A comparative randomized trial to assess the impact of oral contraceptive pretreatment on follicular growth and hormone profiles in GnRH antagonist-treated patients

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BACKGROUND: This randomized controlled trial was designed to assess the impact of oral contraceptive (OC) scheduling with a GnRH antagonist (ganirelix) regimen on the ovarian response of women undergoing recombinant FSH (rFSH) stimulation for IVF, compared with a non-scheduled ganirelix regimen and a long GnRH agonist (nafarelin) protocol. METHODS: A total of 110 women was treated with an OC and ganirelix, 111 with ganirelix alone and 111 with nafarelin. The OC (containing 30 μg ethinylestradiol/150 μg desogestrel) was taken for 14–28 days and stopped 2 days prior to the start of rFSH treatment. Primary efficiency parameters were the number of cumulus-oocyte complexes (per attempt) and the number of grade 1 or 2 embryos (per attempt). RESULTS: In terms of follicular growth and hormone profiles, the OC-scheduled antagonist regimen mimicked the agonist regimen rather than the (non-scheduled) GnRH antagonist regimen. In the OC-scheduled GnRH antagonist group and the nafarelin group (versus the non-scheduled antagonist group), pituitary suppression was more profound at the start of stimulation ($P \leq 0.001$), there was a slower start of follicular growth ($P \leq 0.001$), longer stimulation was required (11.7 and 10.3 days respectively versus 9.4; $P \leq 0.001$), and more rFSH was used (2667 and 2222 IU versus 1966 IU; $P \leq 0.001$). In the three groups, the number of oocytes was similar (13.1, 12.9 and 11.5 respectively; not significant) as well as the number of good quality embryos (5.1, 5.7 and 5.0 respectively; not significant). CONCLUSION: OC treatment prior to the rFSH/ganirelix regimen can be successfully applied to schedule patients, although more days of stimulation and more rFSH are required than with a non-scheduled GnRH antagonist regimen.

Key words: GnRH agonist/antagonist/ganirelix/IVF/nafarelin/oral contraceptive pretreatment

Introduction

GnRH agonists, and more recently GnRH antagonists, are used to prevent endogenous LH surges in women undergoing controlled ovarian stimulation in assisted reproduction. Binding of a GnRH agonist to the receptor initially stimulates FSH and LH release during a short period ('flare-up'), followed by a subsequent reduction of gonadotrophin release. In contrast, GnRH antagonists bind competitively to GnRH receptors, resulting in an immediate suppression of gonadotrophin release.

Ganirelix (Orgalutran®) is a third-generation GnRH antagonist, which is effective at a daily dose of 0.25 mg (Devroey et al., 1998). In comparison with a traditional long GnRH agonist protocol, treatment with a GnRH antagonist starting on stimulation day 5/6 resulted in one or two fewer oocytes, although the number of good quality embryos obtained was similar (Borm and Mannaerts, 2000; van Hooren et al., 2001; Fluker et al., 2001; Hohmann et al., 2003). The lower number of oocytes after GnRH antagonist treatment is most likely related to a slightly different ovarian response in non-suppressed subjects in comparison to pituitary-suppressed subjects after traditional agonist treatment.

Using the same starting dose of recombinant FSH (rFSH) or HMG, the antagonist regimen resulted in faster initial follicular growth but a slightly lower number of follicles on the day of HCG as compared with the long agonist protocol (Albano et al., 2000; Borm and Mannaerts, 2000; Fluker et al., 2001; van Hooren

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et al., 2001; Hohmann *et al.*, 2003). This indicates that a smaller cohort of follicles is recruited in the antagonist regimen. Correspondingly, serum estradiol values were higher at the start of stimulation treatment and lower on the day of HCG. Due to the faster recruitment of follicles in non-suppressed subjects (since they start antagonist treatment not before day 5 of stimulation), a shorter duration of stimulation (and, accordingly, less rFSH or HMG) is required to reach the same HCG criteria. Therefore, advantages of the GnRH antagonist regimen versus the long agonist protocol include the absence of an initial 'flare-up' (no ovarian cyst formation), a considerably reduced treatment duration (more convenient for the patient), and a lower total rFSH or HMG dose needed (225–450 IU less) (Albano *et al.*, 2000; Borm and Mannaerts, 2000; Fluker *et al.*, 2001; van Hooren *et al.*, 2001; Hohmann *et al.*, 2003).

Nevertheless, planning treatment cycles may be more difficult with GnRH antagonists. Ovarian stimulation treatment should start on day 2 or 3 of menses. IVF clinic centres that avoid oocyte retrievals and embryo transfers during weekends prefer to start gonadotrophin treatment at previously planned dates, rather than on day 2 or 3, facilitating scheduling of fertilization procedures. The current randomized trial was designed to investigate the effects of scheduling with oral contraceptives (OC) before starting rFSH and ganirelix treatment for conventional IVF or ICSI. Assuming that OC scheduling may overcome some of the practical drawbacks of GnRH antagonist cycles for those IVF centres which do not operate during weekends, the aim of the study was to investigate the extent to which OC pretreatment suppressed the pituitary, as well as its effects on follicular growth, hormone profiles, and the number of oocytes obtained. The OC-scheduled ganirelix regimen was compared with a non-scheduled ganirelix regimen and with a traditional long protocol with the GnRH agonist nafarelin.

Materials and methods

Subjects

A total of 351 women scheduled for IVF or ICSI were screened and randomized, 117 subjects per treatment group. The main selection criteria were: healthy females of infertile couples, age at time of screening between 18 and 39 years, body mass index between 18 and 29 kg/m^2, body weight ≤90 kg, a normal menstrual cycle with a range of 24–35 days and an intra-individual variation of ±3 days, and willingness to give written informed consent. Exclusion criteria included contraindications for the use of gonadotrophins, endocrine abnormalities (e.g. polycystic ovary syndrome), more than three unsuccessful controlled ovarian stimulation cycles, a history of low or no ovarian response during FSH/HMG treatment, and clinically relevant abnormal laboratory values (including hormones) or medical examination findings. The subjects were randomly assigned to one of three treatment groups by central remote allocation (Interactive Voice Response System). In total, 10 IVF centres participated between June 2000 and May 2002: seven in Australia, one in Denmark, one in Jordan, and one in Norway. The number of participants per centre ranged from 9 to 63. To improve balance, the randomization of subjects to treatment was stratified for type of infertility (primary or secondary), IVF or ICSI, centre, and age. In some centres it proved to be a challenge to implement the study protocol in the clinic routines, because it included three concomitant but very different regimens with respect to drug administration, clinic visits and treatment planning. Therefore, in

some clinics a considerably lower number of patients participated than in others.

