Hormonal profile during the follicular phase in cycles stimulated with a combination of human menopausal gonadotrophin and gonadotrophin-releasing hormone antagonist (Cetrorelix)


1Centre for Reproductive Medicine, University Hospital and Medical School, Dutch-speaking Brussels Free University, Laarbeeklaan 101, 1090 Brussels, Belgium, 2ASTA Medica AG, Frankfurt Main, Germany and 3Department of Obstetrics and Gynecology of the Medical University of Lübeck, Germany

To whom correspondence should be addressed

A third-generation gonadotrophin-releasing hormone antagonist (Cetrorelix) was used during ovarian stimulation in 32 patients undergoing assisted reproduction, in order to prevent the premature luteinizing hormone (LH) surge. In all patients, ovarian stimulation was carried out with two or three ampoules of human menopausal gonadotrophin (HMG), starting on day 2 of the menstrual cycle. In addition, 0.5 mg of Cetrorelix was administered daily from day 6 of HMG treatment until the day of ovulation induction by human chorionic gonadotrophin (HCG). A significant drop in plasma LH concentration was observed within a few hours of the first administration of Cetrorelix (P < 0.005). Moreover, no LH surge was detected at any point in the treatment period in any of the 32 patients. A mean oestradiol concentration of 2122 ± 935 ng/l was observed on the day of the HCG administration, indicating normal folliculogenesis. Like LH, progesterone concentration also dropped within a few hours of the first administration of Cetrorelix (P < 0.005). A 0.5 mg daily dose of Cetrorelix prevented a premature LH surge in all the 32 patients treated.

Key words: GnRH antagonist (Cetrorelix)/in-vitro fertilization/ LH surge/ovarian stimulation

Introduction

Gonadotrophin-releasing hormone agonists have played an important role in assisted reproductive techniques. The reversible suppression of pituitary function has improved the efficacy of gonadotrophin therapy primarily by avoiding the premature LH surge that can be deleterious for normal follicle and oocyte development (Loumaye, 1990; Liu et al., 1992; Smitz et al., 1992). The agonists, after an initial stimulatory effect (flare-up), induce desensitization of the gonadotrophic cells by reducing the number of luteinizing hormone-releasing hormone (LHRH) receptors on the cell membrane (down-regulation) and by the inhibition of second messenger pathways. Depending on the dose and frequency of agonist administration, 2-3 weeks are needed to reach a complete desensitization, and a high dose of gonadotrophin is necessary for ovarian stimulation.

With the introduction of GnRH antagonists, new perspectives have been opened for ovarian stimulation in in-vitro fertilization (IVF) programmes. The competitive blockade of the LHRH receptors leads to an immediate arrest of gonadotrophin secretion, and so the duration of the treatment can be reduced, as well as the number of ampoules of human menopausal gonadotrophin (HMG) used (Diedrich et al., 1994; Olivennes et al., 1994, 1995). Different procedures for antagonist administration have been used in order to suppress the LH surge during ovarian stimulation. Olivennes et al. (1994) used a single administration of GnRH antagonist (Cetrorelix, 5 mg) when plasma oestradiol concentration was between 150 and 200 pg/ml per follicle ≥14 mm diameter. A second injection was performed 2 days later if human chorionic gonadotrophin (HCG) had not been administered in the meantime. A similar protocol was used by Frydman et al. (1991) but with Nal-Glu antagonist. The antagonist was administered when the serum oestradiol concentration was >600 pg/ml and a second injection was repeated 2 days later. A daily administration of 3 or 1 mg as well as 0.5 mg of Cetrorelix, starting on day 7 of ovarian stimulation and continuing until the day of the HCG injection, was used by Diedrich et al. (1994) and Felberbaum et al. (1995a,b). In our study, Cetrorelix was administered daily, at a dose of 0.5 mg, starting on day 6 of HMG treatment. This was the first part of a dose-finding study to evaluate the minimum effective dose of Cetrorelix. The aim of the study was to analyse the concentrations of LH, follicle stimulating hormone (FSH), oestradiol and progesterone during ovarian stimulation with HMG in association with Cetrorelix.

Materials and methods

Subjects

A total of 32 healthy infertile patients between 23 and 39 years of age (32.1 ± 3.1 years, mean ± SD) were included in this study. IVF was performed in four patients, while 28 patients underwent intracytoplasmic sperm injection (ICSI). All patients had regular menstrual cycles ranging in length from 24 to 35 days. They had undergone no more than three previous IVF procedures. The study was approved by the ethical committee of the Brussels Free University, Belgium. All couples were required to sign a written informed consent.

