

Dose-finding study of daily GnRH antagonist for the prevention of premature LH surges in IVF/ICSI patients: optimal changes in LH and progesterone for clinical pregnancy

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BACKGROUND: An optimal range of LH concentrations for achieving pregnancy has not been established. The aim of this study was to investigate the effect of various LH levels induced by different GnRH antagonist doses on IVF outcome. **METHODS:** This was a prospective, single centre study including 144 IVF patients, stimulated with recombinant FSH from cycle day 2, and co-treated with daily GnRH antagonist (antide/Iturelix) (2 mg/2 ml, 1 mg/ml, 0.5 mg/ml, 0.5 mg/0.5 ml or 0.25 mg/ml) from cycle day 7 onwards. Serum samples were taken three times daily. **RESULTS:** Clinical pregnancies were only observed within a particular range of change in LH levels. The upper and lower thresholds for the mean LH area under the curve (AUC), adjusted for the baseline LH level before the antagonist was started (LH AUC^{-S6}; S6 = stimulation day 6) were -2.2 and 12.4 (IU/l) respectively (a negative value = below baseline levels). There were no clinical pregnancies outside these threshold values. Similar results were found for progesterone, the threshold levels of progesterone AUC^{-S6} were 3.98 and -1.21 ng/ml. Moreover, there were no pregnancies with progesterone levels >0.26 ng/ml/follicle on the day of hCG. **CONCLUSIONS:** Excessive or insufficient suppression of LH and progesterone levels during GnRH antagonist administration and high progesterone/follicle on hCG day seems to be associated with impaired clinical pregnancy rates.

Key words: GnRH antagonist/IVF/implantation/ LH/progesterone

Introduction

GnRH antagonist can be used safely to suppress LH levels and to prevent premature LH surges in IVF-stimulated cycles (Huirne and Lambalk, 2001). For many years, long agonist protocols have been the standard treatment regimen for this indication. Recently GnRH antagonists have been introduced in many IVF centres since their use improved patients' convenience in comparison to the long agonist protocol. Three GnRH antagonists have been studied in proper dose-finding studies for their daily use in IVF patients to prevent premature LH surges; ganirelix (Ganirelix dose-finding Study Group 1998), cetrorelix (Albano *et al.*, 1997; Olivennes *et al.*, 1998) and antide (Huirne *et al.*, 2004). In a ganirelix dose-finding study, the implantation rate was inversely associated with the antagonist dose. The highest pregnancy rates were found in the minimal effective dose group (0.25 mg) and low pregnancy rates were especially found in the high dosage groups (2 and 1 mg) (Ganirelix dose-finding Study Group,

1998). The influence of GnRH antagonist on implantation became a matter of debate after the publication of five major comparative studies between GnRH agonists and antagonists in IVF, which reported consequent lower pregnancy rates in the antagonist groups (Albano *et al.*, 2000; Borm and Mannaerts, 2000; European Middle East Orgulatan Study Group, 2001; Fluker *et al.*, 2001). These differences were not statistically significant in the individual studies, but a meta-analysis of these studies showed a significantly lower pregnancy rate of 5% (Al-Inany and Aboulghar, 2002). The mode of LH suppression and the pattern of circulating LH levels vary between GnRH antagonist and GnRH agonist cycles. After an initial period of gonadotrophin hypersecretion, GnRH agonists induce desensitization, mostly resulting in profound and stable LH levels during the entire stimulation period (Janssens *et al.*, 2000; Westergaard *et al.*, 2001). In contrast, the GnRH antagonist treatment regimens allow higher LH levels in the early stimulation period, since

antagonist is usually started from stimulation day 6 onwards. The LH levels rapidly decline after the start of the antagonist treatment, often followed by a gradual increase later in the cycle (Ganirelix dose-finding Study Group, 1998; Oberye *et al.*, 1999; Huirne *et al.*, 2004). Differences in the pattern and the level of LH suppression between agonist and antagonist regimens may play a role in the observed differences in pregnancy rates.

The role of LH on implantation is still not fully elucidated. It has been established that severe suppression of LH using GnRH agonists is associated with impaired IVF outcome (Fleming *et al.*, 1998; Howles, 2000; Balasch *et al.*, 2001; Filicori, 2002) and increased pregnancy loss (Westergaard *et al.*, 2000). A certain LH threshold needs to be achieved for adequate folliculogenesis and steroidogenesis, which is required to provide an appropriate milieu for successful fertilization and implantation (Hillier, 1994; European Recombinant human LH Study Group, 1998; Howles, 2000; Filicori, 2002; Shoham, 2002). On the other hand, there are convincing data suggesting that elevated LH levels are associated with impaired fertilization and pregnancy rates and with higher miscarriage rates (Stanger and Yovich, 1985; Howles *et al.*, 1986; Homburg *et al.*, 1988; Regan *et al.*, 1990; Chappel and Howles, 1991; Shoham, 2002; Tesarik and Mendoza, 2002; Loumaye *et al.*, 2003), the so called 'ceiling' effect (Hillier, 1994). The optimal LH levels to provide an endocrine milieu which results in the highest number of clinical pregnancies in patients undergoing IVF, in protocols with FSH stimulation, are still a matter of debate and have rarely been studied in GnRH antagonist-treated cycles. One recent study demonstrated that exposure to high LH levels in the early follicular phase of GnRH antagonist-treated cycles is associated with a reduced chance of pregnancy (Kolibianakis *et al.*, 2003a). An additional randomized controlled trial in which 257 women were randomized to receive either 150 or 200 IU rFSH per day showed a trend towards higher pregnancy rates, despite lower number of oocytes retrieved, in the lower recombinant (r)FSH dose group in which higher LH levels were found (Out *et al.*, 2004). However, lower early follicular phase LH and estradiol levels after early administration of the GnRH antagonist, stimulation day 1 versus day 6 in 60 patients, did not alter the IVF outcome (Kolibianakis *et al.*, 2003b).