Study design

This was an open-label, randomized, group-comparative, multicentre study to assess the impact of OC pretreatment on follicular growth and hormone profiles in women undergoing GnRH antagonist (ganirelix) treatment and ovarian stimulation for IVF/ICSI. Therefore, the effects of OC-scheduled ganirelix treatment were compared with those of non-scheduled ganirelix treatment and those of treatment with the GnRH agonist nafarelin in a traditional long protocol.

Figure 1 presents an overview of the treatment regimens studied. In the OC-scheduled group, subjects started taking a combined OC pill (30 μg ethinyloestradiol/150 μg desogestrel) Marvelon® (NV Organon, The Netherlands) on day 1 of the menstrual cycle. They took it daily for between 14 and 28 days, depending on the planned start of rFSH treatment.

Treatment with the GnRH antagonist ganirelix (0.25 mg, Orgalutran®; NV Organon, The Netherlands) was started on day 5/6 of rFSH treatment. If no follicles ≥14 mm were observed by ultrasonography on that day, the start of ganirelix was delayed. Injections containing 0.25 mg ganirelix per 0.5 ml were administered s.c. in the thigh, once daily in the morning, until and including the day of HCG administration.

Subjects in the nafarelin group started pretreatment with the GnRH agonist nafarelin (Synarel®; Pharmacia, Australia) on day 21–24 of the preceding cycle. Nafarelin was administered intranasally at a daily dose of 0.8 mg until and including the day of HCG administration.

In all three groups ovarian stimulation was performed with rFSH (follitropin beta, Puregon®; NV Organon, The Netherlands), which was administered s.c. once daily in the morning at a fixed dose of 200 IU during the first 5–6 days. After this period the dosage of rFSH could be adjusted depending on the ovarian response as assessed by ultrasound. Treatment was continued until (and including) the day of HCG administration. In the OC-scheduled ganirelix group, stimulation with rFSH was started 2 days after discontinuation of the OC (irrespective of whether or not menses had started), in the non-scheduled group on day 2–3 of the menstrual cycle and in the nafarelin group after 2–4 weeks of nafarelin treatment [as soon as pituitary down-regulation had been achieved (i.e. serum estradiol ≤50 pg/ml or ≤200 pmol/l); if this stage was not achieved after 4 weeks of nafarelin treatment, the subject discontinued].

HCG, 10 000 IU in 1 ml saline (Pregnyl®, NV Organon, The Netherlands), was administered, either s.c. or i.m., when at least three follicles ≥17 mm or at least one follicle ≥20 mm were observed on ultrasound. In case of risk of ovarian hyperstimulation syndrome (OHSS), the HCG dose was reduced to 5000 IU. Oocyte retrieval was
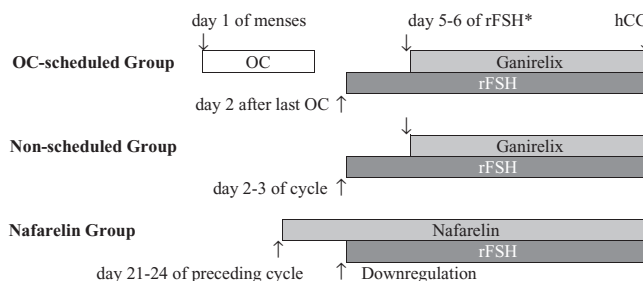
*The start of rFSH was delayed when at day 5/6 of stimulation no follicles ≥14 mm were observed. OC = oral contraceptive. rFSH = recombinant FSH

Figure 1. Treatment regimens using ganirelix (0.25 mg) with oral contraceptive pretreatment (30 μg ethinylestradiol/150 μg desogestrel), ganirelix (0.25 mg) alone, or nafarelin (0.8 mg) for pituitary down-regulation.

performed 30–36 h after HCG administration, followed by IVF or ICSI. No more than three embryos were transferred 2–3 days after oocyte retrieval. Progesterone for luteal support was given daily (doses and administration form as per usual protocol of the participating centre), starting at the latest on the day of embryo transfer, for 2 weeks or up to menses.

The study was approved by the Ethics Committee of each participating centre. All subjects gave written informed consent. The study was performed according to the principles of the Declaration of Helsinki, and the ICH/Good Clinical Practice guidelines. The study was monitored by uniformly trained Clinical Research Associates of Organon with assistance of a contract research organization for the clinics in Perth and Adelaide.

Assessments

Prior to the start of treatment, a physical and gynaecological examination was performed to exclude any abnormality. Blood samples were taken for routine biochemistry, haematology, and hormonal parameters. A pregnancy test (urinary HCG) was performed. Blood samples for hormone assessments were taken just before the first rFSH injection (treatment day 1) and at least once every 2 days from day 5/6 of rFSH treatment (in the antagonist groups just before ganirelix injection) up to and including the day of HCG. Serum FSH, LH, estradiol, and progesterone values were determined by means of the automated Wallac AutoDelfia Fluoroimmunoassay system (PerkinElmer Inc., Wellesley MA, USA) at a central laboratory (ABL BV, Assen, The Netherlands). The maximum intra-assay and inter-assay coefficients of variation were 3.3% for FSH, 3.4% for LH, 4.9% for estradiol, and 4.3% for progesterone. To measure follicular development, ultrasonography was performed at least once every two days from day 5/6 of rFSH treatment up to and including the day of HCG. Other parameters assessed were treatment failure (defined as the number of subjects who did not have an HCG injection or who received an HCG injection because of premature luteinization), number of LH rises (LH ≥10 IU/l), number of oocytes retrieved, number of good quality embryos [grade 1 (defined as excellent: no fragmentation) and grade 2 (defined as good: 1–20% fragmentation)], fertilization rate, implantation rate, and ongoing pregnancy rate (assessed by ultrasound ≥12–16 weeks after embryo transfer).

Statistical analyses

The efficacy analyses were based on an intention-to-treat principle, whereas the safety parameters were based on the actual treatments received. For the intent-to-treat (ITT) analyses, all randomized subjects who received at least one dose of the OC, rFSH, or GnRH analogue were grouped according to the treatment they should have received by randomization (even if actual treatment was different). For the safety analyses, all subjects who received at least one dose were grouped according to the actual treatment they received [all-subjects-treated (AST) group].

