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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

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In this prospective and randomized study, 188 patients received the luteinizing hormone-releasing hormone (LHRH) antagonist cetrorelix, and 85 patients the LHRH agonist buserelin to prevent endogenous luteinizing hormone (LH) surges during ovarian stimulation in in-vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles. Ultimately, 181 patients (96.3%) in the cetrorelix group, and 77 (90.6%) in the buserelin group, reached the day of the human chorionic gonadotrophin (HCG) injection. The mean number of human menopausal gonadotrophin (HMG) ampoules administered and the mean number of stimulation days with HMG were significantly less in the cetrorelix group than in the buserelin group ($P < 0.01$). A rise in LH and progesterone concentrations was observed in three of the 188 patients (1.6%) who received cetrorelix. On the day of the HCG administration, more follicles of a small diameter (11–14 mm) were observed in the buserelin group than in the cetrorelix group ($P = 0.02$) and the mean serum oestradiol concentration was significantly higher in patients who received buserelin than in those who received cetrorelix ($P < 0.01$). Similar results were observed in fertilization, cleavage and pregnancy rates in the two groups. In conclusion, the use of the LHRH antagonists might be considered more advantageous because of the short-term application needed to inhibit gonadotrophin secretion, so allowing a reduction in the treatment time in a clinically significant manner.

Key words: human menopausal gonadotrophins/LHRH antagonist/cetrorelix/LHRH agonist, buserelin

Introduction

For 15 years, luteinizing hormone-releasing hormone (LHRH) agonists have been used in combination with exogenous gonadotrophins for ovarian stimulation (Porter *et al*., 1984; Shaw *et al*., 1985). These agents have certainly played an important role in the control of premature endogenous luteinizing hormone (LH) surge and in reducing the cycle cancellation rate, with a consequent improvement of pregnancy rate per cycle (MacLachlan *et al*., 1989). With the LHRH agonists used in the long protocol, however, pituitary desensitization is achieved only after 2 or 3 weeks of treatment, because of the initial stimulatory effect that may also lead to ovarian cyst formation (Feldberg *et al*., 1989; Ben-Rafael *et al.*, 1990). The use of LHRH antagonists overcomes these disadvantages, because they cause an immediate suppression of gonadotrophin secretion, without the initial stimulatory effect (Hall, 1993). The ability of new LHRH antagonists, cetrorelix (ASTA Medica AG, Frankfurt Main, Germany) and ganirelix (Organon, Oss, The Netherlands), to inhibit premature LH surges during ovarian stimulation has already been reported (Diedrich *et al*., 1994; Olivennes *et al*., 1994, 1995; Albano *et al*., 1996, 1997; The ganirelix dose-finding group, 1998). Recent dose-finding studies have defined the minimal effective dose of cetrorelix able to prevent premature endogenous LH surges during ovarian stimulation either as a single (Olivennes *et al*., 1998) or as a daily injection (Diedrich *et al*., 1994; Albano *et al*., 1997). The present prospective and randomized controlled study was designed to evaluate the efficacy and safety of the LHRH antagonist cetrorelix using the LHRH agonist buserelin (Suprecur®; Hoechst AG, Frankfurt, Germany) as a control group, in patients undergoing ovarian stimulation.

Materials and methods

In this phase III multicentre European prospective and randomized study, 198 patients were randomized in the cetrorelix group and 95 patients in the buserelin group after fulfilling the following inclusion criteria: age ≤39 years, regular menstrual cycle ranging from 24 to 35 days, normal ovarian function as detected by basal serum follicle stimulating hormone (FSH) concentration (FSH ≤10 IU/l), normal

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morphology of the ovaries and of the uterus as assessed by vaginal ultrasound, and no more than three previous in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) procedures. Randomization was performed using a 2:1 ratio (cetrorelix:buserelin) by a centralized telephone procedure, as soon as all relevant screening information was available.