Study design

Before the inclusion of a patient in the study, a general physical examination, gynaecological examination and haematological, biochemical and urine analyses were performed. Hormonal evaluation of FSH, LH, oestradiol and progesterone was performed in the early
fOLLICULAR phase of the menstrual cycle in order to evaluate the functioning of the ovaries. An ultrasound examination was also performed to investigate any abnormalities of the ovaries or of the uterus. Ovarian stimulation was carried out with HMG (Humegon from Organon, Oss, The Netherlands, Menegon from Ferrong, Kiel, Germany and Pergonal from Serono, Genève, Switzerland) 14 patients started with two ampoules (150 IU) and 18 patients with three ampoules (225 IU) per day from day 2 of the menstrual cycle. From day 6 of HMG treatment (day 7 of the menstrual cycle), the dose of HMG was adjusted according to the individual ovarian response to the stimulation, by assessment of oestradiol values and ultrasound measurement of follicles. From day 6 onwards (day 7 of the menstrual cycle), 0.5 mg of Cetrorelix (ASTA Medica AG, Frankfurt Main, Germany) was also administered s.c. in the anterior abdominal wall daily up to and including the last day of HMG administration. Both HMG and Cetrorelix were administered simultaneously in the morning. Ovulation was induced with 10 000 IU of HCG (Pregnyl) when at least three follicles with a mean diameter of ≥17 mm were observed. Oocyte retrieval was performed by transvaginal needle-guided ultrasound aspiration 36 h after HCG injection. Classification of cumulus–oocyte complexes and embryos was performed as previously described (Staessen et al., 1989). A maximum of three embryos was replaced into the uterus 2 days after oocyte retrieval and supernumerary embryos were cryopreserved. Since no evidence for disturbance of the luteal phase has been detected with the use of the antagonists (Ditkoff et al., 1991), no luteal phase support was given to the first six patients. All these patients, however, showed bleeding in the midluteal phase and therefore all subsequent subjects received luteal phase support by means of l.m. administration of HCG (1500 IU every 4 days if oestradiol <2000 ng/l).

Ultrasonography and hormone assays
During the treatment, transvaginal ultrasound was performed daily from the first day of Cetrorelix injection (day 6 of HMG treatment) in order to detect the growth of follicles. Serum FSH, LH, oestradiol and progesterone concentrations were measured once daily during HMG treatment and twice daily during Cetrorelix administration (just before the antagonist and the HMG injections in the morning, and in the afternoon between 1400 and 1700 h), using radioimmunoassays as previously described by Smitz et al. (1988).

Statistical analysis
Statistical analysis for comparison of means was performed using one-way analysis of variance or Student’s t-test. Statistical significance was defined as P < 0.05.

Results
Stimulation outcome and preliminary results of IVF and ICSI
In 32 patients ovarian stimulation was carried out by administration of either two ampoules (150 IU) or three ampoules (225 IU) of HMG (14 and 18 patients respectively) starting on day 2 of the menstrual cycle. Cetrorelix was administered at a daily dose of 0.5 mg from day 7 of the menstrual cycle (day 6 of HMG treatment) until ovulation was induced. On the day of Cetrorelix administration, no statistical difference was observed between the groups receiving two or three ampoules of HMG as regards serum LH (4.3 ± 2.9 and 7.4 ± 8.0 IU/l) and oestradiol concentrations (483.5 ± 408.5 and 592.0 ± 386.8 ng/l respectively). In four patients who started with three ampoules of HMG, an increase of LH was observed before Cetrorelix administration. So far, a high dose of HMG has not appeared to postpone or block the increase of serum LH. A median of 33 ampoules was needed per patient, although a large individual variation was noted within the group (16–70 ampoules). The median number of stimulation days was 11 (range 9–18), with a median of 5 stimulation days with Cetrorelix (range 2–11). On the day of HCG injection the mean (± SD) oestradiol concentration was 2122 ± 935 ng/l (conversion factor to SI unit 3.671) and the mean number of follicles ≥17 mm in diameter was 6.9 ± 3.0, indicating normal folliculogenesis. A total of 344 cumulus–oocyte complexes was retrieved after follicular aspiration, a mean of 12.4 ± 7.2 per patient. Fertilization rates were 55.7 and 72.2% in IVF and ICSI cycles respectively.