Primary efficacy parameters were the number of cumulus–oocyte complexes (per attempt) and the number of grade 1 or 2 embryos (per attempt). 'Per attempt' means that if a subject did not reach a certain stage in IVF treatment, zero values were imputed (e.g. if the particular subject did not have oocyte retrieval, then the number of oocytes, embryos, etc. was set to zero and the pregnancy outcome was set negative).

This study was designed to evaluate whether ganirelix with and without OC pretreatment is at least as effective as the nafarelin regimen. A difference up to three oocytes between the treatments was considered acceptable. Two-sided 95% confidence intervals were used for the difference for ganirelix with or without the OC minus nafarelin, using the appropriate contrasts in an analysis of variance

(ANOVA) model, with treatment and centre as strata (fixed effects). Assuming that the SD of the number of oocytes is 6.4, a sample size of ≥100 subjects per treatment arm (i.e. a total sample size of 300) was needed. Using this sample size, a difference of more than three oocytes in favour of nafarelin versus both ganirelix groups can be ruled out with a probability of 80%. The number of good quality embryos (grade 1 or 2) per attempt was analysed in the same way as the number of cumulus–oocyte complexes retrieved, with treatment and centre as strata (fixed effects).

For the following, secondary parameters, the pairwise differences between the treatment groups were statistically tested by ANOVA: the number and size of follicles, as well as serum hormone values (FSH, LH, estradiol, progesterone), at the different stages of ovarian stimulation (day 1, day 5/6, day 7/8, and the day of HCG injection or 1 day before); duration of rFSH treatment; total rFSH dose; the number of cumulus–oocyte complexes retrieved; the number of good quality embryos; and implantation rate. Ongoing pregnancy rates and incidences of LH rises, respectively, were tested between the treatment groups with Fisher's exact tests. For all other parameters, summary statistics were calculated. Since the multiple analyses of secondary parameters were exploratory, no correction for multiple statistical testing was applied.

Results

Subject characteristics and treatment failures

The three treatment groups were similar with respect to age, height, weight, and body mass index (Table I). The majority (92.5%) of the subjects were Caucasian. No relevant differences were found between the treatment groups for the duration and causes of infertility.

Table II presents the number of patients per treatment stage. Of the 351 subjects randomized, 332 subjects started treatment (i.e. OC, rFSH and/or GnRH analogue). The remaining 19 subjects did not start any treatment, five because of 'spontaneous pregnancy' (two subjects in the OC-scheduled group and three in the non-scheduled ganirelix group). Ninety per cent of all subjects randomized were treated with HCG, and 81% had an embryo transfer. About 50% of the couples had conventional IVF, and 50% had ICSI. The main reasons for treatment failure were insufficient ovarian response, risk of OHSS, the occurrence of adverse events, and an insufficient number of good quality embryos (Figure 2). The most cancellations were seen

Table I. Subject characteristics by treatment group (intention to treat)

Characteristic	OC/ganirelix (n = 111)	Ganirelix (n = 110)	Nafarelin (n = 111)
Age (years) [mean (SD)]	32.7 (3.9)	32.1 (3.7)	32.2 (4.0)
Body mass index (kg/m²) [mean (SD)]	23.3 (3.0)	23.4 (3.0)	24.2 (3.6)
Duration of infertility (years) [mean (SD)]	3.8 (2.7)	3.7 (2.7)	4.3 (3.1)
Primary infertility (%)	44.1	47.3	46.8
Main causes of infertility (%)			
Male factor only	39.6	44.5	30.6
Tubal factor only	23.4	15.5	23.4
Tubal and male factor	1.8	5.5	6.3
Endometriosis only	7.2	6.4	9.0
Unknown	22.5	18.2	21.6

OC = oral contraceptive.

Table II. Disposition of subjects[*n* (%)]

Treatment stage	OC/ganirelix	Ganirelix	Nafarelin	Total
Randomized	117 (100)	117 (100)	117 (100)	351 (100)
Started OC treatment	110[a] (94.0)	–	–	
Started nafarelin treatment	–	–	111 (94.9)	
Started rFSH treatment	110 (94.0)	110 (94.0)	105 (89.7)	325 (92.6)
Started ganirelix treatment	109 (93.2)	108 (92.3)	–	
HCG injection	109 (93.2)	105 (89.7)	101 (86.3)	315 (89.7)
Oocyte retrieval	107 (91.5)[b]	105 (89.7)	101 (86.3)	313 (89.2)
IVF/ICSI	52/52 (88.9)	48/52 (85.5)	53/45 (83.8)	153/149 (86.0)
Embryo transfer	102 (87.2)	95 (81.2)	88 (75.2)	285 (81.2)

[a]One subject, allocated to the oral contraceptive (OC)-scheduled group, did not receive the OC. She was thus included in the OC-scheduled group in the intent-to-treat analysis and in the non-scheduled group in the all-subjects-treated analysis.
[b]Two subjects had HCG but no oocyte retrieval: one due to failure in the preparation of HCG and the other due to insufficient compliance with the medication.
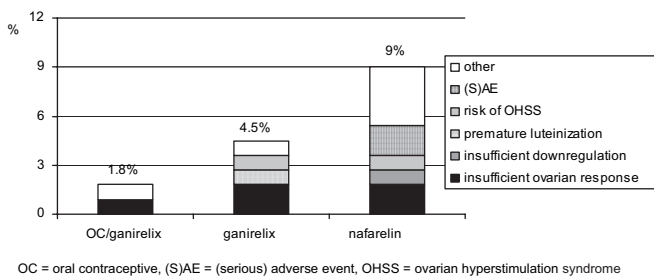rFSH = recombinant FSH.

OC = oral contraceptive, (S)AE = (serious) adverse event, OHSS = ovarian hyperstimulation syndrome

Figure 2. Percentage of treatment failures (i.e. cancellations before HCG administration), by reason (intention to treat).

in the nafarelin group (9%) and the fewest in the OC-scheduled group (1.8%).

Total dose of rFSH and duration of treatment

The mean total rFSH dose in subjects with an HCG injection was 2667 IU in the OC-scheduled ganirelix group versus 1966 IU in the non-scheduled group and 2222 IU in the nafarelin group. The average daily rFSH dose was 200 IU in all three treatment groups. The mean number of rFSH treatment days was longest in the OC-scheduled group (11.7) and shortest in the non-scheduled group (9.4). All pairwise treatment differences in total rFSH dose and duration of rFSH treatment were statistically significant ($P \leq 0.001$).

