

# Treatment with the gonadotrophin-releasing hormone antagonist ganirelix in women undergoing ovarian stimulation with recombinant follicle stimulating hormone is effective, safe and convenient: results of a controlled, randomized, multicentre trial

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A multicentre, open-label, randomized study of the gonadotrophin-releasing hormone (GnRH) antagonist ganirelix (Orgalutran®/Antagon™) was performed in women undergoing ovarian stimulation with recombinant FSH (rFSH: Puregon®). The study was designed as a non-inferiority study using a long protocol of buserelin (intranasal) and rFSH as a reference treatment. A total of 730 subjects was randomized in a treatment ratio of 2:1 (ganirelix:buserelin) using an interactive voice response system which stratified for age, type of infertility and planned fertilization procedure [IVF or intracytoplasmic sperm injection (ICSI)]. The median duration of GnRH analogue treatment was 5 days in the ganirelix group and 26 days in the buserelin group, whereas the median total rFSH dose was 1500 IU and 1800 IU respectively. In addition, in the ganirelix group the mean duration of stimulation was 1 day shorter. During ganirelix treatment the incidence of LH rises (LH ≥10 IU/l) was 2.8% versus 1.3% during rFSH stimulation in the buserelin group. On the day of triggering ovulation by human chorionic gonadotrophin (HCG), the mean number of follicles ≥11 mm diameter was 10.7 and 11.8, and the median serum oestradiol concentrations were 1190 pg/ml and 1700 pg/ml in the ganirelix and buserelin groups respectively. The mean number of oocytes per retrieval was 9.1 and 10.4 respectively, whereas the mean

number of good quality embryos was 3.3 and 3.5 respectively. The fertilization rate was equal in both groups (62.1%), and the same mean number of embryos (2.2) was replaced. The mean implantation rates were 15.7% and 21.8%, and the ongoing pregnancy rates per attempt were 20.3% and 25.7% in the ganirelix and buserelin groups respectively. Evaluation of all safety data indicated that the ganirelix regimen was safe and well tolerated. The overall incidence of ovarian hyperstimulation syndrome was 2.4% in the ganirelix group and 5.9% in the reference group. The results of this study support a safe, short and convenient treatment regimen of ganirelix, resulting in a good clinical outcome for patients undergoing ovarian stimulation for IVF or ICSI.

*Key words:* buserelin/ganirelix/GnRH agonist/GnRH antagonist/ICSI/IVF/ovarian stimulation/recombinant FSH

## Introduction

Ganirelix is the active ingredient of Orgalutran® and Antagon™, a gonadotrophin-releasing hormone (GnRH) antagonist preparation developed for the prevention of premature LH surges in women undergoing ovarian stimulation. In comparison with native GnRH, ganirelix has substituted amino acids at positions 1, 2, 3, 6, 8 and 10, which results in a potent antagonist with only minimal histamine-releasing properties (Rabinovici *et al.*, 1992; Nelson *et al.*, 1995) and high aqueous solubility. The latter property is reflected by the high absolute bioavailability (F) of ganirelix being more than 90% after s.c. injection (Oberyé *et al.*, 1999a).

In current practice, GnRH agonists are used to suppress endogenous gonadotrophins during ovarian stimulation (Loumaye, 1990). However, agonists initially stimulate the release of gonadotrophins (flare-up), and complete pituitary suppression is only achieved after 2–3 weeks pretreatment when pituitary desensitization occurs due to receptor down-regulation.

The introduction of a GnRH antagonist such as ganirelix allows a short and simple treatment regimen for IVF patients undergoing ovarian stimulation, since antagonists immediately suppress gonadotrophins by blocking the GnRH receptor, and thus treatment may be restricted to those days when a premature LH surge is likely to occur. Studies in healthy female volunteers and IVF patients have shown that steady-state concentrations of ganirelix are reached within 2–3 days of treatment, and that maximal suppression of endogenous LH occurs about 4 h after each injection (Oberyé *et al.*, 1999b). Moreover, after

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discontinuation, a rapid recovery of pituitary function (Gordon *et al.*, 1990) was observed, also due to the relative short elimination half-life (about 13 h) of ganirelix. The additional anticipated advantages of antagonist treatment in ovarian stimulation programmes are a reduction of the use of gonadotrophins, a lower risk for developing ovarian hyperstimulation syndrome (OHSS), and the ability to use a bolus injection of a GnRH agonist to trigger a midcycle LH surge for final follicular maturation (Olivennes *et al.*, 1996). Moreover, in cases of multiple follicles and excessively high oestradiol concentrations, the use of GnRH agonist instead of human chorionic gonadotrophin (HCG) is thought to prevent the clinical manifestation of OHSS (Itskovitz *et al.*, 1991). If nevertheless, the IVF cycle is cancelled, ganirelix treatment may be continued to prevent spontaneous ovulation as well as signs and symptoms of OHSS (De Jong *et al.*, 1998).

The third-generation GnRH antagonists cetrorelix and ganirelix have both been applied in a multiple-dose regimen in women undergoing ovarian stimulation. Clinical research of cetrorelix started off with relatively high daily dosages of 3 and 1 mg (Diedrich *et al.*, 1994; Felberbaum *et al.*, 1995), but finally the lowest effective daily dose of cetrorelix appeared to be 0.25 mg (Albano *et al.*, 1997, 1999). To select the minimal effective daily dose of ganirelix, a multicentre, double-blind, randomized, dose-finding study was performed in 333 women including six different dosages ranging between 0.0625 and 2 mg (Ganirelix dose-finding study group, 1998; Itskovitz-Eldor *et al.*, 1998). In this study, patients were treated with a fixed dose of 150 IU rFSH for 5 days before starting ganirelix. The study revealed that a daily dose of 0.25 mg ganirelix prevented LH from rising above 10 IU/l during stimulation, and resulted in a good clinical outcome, i.e. the ongoing pregnancy rate was 34% per attempt (23/68) and 37% per transfer (23/62). Moreover, as in other studies (Fujimoto *et al.*, 1997; Oberyé *et al.*, 1999b), serum ganirelix concentrations increased in a linear dose-proportional manner, and serum LH decreased in a dose-proportional manner, indicating that the degree of pituitary suppression can be adjusted by changing the ganirelix dose.

In the current study the efficacy and safety of a multiple-dose regimen administering 0.25 mg ganirelix daily was assessed in a randomized study in women undergoing ovarian stimulation with rFSH for IVF or intracytoplasmic sperm injection (ICSI).