The study was approved by the Ethical Committees of the seven European centres involved. All couples were required to sign a written informed consent. The trial was performed in accordance with the principles of the Declaration of Helsinki and the European Note for Guidance on Good Clinical Practice. Prior to the start of stimulation, 10 patients in each group withdrew from the study, leaving totals of 188 and 85 patients in the cetrorelix and buserelin groups respectively.

In the cetrorelix group, ovarian stimulation was carried out with human menopausal gonadotrophins (HMG, Humegon®; Organon, Oss, The Netherlands; Menogon®; Ferring, Kiel, Germany; Pergonal®; Serono, Geneva, Switzerland), starting with two ampoules (150 IU) on day 2 or 3 of the menstrual cycle for 5 days. The dosage was adjusted thereafter, according to the individual ovarian response to the stimulation, as assessed by oestradiol values and ultrasound measurements of the follicles. Cetrorelix 0.25 mg was administered s.c. daily, starting from day 6 of the HMG treatment, up to and including the day of human chorionic gonadotrophin (HCG) administration. In the buserelin group, patients received daily doses of 4×150 µg of buserelin administered intranasally, starting in the mid-luteal phase of the menstrual cycle preceding the ovarian stimulation cycle, for 2 or 3 weeks. When pituitary desensitization was achieved, ovarian stimulation was started with two ampoules of HMG as described in the cetrorelix group. Prerequisites to starting with HMG were: oestradiol ⩽50 pg/ml, progesterone ⩽1 ng/ml, FSH ⩽10 IU/l, LH ⩽10 IU/l, and no ovarian cyst with a diameter ⩾2 cm. Treatment with buserelin was continued up to and including the day of HCG administration. Final oocyte maturation was induced with 10 000 IU of HCG when at least one follicle with a mean diameter ⩾20 mm was observed and the serum oestradiol concentration was ⩾1200 pg/ml. To avoid the ovarian hyperstimulation syndrome (OHSS), HCG was not administered and the cycle was cancelled in case of the presence of more than 12 follicles with a mean diameter ⩾15 mm and/or an oestradiol concentration ⩾4000 pg/ml.

During the treatment, transvaginal ultrasound was performed on day 1 and on day 6 of the HMG treatment, optionally from day 6 of the HMG treatment onwards, and on the day of HCG administration in order to assess the follicular growth. Blood samples were taken for the measurement of FSH, LH, oestradiol and progesterone at each centre's laboratory, on the day of screening, on day 1 of HMG administration, and daily starting from day 6 of HMG administration. Furthermore, hormonal analysis was performed on the day of oocyte retrieval and embryo transfer, and on day 6 or 8 after embryo transfer. Additional serum samples were collected from each patient and frozen at –20°C to be analysed at the central clinical laboratory of ASTA Medica AG, Frankfurt, Germany.

Oocyte retrieval was performed by transvaginal needle-guided ultrasound aspiration 36 h after HCG injection. A maximum of three embryos was replaced into the uterine cavity 2 or 3 days after oocyte retrieval, and supernumerary embryos were cryopreserved for later use (Van Steirteghem *et al*., 1994). All patients received luteal phase support, either by HCG injection (if serum oestradiol concentrations were <2000 pg/ml) or by natural micronized progesterone given intravaginally according to the centres' rules. Clinical pregnancy was determined by ultrasound demonstration of a gestational sac and a fetus with cardiac activity.

Statistical methods

All statistical evaluations and analyses were performed using SAS 6.09 (SAS Institute Inc., SAS Campus Drive, Cary, NC, USA).

One-sided 95% lower confidence limits (CL) were calculated (Pearson-Clopper, 1985) for success rate (percentage of patients reaching the day of HCG). Mantel–Haenzel tests adjusted for centres were used for comparisons of rates except for OHSS, miscarriage, and ectopic pregnancy rates where, due to low incidence, no centre-adjusted analysis was performed but rather Fisher's exact test. For all other comparisons, the Wilcoxon rank test stratified by centre was used.

Results

All analyses were performed only on subjects randomized into the study and having received at least one dose of HMG and at least one dose of cetrorelix or buserelin, respectively.