The mean duration of ganirelix administration was similar in the scheduled and non-scheduled groups (4.6 and 4.4 days respectively). However, the mean starting day of ganirelix was considerably later in the OC-scheduled group (day 7.1 of rFSH treatment) than in the non-scheduled group (day 4.9 of rFSH treatment). Nafarelin was used on average for 27.0 days, ~22 more days than ganirelix was used. In the scheduled group, the oral contraceptive was used on average for 16.7 days.

Follicle growth

Figure 3 presents the mean number of follicles ≥11 mm during stimulation. Figure 4 presents the number and sizes of follicles on the day of HCG injection (or one day before). Early during stimulation (day 5/6), the total number of follicles was highest
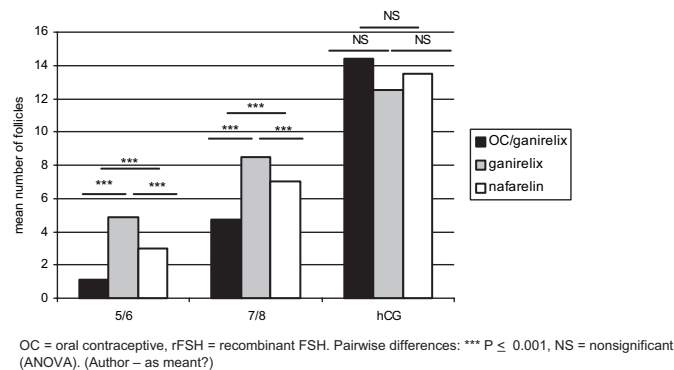

OC = oral contraceptive, rFSH = recombinant FSH. Pairwise differences: *** $P \leq 0.001$, NS = nonsignificant (ANOVA). (Author – as meant?)

Figure 3. Mean number of follicles (≥11 mm) on day 5/6, day 7/8 of rFSH stimulation treatment and on the day of (or one day before) HCG injection (intention to treat).
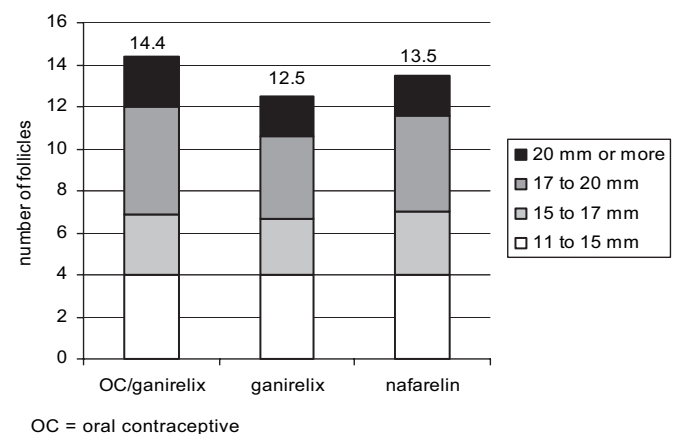

OC = oral contraceptive

Figure 4. Mean number and size of follicles on the day of (or one day before) HCG injection (intention to treat).

($P < 0.001$) in the non-scheduled group and lowest in the OC-scheduled group. At the end of stimulation, the number of follicles was similar in all three treatment groups. Figure 3 illustrates that follicular growth started more slowly in the OC-scheduled group, whereas follicular growth started relatively fast in the non-scheduled ganirelix group. At the end of stimulation,

the mean (SD) number of follicles ≥11 mm was similar in all three groups [14.4 (7.2), 12.5 (8.2), and 13.5 (7.4) in the OC/ganirelix, ganirelix and nafarelin groups respectively]. For the number of largest follicles (≥20 mm) the pairwise comparisons did not show any statistically significant differences at any stage of stimulation. The development of large follicles (≥17 mm) was significantly faster in the non-scheduled ganirelix group. However, at the end of stimulation the number of these follicles (≥17 mm) was highest in the OC-scheduled ganirelix group, which was statistically significant.

LH rises

In total, 20 subjects experienced an LH rise (LH ≥10 IU/l during any of the assessments) and 12 out of these 20 subjects had a concomitant progesterone rise (≥ 3.18 nmol/l) (see Table III). Significantly more LH rises were observed in the non-scheduled ganirelix group as compared with the scheduled group and the nafarelin group ($P < 0.001$), and the majority of those occurred before the ganirelix treatment was started. Fourteen subjects with an LH rise, all from the non-scheduled ganirelix group, had embryo transfer; and an ongoing pregnancy was established in two of them.

Serum hormone profiles

Median serum LH, FSH, estradiol (E_2) and progesterone values during ovarian stimulation are presented in Figure 5. On day 1 of stimulation, serum LH and FSH levels indicate that the degree of pituitary suppression is more profound in the OC-scheduled ganirelix group (0.9 and 1.6 IU/l respectively) than in the nafarelin group (2.1 and 3.8 IU/l respectively); there is obviously no pituitary suppression in the non-scheduled ganirelix group (4.2 and 6.3 IU/l respectively). During the first 6 days of stimulation, serum LH levels declined in the non-scheduled group and the nafarelin group whereas serum LH values in the OC-scheduled group tended to increase. On day 5/6 of stimulation, these LH values were 1.6, 1.7 and 1.0 IU/l in the scheduled, non-scheduled and nafarelin groups; and on the day of hCG, they were 0.6, 1.4 and 1.1 IU/l respectively. Serum FSH reached similar levels across the three groups from day 6 of stimulation onward. At the end of stimulation, median serum FSH levels were lowest in the non-scheduled group (10.1 IU/l versus 11.5 and 11.0 IU/l in the scheduled and nafarelin groups respectively). During stimulation, an increase of serum (E_2) was observed in all three treatment groups. However, in the initial phase of stimulation serum E_2 values were considerably higher in the non-scheduled ganirelix group than in the other groups, reaching values of 417 pg/ml on day 5/6 of stimulation versus 181 and 208 pg/ml in the scheduled and nafarelin groups. At the end of stimulation, serum E_2 levels increased more rapidly in the nafarelin group, reaching a median value of 2130 pg/ml versus 1530 pg/ml in the OC-scheduled and 1490 pg/ml in the non-scheduled ganirelix groups. Median serum progesterone values were also higher in the non-scheduled ganirelix group during the first 7/8 days of stimulation (i.e. 0.75 ng/ml versus 0.55 and 0.65 ng/ml in the scheduled and nafarelin groups). At the end of stimulation, the serum progesterone values were similar in the three groups (i.e. 1.14, 0.98 and 1.07 ng/ml in the scheduled, non-scheduled, and nafarelin groups respectively).