In a previous study we demonstrated that various endogenous LH levels can be induced by different doses of repeated GnRH antagonist injections in IVF/ICSI patients undergoing ovarian stimulation with rFSH (Huirne *et al.*, 2004). The aim of the present study was to examine the effect of various LH concentrations, induced by different GnRH antagonist doses, on the outcome of IVF.

Materials and methods

Patients

All patients scheduled for IVF or ICSI treatment in our department during a period of 7 months were assessed for eligibility. In this period, 660 IVF cycles were performed in 290 patients; of these, 158 patients were eligible and 153 were asked to participate. A total

of 144 patients was included. All patients were aged between 21 and 39 years, had spontaneous regular menstrual cycles between 25 and 35 days, two ovaries, a normal uterine cavity, a body mass index $\leq 30 \text{ kg/m}^2$ and had had at least two spontaneous menstruations since the last clomiphene citrate or gonadotrophin treatment. Women with elevated hormone levels (FSH $\geq 10 \text{ IU/l}$ or LH $\geq 8 \text{ IU/l}$ or prolactin levels ($\geq 800 \text{ mIU/l}$) on cycle day 2 or 3 were excluded from participation. Patients with polycystic ovarian syndrome (defined as oligomenorrhoea and elevated LH levels or signs of hyperandrogenism) were also excluded. Also excluded were patients with abnormal haematological or biochemical parameters, patients with any previous assisted reproduction cycle with fewer than three oocytes, known allergy or hypersensitivity to human gonadotrophin preparations or GnRH analogues. The protocol was approved by the Committee on Ethics of Research involving Human Subjects of the VUMC, Amsterdam, The Netherlands. All participants signed informed consent forms.

Study design

A phase II, single centre study, conducted in two phases—a double-blind phase with two parallel treatment groups—was followed by an open phase. In the double-blind phase, 60 patients were randomized to two different treatment groups (A: 2 mg/2 ml; B: 1 mg/ml). To improve patients' convenience, the 2 mg in group A was given as two injections of 1 mg/ml antide, one injection in the morning and one in the evening, since we expected that one injection with a volume of 2 ml would be too painful. Patients in group B received placebo in the morning and 1 mg/ml antide in the evening. Since none of the two groups turned out to be a failure group (i.e. with two or more LH surges) we decided to add an open phase in which three additional treatment groups with lower antide dosages were studied (0.5 mg/0.5 ml, 0.5 mg/ml, 0.25 mg/ml). The additional arms were added in a consecutive order and patients were enrolled in a chronological fashion. New evidence which became available after the start of this study suggested that the bioavailability of antide increases after dilution in larger volumes of glucose 5% (data on file: Serono International, Geneva). Therefore in two groups (0.5 and 0.25 mg/ml) antide was diluted in larger volumes of glucose 5% solution: 0.5 and 0.25 mg in 1 ml respectively. This means that in two arms, 0.5 mg antide was administered but in group C it was diluted in 1.0 ml glucose 5% solution and in group D it was diluted in 0.5 ml glucose 5% solution.

Each group was intended to contain 30 patients unless more than one LH surge occurred, which according to the protocol led to the discontinuation of that particular dose group and was considered to be a failure dose. More than one LH surge per 30 patients was considered to be unacceptable for clinical use in IVF patients. Additional observations were performed, to search for optimal GnRH antagonist and/or LH levels. We made scatter plots to see whether an optimal range could be found for clinical pregnancy.

Masking

Treatment packs for the double-blind phase of the study were prepared according to the randomization list by Serono International (Geneva, Switzerland). Patient packs, containing antide/placebo or antide/antide vials, were labelled with unique study identification numbers, provided by Serono International (Geneva, Switzerland), placebo vials contained a sterile isotonic aqueous solution. When eligible, patients were enrolled into the study by one of the two responsible trained researchers and received a unique study number in a chronological order at the start of the first stimulation day. The code was not known to the executors of the study. Assignment to

group A or B was therefore double-blind, assignment to group C, D or E depending on the chronological entry of the study.

Treatment protocol

On day 2 or 3 of a spontaneous menstruation, rFSH (Gonal-F®; Serono, Switzerland) was given as a single daily s.c. injection. The starting dose varied between 150 and 300 IU, depending on previous ovarian response, but was fixed for the first 5 days. After this period, depending on ovarian response as assessed by daily ultrasound, the rFSH dose could be adjusted. All antide, placebo and rFSH injections from stimulation day 6 (S6) onwards were given subcutaneously, by a trained research professional. From stimulation day 6 onward, up to and including the day of rhCG (rhCG; Ovitrelle®; Serono) administration, daily antide was started. rhCG was administered as soon as one follicle was ≥ 18 mm and three follicles were ≥ 16 mm. Thirty-six hours after rhCG administration, oocyte retrieval was performed transvaginally and ultrasound-guided. The oocyte retrieval was followed by IVF with or without ICSI; a maximum of three embryos was replaced 2–3 days thereafter. Luteal support (200 mg progesterone vaginally, three times daily) was started 1 day after oocyte retrieval until the third week of pregnancy or a negative pregnancy test.