A total of 188 patients, aged 31.9 ± 3.7 years (mean ± SD) and a total of 85 patients, aged 31.6 ± 3.8 years, were analysed in the cetrorelix and buserelin groups respectively. Ultimately, 181 patients in the cetrorelix group (96.3%; 95% CL: 93.1%) and 77 patients in the buserelin group (90.6%; 95% CL: 83.7%) reached the day of the HCG injection (primary end-point). In seven patients of the cetrorelix group, HCG was not administered because they had a poor ovarian response ($n = 3$), were at risk of ovarian hyperstimulation ($n = 3$), or had a premature LH rise (10.6 IU/l) with a concomitant progesterone rise (1.0 ng/ml) during ovarian stimulation ($n = 1$). In the buserelin group, HCG was not administered in eight patients because they had a poor ovarian response ($n = 3$) or were at risk of ovarian hyperstimulation ($n = 5$).

The stimulation outcome in the two groups of patients who reached the day of HCG is shown in Table I. The number of HMG ampoules administered was significantly less in the cetrorelix group than in the buserelin group, as was the duration of the ovarian stimulation with HMG ($P < 0.01$). On the day of the HCG administration, more follicles of a small diameter (11–14 mm) were observed in the buserelin group than in the cetrorelix group ($P = 0.02$) and the mean serum oestradiol concentration was significantly higher in patients who received buserelin than in those who received cetrorelix ($P < 0.01$).

The median serum LH concentrations during ovarian stimulation were higher, although not statistically different, in the cetrorelix than in the buserelin group, before the antagonist administration. However, serum LH concentrations became similar in the two groups after cetrorelix administration (Figure 1). In the cetrorelix group, one patient had an increase in serum LH concentration (10.6 IU/l) with a concomitant progesterone rise (1.4 ng/ml) before cetrorelix administration, on day 6 of the HMG treatment. However, decreases in LH (1.6 IU/l) and progesterone (0.97 ng/ml) concentrations were observed after the first cetrorelix injection. Eight cumulus–oocyte complexes (COC) were retrieved and six two pronuclear (2PN) oocytes were obtained in this patient. No pregnancy occurred after the replacement of two excellent embryos. Moreover, in six patients (3.2%) of the cetrorelix group, rises in serum LH concentration of 10, 16, 11, 11.7, 12.5 and 10.5 IU/l respectively, with no concomitant serum progesterone rise were observed before the first cetrorelix administration. A decrease in serum LH concentration however, was detected on the day after the first cetrorelix injection in all patients (4.1,

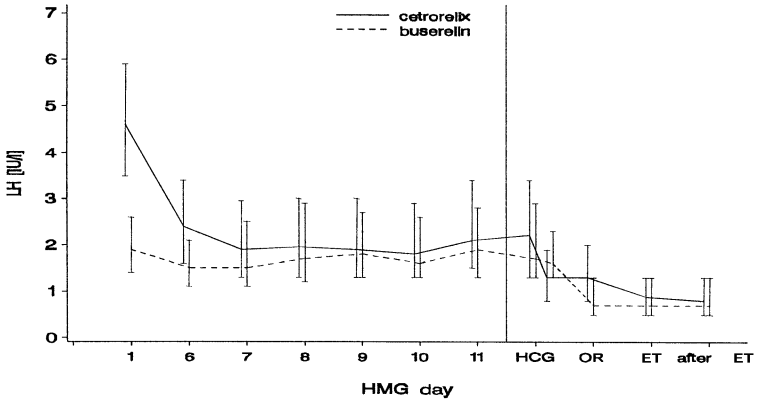

Figure 1. Median concentrations interquartile ranges of serum luteinizing hormone (LH) (IU/l) during the follicular and early luteal phase of ovarian stimulation cycles with the association of human menopausal gonadotrophin and cetrorelix 0.25 mg (—) or buserelin 600 µg (---). HCG = day of human chorionic gonadotrophin administration; HMG = human menopausal gonadotrophin; OR = oocyte retrieval; ET = embryo transfer.