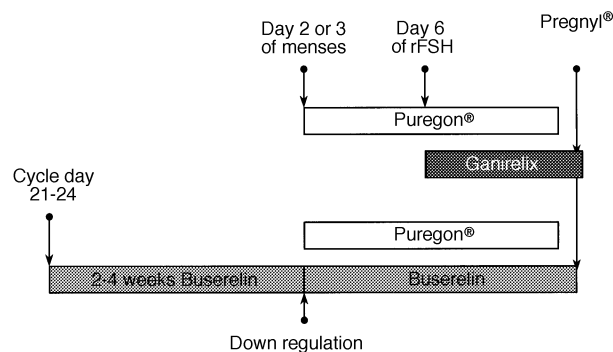
## Materials and methods

### Patients

A total of 730 patients, for whom ovarian stimulation and IVF or ICSI was indicated, was screened and randomized in this study. In total, 20 IVF centres in 10 European countries participated, and the number of randomized patients per centre ranged from 11 to 60. Main inclusion criteria were: age at least 18 years but not older than 39 years; body mass index (BMI) between 18 and 29 kg/m<sup>2</sup>; regular menstrual cycle, ranging from 24 to 35 days.

### Study design

This trial was a phase III, multi-centre, open-label, randomized study to assess the efficacy and safety of the GnRH-antagonist ganirelix



**Figure 1.** Schematic of the treatment regimen with ganirelix (upper part of diagram) and with a long protocol of intranasal buserelin (lower part) in patients undergoing ovarian stimulation with recombinant FSH (rFSH; Puregon®).

in women undergoing ovarian stimulation. Eligible patients were randomized by an interactive voice response system (IVRS) to either treatment with ganirelix (Orgalutran®, Org 37462; NV Organon, Oss, The Netherlands) or buserelin (Suprecur®, Hoechst, Frankfurt Am Main, Germany) in a ratio of 2:1. To improve balance, a minimization method was used for randomizing patients to treatment (Treasure and MacRae, 1999), stratifying for centre age, for primary or secondary infertility, and for IVF or ICSI.

A diagrammatic representation of the applied treatment regimens is shown in Figure 1. Injections of rFSH (Puregon®, NV Organon) and ganirelix were given in the morning. In the ganirelix group, treatment with rFSH was started in patients on day 2 or 3 of the menstrual cycle by a once-daily s.c. injection. After 5 days of rFSH treatment, ganirelix treatment was started by daily s.c. administration in the upper leg. Ganirelix treatment was continued up to and including the day of HCG administration.

In the buserelin reference group, pretreatment with buserelin was started in the midluteal phase (cycle day 21–24) with a daily dose of 0.6 mg intranasally (four puffs per day). Ovarian stimulation was started after 2 weeks if pituitary down-regulation was established (serum oestradiol concentration <50 pg/ml or <200 pmol/l). In case down-regulation was not achieved after 2 weeks, stimulation was postponed and the daily dose of buserelin was doubled to 1.2 mg. The dose of buserelin at which down-regulation was established (0.6 or 1.2 mg) was continued up to the day of HCG. If down-regulation with buserelin was not achieved within 4 weeks, treatment was discontinued.

In both treatment groups, ovarian stimulation was started with a fixed daily dose of 150 IU rFSH for the first five treatment days. From day 6 onwards, the dose of rFSH was adapted depending on the ovarian response as monitored via ultrasonography. On the day of HCG, rFSH was not administered.

HCG (10 000 IU, Pregnyl®, NV Organon) was administered when at least three follicles of  $\geq 17$  mm diameter were observed, and 30–36 h thereafter oocyte retrieval was performed. Oocyte retrieval was followed by IVF or ICSI, and no more than three embryos were to be replaced 2–5 days thereafter. Luteal phase support was given according to the clinics' routine practice, and was started no later than the day of embryo transfer.

### Assessments

In the ganirelix group prior to the start of rFSH, and in the buserelin group prior to the start of buserelin, an HCG test was to be performed to exclude pregnancy. When bleeding did not occur within 2 weeks after starting buserelin treatment, an additional HCG test was performed. Just before the first injection of rFSH, a blood sample

was taken for hormone assessment and ultrasonography (USS) was performed. From rFSH treatment day 6 up to and including HCG administration, the patient returned to the clinic for USS and blood sampling before ganirelix administration once every 2 days. Serum FSH, LH, oestradiol and progesterone were assessed by a central laboratory by means of fluoro-immunoassay (Delfia®, Wallac OY, Finland).

Local tolerance was assessed by the patient at 1, 4 and 24 h after each ganirelix injection. The subject was asked to record on a diary card the score (none, mild, moderate or severe) for five different parameters, i.e. bruising, swelling, pain, itching and redness.

**Statistical methods**

The study was designed as a non-inferiority trial to test whether the combination of efficacy, safety and convenience of ganirelix treatment was clinically equivalent to the current care, i.e. GnRH agonist treatment in a long protocol. As a large previous study with Puregon and intranasal buserelin in a long protocol had an excellent clinical outcome (Out *et al.*, 1995), this regimen was selected as the reference treatment in the current study. Data from the intent-to-treat (ITT) group were used for efficacy analysis, and data from the all-subjects-treated (AST) group were used for safety analysis. The ITT group and the AST group consisted of all patients randomized who started treatment. Patients in the ITT group were grouped according to the treatment they should have received by randomization, whereas patients in the AST group were grouped according to the actual treatment they received.

**Efficacy analysis**

A total of 701 subjects started treatment with rFSH or buserelin and was included in the ITT group. Of the treated subjects, 463 were randomized to the ganirelix group and 238 to the buserelin group. One subject was randomized to buserelin, but was treated with the ganirelix regimen, and one subject was randomized to buserelin and received during buserelin treatment also three injections of ganirelix. Since both subjects were intended to receive buserelin, they were included in the ITT group of buserelin. One subject with a spontaneous pregnancy who started buserelin treatment was also included in the ITT group. Main efficacy parameters were treatment failure, number of cumulus–oocyte complexes, number of good quality embryos, and ongoing pregnancy rate. For patients treated with both IVF and ICSI, oocyte quality was not analysed.

The estimated difference of ganirelix and buserelin in ongoing pregnancy rate was compared with the margin of –5%. For cumulus–oocyte complexes, the lower one-sided 97.5% confidence limit of the treatment difference was compared with the equivalence margin of –3 oocytes. For continuous efficacy variables, adjusted-for-centre treatment means and their differences were calculated, using a weighted average over the centres based on the Cochran–Whitehead method (Whitehead and Whitehead, 1991). For the ongoing pregnancy outcome the Cochran–Mantel–Haenszel weights (Cochran, 1954) were used. Lower one-sided 97.5% confidence limits of the adjusted-for-centre treatment differences between ganirelix and buserelin were calculated for the number of oocytes, good quality embryos, and the ongoing pregnancy rate. For the rate of study medication treatment failure in each group, a one-sided 97.5% confidence limit was calculated based on the binomial distribution.