Assessments

One to three months before randomization, serum samples were taken to assess hormone levels (FSH, LH, estradiol, progesterone and prolactin), taken on cycle day 2 or 3. On stimulation day 1 (S1), before any study drug was administered, a blood sample was taken to perform a pregnancy test and to assess FSH, LH, estradiol (E₂) and progesterone levels and a transvaginal ultrasound was performed to measure follicular activity, endometrium thickness and to exclude the presence of cysts. During antide administration, three samples per day were taken (in the morning before any injection, in the evening prior to Gonal-F or antide injection, and 20–84 min later), to assess serum levels (FSH, LH, E₂, progesterone and antide). The potential variation in timing of the evening post-injection blood sampling was intended to allow pharmacokinetic and pharmacodynamic modelling (data not shown). The mean sample time was 34.6 (SD 3.9) min after antide injection (range of the mean per patient varied from 30 to 53 min). Transvaginal ultrasound was performed daily to assess follicular development and endometrium thickness. On the day of embryo transfer, serum samples were taken to measure the antide level, and 7–11 days after oocyte retrieval to measure the levels of progesterone and antide. Finally, 23–25 days after oocyte retrieval, serum hCG levels were measured. If positive, a vaginal ultrasound was performed 35 and 42 days following rhCG administration, to record the number of fetal sacs and fetal heart activity. Ultrasound was repeated at a gestational age of 12 weeks.

Serum assessment

Blood samples were processed to serum immediately after collection and stored at -20°C . Routine haematology, biochemistry and urine assessment were performed by the local laboratory (The Central Laboratory of the VUMC) using commercially available immunometric assays. LH and FSH levels before inclusion, in morning samples, were assessed by the local laboratory using immunometric assay kits (Amerlite; Amersham, UK). Patients were excluded for high LH and FSH values on cycle day 3, respectively >8 and >10 IU/l, using immunometric assay kits (Amerlite). Half-way through the study we were forced to change the assay, since Amerlite assays were no longer available. We decided to use Delphia

(Finland) assays. During the transition period of the assays, we assessed LH levels using both assays in 89 patients. Excellent correlation was observed between the two assays for the measurement of LH ($r = 0.981$) and FSH ($r = 0.996$). A regression analysis revealed that the coefficient of LH was 1.24 using Delphia compared with Amerlite, thus the LH threshold level of 8 IU/l assessed by Amerlite was equivalent to 9.9 IU/l if assessed by Delphia assay. In addition, the coefficient of FSH was 1.28, thus the FSH threshold level of 10 IU/l assessed by Amerlite was equivalent to 12.8 IU/l if assessed by Delphia assay.

For definitive analyses of all hormone and antide levels, as presented in this report, all serum samples (taken three times daily) were assessed retrospectively by LCG Bioscience Services Ltd. E₂ was measured using Sorin Radioimmunoassay, progesterone using DPC Coat-a-Count radioimmunoassay solid phase coated tube separation, FSH and LH using Serono MAIAclone IRMA. The lower limit of quantification for LH was 1 IU/l. For the retrospective analyses, we defined an LH surge as LH > 12.4 IU/l and progesterone > 2 ng/ml in one or more samples, taking all samples (three times daily) into account from S6 until hCG administration day, equivalent to the threshold levels using the Delphia assays. The retrospective centralized analysis of serum antide levels was performed by Woods Assay (radioimmunoassay); all samples were analysed in triplicate; 1 mg/l was the limit of quantification.

Outcome measures

Drug requirements, stimulation results, IVF outcome and its relationship to serum hormone and antide levels.

Statistical analyses

Treatment groups were compared depending on the nature of the variables, i.e. analysis of variance (ANOVA) or analysis of covariance (ANCOVA), χ^2 -test, Fisher's exact test or non-parametric ranking methods such as Kruskal–Wallis and Mann–Whitney *U*-tests. Results are reported as mean \pm SD. Correlations were calculated according to Pearson's correlation coefficient. $P < 0.05$ was considered to be statistically significant. Analyses were performed on all subjects who were randomized or received Gonal-F (all patients who were included in the study) unless otherwise reported. The number of patients included in A, B, C, D and E were 30, 30, 31, 23 and 30 respectively. An overall dose–response test for linear trend with the treatment groups was performed on all efficacy data. For continuous normally distributed data, a linear contrast of the treatment groups was tested in a one-way ANOVA; for ordinal (ordered categorical) data, a Jonckheere–Terpstra test and for binary data a Cochran–Armitage test were used.

This pilot study was not powered to calculate pregnancy rates, but group size was based on clinically relevant arguments. More than one LH surge per 30 patients was considered to be unacceptable for clinical use in IVF patients. Therefore the number of patients was intended to be 30 per group unless more than one LH surge occurred. This study employs patients included in a previous dose-finding study in which IVF outcome and hormones were examined (Huirne *et al.*, 2004).

Total exposure to antide and hormone levels was expressed as area under the curves (AUC) during antagonist administration [i.e. from stimulation day 6 (S6) to hCG administration day]. For this calculation, the sum of the mean daily levels (= sum of three samples per day/3) of all days during antide treatment was taken. To calculate the induced change in serum levels in comparison to the basal level on S6 (the time-point at which the antagonist was started), AUC was calculated after subtraction of the basal level on S6 of all samples, defined as $\text{AUC}^{-\text{S6}}$ (see Figure 1).