Table I. Stimulation outcome[a] in patients treated with human menopausal gonadotrophin (HMG) in association with cetrorelix 0.25 mg or buserelin 600 µg

	Cetrorelix	Buserelin	*P*
Number of patients[b]	188	85	
Age (years)[b]	31.9 ± 3.7	31.6 ± 3.8	NS
Days of analogue treatment[b]	5.7 ± 2.3	26.6 ± 3.2	< 0.001
Number of patients who reached the day of HCG (%)	181 (96.3)	77 (90.6)	NS
Number of HMG ampoules[c]	23.6 ± 8.5	25.6 ± 7.6	< 0.01
Days of HMG treatment[c]	10.6 ± 2.3	11.4 ± 1.8	< 0.01
Number of follicles on the day of HCG injection[c]			
11–14 mm	3.2 ± 2.6	4.3 ± 3.3	0.02
15–19 mm	5.4 ± 3.3	6.2 ± 3.4	NS
⩾20 mm	1.9 ± 1.5	1.8 ± 1.7	NS
17β-oestradiol the day of HCG injection (pg/ml)[c]	1625 ± 836	2082 ± 1049	< 0.01

[a]Values are mean ± SD.
[b]Patients randomized and having received at least one dose of HMG and at least one dose of cetrorelix or buserelin.
[c]Analysis performed in patients who reached the day of HCG.
HCG = human chorionic gonadotrophin; NS = not significant.

2.1, 3.9, 6.0, 7.6 and 1.8 IU/l respectively). In all but one of these patients (who did not receive HCG and did not have oocyte retrieval because she was at risk of OHSS), eight, 11, 18, 19 and 14 COC were retrieved, and seven, one, 13, three and three embryos respectively were obtained. After the transfer of at least one good quality embryo in all these patients, two clinical pregnancies with the delivery of three healthy children occurred. Unexpectedly, in eight patients (4.3%) a premature LH rise with a concomitant progesterone rise was observed during the cetrorelix administration, according to the local laboratory results. In only three of these eight patients were serum LH and progesterone rises confirmed by the central laboratory results. In one of the eight patients, HCG was not administered; in seven patients HCG was administered and 3.9 ± 2.7 (mean ± SD) COC were retrieved. The mean (± SD) number of 2PN oocytes in these patients was 2.3 ± 1.1. At least one good quality embryo was transferred in five of the seven patients, but no pregnancy occurred. In two patients, embryo transfer was not performed. In the buserelin group, a rise in serum LH concentration (11 IU/l) was also observed in one patient during the ovarian stimulation (day 6 HMG) with no concomitant progesterone rise. However, the LH concentrations decreased to a median value of 3.4 IU/l in the following 3 days of the ovarian stimulation. In this patient, 13 oocytes were fertilized after the retrieval of 24 COC. A clinical pregnancy occurred after the transfer of two good quality embryos, with delivery of a healthy child.

Of the 181 patients receiving HCG in the cetrorelix group, 178 (98.3%) underwent oocyte retrieval. Three patients did not undergo oocyte retrieval because an impaired ovarian response was observed. In these three patients, intrauterine insemination was performed 36 h after HCG administration, as tubal patency was documented. A single pregnancy ensued in one of the three patients. In the buserelin group, all patients who received HCG underwent oocyte retrieval.

The mean number of COC and the mean number of 2PN oocytes were significantly lower in the cetrorelix than in the buserelin group ($P \leqslant 0.01$). However, the fertilization and cleavage rates were similar in the two groups (Table II). The results in terms of clinical outcome in the cetrorelix and in the buserelin groups are summarized in Table III. There were

