+4.70$).

Table 2. Follicular development in the antagonist and the agonist group during ovarian stimulation

	Antagonist group (%)	Agonist group (%)	Difference % (95% CI)
At least 12 mm			
Day 1	0 (0)	0 (0)	0 (-6.9 to $+12.8$)
Day 3	0 (0)	0 (0)	0 (-6.9 to $+12.8$)
Day 5	8 (30.8)	3 (5.8)	25 ($+7.6$ to $+44.6$)
Day 7	14 (53.8)	18 (34.6)	19.2 (-3.6 to $+40.1$)
Day 8	15 (57.7)	19 (36.5)	21.1 (-2.0 to $+41.6$)
At least 15 mm			
Day 1	0 (0)	0 (0)	0 (-6.9 to $+12.8$)
Day 3	0 (0)	0 (0)	0 (-6.9 to $+12.8$)
Day 5	2 (7.7)	0 (0)	7.7 (-1.1 to $+24.1$)
Day 7	9 (34.6)	5 (9.6)	25.0 ($+6.2$ to $+44.9$)
Day 8	12 (46.2)	10 (19.2)	26.9 ($+5.4$ to $+7.1$)
At least 18 mm			
Day 1	0 (0)	0 (0)	0 (-6.9 to $+12.8$)
Day 3	0 (0)	0 (0)	0 (-6.9 to $+12.8$)
Day 5	0 (0)	0 (0)	0 (-6.9 to $+12.8$)
Day 7	0 (0)	0 (0)	0 (-6.9 to $+12.8$)
Day 8	9 (34.6)	1 (1.9)	32.7 ($+15.4$ to $+51.9$)

Values in the tables represent the number (%) of women who had at least one follicle whose size was within the set criteria.
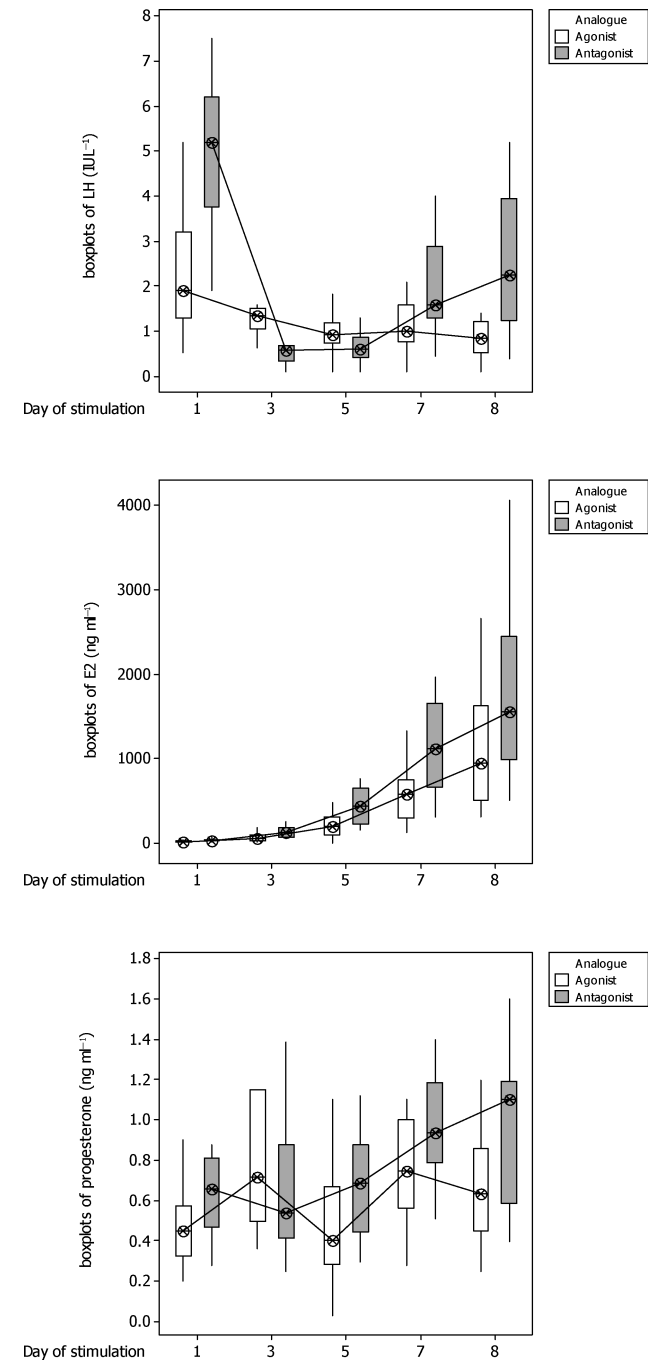
Figure 1. Boxplots of LH, E2 and Progesterone in the antagonist and the agonist group

No significant differences were observed regarding achievement of pregnancy. Biochemical, clinical (sac with fetal heart at 7 weeks) and ongoing pregnancy rates (sac with fetal heart at 12 weeks) in the two groups compared are shown in Table 4. Moreover, no significant difference was present in implantation rate between the agonist and the antagonist group [29 (76) versus 25% (33), respectively; $P = 0.213$].