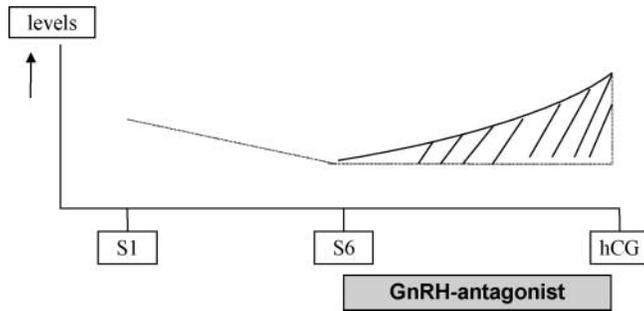


Figure 1. The method of area under the curve (AUC) calculation of the change in hormone levels. S1 = stimulation day 1; S6 = stimulation day 6.

Results

The general results (i.e. basic characteristics and cancellation rates), pharmacokinetic and the induced hormone levels of this antide dose-finding study were reported elsewhere (Huirne *et al.*, 2004). The ultrasound findings during

stimulation are presented in Table I and the IVF outcome in Table II. There were no differences in number of mature follicles (≥ 11 mm), mean follicular size and endometrium thickness on the day of hCG administration between the various dose groups (see Table I). Also no differences were found in number of oocytes retrieved or mean number of metaphase II oocytes per patient; the latter was only assessed and analysed in ICSI. Fertilization rate, total number of embryos, good quality embryos (grade I and II) and number of embryos transferred were also not different. There was a tendency toward higher pregnancy and implantation rates in the middle dose groups (0.5 and 1.0 mg/ml; not significant). None of the patients in this study had moderate or severe symptoms associated with OHSS necessitating hospitalization.

In contrast to the serum hormone levels, none of the IVF outcome parameters was linearly related to the dose groups or antide levels. The relationship between the AUC of antide and LH during antide administration and the occurrence of clinical pregnancies is plotted in Figure 2. No pregnancies

Table I. Late follicular phase ultrasound measurements

Ultrasound measurements	Antide treatment group (daily dose)					P
	A (2 mg/2 ml)	B (1 mg/ml)	C (0.5 mg/ml)	D (0.5 mg/0.5 ml)	E (0.25 mg/ml)	
No. of patients	30	30	31	23	30	
No. of follicles ≥ 11 mm, S6	3.1 (3.0)	2.8 (1.6)	3.8 (2.5)	3.0 (2.6)	3.9 (3.2)	0.42
No. of follicles ≥ 11 mm, hCG ^a	10.5 (4.8)	10.6 (3.6)	9.8 (3.2)	9.7 (4.0)	10.8 (4.3)	0.92
No. of follicles ≥ 15 mm, hCG ^a	5.6 (2.5)	6.0 (2.5)	5.4 (1.6)	5.5 (2.3)	6.0 (2.3)	0.92
No. of follicles ≥ 17 mm, hCG ^a	3.5 (1.4)	4.0 (1.7)	3.1 (0.9)	3.5 (1.4)	4.2 (2.6)	0.13
Endometrial thickness, hCG ^a	10.8 (2.3)	10.7 (2.0)	10.9 (1.8)	10.2 (1.6)	10.9 (2.1)	0.77

Values are means (\pm SD). Analyses were performed using Kruskal–Wallis tests, except for the endometrial thickness, where we used ANOVA.

Analyses were performed on all patients included in the study, unless otherwise indicated.

S6 = stimulation day 6; hCG = day of hCG.

a = analyses restricted to the patients who received hCG.

Table II. IVF outcome

IVF outcome	Antide treatment group (daily dose)					P
	A (2 mg/2 ml)	B (1 mg/ml)	C (0.5 mg/ml)	D (0.5 mg/0.5 ml)	E (0.25 mg/ml)	
No. of patients	30	30	31	23	30	
No. of oocytes	10.8 (6.8)	10.4 (5.2)	10.1 (5.1)	9.9 (4.2)	10.8 (5.9)	0.99
No. of metaphase II oocytes ^a	7.6 (5.3)	8.3 (5.3)	7.9 (4.3)	5.2 (2.8)	8.0 (3.5)	0.45
Fertilization rate ^b	0.57 (0.3)	0.62 (0.2)	0.65 (0.3)	0.53 (0.2)	0.65 (0.3)	0.20
No. of embryos	5.5 (4.8)	5.9 (3.4)	5.4 (3.5)	4.5 (3.6)	6.3 (5.0)	0.60
No. of good quality embryos ^c	3.5 (3.5)	4.2 (2.5)	3.3 (2.3)	2.8 (3.2)	4.5 (3.5)	0.05
No. of embryos transferred/embryo transfer	1.9 (0.5)	2.1 (0.4)	2.0 (0.3)	2.0 (0.3)	1.9 (0.4)	0.29
Implantation rate ^d (%)	6.7	13.8	16.1	7.9	8.9	0.50
Biochemical pregnancies ^e (%)	3 (10.0)	10 (33.3)	10 (32.3)	3 (13.0)	5 (16.7)	0.08
Clinical pregnancies ^f (%)	3 (10.0)	6 (20.0)	8 (25.8)	3 (13.0)	4 (13.3)	0.48
Ongoing pregnancies ^g (%)	3 (10.0)	6 (20.0)	7 (22.6)	3 (13.0)	4 (13.3)	0.65

Values are mean (\pm SD).

Analyses were performed on all patients included in the study, unless otherwise indicated. Kruskal–Wallis tests were used, except for % IVF and pregnancy parameters: χ^2 or Fisher's exact tests.

^aAnalyses only in ICSI patients.

^bFertilization rate = 2PN fertilized oocytes/number of oocytes inseminated.

^cNumber of grade I and II embryos.

^dThe mean implantation rate = sum of all individual implantation rates/number of subjects; implantation rate = number of gestational sacs per subject/number of embryos transferred per subject.