Table II. Fertilization and cleavage outcome in the two groups of patients

	Cetrorelix	Buserelin	*P*
No. of started cycles	188	85	–
No. of patients with OR	178[a]	77	–
No. of patients with ⩾1 fertilized oocyte	166	72	–
No. of COC			
Total number	1398	816	
Mean number per patient[b,c]	8.0 ± 4.9	10.6 ± 6.6	< 0.01
No. of 2PN oocytes			
Total number (% of COC)	750 (53.6)	432 (52.9)	
Mean number per patient[b]	4.5 ± 3.3	6.0 ± 4.1	0.01
No. of cleaved embryos (% of 2PN)	671 (89.5)	345 (79.9)	
No. of excellent (% of all cleaved)	235 (35.0)	94 (27.2)	
No. of good (% of all cleaved)	321 (47.8)	154 (44.6)	
No. of fair (% of all cleaved)	115 (17.1)	97 (28.1)	
No. of embryo transfers (% started cycle)	157 (83.5)	67 (78.8)	NS
No. of embryos replaced			
Total number	343	147	
Mean number per patient[b]	2.2 ± 0.6	2.2 ± 0.6	NS
No. of frozen embryos/oocyte			
Total number	197	153	
Mean number per patient[b]	1.1 ± 2.3	2.0 ± 3.4	NS

[a]Oocytes were obtained in 175 patients.
[b]Values are mean ± SD.
[c]Mean number per patient with at least one fertilized oocyte.
COC = cumulus–oocyte complex; NS = not significant; OR = oocyte retrieval; 2PN = two pronuclear.

Table III. Clinical outcome in the two groups of patients

	Cetrorelix	Buserelin	*P*
No. of started cycles	188	88	
No. of patients with oocyte retrieval	178	77	
No. of embryo transfers (% started cycle)	157 (83.5)	67 (78.8)	NS
No. of clinical pregnancies (% started cycle)	42 (22.3)[a]	22 (25.9)	NS
No. of miscarriages	7	2	
No. of ectopic pregnancies	1	0	
No. of deliveries (% started cycle)	34 (18.1)	19 (22.4)	NS
Singletons	26[b]	17	
Twins	8	2	
No. of children born (% embryos replaced)	42 (12.2)	21 (14.3)	

[a]Including one patient who underwent intrauterine insemination because of a poor ovarian response.
[b]One stillbirth.
NS = not significant.

42 conception cycles in the cetrorelix group (22.3%), including seven miscarriages and one ectopic pregnancy, resulting in 34 deliveries (18.1%) and 42 children born. In the buserelin group, 22 conception cycles (25.9%) were obtained, including two miscarriages, resulting in 19 deliveries (22.4%) and 21 children born. The outcome of one clinical pregnancy is unknown. The life birth rate (number of children born per embryos replaced) was 12.2% in the cetrorelix group and 14.3% in the buserelin group. These data were not significantly different.

The incidence of OHSS grade II and III (WHO classification), was significantly higher in the buserelin group (5/77 patients = 6.5%) than in the cetrorelix group (2/181 patients = 1.1%) ($P = 0.03$).

Discussion

In this prospective and randomized study, the number of patients reaching the day of HCG in the cetrorelix and buserelin groups was similar (Table I). The mean number of treatment days with the LHRH antagonist cetrorelix was only 5.7, while that with the LHRH agonist buserelin was 26.6. This may be considered a clear benefit in terms of patients' comfort.

In previous non-controlled studies, an advantage in reducing the number of HMG ampoules in cycles stimulated with the association of gonadotrophins and LHRH antagonists has been postulated (Diedrich *et al*., 1994; Olivennes *et al*., 1994). However, in a phase II dose-finding study, where ovarian stimulation was carried out with HMG in combination with the LHRH antagonist cetrorelix 0.25 mg, a mean (± SD) of

33.4 ± 8.1 ampoules was used (Albano *et al*., 1997). It is well known that a long desensitization protocol using LHRH agonists requires a large number of HMG ampoules varying from 30 to ⩾40 (MacLachlan *et al*., 1989; Smitz *et al*., 1992). In the present study, significantly fewer ampoules of HMG were used in patients treated with cetrorelix than in those treated with buserelin (23.6 ± 8.5 versus 25.6 ± 7.6 respectively; $P < 0.01$). However, it must be noted that a small number of ampoules was used in both groups. This may be associated with the fact that a fixed dose of 2 ampoules of HMG was used for the first 5 days of treatment in both groups, and suggests that a 'softer' approach to ovarian stimulation may decrease the number of HMG ampoules used, even with a long desensitization protocol, at least using a nasal spray preparation. Similar results were observed in a large randomized dose-finding study where the LHRH antagonist ganirelix was used in association with recombinant FSH. In the 0.25 mg group, 22 ampoules of recombinant FSH were used for ovarian stimulation (The ganirelix dose-finding group, 1998).