Number of oocytes and good quality embryos

The mean number of oocytes per attempt was 13.1 (SD 7.8) in the OC-scheduled ganirelix group, 11.5 (SD 7.6) in the non-scheduled ganirelix group, and 12.9 (SD 8.7) in the nafarelin group. The pairwise statistical comparisons did not reveal any differences (Table IV): the difference of OC/ganirelix minus nafarelin was estimated as 0.09 (95% CI: −2.00 to 2.18) and the difference of ganirelix minus nafarelin was estimated as −1.56 (95% CI: −3.66 to 0.54). The maturity of the oocytes retrieved was comparable in all three groups (see Table IV).

Statistical analyses (see Table IV) indicated that the number of grade 1–2 embryos was similar in all three treatment groups: the difference of OC/ganirelix versus nafarelin was estimated as −0.44 (95% CI: −1.54 to 0.65) and of ganirelix versus nafarelin as −0.66 (95% CI: −1.76 to 0.44).

Clinical outcome

Table IV presents the summary statistics of the efficacy parameters. The fertilization rate was similar in all treatment groups and ranged from 61.2 to 66.7%. The mean total number of embryos transferred was 2.0 (range: 1–3) in all treatment groups. The mean implantation rate for subjects with an embryo transfer was lowest (12.3%) in OC/ganirelix and highest in the nafarelin group (21.6%) (pairwise comparison between OC/ganirelix and nafarelin: $P = 0.03$). The differences between the ongoing pregnancy rates per attempt were not statistically significantly different between the OC-scheduled group (16.2%), the non-scheduled ganirelix group (20.9%) and the nafarelin group (23.9%).

Table III. LH/progesterone (P) rises during ovarian stimulation treatment (intention to treat)

	OC/ganirelix ($n = 111$)		Ganirelix ($n = 110$)		Nafarelin ($n = 111$)	
	Without P rise	With P rise	Without P rise	With P rise	Without P rise	With P rise
Before GnRH analogue treatment	0	1 (0.9)	5 (4.5)	8 (7.3)	–	–
During GnRH analogue treatment	0	1 (0.9)	2 (1.8)	2 (1.8)	1 (0.9)	0
Total no. of LH rises	2 (1.8)[a]		17 (15.4)[a,b]		1 (0.9)[b]	

Values in parentheses are percentages.
LH rise: LH value ≥10 IU/l; progesterone rise: progesterone value ≥1 ng/ml (≥3.18 nmol/l).
[a,b]Pairwise differences: [a]OC/ganirelix versus ganirelix: $P < 0.001$; [b]nafarelin versus ganirelix: $P < 0.001$; OC/ganirelix versus nafarelin: non-significant (χ^2-test).
OC = oral contraceptive.
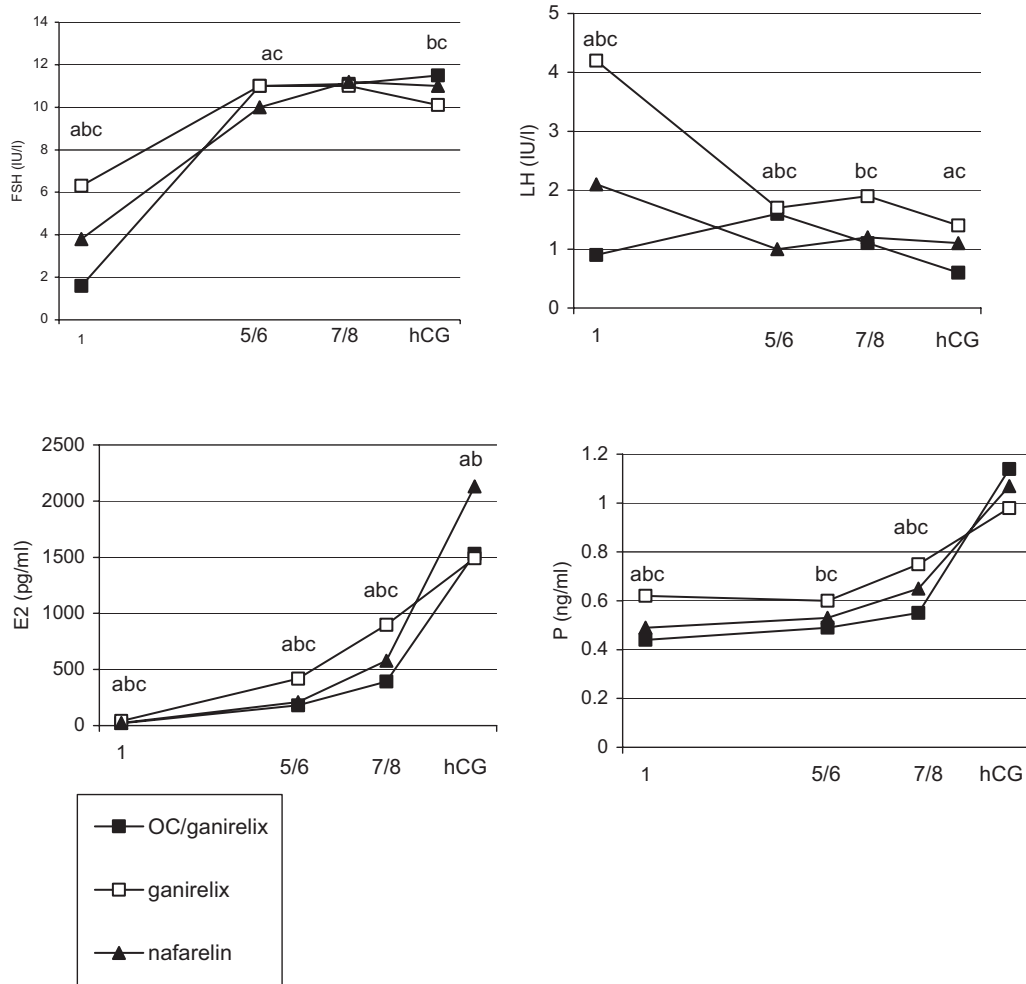
OC = oral contraceptive, rFSH = recombinant FSH, E$_2$ = oestradiol, P = progesterone. Pairwise differences: *a* = OC/ganirelix versus nafarelin statistically significant (P ≤ 0.05), *b* = ganirelix versus nafarelin statistically significant (*P* ≤ 0.05), c = OC/ganirelix versus ganirelix statistically significant (*P* ≤ 0.05). Oestradiol: 1 pg/ml = 3.671 pmol/l; progesterone 1 ng/ml = 3.18 nmol/l.