^eBiochemical pregnancy rate (PR) = hCG >50 IU/l.

^fClinical pregnancy rate = intrauterine pregnancy with at least one fetus with heart activity.

^gOngoing pregnancy = intrauterine pregnancy with proof of at least one vital fetus at 12–16 weeks after embryo transfer.

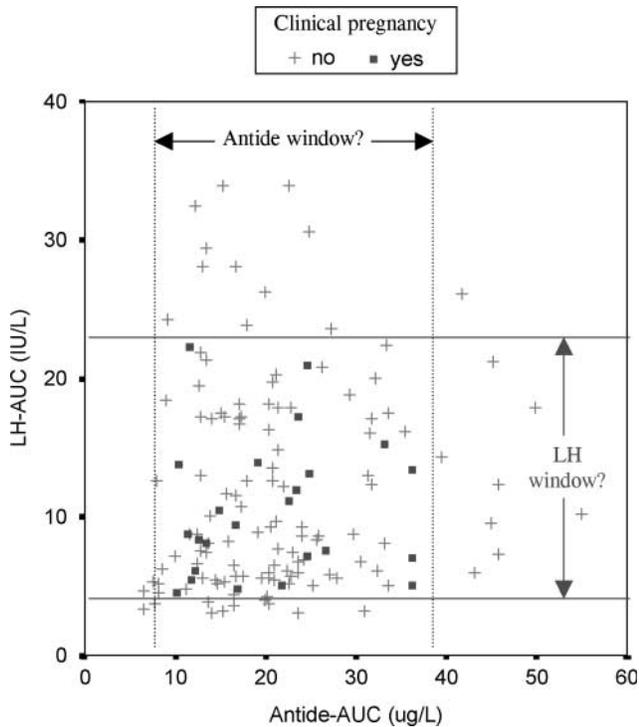


Figure 2. LH and Antide AUC levels in relation to clinical pregnancy

were observed in relation to either very high or very low LH or antide levels, suggesting an optimal window for total exposure of these compounds for the occurrence of pregnancy. The upper and lower thresholds for the total LH AUC were 22.3 and 4.47 respectively. The total amount of LH secreted was the highest with the lowest antide serum levels, while the lowest LH AUC values were found in association with the highest antide AUC values. Variation in the AUC values was higher in comparison to the absolute LH and antide levels per day, which showed better correlation (Huirne *et al.*, 2004). The upper and lower levels for antide AUC were 10.2 and 36.3 ($\mu\text{g/l}$) respectively. Scatter plots of the LH and antide levels on S7 and S8 and the day of hCG did not show any relationship or threshold level for the occurrence of clinical pregnancies (data not shown).

To study the effect of the induced change in LH during antide administration, we calculated the $\text{LH AUC}^{-\text{S6}}$. Clinical pregnancies could only be observed within a narrow range of induced change in LH levels ($\text{LH AUC}^{-\text{S6}}$) (see Figure 3), indicating the existence of an optimal window of LH changes (either an increase or decrease in relation to endogenous baseline levels) to obtain clinical pregnancy. No clinical pregnancies were observed if the LH levels increased too much ($\text{LH AUC}^{-\text{S6}} > 12.4 \text{ IU/l}$), or if LH decreased too much ($\text{LH AUC}^{-\text{S6}} < -2.2 \text{ IU/l}$) (a negative value means that the levels are decreased in comparison to the baseline levels). Based on these $\text{LH AUC}^{-\text{S6}}$ threshold levels, outside which no pregnancies occurred, we divided the patients retrospectively into three groups: a lower group with $\text{LH AUC}^{-\text{S6}} < -2.2 \text{ (IU/l)}$, a middle group with $\text{LH AUC}^{-\text{S6}} \geq -2.2$ and $\leq 12.4 \text{ (IU/l)}$, and a higher group with LH

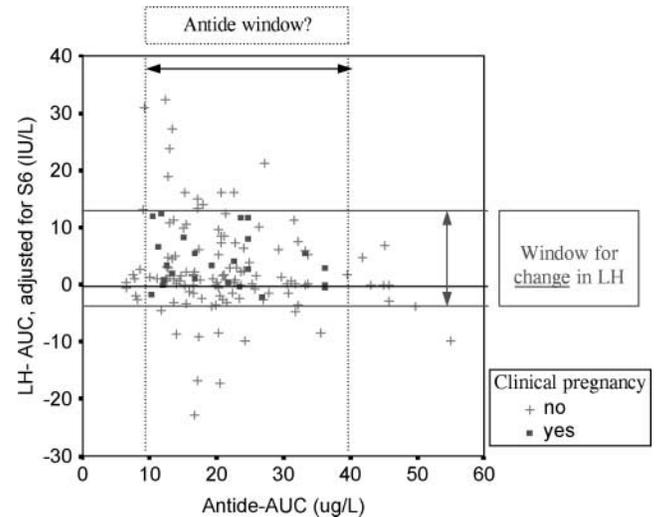


Figure 3. Change in LH: expressed as $\text{LH AUC}^{-\text{S6}}$ in relation to clinical pregnancy

$\text{AUC}^{-\text{S6}} > 12.4 \text{ (IU/l)}$. There were no differences in baseline characteristics and baseline hormone levels (see Table III). Also no differences were found with respect to duration of treatment, rFSH requirements, E_2 levels or FSH levels (data not shown). Additionally, the number and quality of oocytes, fertilization rate, number and quality of embryos and number of embryos replaced were also not different between the three groups (see Table III). On the basis of our selection criteria for the three groups, changes in LH and progesterone levels were different between the three groups (see Table III).