Discussion

This study has shown that initiation of GnRH antagonist on Day 1 of stimulation for IVF when compared with the

Table 4. Achievement of pregnancy in the agonist and the antagonist groups

	Antagonist group $(n) = 26$	Agonist group $(n = 52)$	Difference 95% CI	P (Fisher's exact test)
Biochemical pregnancy % (n)	76.9 (20)	67.3 (35)	9.6 (-12.4 to $+27.7$)	0.439
Clinical pregnancy % (n)	57.7 (15)	61.5 (32)	-3.8 (-26.1 to $+17.7$)	0.808
Ongoing pregnancy % (n)	46.2 (12)	48.1 (25)	-1.9 (-23.7 to $+20.6$)	0.999

long-agonist protocol is associated with a more rapid follicular development, an earlier rise in E_2 levels and significantly higher levels of progesterone. These differences are accompanied by significantly lower LH levels in the early follicular phase and significantly higher LH levels in the late follicular phase in the antagonist group. The exposure to hormonal levels during the above period, as measured by the area under the curve, was higher in the antagonist when compared with the GnRH agonist group for LH and progesterone, but not significantly so. In contrast, a significantly higher exposure to E_2 was present in the antagonist when compared with the agonist group.

The current study provides hormonal and follicular development data between a long agonist protocol and an antagonist scheme of stimulation in which GnRH antagonist is started on Day 1 of stimulation, in women with PCOS undergoing IVF. Randomized patients were stimulated with the same starting dose of rec FSH and E_2 levels were not considered for timing HCG administration, which was performed as soon as greater than or equal to three follicles of 17 mm were present. As a result, duration of stimulation reflects only follicular development.

Currently, two comparative studies have been published in PCOS patients between GnRH agonists and antagonists (Hwang *et al.*, 2004; Bahceci *et al.*, 2005). The stimulation protocols used in those studies do not allow meaningful comparisons to be performed with the current study. Similar to the present study, Bahceci *et al.* (2005) compared in a randomized trial GnRH agonists with antagonists in PCOS patients. Although a long agonist protocol was used in both the studies, in contrast to the current study, initiation of GnRH antagonist was performed in a flexible manner by the presence of a follicle of 14 mm. The antagonist protocol used by Hwang *et al.* (2004) was more similar to the one used in the current study. The GnRH antagonist cetrorelix was started one day prior to initiation of stimulation with HMG and was compared with the long agonist protocol in 60 PCOS patients. However, the different type of gonadotrophins administered as well as the different type of antagonist and the variable dose with which it was used during stimulation, makes comparison of hormonal and follicular data between the two studies difficult.

In the current study, stimulation in the agonist and the antagonist group started with down-regulated endogenous LH levels. In the agonist group this occurred after 2 weeks of

agonist administration, while in the antagonist group LH was suppressed immediately, a few hours after antagonist administration (The ganirelix dose-finding study group, 1998). Although it cannot be excluded that the significant differences observed in the degree of LH suppression between the two groups compared (Table III) might be responsible partially for the differences observed in follicular development, this appears to be unlikely. It has been suggested that LH assists in follicular development (Filicori *et al.*, 2002) and thus at least in the early follicular phase. Follicular development should be expected to be better in the agonist group that showed significantly higher endogenous LH levels.

Alternatively, the distinct patterns of follicular development might be attributed to the different endocrine environment during the phase preceding the initiation of stimulation in each of the two groups compared. OCP, which is administered in the antagonist group, is known to suppress endogenous gonadotrophin levels. However, stimulation in the antagonist group did not start immediately after OCP discontinuation but only after menstruation had occurred. As menstruation following OCP discontinuation, occurs usually after 5 days, normal endogenous gonadotrophins are expected to be present at rec FSH initiation in the antagonist group. In the current study, this was true for FSH and LH levels which were not suppressed prior to initiation of antagonist (Table 3). On the other hand, in the agonist group the preceding phase is characterized by suppression of gonadotrophins due to the analogue use following an initial OCP administration and stimulation starts with suppressed endogenous gonadotrophins (Table 3). These data suggest that in PCOS patients treated by IVF the continuous suppression of endogenous gonadotrophins in the phase preceding initiation of stimulation might be associated with a slower pattern of follicular development and lower E_2 levels.

Nevertheless, similar numbers of cumulus-oocyte complexes were retrieved in the agonist and the antagonist group by prolonging the follicular phase in the agonist group, in which a slower follicular development was observed. However, this was accompanied by an increased incidence in the occurrence of moderate to severe OHSS. The reduced OHSS incidence in the antagonist group is a result of a subgroup analysis and as such it serves mainly to generate a hypothesis to be tested in future studies. If confirmed, it might be of importance, in selecting the preferred protocol of treatment for high-risk patients with OHSS such as those with PCOS.

In the two existing studies comparing GnRH agonists with antagonists in PCOS patients, a similar trend for OHSS occurrence was observed. Although no severe OHSS cases were reported in the study by Hwang *et al.* (2004), embryo transfer was cancelled due to high risk of OHSS in three patients in the agonist group while embryo transfer was not cancelled for the same reason in the antagonist group. In the study by Bahceci *et al.* (2005), no significant difference was observed in OHSS occurrence (grade I–II), although this was again higher in the agonist group (7.1 versus 5.0%, respectively).

A different pattern in the levels of LH was also observed during ovarian stimulation with significantly higher LH levels in the antagonist group in the late follicular phase (Days 7

and 8). These might be related to the significantly higher levels of E_2 in the antagonist when compared with the agonist group during these days, through a positive feedback mechanism. Alternatively, the degree of suppression with GnRH antagonist might not be adequate to maintain LH levels suppressed to a similar extent as the GnRH agonist group. The adequacy of LH suppression using the standard dose of 0.25 mg daily has been put in doubt recently (Messinis *et al.*, 2005).

In conclusion, the current study suggests that in PCOS patients undergoing IVF, initiation of GnRH antagonist concomitantly with rFSH when compared with the long agonist protocol is associated with an earlier follicular development, and a different hormonal environment during the follicular phase when compared with the long agonist protocol. In these high-risk for OHSS patients, the Day 1 antagonist protocol appears promising and its effect on ongoing pregnancy/live birth rates should be investigated in large-scale studies.

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