Hormonal profiles during ovarian stimulation
Luteinizing hormone
In all patients, plasma LH concentration showed a significant decrease within only 9 h of the first dose of Cetrorelix administration, from 6.1 ± 6.4 IU/l (mean ± SD) before the first injection (day 7, morning sample) to 2 ± 1.2 IU/l a few hours after the injection (day 7, afternoon sample) (P < 0.005). Plasma LH remained constantly low during the next 5 days of observation (Figure 1). In five patients a high LH concentration was observed on day 7 in the morning (19.4 ± 6.3 IU/l). However, there was no concomitant progesterone rise (progesterone <1 μg/l). In these patients a drastic decrease of LH concentration (3.6 ± 1.4 IU/l) was also observed on the day of the first Cetrorelix injection (P < 0.05).

Follicle stimulating hormone
No evidence of FSH decrease was seen after the first administration of Cetrorelix, but an increasing concentration of FSH was observed consistently 9 h after each administration of HMG and Cetrorelix (Figure 1).

17β-Oestradiol
Figure 1 shows a regularly increasing concentration of oestradiol during the ovarian stimulation with HMG and Cetrorelix. A plateau in plasma oestradiol concentration (544 ± 393 ng/l on the morning sample versus 548 ± 407 ng/l on the afternoon sample; conversion factor to SI unit 3.671) was observed after the first Cetrorelix administration (day 7, evening). Increase of oestradiol concentration was regular until the day of HCG administration (Figure 2).

Progesterone
Like LH, plasma progesterone concentration also dropped significantly within 9 h of the first administration of Cetrorelix, from 0.27 ± 0.2 μg/l (conversion factor to SI unit 3.180) before the injection (day 7, morning) to 0.12 ± 0.09 μg/l after the injection (day 7, evening) (P < 0.005; Figure 1).

Discussion
For the last decade, GnRH agonists have been routinely used in ovarian stimulation in order to provide a more comfortable monitoring of follicular growth and to prevent a spontaneous LH surge (Wildt et al., 1986; Neveu et al., 1987). However, because of the initial stimulatory phase (flare-up effect), a
Figure 1. Mean concentration and SD of FSH, LH, oestradiol and progesterone (µg/l) during ovarian stimulation with human menopausal gonadotrophin in combination with Cetrorelix.
*Denotes a significant decrease of LH and progesterone, observed after the first injection of 0.5 mg of Cetrorelix (P < 0.005).

activity has become available (Rivier et al., 1986; Muller et al., 1994; Reissmann et al., 1995). GnRH antagonists bind competitively to the gonadotrophic cell receptors, preventing the stimulatory effect of endogenous LHRH on the pituitary cells. This mechanism of action has the advantage of producing no flare-up effect, so that the initial stimulatory phase can be avoided and the duration of the treatment can be reduced, as reported by some authors (Diedrich et al., 1994; Olivennes et al., 1994, 1995). In contrast with these studies, we have not, so far, observed a reduction in the numbers of HMG ampoules used. A median of 33 ampoules was needed per patient. However, a large individual variation was noted within the group (16–70 ampoules), associated with a poor ovarian response in some patients. Large control studies are necessary in order to define the exact number of ampoules needed. In natural cycles, a single administration of the antagonist during the late follicular phase delayed the LH surge (Bouchard et al., 1990; Ditkoff et al., 1991; Leroy et al., 1994). Previous studies have also shown the efficacy of GnRH antagonists in eliminating the risk of endogenous LH surges in ovarian stimulation cycles (Frydman et al., 1991; Cassidenti et al., 1992; Diedrich et al., 1994; Olivennes et al., 1994, 1995). In our study, a significant drop of LH concentration was observed within 9 h after the first Cetrorelix injection, and this remained consistently low until HCG administration (Figure 1). Furthermore, five patients revealed a rise in LH concentration (>10 IU/l) before the Cetrorelix administration without a concomitant progesterone rise. In this group of patients oestradiol concentration was 888.8 ± 352.6 ng/l before the Cetrorelix administration, while in patients who did not have an increase of LH, the oestradiol concentration was lower, although not significantly (480.8 ± 372.4 ng/l). A significant drop in the LH concentration was also detected in the same five patients after the antagonist administration. The increase in LH concentration may be related to the high oestradiol concentration as a consequence of HMG given.