Figure 5. Serum hormone levels (median values) on day 1, day 5/6, day 7/8 of rFSH stimulation treatment and on the day of (or one day before) HCG injection (intention to treat).

Safety and tolerance

The number of subjects who reported one or more adverse events after the start of GnRH analogue treatment is presented in Table V. Fifteen subjects reported a total of 18 serious adverse events (nine events in eight subjects of the scheduled group, three events in three subjects of the non-scheduled group, and six events in four subjects of the nafarelin group). In four of these cases, the investigator considered the serious adverse events to be at least possibly related to the study drug: i.e. one ovarian cyst in the OC/ganirelix group, two cases of ovarian hyperstimulation syndrome (one in the OC-scheduled group and one in the nafarelin group), and one case of ectopic pregnancy in the non-scheduled group. All women recovered from these events. The incidence of (drug-related) adverse events was highest in the nafarelin group. During treatment with nafarelin, seven subjects (6.3%) discontinued treatment because of adverse events, versus none (0%) and three subjects (2.8%) in the scheduled and non-scheduled ganirelix groups.

Most adverse events were of mild or moderate intensity. Headache and abdominal pain were reported most frequently: headache in 6.5% of the OC-scheduled group, 8.3% of the non-scheduled group, and 36.9% of the nafarelin group. The corresponding values for abdominal pain were 11.1, 8.3 and 12.6% respectively. In the nafarelin group, hot flushes were also reported (in 8.1% of subjects versus 0% in both ganirelix groups).

The incidence of ovarian hyperstimulation syndrome (OHSS) is summarized in Table VI. The study was not powered to show statistically significant differences between the treatment groups, but there were fewer reports in both ganirelix groups (i.e. 2.7% in the OC/ganirelix group and 1.8% in the non-scheduled group versus 5.4% in the nafarelin group). All cases were mild or moderate [grade I or II, WHO classification (WHO Scientific Group, 1973)], with the exception of one severe (grade III) case observed in the nafarelin group.

Table IV. Summary statistics of efficacy parameters (intention to treat)

Parameter	OC/ganirelix (n = 111)	ganirelix (n = 110)	nafarelin (n = 111)	P (comparison between treatment groups, ANOVA)
Total rFSH dose (IU)[a]	2667.0 (880.7)	1965.7 (515.5)	2221.8 (655.3)	≤ 0.001[c]
No. of rFSH treatment days[a]	11.7 (1.9)	9.4 (1.6)	10.3 (1.7)	≤ 0.001[c]
No. of oocytes recovered per attempt	13.1 (7.8)	11.5 (7.6)	12.9 (8.7)	NS[d]
No. of good quality embryos obtained[b]	5.1 (3.8)	5.0 (4.5)	5.7 (4.3)	NS[d]
No. of GnRH analogue treatment days (patients who received HCG)	4.6 (1.6)	4.5 (1.3)	27.0 (3.7)	–
No. of mature oocytes in IVF cycles	12.7 (7.8)	10.6 (6.9)	11.8 (5.7)	–
No. of metaphase II oocytes in ICSI cycles	11.2 (6.3)	9.5 (6.0)	11.3 (6.6)	–
No. of embryos obtained[e]	7.5 (4.8)	7.3 (5.2)	8.2 (5.4)	–
Fertilization rate % (SD)	61.2 (25.7)	66.7 (24.7)	64.7 (23.7)	–
Implantation rate per transfer % (SD)	12.3 (27.3)	17.4 (30.8)	21.6 (33.4)	0.03 (OC/ganirelix versus nafarelin) NS (other pairwise difference)
Miscarriage rate per biochemical pregnancy [n (%)]	1 (3.3)	3 (10.0)	7 (17.1)	–
Ongoing pregnancy rate per attempt [n (%)]	18 (16.2)	23 (20.9)	26 (23.9)	NS[d]

Values are mean (SD) unless otherwise stated.
[a]Among patients who received HCG.
[b]For patients with IVF or ICSI.
[c]All pairwise differences were statistically significant: $P \leq 0.001$.
[d]All pairwise differences were not significant (NS): $P > 0.05$.
[e]Grade 1 (excellent quality: no fragmentation) or grade 2 (good quality: 1–20% fragmentation) embryos, per subject with IVF/ICSI.
OC = oral contraceptive; rFSH = recombinant FSH.

Table V. Number (%) of subjects with various types of adverse events (all subjects treated)

	OC/ganirelix (n = 108[a])	Ganirelix (n = 109[a])	Nafarelin (n = 111[a])
Deaths	0 (0.0)	0 (0.0)	0 (0.0)
Subjects with serious adverse events	8 (7.4)	3 (2.8)	4 (3.6)
Subjects with adverse events causing discontinuation	0 (0.0)	3 (2.8)	7 (6.3)
Subjects with adverse events	44 (40.7)	48 (44.0)	75 (67.6)
Subjects with adverse events of known severe intensity	2 (1.9)	4 (3.7)	8 (7.2)
Subjects with drug-related adverse events[b]	23 (21.3)	19 (17.4)	46 (41.4)

[a]Treated with GnRH analogue.
[b]According to the investigator definitely, probably or possibly related (see text for further descriptions).
OC = oral contraceptive.

Table VI. Number (%) of subjects with ovarian hyperstimulation syndrome (all subjects treated)

	OC/ganirelix (n = 110)	Ganirelix (n = 111)	Nafarelin (n = 111)
Mild (grade I)[a]	1 (0.9)	1 (0.9)	1 (0.9)
Moderate (grade II)[a]	2 (1.8	1 (0.9)	4 (3.6)
Severe (grade III)[a]	0 (0.0)	0 (0.0)	1 (0.9)
Total	3 (2.7)	2 (1.8)	6 (5.4)

[a]Ovarian hyperstimulation as per World Health Organization classification (WHO Scientific Group, 1973).
OC = oral contraceptive.