**Freeze–thaw cycles**

Embryos were frozen in 18 out of the 20 IVF centres; in two German centres only two-pronuclear (2PN) oocytes were frozen which were not included in this analysis. Data were collected for all patients (*n* = 126) who did not become pregnant after replacement of fresh embryos,

**Table I.** Demographics and infertility characteristics

Characteristic	Ganirelix ( <i>n</i> = 463)	Buserelin ( <i>n</i> = 238)
Age (years) <sup>a</sup>	31.9 ± 3.6	31.9 ± 3.8
Body mass index (kg/m <sup>2</sup> ) <sup>a</sup>	23.0 ± 2.9	23.0 ± 2.7
Duration of infertility (years) <sup>a</sup>	4.5 ± 2.7	4.4 ± 2.7
Main causes of infertility (%)		
Male (only)	41.0	38.2
Tubal (only)	29.8	28.2
Unknown	13.8	5.0
Parity (%)		
Primary infertility	56.6	56.3
Secondary infertility	43.4	43.7

<sup>a</sup>Values are mean ± SD.

and for whom spare embryos were cryopreserved. By June 1999, 53 of these patients had had at least one embryo transfer using thawed embryos. The outcome of these first freeze–thaw cycles is presented.

**Safety analysis**

Analysis was performed by means of frequency distributions of the incidence of adverse events, local tolerance outcome, clinically significant abnormal laboratory values and vital signs. For patients treated with the ganirelix regimen, analysed data included all days of stimulation, thus also the first 5 days of rFSH treatment when patients were not yet exposed to ganirelix.

**Results**

**Patient characteristics**

The two treatment groups were similar with respect to age, body height, weight and BMI (see Table I). The overall (*n* = 701) mean age, body height, weight and BMI were 31.9 years, 166.6 cm, 63.8 kg and 23 kg/m<sup>2</sup> respectively. The majority (98%) of patients was Caucasian. No relevant differences were found between the treatment groups for the cause of infertility which was overall 40.1% male factor, 29.2% tubal factor and 15.5% unknown factors. The overall percentage of subjects with primary infertility was 56.5%, and similar in both groups.

**Disposition and cancellations**

In total, 730 subjects were randomized, 486 patients to the ganirelix group and 244 patients to the buserelin group (ratio ~2:1). A total of 701 subjects received rFSH or GnRH analogue treatment. The number of patients per treatment stage is presented in Table II.

In total, 16 subjects (3.5%) in the ganirelix group and 14 subjects (5.9%, including the patient who was randomized to this group but was treated with ganirelix) in the buserelin group had a treatment failure in that they did not receive HCG, or received HCG because of premature luteinization. The overall cancellation rate up to embryo transfer was 13.8 and 12.6% for the ganirelix and buserelin groups respectively. Main reasons for discontinuation of treatment were insufficient ovarian response (3.2 versus 2.5%) and fertilization failure (6.2 versus 4.1%). Overall, eight patients in the ganirelix group and two patients in the buserelin group had intrauterine

**Table II.** Number of patients per treatment stage

	Ganirelix	Buserelin
No. of subjects randomized to group	463	238
Treated with buserelin	–	237 (100)
Treated with rFSH	463 (100)	228 (96)
Treated with ganirelix	460 (99)	2 <sup>a</sup>
HCG for triggering ovulation	448 (97) <sup>b</sup>	224 (95)
Oocyte retrieval	440 (95)	221 (93)
Embryo transfer	399 (86)	208 (88)

<sup>a</sup>Two patients who were randomized to the buserelin group were treated with ganirelix, one of whom received no buserelin at all (see Materials and methods; Efficacy analysis).

<sup>b</sup>Includes one patient who received HCG because of premature luteinization. Values in parentheses are percentages.

HCG = human chorionic gonadotrophin.

insemination instead of oocyte retrieval. In the buserelin group, seven patients (3.0%) were discontinued before starting rFSH due to insufficient down-regulation. In the ganirelix group, two patients (0.4%) were discontinued because of premature luteinization.

#### **Duration of GnRH analogue treatment and total dose of rFSH**

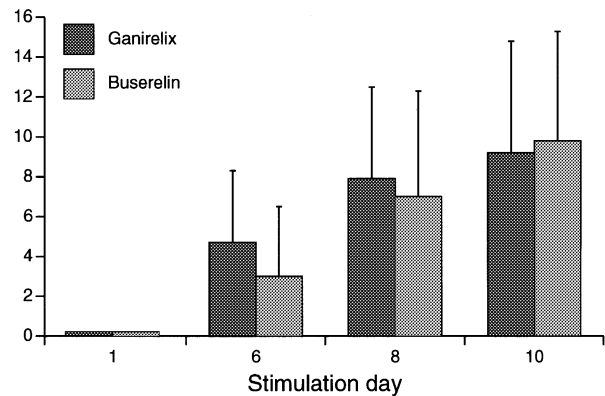
The median (range) duration of GnRH analogue treatment was 5 (2–14) days in the ganirelix group, and 26 (18–44) days in the buserelin group. The total amount of GnRH analogue administered was 1.25 versus 16.2 mg. The median (range) duration of rFSH treatment was 9 (6–18) and 10 (6–19) days respectively. The total amount of rFSH administered was in total 1500 (900–5400) IU and 1800 (900–6450) IU, and the median daily dose was 150 IU/day and 178 IU/day in the ganirelix and buserelin groups respectively.

#### **LH rises ( $\geq 10$ IU/l) before and during ganirelix treatment**

Early LH rises at day 6 of stimulation, before the first ganirelix administration, were observed in 20 patients (4.3%). In these patients serum LH values ranged between 10.4 and 33.4 IU/l and concomitant rises of serum progesterone  $>1$  ng/ml were observed in seven out of these 20 patients. On day 6 of stimulation, this subset of patients had on average 6.8 follicles  $\geq 11$  mm diameter, and their median serum oestradiol concentration was 856 (range 348–1900) pg/ml, indicating that most of these patients were high responders. Due to initiation of ganirelix treatment, endogenous LH rises were effectively cut-down in all 20 patients. Out of the 20 patients, 19 had embryo transfer, and in three patients an ongoing pregnancy was established.

After the day of the first ganirelix injection, 13 women (2.8%) had an LH value  $\geq 10$  IU/l. Peak LH values ranged between 10.1 and 58.1 IU/l, and concomitant rises ( $>1$  ng/ml) of serum progesterone were observed in six patients (1.5%). Seven patients were discontinued before embryo transfer, and none of the other patients became pregnant. In the buserelin group, three patients (1.3%) had an LH rise to  $\geq 10$  IU/l during stimulation. Of these, one patient was discontinued and one patient became pregnant.

Miscarriage did not occur in any of the patients with an

**Mean number of follicles**

**Figure 2.** Number of follicles of  $\geq 11$  mm diameter on stimulation days 1, 6, 8 and 10 of rFSH for patients with at least 9 days of stimulation. Values are mean  $\pm$  SD.

