Hormone Profiles Under Ovarian Stimulation with Human Menopausal Gonadotropin (hMG) and Concomitant Administration of the Gonadotropin Releasing Hormone (GnRH)-Antagonist Cetrorelix at Different Dosages*

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Submitted: June 5, 1995
Accepted: August 29, 1995

Purpose: *The premature LH surge in ART programs seems to be avoided by daily administration of the GnRH-antagonist Cetrorelix during the midcycle phase in controlled ovarian hyperstimulation with hMG. The dosage necessary for sufficient suppression of the pituitary gland is not yet defined.*
Methods: *To elucidate this question three daily dosages (3, 1, 0.5 mg) were administered and the hormone profiles obtained as well as the number of oocytes retrieved, the fertilization rate, and the consumption of HMG were compared.*
Results: *No premature LH surge could be observed at any of the three dosages administered. Both gonadotropins were deeply suppressed. The fertilization rates of the oocytes obtained were 45.3% in the 3-mg group, 53.1% in the 1-mg group, and 67.7% in the 0.5-mg group. The average uses of hMG ampoules were 30 in the 3-mg group, 27 in the 1-mg group, and 26 in the 0.5-mg group.*
Conclusions: *Cetrolix, 0.5 mg/day, administered during the midcycle phase of controlled ovarian hyperstimulation with hMG is enough to prevent completely the premature LH surge. Perhaps even lower dosages would be sufficient. Regarding fertilization rates and use of hMG, the lower dosage seems to be the most favorable.*

KEY WORDS: gonadotropin releasing hormone antagonists; premature luteinizing hormone surge; controlled ovarian hyperstimulation.

*Presented in part at the IXth World Congress on In Vitro Fertilization and Alternate Assisted Reproduction, April 3–7, 1995, Vienna, Austria.

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INTRODUCTION

GnRH, pulsatile-secreted by the mediobasal part of the hypothalamus, plays a pivotal role in control of the ovarian cycle of the adult woman due to its regulatory function on the hypothalamopituitary gonadal axis. After it was isolated and successfully analyzed in 1971 by Schally and Guillemin, it was possible by modification of the molecular structure of this decapeptide to obtain analogue compounds with agonistic and antagonistic effects (1,2). The agonists, after an initial stimulatory effect, the so-called "flare-up," lead to desensitization of the gonadotrophic cells, which seems to be the result of a combination of receptor number down-regulation and uncoupling of GnRH receptors from intracellular effectors and inhibition of gonadotropin biosynthesis (3). In part, also postreceptor mechanisms may be involved whereby increased levels of immunoreactive but biologically inactive LH are secreted (4,5). We call this the "down-regulation" of the pituitary gland (6,7). The antagonists instead produce an immediate effect by competitive blockage of the GnRH receptors (8,9). Without any intrinsic activity of these compounds, the flare-up is completely avoided. The antagonists block the receptors and inhibit their microaggregation and the postreceptor mechanisms are not induced. Within hours the secretion of the gonadotrophic hormones comes down (9). In 1991 Ditkoff *et al.* demonstrated that short-term application of the antagonist Nal–Glu in the midcycle phase of healthy women with normal cycles was able to prevent the midcycle LH peak and, by this, the spontanous ovulation. They applied 50 μg of Nal–Glu per kg body weight per day for 4 days. No LH surge

1058-0468/96/0300-0216$09.50/0

took place and estradiol concentrations as well as follicular growth remained arrested (10). The occurrence of premature LH surges is a main reason for the relatively low efficacy of ovarian stimulation with hMG only in IVF programs. In addition, these LH surges have a negative impact on the quality of the oocytes and embryos and, subsequently, on the rate of pregnancy (11,12). By introducing the GnRH agonists into the stimulation protocols of assisted reproduction technique programs (ART programs), improved synchronization of follicular maturation and an important reduction of premature luteinization, to lower than 2%, was achieved (13). The premature LH surge seems to be avoided as well by daily administration of Cetrorelix from day 7 onward until ovulation induction, what we call the "Lübeck protocol" (Fig. 1), as by single or dual administration around day 9, as published by Olivennes *et al.* (15). In this protocol the antagonist is injected at the time when estradiol reaches 150–200 pg/ml and the follicle size is > 14 mm, which usually is the case on day 9 of the cycle (14,15). Hence the dosage necessary for sufficient suppression of the pituitary gland at this critical moment of controlled ovarian hyperstimulation is not yet defined, as clinical experience with this relatively new compound is not widely spread. To elucidate this question in two subsequent open Phase II studies applying the Lübeck protocol, three dosages (3, 1, and 0.5 mg) were administered and the hormone profiles obtained as well as the number of oocytes retrieved, the fertilization rates, and the consumption of hMG were compared.