Discussion

The current study is the first systematic investigation of the effects of OC scheduling with a GnRH antagonist (ganirelix) regimen in controlled ovarian stimulation. The effects on follicular growth and hormone profiles were compared with those resulting from a non-scheduled GnRH antagonist regimen and those of a traditional GnRH agonist (nafarelin) regimen. The results indicate that an OC-scheduled ganirelix treatment mimics the effects of traditional down-regulation with a long GnRH agonist protocol: as a result of the pituitary suppression caused by the OC, follicular growth starts relatively slowly, which is accompanied by lower serum estradiol levels early on, a longer duration of rFSH stimulation, and a higher total rFSH consumption than in non-scheduled antagonist cycles. The clinical outcome, in this study defined as the number of oocytes per retrieval and the number of grade 1 or 2 embryos per IVF/ICSI attempt, is similar in comparison with non-scheduled antagonist or agonist treatments.

The differences observed between the non-scheduled antagonist regimen and the long down-regulation protocol were well in accordance with observations from previous studies (Borm and Mannaerts, 2000; Fluker et al., 2001; van Hooren et al., 2001; Hohmann et al., 2003). Ganirelix was used for only 4.5 days, whilst in the long protocol the agonist was used on average for 27.0 days. In the non-scheduled ganirelix cycles, the cumulative use of rFSH was on average 250 IU lower than in the agonist cycles, which compares well to the 225–450 IU lower total FSH doses required in antagonist cycles in previous studies (Borm and Mannaerts, 2000; Fluker et al., 2001;

van Hooren *et al.*, 2001; Hohmann *et al.*, 2003). Less exogenous FSH needs to be administered in non-scheduled ganirelix cycles, since endogenous FSH production is not suppressed until day 5/6. This was also reflected in the hormone profiles at the start of the cycle: FSH and LH levels were considerably higher in the ganirelix group than in the nafarelin group. Follicular development was initially faster in the ganirelix group than in the nafarelin group but slowed towards the end of stimulation. Ultimately, the number of follicles ≥11 mm was similar, but the final cohort of large follicles (≥17 mm) was smaller in the non-scheduled group.

The incidence of premature LH rises, with or without a concomitant progesterone rise, was relatively high (15.4%) and in accordance with an earlier study that used a higher rFSH starting dose (Fluker *et al.*, 2001). Most of these LH rises were observed prior to the start of ganirelix treatment. The total number of oocytes recovered and the number of good quality embryos were similar in the two groups. The cancellation rates due to side-effects were low in the ganirelix group; and hot flushes, associated with the down-regulation in a long down-regulation protocol, were not reported by these patients. The incidence of ovarian hyperstimulation syndrome was very low in the non-scheduled ganirelix group (1.8%) compared to the nafarelin group (5.4%).

Scheduling of antagonist cycles with 14–28 days of OC treatment had a marked impact on the hormone profiles and follicular development, although the final outcome in terms of the number of oocytes and good quality embryos was similar. The pituitary suppression due to OC use resulted in very low FSH and LH levels at the start of the cycle, even lower than those seen in the long down-regulation group. Consequently, more rFSH was needed in the OC-scheduled ganirelix group: they received on average 700 IU more rFSH than did non-scheduled ganirelix patients and 445 IU more than did nafarelin patients. Their stimulation lasted 2.3 days longer than in non-scheduled patients, whilst the start of ganirelix treatment was 2 days later. Two other studies into OC-scheduling with an antagonist regimen suggest that the increase in rFSH consumption can be limited by prolonging the gap between the discontinuation of OC treatment and the start of stimulation: in these studies, the gap was 4–5 days instead of the 1–2 days used in the current study (Doody *et al.*, 2001; Van Loenen *et al.*, 2002).

In the OC-scheduled group, the initially low LH levels increased gradually, from the time the OC had been stopped until the ganirelix administration was started, on average on day 7. The FSH levels in the OC-scheduled patients were rather similar to those in the two other groups from day 6 stimulation onwards, due to the daily administration of exogenous FSH. The FSH levels on the day of hCG administration were related to the amount of rFSH used and were accordingly highest in the scheduled group.

Serum estradiol levels were initially low but reached levels comparable to those in the non-scheduled group at the end of stimulation. The hormone profiles reflected the pattern of follicular growth observed in the scheduled ganirelix group: due to the pituitary suppression at the start of the cycle, follicular growth started more slowly than in non-scheduled cycles, which was followed by a more rapid growth at the end of

stimulation. This pattern mimicked that observed in the nafarelin patients and can be explained by the combined effects of pituitary suppression at the start of the cycle and longer stimulation using more rFSH during the cycle.

Ultimately, the number of oocytes recovered was similar. The estradiol levels on the day of HCG in the OC-scheduled ganirelix cycles deviated from the nafarelin group and were in line with the levels observed in the non-scheduled ganirelix group. The lower levels did not correspond with the rates of follicular growth observed in the non-scheduled ganirelix group, and they may be indicative of a lower estradiol concentration per follicle in GnRH antagonist-treated patients, as has been suggested previously (Garcia-Velasco *et al.*, 2001).

The percentage of treatment failures (cancellations prior to HCG administration) was very low in the scheduled ganirelix group (1.8%) as compared with the traditional long protocol group (9%). This could partly be attributed to the higher incidence of adverse events in the nafarelin group and a lower incidence of insufficient ovarian response in the OC-scheduled group. However, a relatively high number of subjects in the nafarelin group discontinued because of 'other reasons', not related to the treatment. Therefore, the relatively high risk of treatment failure in this group was partly biased. Finally, the addition of OC pretreatment to ganirelix cycles appeared to reduce the occurrence of LH rises, approaching the percentages obtained with a traditional GnRH agonist protocol (1.8 and 0.9% respectively). This can be attributed to the suppression of pituitary LH production by the OC prior to ovarian stimulation.