**Table III.** Number and size of follicles (mean  $\pm$  SD) grouped according to diameter and serum hormone values (median with 5% and 95% percentiles) on the day of HCG administration. Restricted to patients who received an HCG injection

	Ganirelix (n = 448) <sup>a</sup>	Buserelin (n = 224)
Follicles (n)		
$\geq 11$ mm	10.7 $\pm$ 5.3	11.8 $\pm$ 5.4
$\geq 15$ mm	7.7 $\pm$ 4.0	8.3 $\pm$ 3.9
$\geq 17$ mm	4.9 $\pm$ 2.6	5.2 $\pm$ 2.3
Hormones		
FSH (IU/l)	7.7 (5.0–14.1)	8.4 (5.4–17.6)
LH (IU/l)	1.6 (<0.6–6.9)	1.5 (<0.6–4.4)
Oestradiol (pg/ml)	1190 (373–3105)	1700 (527–4070)
Progesterone (ng/ml)	0.7 (0.4–1.6)	0.7 (0.4–1.6)
Oestradiol/follicle (pg/ml)	111	144

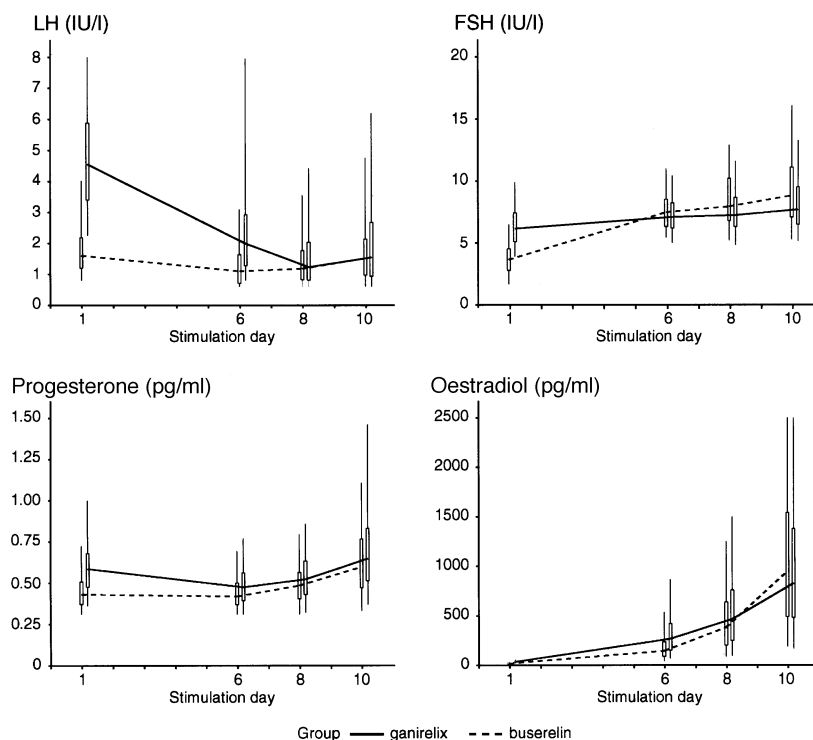
<sup>a</sup>Includes one patient who received HCG because of premature luteinization.

early or late rise of serum LH after confirmation of an early clinical pregnancy.

#### **Follicle growth**

The mean ( $\pm$  SD) number of follicles  $\geq 11$  mm diameter measured on days 6, 8 and 10 of stimulation for patients with at least 9 days of stimulation are presented in Figure 2. The number and size of follicles measured at the day of HCG administration are summarized in Table III.

Comparison of the number and size of growing follicles indicates a different follicle growth pattern in the ganirelix group than in the buserelin group. At day 6 of stimulation, after 5 days of 150 IU rFSH per day, absence of follicular growth (no follicles  $\geq 11$  mm diameter) was observed in 13.2% of patients treated with the ganirelix regimen, whereas this incidence was 31.5% in the buserelin reference group. Accordingly, initial follicular growth appeared to be more rapid in the ganirelix group than in the buserelin group, as indicated by the mean number of follicles  $\geq 11$  mm diameter on day 6 of stimulation (4.7 versus 3.0; Figure 2). On day 8 of stimulation, the difference between the treatment groups was less apparent, and on the day of HCG the opposite was observed in that the number of follicles  $\geq 11$  mm was smaller



**Figure 3.** Serum hormone concentrations on stimulation days 1, 6, 8 and 10 of rFSH for patients with at least 9 days of stimulation. The boxes indicate the 75% and 25% percentiles, the vertical lines indicate the 95% and 5% percentiles, and median values are connected.

in the ganirelix group than in the buserelin group (10.7 versus 11.8). Comparison of follicle sizes between the two treatment groups indicates that this difference was mainly due to fewer small follicles in the ganirelix group (Table III), as also reflected by the comparable mean number of follicles  $\geq 17$  mm at the day of HCG administration (4.9 versus 5.2).

### Serum hormone concentrations

Median serum FSH, LH, oestradiol and progesterone concentrations measured at days 1, 6, 8 and 10 of stimulation for patients with at least 9 days of stimulation are presented in Figure 3. At the start of rFSH stimulation (day 1), serum hormone concentrations were higher in the ganirelix group than in the buserelin group, and represented normal levels as measured at day 2 to 3 of the menstrual cycle and after pituitary down-regulation respectively. On day 6 of stimulation, serum FSH and LH concentrations had become similar in both treatment groups, probably due to a negative feedback by initial rising oestradiol concentrations in the ganirelix group. Median serum oestradiol concentrations rose from 38 pg/ml on day 1 to 358 pg/ml on day 6 of stimulation in the ganirelix group, and from 19 pg/ml to 160 pg/ml in the buserelin group. This increase was in accordance with the number of growing follicles in each group. From day 6 to the day of HCG, serum FSH increased by 0.5 IU/l in the ganirelix group and by 1 IU/l in the buserelin group. Predose concentrations of serum LH in patients treated with ganirelix were comparable with those measured in the buserelin group. After day 6 of stimulation up to the day of HCG, serum oestradiol concentrations followed the pattern of follicular growth in that on day 8 the oestradiol concentrations were only 128 pg/ml higher in the ganirelix

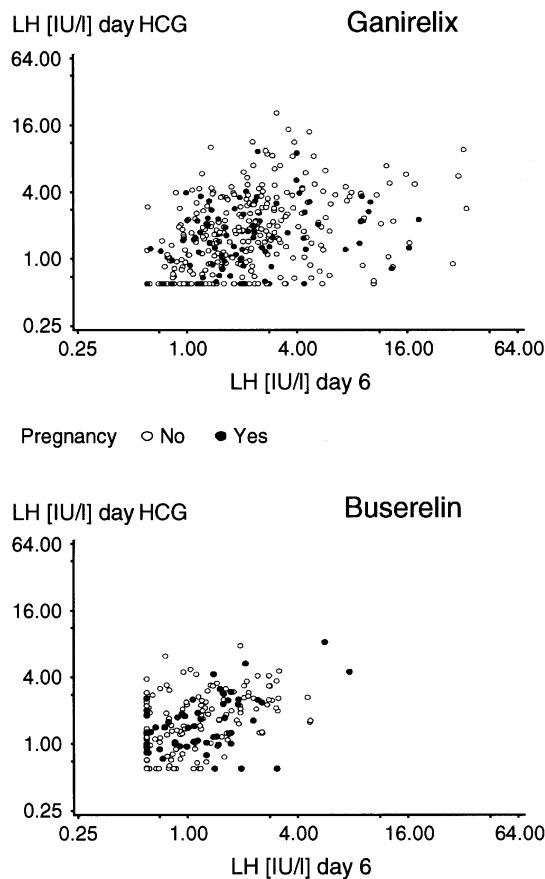
group than in the buserelin group, whereas on the day of HCG (see Table III) the opposite was observed, with serum oestradiol concentration being about 500 pg/ml lower in the ganirelix group than in the buserelin group (1190 versus 1700 pg/ml). Finally, serum oestradiol content per follicle ( $\geq 11$  mm) was lower in the ganirelix group than in the buserelin group.