LH is related to estradiol and progesterone production (Pearson's correlation between LH AUC and E_2 AUC is 0.40; $P < 0.001$; between LH AUC and progesterone AUC is 0.55; $P < 0.001$; between $\text{LH AUC}^{-\text{S6}}$ and progesterone $\text{AUC}^{-\text{S6}}$ is 0.55; $P < 0.001$; and between LH AUC and progesterone/follicle and is 0.39; $P < 0.001$). In an attempt to obtain information on individual association of these parameters with clinical pregnancy, we created scatter plots of these individual parameters in relation to the $\text{LH AUC}^{-\text{S6}}$ (see Figure 4). We did not observe any optimal area for change in E_2 levels, expressed as ' $\text{E}_2 \text{ AUC}^{-\text{S6}}$ ' (see Figure 4a). In contrast, the change in progesterone, expressed as ' $\text{progesterone AUC}^{-\text{S6}}$ ', showed clear upper and lower thresholds for the occurrence of clinical pregnancies, 3.98 and -1.21 ng/ml respectively (Figure 4b). To correct for the effect of the number of follicles, we calculated the E_2 level per mature follicle on the day of hCG day ($\text{E}_2/\text{follicle} \geq 11 \text{ mm}$) as plotted in Figure 5a. These E_2 levels/mature follicle did not show any optimal level for the occurrence of clinical pregnancies, in agreement with the absolute E_2 levels or change in E_2 levels. To obtain a parameter for premature luteinization, adjusted for the total number of follicles, we calculated the progesterone levels per follicle $\geq 11 \text{ mm}$ on the day of hCG (progesterone/follicle) (see Figure 5b). For the progesterone/follicle on hCG day, a clear upper threshold with respect to the occurrence of clinical pregnancy was seen. No pregnancies occurred if the progesterone level was $> 0.26 \text{ ng/ml/follicle}$.

Table III. Baseline characteristics of three groups with different changes in LH levels, expressed as LH AUC^{-S6}

Baseline characteristics	Change in LH: LH AUC ^{-S6} (IU/l)			P
	Lower group < -2.2	Middle group ≥ -2.2 and ≤ 12.4	Higher group > 12.4	
No. of patients	24	106	14	
Age (years)	34.3 (3.3)	32.9 (3.5)	31.8 (3.2)	0.09
Body mass index (kg/m ²)	22.6 (2.7)	23.5 (2.9)	25.1 (3.8)	0.11
Duration of fertility (years)	4.4 (3.4)	4.3 (3.1)	3.3 (2.1)	0.63
No. of follicles < 11 mm, S1	7.5 (4.9)	7.6 (4.0)	8.7 (5.5)	0.85
LH, CD2/3 ^a	3.9 (1.6)	3.4 (1.2)	4.1 (1.1)	0.06
FSH, CD2/3 ^a	9.0 (5.1)	7.9 (2.5)	7.7 (2.3)	0.72
E ₂ , CD2/3 ^a	323 (547)	211 (237)	239 (133)	0.25
Progesterone, CD2/3 ^a	2.8 (1.3)	2.8 (2.0)	5.5 (10)	0.48
Changes in LH and progesterone				
LH AUC ^{-S6}	-6.7 (5.5)	2.9 (3.8)	19.3 (6.8)	<0.01
Progesterone AUC ^{-S6}	0.006 (2.3)	0.88 (1.8)	3.3 (2.8)	<0.01
IVF outcome parameters				
No. of oocytes	10.5 (7.0)	10.5 (5.2)	9.8 (5.0)	0.78
No. of metaphase II oocytes ^b	8.4 (4.7)	7.1 (4.3)	8.6 (3.5)	0.32
No. of embryos	6.1 (5.1)	5.5 (3.9)	5.2 (3.8)	0.94
No. of good quality embryos ^c	4.0 (3.4)	3.7 (3.1)	3.4 (2.2)	0.84
No. of embryos replaced	1.9	2.0	1.9	0.31
Implantation rate ^d	0	13.2 %	0	
No. of clinical pregnancies (%) ^e	0 (0)	24 (29)	0 (0)	

Values are means (± SD).

Analyses are performed on all patients included in this study, unless otherwise stated. LH AUC^{-S6} = LH area under the curve, adjusted for baseline levels on stimulation day 6. The threshold values for change in LH levels (LH AUC^{-S6}) were based on the occurrence of clinical pregnancy (see Figure 3).

^aCD2/3 = baseline value on cycle day 2 or 3 before any medication was given.

^bAnalyses only in ICSI patients.

^cNumber of grade I and II embryos.

^dThe mean implantation rate = sum of all individual implantation rates/number of subjects; implantation rate = number of gestational sacs per subject/number of embryos transferred per subject.

^eClinical pregnancy rate = intrauterine pregnancy with at least one fetus with heart activity. P-values were calculated using Kruskal–Wallis or Fisher’s exact tests.