Recently, cetrorelix has been used in natural cycles in patients undergoing IVF or ICSI procedure (Rongières-Bertrand *et al*., 1999). Cetrorelix (0.5 or 1 mg) was administered as a single injection when the serum oestradiol concentration was 100–150 pg/ml and a leading follicle of 12–14 mm was observed echographically. To avoid a reduction in serum oestradiol concentration after the antagonist administration, 150 IU of HMG were administered at the time of the first cetrorelix injection until the day of the HCG administration. The authors observed a very low cancellation rate (9%) compared with previous studies of natural cycles where 30% cancellation rate was reported. Moreover, a mean of 4.7 ± 1.4 HMG ampoules were used. Therefore, this treatment seems to be promising to simplify IVF/ICSI treatments, and to reduce the cost and the risk of OHSS.

The mean serum oestradiol concentration on the day of HCG injection was significantly higher in the buserelin group than in the cetrorelix group ($P < 0.01$), presumably due to the larger number of small follicles observed in the buserelin group ($P = 0.02$) (Table I). This finding may be the reason for the higher incidence of OHSS observed in the buserelin than in the cetrorelix group. A more profound pituitary suppression in the cetrorelix group than in the buserelin group may not be postulated as the serum LH concentration was similar in both groups (Figure 1). In a controlled study which was conducted to compare the efficacy of the LHRH agonist leuprolide acetate with that of the LHRH antagonist Nal-Glu in suppressing LH secretion during ovarian stimulation in IVF, a higher serum oestradiol concentration was also observed in the LHRH agonist group (Minaretzis *et al*., 1995).

Earlier studies have shown that the treatment with LHRH agonists reduces the incidence of endogenous LH surges to <2% (Wildt *et al*., 1986; Loumaye, 1990). In a previous dose-finding study, 0.25 mg of cetrorelix was shown to be the minimal effective dose able to prevent LH surges during ovarian stimulation (Albano *et al*., 1997). Unexpectedly, in the present study, eight patients (4.3%) in the cetrorelix group had an increase in serum LH concentration with a concomitant progesterone rise before HCG administration, according to the local laboratory's values. However, in only three patients (1.6%) were these results confirmed by the central laboratory. In one of the eight patients, one injection of cetrorelix was missed during ovarian stimulation. No pregnancies occurred in these eight patients. There is some evidence that high LH concentrations during the follicular phase of ovarian stimulation cycles have a negative impact on fertilization and implantation rates (Stanger and Yovich, 1985; Howles *et al*., 1987). In the present study, six patients in the cetrorelix group (3.2%) had a rise in serum LH concentration with no concomitant progesterone rise before the first cetrorelix administration. In these patients the antagonist was able to induce a decrease in LH levels after its injection. Embryo transfer was performed in all patients but one, and two clinical pregnancies occurred. This might suggest the irrelevance of a transient rise in LH on the quality and/or the maturity of the oocytes and on the IVF/ICSI outcome

Although the mean number of COC and 2PN was significantly lower in the cetrorelix group than in the buserelin group, the mean number of embryos available for transfer and for freezing was similar in both groups. This outcome is associated with the similar results obtained in terms of fertilization and cleavage rate in the two groups.

An apparent difference in pregnancy and delivery rate between the buserelin and cetrorelix groups was not statistically significant. Moreover, the percentage of babies born was similar in the two groups. Further investigations are necessary to confirm whether or not there is a significant difference in pregnancy rates.

In conclusion, the use of the LHRH antagonists may be considered more advantageous because of the short-term application required to inhibit gonadotrophin secretion and so allow a reduction in the treatment time in a clinically significant manner. Furthermore, the risk of OHSS appears to be reduced after the use of the LHRH antagonist cetrorelix, and this may be associated with the short treatment period with HMG.

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