Individual serum LH values measured on day 6 and measured on the day of HCG were plotted in Figure 4. Comparison of the predose values in the ganirelix group with LH values measured during down-regulation with buserelin, indicates a larger variability in the ganirelix group. A possible relationship between serum LH and pregnancy outcome (Figure 4) or between serum oestradiol and pregnancy outcome (data not shown) was not revealed.

### Treatment outcome

The mean number of oocytes recovered, their quality and the mean number of embryos obtained and their quality in each treatment group are given in Table IV. In comparison with buserelin treatment, ganirelix treatment resulted in one preovulatory follicle less (see above) and, as a consequence, one cumulus-oocyte complex less was recovered at oocyte retrieval. The estimated treatment difference was  $-1.0$  oocyte (lower 97.5% confidence limit  $-1.8$ ) which was within the equivalence margin of  $-3$  oocytes.

In total, 357 patients had IVF and 291 patients had ICSI, whereas 10 patients had both IVF and ICSI (1.5 versus 1.3% in the ganirelix and buserelin groups respectively). The mean ( $\pm$  SD) number of metaphase II oocytes recovered in ICSI patients was 83% in each group, i.e.  $7.1 \pm 4.2$  versus  $8.5 \pm 5.2$ , and the overall fertilization rate was 62.1% in each group.



**Figure 4.** Concentrations of serum LH in individual patients on day 6 of stimulation versus serum LH on the day of HCG. Filled points represent values of patients who became pregnant.

**Table IV.** Number of cumulus–oocytes complexes recovered per attempt and per oocyte retrieval, number and quality of embryos obtained, and number of transferred embryos. Values are presented as mean  $\pm$  SD

	Ganirelix	Buserelin
Cumulus–oocyte complexes/attempt	$n = 463$ $8.7 \pm 5.6$	$n = 238$ $9.7 \pm 6.2$
Number of oocytes before IVF	$n = 234$ $9.6 \pm 5.7$	$n = 123$ $10.3 \pm 5.7$
Number of oocytes before ICSI	$n = 196$ $8.6 \pm 4.8$	$n = 95$ $10.3 \pm 5.9$
Cumulus–oocyte complexes/retrieval	$n = 440$ $9.1 \pm 5.4$	$n = 221$ $10.4 \pm 5.8$
Embryos	$n = 463$	$n = 238$
Total	$6.0 \pm 4.5$	$7.1 \pm 5.2$
Good quality	$3.3 \pm 3.0$	$3.5 \pm 3.2$
Frozen	$1.1 \pm 2.3$	$1.3 \pm 2.5$
Replaced	$2.2 \pm 0.6$	$2.2 \pm 0.6$

ICSI = intracytoplasmic sperm injection.

In addition, the number of good quality embryos obtained was comparable between the groups, and the estimated treatment difference was only  $-0.1$  embryo (lower 97.5% confidence limit  $-0.4$ ). At transfer, in each group a mean of 2.2 embryos were replaced, including a mean of 2.0 and 1.9 good quality embryos in the ganirelix and buserelin groups respectively.

The clinical outcome of all patients receiving embryo transfer, expressed per attempt and per embryo transfer, is

**Table V.** Clinical outcome as percentage per attempt and per transfer in patients treated with the ganirelix regimen and with a long protocol of buserelin

	Ganirelix	Buserelin
No. of patients receiving embryo transfer	399	208
Implantation rate (%)	15.7	21.8
Miscarriage rate (%)	12.0	13.9
Vital pregnancies		
Per attempt (%)	21.8	28.2
Per transfer (%)	25.1	31.7
Ongoing pregnancy rate		
Per attempt (%)	20.3	25.7
Per transfer (%)	23.3	29.0
Ongoing pregnancies		
Singletons (%)	76.2	68.9
Twins (%)	20.2	26.2
Triplets (%)	3.2	3.3

shown in Table V. Although a similar number of embryos was replaced in each group, the implantation rate (number of gestational sacs divided by the number of replaced embryos) was relatively lower in the ganirelix group (15.7 versus 21.8%), whereas the miscarriage rate per clinical pregnancy was comparable (12.0 versus 13.9%). Accordingly, the vital and ongoing pregnancy rate tended to be lower in the ganirelix group than in the buserelin group. For the ITT group, the ongoing pregnancy rate per attempt was 20.3% in the ganirelix group and 25.7% in the buserelin group (including one spontaneous pregnancy in the buserelin group). The estimated difference for the ongoing pregnancy rate was at the margin of  $-5\%$ . When comparing the ongoing pregnancy rate per study site, this difference ranged from 26.3% in favour of ganirelix to 28.6% in favour of buserelin. The ongoing pregnancy rate per attempt for patients ( $n = 337$ ) treated at study sites ( $n = 10$ ) that had previous experience with the ganirelix regimen was similar, i.e. 24.2% in the ganirelix group and 23.6% in the buserelin group, whereas this rate was respectively 16.5 and 27.5% for patients ( $n = 363$ ) treated in study sites ( $n = 10$ ) that had applied the ganirelix regimen for the first time.

Overall, 94 and 61 ongoing pregnancies respectively were established at 12–16 weeks after embryo transfer. The multiple pregnancy rate was 23.4% in the ganirelix group and 29.5% in the buserelin group.

#### Outcome of subsequent freeze–thaw cycles

The mean ( $\pm$  SD) number of embryos frozen was  $1.1 \pm 2.3$  and  $1.3 \pm 2.5$  in the ganirelix and buserelin groups respectively. The first frozen–thawed embryo cycles ( $n = 53$ ) performed within one year after study completion resulted in three miscarriages, one induced abortion and 10 ongoing pregnancies (12–16 weeks after embryo transfer). Seven pregnancies (20.0%) were established in patients previously treated with ganirelix, and three pregnancies (16.7%) were established in patients treated with buserelin. The overall ongoing pregnancy rate was 18.9% (see Table VI).