The implantation and pregnancy rates were numerically lower, in particular in the OC-scheduled group. The borderline significance of the difference in implantation rate between the OC-scheduled group and the nafarelin group ($P = 0.03$) disappears if correction for multiple statistical testing is applied. It has been suggested in previous studies, however, that there might be a tendency towards lower implantation rates in antagonist-treated IVF cycles as compared with agonist-treated cycles (Borm and Mannaerts, 2000; Fluker *et al.*, 2001). The current study does not exclude this possibility, in particular with respect to the results in the OC-scheduled group. In that group, ovarian stimulation was started 2 days after discontinuation of the OC, irrespective of whether a withdrawal bleed had begun. It can be speculated that in some patients the endometrial proliferation was therefore lagging behind at the time of oocyte retrieval, affecting the outcome of the cycle. It may also be possible that the profound suppression of LH, as observed in this group, affects reproductive outcome (Filicori, 1999; Filicori *et al.*, 2002) or that the prolongation of the follicular phase associated with the longer duration of stimulation affects endometrial receptivity (Kolibianakis *et al.*, 2004). A longer gap between discontinuation of OC treatment and the start of ovarian stimulation would address these issues. Indeed, results of other, smaller studies, which used a gap of 4–5 days, have produced more favourable pregnancy rates (Doody *et al.*, 2001; Obruca *et al.*, 2001; Kelly *et al.*, 2002; Kenigsberg *et al.*, 2002; Meldrum *et al.*, 2002; Van Loenen *et al.*, 2002). The optimal gap and the impact of OC scheduling on implantation and pregnancy rates need to be explored in future, appropriately powered studies.

In summary, OC scheduling of a GnRH antagonist protocol results in follicular growth and hormone profiles that are closer to those observed in GnRH agonist protocols than in non-scheduled GnRH antagonist protocols. The number of premature LH rises was low compared to that in non-scheduled GnRH antagonist cycles. The three regimens produced similar numbers of oocytes and good quality embryos. The two ganirelix regimens were associated with a lower incidence of adverse drug reactions, and potentially of ovarian hyperstimulation syndrome. However, the greater convenience of OC scheduling might have been offset by the need for longer stimulation and more rFSH than with a non-scheduled regimen.

References

Albano C, Felberbaum RE, Smitz J, Riethmuller-Winzen H, Engel J, Diedrich K and Devroey P (2000) Ovarian stimulation with HMG: results of a prospective randomized phase III European study comparing the luteinizing hormone-releasing hormone (LHRH)-antagonist cetrorelix and the LHRH-agonist buserelin. European Cetrorelix Study Group. Hum Reprod 15,526–531.

Borm G and Mannaerts B; European Orgalutran Study Group (2000) 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. Hum Reprod 15,1490–1498.

Devroey P, Abyholm T, Diedrich K, de Jong D, Hillensjo T, Hedon B, Itskovitz-Eldor J, Kahn J, Naether O, Olivennes F et al (1998) A double-blind, randomized, dose-finding study to assess the efficacy of the gonadotrophin-releasing hormone antagonist ganirelix (Org 37462) to prevent premature luteinizing hormone surges in women undergoing ovarian stimulation with recombinant follicle stimulating hormone (Puregon™). Hum Reprod 13, 3023–3031.

Doody KJ, Langley M, Marek D and Doody K (2001) Oral contraceptive pretreatment for IVF cycles employing recombinant FSH and a GnRH antagonist. Abstract no. P-373. Fertil Steril 76(3 Suppl 1), S236.

Filicori M (1999) The role of luteinizing hormone in folliculogenesis and ovulation induction. Fertil Steril 71,405–414.

Filicori M, Cognigni GE, Samara A, Melappioni S, Perri T, Cantelli B, Parmegiani L, Pelusi G and DeAloysio D (2002) The use of LH activity to drive folliculogenesis: exploring uncharted territories in ovulation induction. Hum Reprod Update 8,543–557.

Fluker M, Grifo J, Leader A, Levy M, Meldrum D, Muasher SJ, Rinehart J, Rosenwaks Z, Scott RT, Jr., Schoolcraft W et al (2001) Efficacy and safety of ganirelix acetate versus leuprolide acetate in women undergoing controlled ovarian hyperstimulation. Fertil Steril 75,38–45.

Garcia-Velasco JA, Isaza V, Vidal C, Landazabal A, Remohi J, Simon C and Pellicer A (2001) Human ovarian steroid secretion in vivo: effects of GnRH agonist versus antagonist (cetrorelix). Hum Reprod 16,2533–2539.

Hohmann FP, Macklon NS and Fauser BC (2003) A randomized comparison of two ovarian stimulation protocols with gonadotropin-releasing hormone (GnRH) antagonist cotreatment for in vitro fertilization commencing recombinant follicle-stimulating hormone on cycle day 2 or 5 with the standard long GnRH agonist protocol. J Clin Endocrinol Metab 88,166–173.

Kelly MP, Shapiro D, Tillotson D and Batzer F (2002) Clinical outcomes and cycle characteristics of donor oocyte cycles using the GnRH analogues ganirelix or leuprolide. Fertil Steril 78(3 Suppl 1), S118–S119, Abstract P-10.

Kenigsberg D, Brenner S and Nee G (2002) Initial experience with a GnRH antagonist in good prognosis patients undergoing IVF treatment. Abstract P-1. Fertil Steril 77(4 Suppl 3), S12–S13.

Kolibianakis EM, Albano C, Camus M, Tournaye H, Van Steirteghem AC and Devroey P (2004) Prolongation of the follicular phase in in vitro fertilization results in a lower ongoing pregnancy rate in cycles stimulated with recombinant follicle-stimulating hormone and gonadotropin-releasing hormone antagonists. Fertil Steril 82,102–107.

Meldrum D, Scott R, Levy MJ, Alper M and Noyes N (2002) A pilot study to assess oral contraceptive (OC) pretreatment in women undergoing controlled ovarian hyperstimulation (COH) in ganirelix acetate cycles. Fertil Steril 78(3 Suppl 1), S176, Abstract P-182.

Obruca A, Fischl F and Huber J (2001) Programming oocyte retrieval using oral contraceptive pretreatment before ovarian stimulation with a GnRH antagonist (Cetrotide) protocol. Abstract O-217. Hum Reprod 16(Suppl 1),89.

van Hooren HG, Fischl F, Aboulghar, Mares, Nicollet B, Behre HM, van D, V, Simon A, Kilani Z, Barri PN et al (2001) Comparable clinical outcome using the GnRH antagonist ganirelix or a long protocol of the GnRH agonist triptorelin for the prevention of premature LH surges in women undergoing ovarian stimulation. Hum Reprod 16,644–651.

Van Loenen ACD, Pirard C, Donnez J, Huirne AF, Schats R and Lambalk CB (2002) Recombinant follicle stimulating hormone (R-FSH) versus recombinant luteinizing hormone (R-LH) and R-FSH treatment in combination with cetrorelix after oral contraceptive programming in IVF/ICSI: a feasibility study. Fertil Steril 78(3 Suppl 1), S46, Abstract O-119.

WHO Scientific Group (1973) Agents stimulating gonadal function in human. World Health Organization, Geneva.