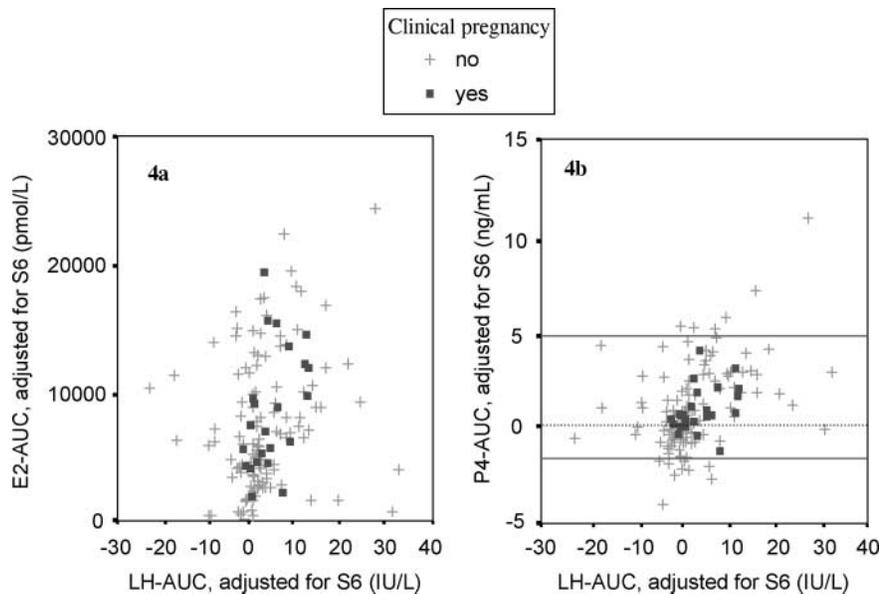


Figure 4. Change in oestradiol and progesterone levels, expressed as E2-AUC^{-S6} and P4-AUC^{-S6} in relation to LH levels and clinical pregnancy

Discussion

The primary aim of this paper was to assess IVF outcome parameters and the optimal range for induced LH levels induced by different doses of daily GnRH antagonist administration. No significant differences were found in IVF outcome parameters between the various treatment groups in

which dosages varied between 2.0 mg/2 ml and 0.25 mg/ml antide. Number of follicles, mature follicles, oocytes, quality of oocytes, number of metaphase II oocytes, and number and quality of embryos were all similar between the various treatment groups. With respect to the clinical pregnancies, again no significant differences could be found between the dose

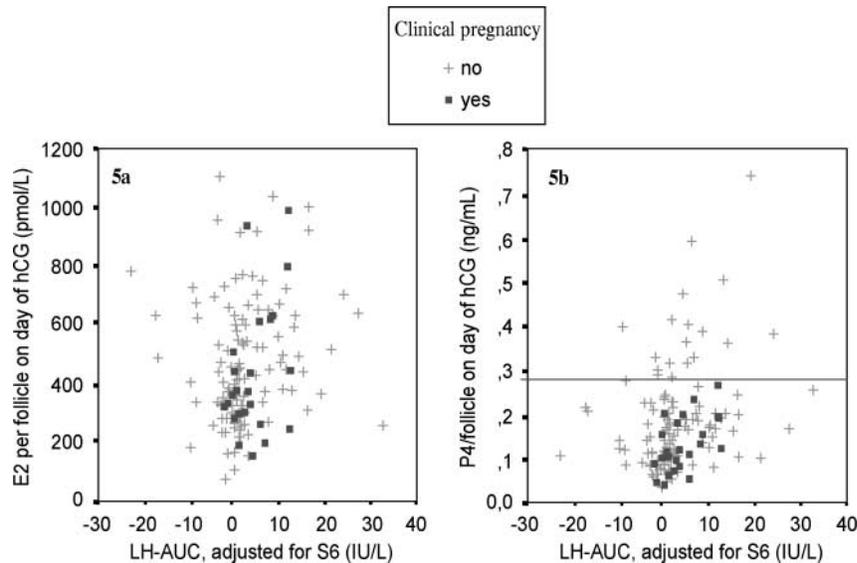


Figure 5. E2/follicle and P4/follicle on the day of hCG administration in relation to LH levels and clinical pregnancy

groups, although the middle dose groups (0.5 and 1.0 mg/ml) seemed to be optimal for clinical and ongoing pregnancy rates. These results compare well with a ganirelix dose-finding study (Ganirelix dose-finding Study Group, 1998).

In previous studies it was demonstrated that different endogenous LH levels can be induced by different GnRH antagonist dosages (Duijkers *et al.*, 1998; Ganirelix dose-finding Study Group, 1998; Obery *et al.*, 1999; Huirne *et al.*, 2004). Thus, different GnRH antagonist dosages can be used as an instrument to induce deliberately different LH levels. To test our hypothesis that the induced differences in LH levels may be responsible for the observed optimal GnRH antagonist range rather than the antagonist level itself, we analysed in detail the relationship between LH levels, LH AUC levels and pregnancy rates. In line with the LH window postulated by others (Hillier, 1994; Shoham, 2002), we found an optimal area in LH AUC for the occurrence of a clinical pregnancy. No pregnancies were observed when the total LH AUC (calculated out of three samples taken per day) was >22.3 or <4.47 IU/l. The absolute LH levels taken on a single sample day, either day 7, 8 or hCG day, did not show any clear relationship with pregnancy rate. These findings are in line with an earlier report (Balasch *et al.*, 2001). Apparently, the parameter LH AUC associates more strongly with pregnancy rates than the absolute LH level taken in one single sample. Possibly, the AUC during the entire antagonist treatment period is a better representative of LH exposure. The association between LH AUC levels and pregnancy rates became clearer when the changes in LH levels (expressed as LH AUC, adjusted for the baseline LH levels) were taken into account. Apparently, too large changes—either increases or decreases in LH levels during the antagonist administration period—rather than absolute AUC levels, are associated with a decreased chance of clinical pregnancy. These findings were independent of baseline characteristics and other parameters of IVF outcome (see Table III). The occurrence of a pregnancy is effected by many factors and vari-