#### Safety and tolerance

The number of subjects who experienced at least one adverse experience was 125 (26.9%, i.e. 125/465) in the ganirelix

**Table VI.** Outcome of 53 first freeze–thaw cycles

	Ganirelix	Buserelin	Overall
No. of first attempts	35	18	53
Embryos (mean)			
Good quality	1.6 <sup>a</sup>	1.9 <sup>a</sup>	1.7
Transferred	2.1 <sup>a</sup>	2.4 <sup>a</sup>	2.2
No. of miscarriages	2	1	3
Induced abortions ( <i>n</i> )	1	0	1
No. of ongoing pregnancies	7	3	10
Pregnancy rate (%)	20.0	16.7	18.9

<sup>a</sup>Data are missing for one cycle.

group, and 74 (31.4%, i.e. 74/236) in the buserelin group. The most frequently reported experiences were headache, abdominal pain (gynaecological), OHSS and miscarriages. Treatment discontinuation because of an adverse experience occurred for one patient in the ganirelix group (0.2%) due to the risk for developing OHSS, and for one patient (0.4%) in the buserelin group because of spontaneous ovulation before oocyte retrieval. The number of subjects with possible or probable drug-related experiences was 11 (2.4%) in the ganirelix group, and nine (3.8%) in the buserelin group. In the ganirelix group, 18 patients (3.9%) were hospitalized because of an adverse experience, i.e. ectopic pregnancy (*n* = 4), OHSS (*n* = 4), miscarriage (*n* = 6), threatening abortion (*n* = 1), hyperemesis (*n* = 1), urinary retention (*n* = 1) and abdominal pain (gynaecological) (*n* = 1). In the buserelin group, 11 patients (4.6%) were hospitalized because of ectopic pregnancy (*n* = 1), OHSS (*n* = 6 including 1 case of enteritis) and miscarriage (*n* = 4). All these adverse experiences were indicated as not, or unlikely to be, drug-related.

The incidence of OHSS was two-fold lower in the ganirelix group than in the buserelin group. Eleven subjects (2.4%) in the ganirelix group and 14 subjects in the buserelin group (5.9%) experienced signs and symptoms related to OHSS. For two pregnant patients in the ganirelix group OHSS was graded as severe; all other cases were of moderate or mild intensity.

The local tolerance outcome indicated that ganirelix administered daily by the s.c. route was well tolerated. The percentage of patients with at least one moderate or severe local tolerance reaction (skin redness, swelling, bruising, pain or itching) during ganirelix treatment was 16.6, 2.0 and 2.7%, at 1, 4 and 24 h after the injection respectively. Most frequently reported were moderate or severe skin redness (9.5%) or swelling (9.5%) at 1 h after injection, but by 4 h after injection these reactions had mostly disappeared. At 24 h after injection, bruising (moderate or severe) was most frequently reported (2.5%). None of the patients had to discontinue ganirelix treatment because of a hypersensitivity reaction or because of a drug-related adverse experience.

## Discussion

The ganirelix regimen is a new treatment option for patients undergoing ovarian stimulation, which largely reduces the duration of GnRH analogue treatment and prevents adverse events related to flare-up or down-regulation induced by GnRH

agonists. In addition to its convenience, the regimen appeared to be safe and well-tolerated.

Ganirelix is used to prevent premature LH surges occurring during ovarian stimulation. However, in the literature no clear definition on an LH surge has been provided, and therefore data analysis was based primarily on the incidence of LH rises  $\geq 10$  IU/l and additionally on concomitant rises of serum progesterone 1 ng/ml, indicating premature luteinization. During ganirelix treatment the overall incidence of LH rises was 2.8%, and only in 1.5% of the cases was a concomitant rise in progesterone concentration observed, demonstrating the effective suppression of endogenous LH during ovarian stimulation.

In this efficacy trial, ganirelix treatment was started on day 6 of stimulation, since the previous dose-finding study (Ganirelix dose-finding study group, 1998) demonstrated that during the first days of stimulation, median serum LH concentrations decrease and nadir LH concentrations are reached at day 5 of stimulation. This initial suppression of endogenous LH is thought to be established by a negative feedback of rising oestradiol concentrations during the first days of stimulation. When follicular growth is progressing and oestradiol concentrations become as high as in the late follicular phase of the normal menstrual cycle, then the reverse occurs and the risk for a premature LH surge becomes imminent (Filicori *et al.*, 1986). In the current study, early LH rises before the first ganirelix administration occurred in 20 patients who were high responders, i.e. stimulation resulted in more rapid initial follicular growth and in a more pronounced rise of serum oestradiol as compared with the overall treatment group. Even though the clinical outcome of this small subset of patients was good, early LH rises may be prevented by starting ganirelix treatment on day 5 instead of day 6 of stimulation. On the other hand, 13.2% of all patients did not show any follicles  $\geq 11$  mm diameter at day 6 of stimulation; in these lower responders exposure to ganirelix may be limited by delaying the start of treatment up to the moment of actual follicle growth.

In the current study, duration of treatment was short, i.e. on average 9 days with rFSH including 5 days of ganirelix treatment. Since initial growth of follicles was more rapid and endogenous FSH concentrations were only partly suppressed (during the late follicular phase), the duration of rFSH treatment was one day shorter and the amount of rFSH required was lower in the ganirelix group. Since the number of subjects without any follicles  $\geq 11$  mm diameter on day 6 of stimulation was twice as high in the long protocol of buserelin, the ganirelix regimen might be of special benefit for poor-responders, who are frequently treated with a short flare-up protocol of GnRH agonist (Frydman *et al.*, 1988).

Comparison of the number and size of follicles indicated that, in the ganirelix group, initial follicular growth was faster but the final cohort of growing follicles was smaller and produced on average less oestradiol, which is explained by the different endocrine status of the patient at the start of stimulation. In view of this different follicular pattern, rFSH dose adjustments in patients treated with the ganirelix regimen should be based on the number and size of growing follicles,

rather than on the amount of circulating oestradiol. The smaller cohort of follicles and the lower oestradiol concentrations are in good agreement with the lower incidence (less than half) of OHSS in the ganirelix group.

The possible direct effect of GnRH antagonists on follicle growth, steroidogenesis, oocyte or embryo quality or implantation is of specific interest, since GnRH receptors have been identified in human granulosa-lutein cells (Latouche *et al.*, 1989; Brus *et al.*, 1997), and might be present in uterine endometrial tissue (Raga *et al.*, 1998), although their function and interaction with GnRH or GnRH analogues is not (yet) understood (Ikeda *et al.*, 1997). Recent studies *in vitro* with human granulosa cells have demonstrated that neither ganirelix nor cetrorelix exert any significant action on ovarian steroidogenesis (Ortmann *et al.*, 1999; Verboost *et al.*, 1999), and in the ganirelix dose-finding study no difference was noted in the number or size of follicles of patients treated in the six different dose groups (Ganirelix dose-finding study group, 1998).