In contrast with other studies, where plasma FSH concentration slightly decreased simultaneously with LH concentration (Diedrich et al., 1994; Olivennes et al., 1994), our study did not reveal a decrease in FSH after the administration of the antagonist (Figure 1). This may be associated with the lower dose of antagonist used, or possibly, to the influence of the...
exogenous FSH used. Recently, Olivennes et al. (1995), using a lower dose of the antagonist (3 mg), observed no decrease in FSH concentration after the injection of the antagonist, in contrast with their previous study in which they used 5 mg (Olivennes et al., 1995). In our study, the FSH concentration increased after the HMG administration, coinciding with the blood samples taken in the afternoon. Indeed, the blood sample obtained in the morning before the HMG injection showed a decrease in FSH concentration which was probably due to the plasma half-life of exogenous FSH.

In a study conducted by Olivennes et al. (1994), a drop in oestradiol concentration was observed after the Cetrorelix injection (5 mg), probably owing to the decrease in LH-controlled androgen production. However, in a more recent study, the same group (Olivennes et al., 1995) did not observe a decrease in oestradiol secretion when the dose of HMG was increased on the day of the antagonist administration. Moreover, a lower dose of the antagonist was used in the same study (3 mg), so that the absence of oestradiol decrease could be attributed either to the reduction in the dose of the antagonist or to the increased number of ampoules of HMG administered (two versus three ampoules). In our study we observed a plateau of oestradiol concentration only after the first Cetrorelix injection (Figure 1). Nevertheless, adequate follicular development was noticed, indicated by a constant increase in the oestradiol concentration until the day of the HCG administration, as shown in Figure 2. With regard to progesterone secretion, a significant decrease was observed after the first Cetrorelix injection. However, the progesterone concentration increased gradually during the late follicular phase, probably owing to an increased LH-receptor sensitivity related to elevated oestradiol concentration (Figure 1).

It is an ongoing debate whether daily administration of the antagonist is preferable to a single or dual injection (Olivennes et al., 1994, 1995). From a practical viewpoint, the daily administration is more convenient, since there is no risk of missing an endogenous LH surge and also there is no need to measure the LH plasma concentration each day. Since in daily injection a total dose of 2.5 mg was given over 5 days (0.5 mg per day), approximately the same amount was administered as in the single-dose administration (3 mg). Prevention of LH surge was the primary end-point in this study and it was successfully achieved. The efficacy results show that Cetrorelix, in combination with HMG, stimulates normal multiple follicular development, as demonstrated by the number of pre-ovulatory follicles, the increase in oestradiol secretion and the numbers of oocytes recovered. However, the number of HMG ampoules used was not reduced in this study. This probably reflects a poor ovarian response from some patients, as indicated by the large inter-individual variation.

A prospective study comparing patients treated with HMG in association with Cetrorelix and HMG alone is necessary to assess the efficacy of Cetrorelix in preventing the LH surge. However, the results of a recent randomized assessor-blind study conducted in our centre, comparing the effect of two different gonadotrophins without the association of GnRH analogues in ovarian stimulation cycles, suggest it is difficult to prevent an LH surge. In 36% of the cycles, a premature rise of LH and/or progesterone was observed. These cycles were not cancelled; however, the incidence of fertilization failure was relatively high (23%), indicating compromised oocyte quality (Devroey et al., 1995). The difficult interpretation of increased serum LH concentrations in the late follicular phase indicates the need for assessment of the use of GnRH antagonists.

In conclusion, daily administration of 0.5 mg of Cetrorelix prevented the occurrence of an endogenous LH surge in all the patients treated. This indicates that 0.5 mg of Cetrorelix, administered daily, is adequate for ovarian stimulation.

Acknowledgements

The authors wish to thank the clinical, paramedical and laboratory staff of the Centre for Reproductive Medicine, Brussels, Belgium. Furthermore we are very grateful to the study coordinators Mrs Andrea De Brahant and Mrs Marleen Magnus and also to Mrs Pascale Haegeman and Mrs Anne Prothmann. The authors also thank M.R. Winter of the Languages Education Center for correcting the manuscript. Grants from the Belgium Foundation for Medical Research are kindly acknowledged.

References


C. Albano et al.


Received on March 18, 1996, accepted on July 10, 1996