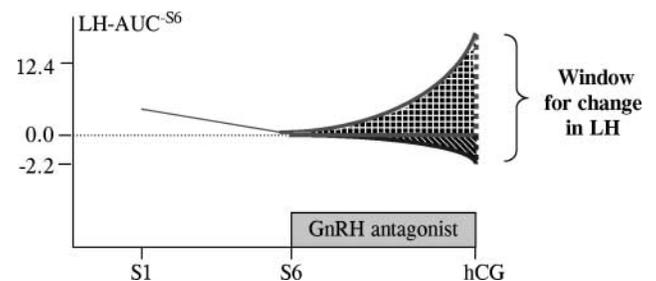


Figure 6. The interpretation of change in LH levels ($\text{LH-AUC}^{-\text{S6}}$) in relation to clinical pregnancy. S1 = stimulation day 1; S6 = stimulation day 6.

ables other than LH and progesterone. The only significant covariant to clinical pregnancy in a univariate analyses was change in LH levels ($P = 0.006$).

Thus changes in the endocrine milieu seem to be important for the IVF outcome in terms of clinical pregnancy. Figure 6 gives our interpretation of these results. The physiological basis for the association of the increased change in LH levels and lower pregnancy rates is not clear. It may interfere with the correct sequence of maturational changes and right synchronization between nuclear and cytoplasmic maturation (Mattioli and Barboni, 2000; Luborsky *et al.*, 2002) but it may also cause advanced endometrium maturation (Kolibianakis *et al.*, 2002, 2003c).

Caution is needed to extrapolate our findings in general to GnRH antagonist studies using the minimum effective dose for the prevention of untimely LH surges in IVF stimulated cycles, since we induced extreme (high and low) hormone levels. This probably explains in part why others do not find an association between LH levels and IVF outcome, with a smaller range of the LH levels in these studies (Balasch *et al.*, 2001; Balasch and Fabregues, 2002).

LH is required to produce progesterone by luteinizing the granulosa cells and estradiol by producing androstendione as a substrate for estradiol. In a previous study we demonstrated

that LH levels in GnRH antagonist cycles were related to progesterone and estradiol (Huirne *et al.*, 2004). In an attempt to obtain information on the individual association of these parameters with clinical pregnancy, we performed scatter plots of these parameters with changes in LH levels and studied the occurrence of clinical pregnancy. Similar to the observed window for changes in LH, changes in progesterone levels also seemed to be closely related to the occurrence of clinical pregnancy. Absolute progesterone levels on hCG day were not correlated with clinical pregnancy. This is consistent with the findings of others (Urman *et al.*, 1999). However, in an observational study, progesterone levels > 1.2 ng/ml on the day of hCG as a parameter of premature luteinization were associated with lower pregnancy and implantation rates (Bosch *et al.*, 2003).

Absolute progesterone levels are related to the total amount of luteinized granulosa cells, thus total number of follicles may be a confounder for the absolute progesterone levels on the day of hCG. Therefore we corrected the absolute progesterone levels on the day of hCG for the total number of follicles (progesterone/follicle), as a parameter for premature luteinization. This parameter showed a clear ceiling effect for the occurrence of clinical pregnancy. No pregnancy occurred in our study when the progesterone levels/follicle on the day of hCG exceeded a certain threshold (0.26 ng/ml/follicle).

Since LH and progesterone were closely correlated in the current study, we could not discriminate which of the two should be held responsible for the observed differences in pregnancy rates. No association was found for changes in estradiol levels nor for the absolute estradiol levels on the day of hCG administration with or without correction for the total number of mature follicles on that day. Similar results were found in other studies using GnRH antagonists (Ganirelix dose-finding Study Group, 1998). Altogether it seems to be that changes in LH and/or progesterone levels play a specific key role in the occurrence of clinical pregnancy rates in GnRH antagonist-treated IVF patients. Apparently a certain stability of these hormones associates with clinical pregnancy. These findings are in agreement with the observed lower pregnancy rates in the group with higher LH levels, in which hCG was administered 2 days later than a control group in GnRH antagonist-treated patients (Kolibianakis *et al.*, 2003d). We speculate that our findings may have implications particularly for specific patient groups which are sensitive for high fluctuations in LH levels. For example, in patients with extreme low or high body mass indexes, the minimum effective dose may result in respectively too strong or insufficient suppression of LH levels. In addition, patients with high baseline LH levels (polycystic ovarian syndrome or patients with diminished ovarian reserve) may be more prone to larger changes (decreases) in LH levels. Based on our results, it is expected that depending on the level of LH and progesterone suppression during antagonist suppression, some patients will benefit from adjustment of the GnRH antagonist dose or addition of LH. These findings are in line with a study, using GnRH agonists, demonstrating that addition of LH was beneficial

for patients who had a very low endogenous LH level and detrimental for those with high endogenous LH levels (Loumaye *et al.*, 2003). The possibility of improving pregnancy rates in GnRH antagonist-treated cycles by adaptation of the GnRH antagonist dose or the addition of LH where the LH levels are inappropriate, or using a treatment regimen resulting in stable LH levels, should be studied in a prospective manner.

In conclusion, this is the first study demonstrating that the dynamics of LH and progesterone play a critical role in implantation when GnRH antagonist is used. No pregnancies occurred when the LH and progesterone changed too much (either increase or decrease) during GnRH antagonist administration due to insufficient or too high dosages of the GnRH antagonist. Moreover, a clear ceiling effect of high (absolute) progesterone levels per follicle could be observed. Further studies are required to investigate whether pregnancy rates can be improved by changing the treatment strategies, which result in stable and appropriate LH levels during the IVF cycle.

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