In comparison with buserelin treatment in a long protocol, the ganirelix regimen resulted in one preovulatory follicle less and, as a consequence, one cumulus-oocyte complex less was recovered at oocyte retrieval. This difference is within the preset equivalence margin of -3 oocytes, and is thought to be related to the short regimen rather than ganirelix *per se*. The recovery of fewer oocytes is a well-described phenomenon of the short protocol of GnRH agonists in comparison with the long protocol (Tan *et al.*, 1992; Cramer *et al.*, 1999). Overall, the ganirelix regimen resulted in the recovery of good quality oocytes as reflected by the percentage of metaphase II oocytes in ICSI patients (83% in each group), the high fertilization rate (62.1% in each group), and the number of good quality embryos, which was comparable with the reference group. The latter finding suggests a higher recovery of good quality embryos in the ganirelix regimen than in the buserelin group, which is in good agreement with the outcome of a previous small study which compared the antagonist Nal-Glu (5 mg/day) with the agonist leuprolide acetate (Minaretzis *et al.*, 1995). The recovery of good quality oocytes and embryos is further supported by the good success rates of replaced frozen embryos collected in the dose-finding study of ganirelix (Kol *et al.*, 1999), as well as in this study.

In the current trial, on average only 2.2 embryos were replaced, which is in line with the current standard practice in several European IVF centres not to replace more than two good quality embryos, in order to prevent multiple pregnancies. In comparison with a large previous multi-centre study of Puregon, using the same stimulation regimen with buserelin, the ongoing pregnancy rate per attempt was very similar to the outcome of the ganirelix regimen (Out *et al.*, 1995). Although the clinical outcome is considered good (ongoing pregnancy rate per attempt was 20.3%), in the current study the pregnancy rate tended to be higher in the reference group. In view of the limited study power, it cannot be excluded that this tendency is related to chance. However, using the same multiple dose regimen and also intranasal buserelin in a long protocol as control, others (Felberbaum, 1999) reported a vital pregnancy rate per transfer of 27% (compared with 25%

in this study) in patients treated with human menopausal gonadotrophin (HMG) and 0.25 mg GnRH antagonist cetrorelix ( $n = 188$ ) and of 33% (cf. 32% in this study) in patients treated with the agonist ( $n = 85$ ). Therefore, several other factors that may influence clinical outcome should be considered. For instance, study sites that participated in the previous ganirelix dose-finding study provided a favourable outcome more often for the ganirelix regimen compared with study sites who participated for the first time. This indicates that some clinical experience with the new ganirelix regimen might contribute positively to the success rate. In addition, the regimen applied has not been selected based on prospective research, and further optimization of the regimen might appear beneficial. In the current applied regimen, stimulation was started on day 2 or 3 of the follicular phase; thus serum gonadotrophin and steroid concentrations were higher than after down-regulation in the reference group. High concentrations of serum hormones are especially established in patients who receive the GnRH-agonist flare-up protocol, and these were reported to have a lower clinical pregnancy rate, which is thought to be related to a higher oestradiol concentration before HCG administration, and a higher production of oestradiol per oocyte recovered (Cramer *et al.*, 1999). In the ganirelix group, the opposite was shown in that before HCG administration oestradiol concentrations were lower, and also the oestradiol production per follicle was lower in the ganirelix group than in the buserelin group. Although in the current study neither serum LH nor oestradiol concentrations appeared to be predictive factors for pregnancy (Loumaye *et al.*, 1997), it cannot be excluded that the hormonal milieu of the current ganirelix regimen is less favourable for a certain subset of patients. If so, patients might benefit from postponing HCG administration and allowing follicles to grow larger, or to pretreat patients with oral contraceptives which would also allow patient scheduling. Thus, further optimization of treatment, as well as clinical experience with the antagonist regimen, might further optimize clinical outcome in the near future.

Overall, it may be concluded that ganirelix introduces a new treatment option for patients undergoing ovarian stimulation for IVF or ICSI which is safe, short and simple. The clinical outcome was good, and the ongoing pregnancy rate was within the range of pregnancy rates of a long-protocol GnRH-agonist, the latter being supported by many years of clinical experience.

## References

- Albano, C., Smitz, J., Camus, M. *et al.* (1997) Comparison of different doses of gonadotrophin-releasing hormone antagonist cetrorelix during controlled ovarian hyperstimulation. *Fertil. Steril.*, **67**, 917-922.
- Albano, C., Smitz, J., Tournaye H. *et al.* (1999) Luteal phase and clinical outcome after human menopausal gonadotrophin/gonadotrophin releasing hormone antagonist treatment for ovarian stimulation in in-vitro fertilization/intracytoplasmic sperm injection cycles. *Hum. Reprod.*, **14**, 1426-1430.
- Brus, L., Lambalk, C.B., de Koning J. *et al.* (1997) Specific gonadotrophin-releasing hormone analogue binding predominantly in human luteinized follicular aspirates and not in human pre-ovulatory follicles. *Hum. Reprod.*, **12**, 769-773.
- Cochran, W. (1954) The combination of estimates from different experiments. *Biometrics*, **10**, 101-129.
- Cramer, D.W., Powers, D.R., Oskowitz, S.P. *et al.* (1999) Gonadotropin-releasing hormone agonist use in assisted reproduction cycles: the influence of long and short regimens on pregnancy rates. *Fertil. Steril.*, **72**, 83-89.



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- De Jong, D., Macklon, N.S., Mannaerts B. *et al.* (1998) High dose gonadotrophin-releasing hormone antagonist (ganirelix) may prevent ovarian hyperstimulation syndrome caused by ovarian stimulation for in-vitro fertilization. *Hum. Reprod.*, **13**, 573–575.
- Diedrich, K., Diedrich, C., Santos, E. *et al.* (1994) Suppression of the endogenous luteinizing hormone surge by the gonadotrophin-releasing hormone antagonist cetrorelix during ovarian stimulation. *Hum. Reprod.*, **9**, 788–791.
- Felberbaum, R. (1999) Cetrorelix in controlled ovarian stimulation for ART: results of phase III, multiple dose treatment. *Gynecol. Endocrinol.*, **13**, Abstract 028.
- Felberbaum, R., Reissman, T.K., Kupker, W. *et al.* (1995) Preserved pituitary response under ovarian stimulation with HMG and GnRH-antagonists (Cetrorelix) in women with tubal infertility. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, **61**, 151–155.
- Filicori, M., Santoro, N., Merriam, G.R. *et al.* (1986) Characterization of the physiological pattern of episodic gonadotropin secretion throughout the human menstrual cycle. *J. Clin. Endocrinol. Metab.*, **62**, 1136–1144.
- Frydman, R., Belaisch-Allert, J., Parneix, I. *et al.* (1988) Comparison between flare-up and down regulation effects of luteinizing hormone-releasing hormone agonists in an *in vitro* fertilization program. *Fertil. Steril.*, **50**, 471–475.
- Fujimoto, V.Y., Monroe, S.E., Nelson, L.R. *et al.* (1997) Dose-related suppression of serum luteinizing hormone in women by a potent new gonadotrophin-releasing hormone antagonist (ganirelix) administered by intranasal spray. *Fertil. Steril.*, **67**, 469–473.
- Ganirelix dose-finding study group (1998) A double-blind, randomized, dose-finding study to assess the efficacy of the GnRH-antagonist ganirelix (Org 37462) to prevent premature luteinizing hormone surges in women undergoing controlled ovarian hyperstimulation with recombinant follicle stimulating hormone. *Hum. Reprod.*, **13**, 3023–3031.
- Gordon, K., Danforth, D.R., Williams, R.F. *et al.* (1990) A novel regimen of gonadotrophin-releasing hormone (GnRH) antagonist plus pulsatile GnRH: controlled restoration of gonadotrophin secretion and ovulation induction. *Fertil. Steril.*, **54**, 1140–1145.
- Ikeda, M., Taga, M., Kurogi, K. and Minaguchi, H. (1997) Gene expression of gonadotrophin-releasing hormone, but not its receptor, in human endometrium and decidua. *Mol. Endocrinol.*, **13**, 165–168.
- Itskovitz, J., Boldes, R., Levron, J. *et al.* (1991) Induction of preovulatory luteinizing hormone surge and prevention of ovarian hyperstimulation syndrome by gonadotrophin-releasing hormone agonist. *Fertil. Steril.*, **56**, 213–220.
- Itskovitz-Eldor, J., Kol, S., Mannaerts, B. and Coelingh Bennink, H. (1998) First established pregnancy after controlled ovarian hyperstimulation with recombinant follicle stimulating hormone and GnRH antagonist ganirelix (Org 37462). *Hum. Reprod.*, **13**, 294–295.
- Kol, S., Lightman, A., Hillensjö, T. *et al.* (1999) High doses of GnRH antagonist in IVF do not adversely effect the outcome of subsequent freeze-thaw cycles. *Hum. Reprod.*, **14**, 2242–2444.
- Latouche, J., Crumerolle-Arias, M., Jordan, D. *et al.* (1989) GnRH receptors in human granulosa cells: anatomical localization and characterization autoradiographic study. *Endocrinology*, **125**, 1739–1741.
- Liu, H.C., Lai, Y.M., Davis, O. *et al.* (1992) Improved pregnancy outcome with gonadotrophin-releasing hormone agonist (GnRHa) stimulation is due to improvement in oocytes quantity rather than quality. *J. Assist. Reprod. Genet.*, **9**, 338–344.
- Loumaye, E. (1990) The control of endogenous secretion of LH by gonadotrophin-releasing hormone agonists during ovarian hyperstimulation for in-vitro fertilization and embryo transfer. *Hum. Reprod.*, **5**, 357–376.
- Loumaye, E., Engrand, P., Howles, C. and O'Dea, L. (1997) Assessment of the role of serum luteinizing hormone and estradiol response to follicle-stimulating hormone on *in vitro* fertilization treatment outcome. *Fertil. Steril.*, **67**, 889–899.
- Minaretzis, D., Alper, M.M., Oskowitz, S.P. *et al.* (1995) Gonadotrophin-releasing hormone antagonist versus agonist administration in women undergoing controlled ovarian hyper-stimulation: cycle performance and in vitro steroidogenesis of granulosa-lutein cells. *Am. J. Obstet. Gynecol.*, **172**, 1518–1525.
- Nelson, L.R., Fujimoto, V.Y., Jaffe R.B. *et al.* (1995) Suppression of follicular phase pituitary-gonadal function by a potent new gonadotrophin-releasing hormone antagonist with reduced histamine-releasing properties (ganirelix). *Fertil. Steril.*, **63**, 963–969.
- Oberý, J.J.L., Mannaerts, B.M.J.L., Kleijn, H.J. and Timmer, C.J. (1999a) Pharmacokinetic and pharmacodynamic characteristics of ganirelix (Orgalutran): Part I. Absolute bioavailability of 0.25 mg ganirelix after single subcutaneous injection in healthy female volunteers. *Fertil. Steril.*, **72**, 1001–1005.
- Oberý, J.J.L., Mannaerts, B.M.J.L., Huisman, J.A.M. and Timmer, C.J. (1999b) Pharmacokinetic and pharmacodynamic characteristics of ganirelix (Orgalutran): Part II. Dose-proportionality and gonadotropin suppression after multiple doses of ganirelix in healthy female volunteers. *Fertil. Steril.*, **72**, 1006–1012.
- Olivennes, F., Fanchin, R., Bouchard, P. *et al.* (1996) Triggering of ovulation by a gonadotrophin-releasing hormone (GnRH) agonist in patients pretreated with a GnRH antagonist. *Fertil. Steril.*, **66**, 151–153.
- Ortmann, O., Weiss, J.M., Gurke, E.M. *et al.* (1999) Ovarian effects of GnRH antagonists. *Gynecol. Endocrinol.*, **13**, Abstract 102.
- Out, H.J., Mannaerts, B.M.J.L., Driessen, S.G.A.J. *et al.* (1995) A prospective, randomised, assessor-blind, multicentre study comparing recombinant and urinary follicle stimulating hormone (Puregon versus Metrodin) in in-vitro fertilization. *Hum. Reprod.*, **10**, 2534–2540.
- Rabinovici, J., Rothman, P., Monroe, S.E. *et al.* (1992) Endocrine effects and pharmacokinetic characteristics of a potent new gonadotrophin-releasing hormone antagonist (ganirelix) with minimal histamine-releasing properties: studies in postmenopausal women. *J. Clin. Endocrinol. Metab.*, **75**, 1220–1225.
- Raga, F., Casan, E.M., Krussel, J.S. *et al.* (1998) Quantitative gonadotrophin-releasing hormone gene expression and immunohistochemical localization in human endometrium throughout the menstrual cycle. *Biol. Reprod.*, **59**, 661–669.
- Tan, S.L., Kingsland, C., Campbell, S. *et al.* (1992) The long protocol of administration of gonadotrophin-releasing hormone agonist is superior to the short protocol for ovarian stimulation for *in vitro* fertilization. *Fertil. Steril.*, **57**, 810–814.
- Treasure, T. and MacRae, K.D. (1999) Minimisation: the platinum standard for trials? *Br. Med. J.*, **317**, 362–363.
- Verboost, P.M., Smits, J., Peddemors, C. *et al.* (1999) Effects of GnRH-agonist and antagonists on steroid production in rat and human granulosa cells. *Gynecol. Endocrinol.*, **13**, Abstract 101.
- Whitehead, A. and Whitehead, J. (1991) A general parametric approach to the meta-analysis of randomized clinical trials. *Stat. Med.*, **10**, 1665–1677.

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