MATERIALS AND METHODS

After giving formal consent, 35 patients, all suffering from tubal infertility, were enrolled according to the following inclusion criteria: infertility caused by tubal functional disturbance, patient age between 18 and 37 years, no more than two previous inductions of ovulation, a regular menstrual cycle between 24 and 35 days, a normal uterus and functional ovaries, good general health conditions, body weight between ±/−25% of the Broca Index, and no male infertility or endocrine abnormalities observed. Starting on cycle day 2 they were treated with 150 IU follicle stimulating hormone (FSH) and 150 IU luteinizing hormone (LH) per day (Pergonal; Serono, Unterschleißheim, Germany). From cycle day 7 until induction of ovulation, 12 patients were treated with 3 mg Cetrorelix s.c./day (mean age: 32.3 ± 2.5). As no premature LH surge could be observed, 12 patients received 1 mg Cetrorelix/day (mean age: 32 ± 2.5), and another 11 patients 0.5 mg Cetrorelix/day (mean age: 30.8 ± 2.6). On day 5 the dose of hMG was adjusted to the individual ovarian response of the patient to the stimulation as assessed by estradiol values and measurement of follicles. This treatment was continued until induction

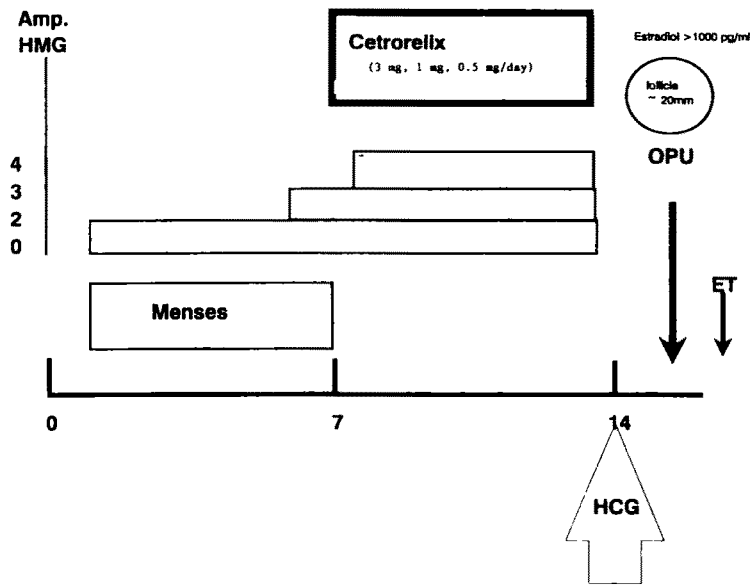

Fig. 1. Ovarian stimulation with hMG and concomitant administration of GnRH antagonist (Cetrorelix); the "Lübeck protocol."

of ovulation with 10,000 IU hCG i.m. (Predalon; Organon, Oss, Holland), given when the leading follicle reached a diameter of 18–20 mm, measured by transvaginal ultrasound, and when estradiol values indicated a satisfactory follicular response. During the treatment cycles blood samples were drawn daily for measurements of estradiol, progesterone, LH, and FSH. Transvaginal oocyte retrieval was performed 36 hr after the HCG injection. IVF and embryo transfer were carried out as described previously (16). For luteal support we administered 5000 IU of hCG i.m. on days 2 and 5 after follicular puncture. During the treatment cycles blood samples were drawn daily for measurements of estradiol, progesterone, LH, and FSH performed by enzyme immunoassays.

RESULTS

No premature LH surge was observed. All cycles could be evaluated. Only one ovulation induction had to be canceled due to an extremely low response in the 0.5-mg group. Nevertheless, the hormone profiles of this patient were included in the statistical analysis. The mean courses in the three dosage groups of FSH and LH were quite similar, with a profound suppression of both gonadotropins (Figs. 2 and 3) In the case of LH, concentrations below 2 mIU/ml were achieved at cycle day 12. In the case of estradiol there was a distinctly higher increase in concentration in the group treated with 0.5 mg Cetrorelix/day, reaching an average maximum of 2164.91 + 2102.93 pg/ml on cycle day 10, compared to 852.25 + 325.19 pg/ml in the 3-mg group and 1022.5 + 602.86 pg/ml in the group treated with 1 mg Cetrorelix per day. (Fig. 4, Table I). Progesterone levels in the luteal phase were suprisingly lower with a lower dose of antagonists than in the 3-mg group, reaching a serum concentration at cycle day 20 of 209.41 + 76.34 ng/ml in the 3-mg group and 129.08 + 70.59 and 134 + 97.23 ng/ml in the 0.5-mg group (Fig. 5). The fertilization rates of the recovered oocytes were 45.3% in the 3-mg group, 53.2% in the 1-mg group, and 67.7% in the 0.5-mg group. In the 3-mg group 106 oocytes were recovered and 30 embryos were obtained, 36.7% of them being excellent according to morphological microscopic criteria (17). In the 1-mg group 94 oocytes were collected and 28 embryos were obtained, 53.6% being excellent. In the 0.5-mg group 127 oocytes were recovered and 27 embryos were obtained, 37% of them being excellent. Regarding the cleavage rate there are no data available, as we are forced by the German "Embryonenschutzgesetz" to decide at the pronucleus stage which embryos will be transfered and which will not (18) (Table II).

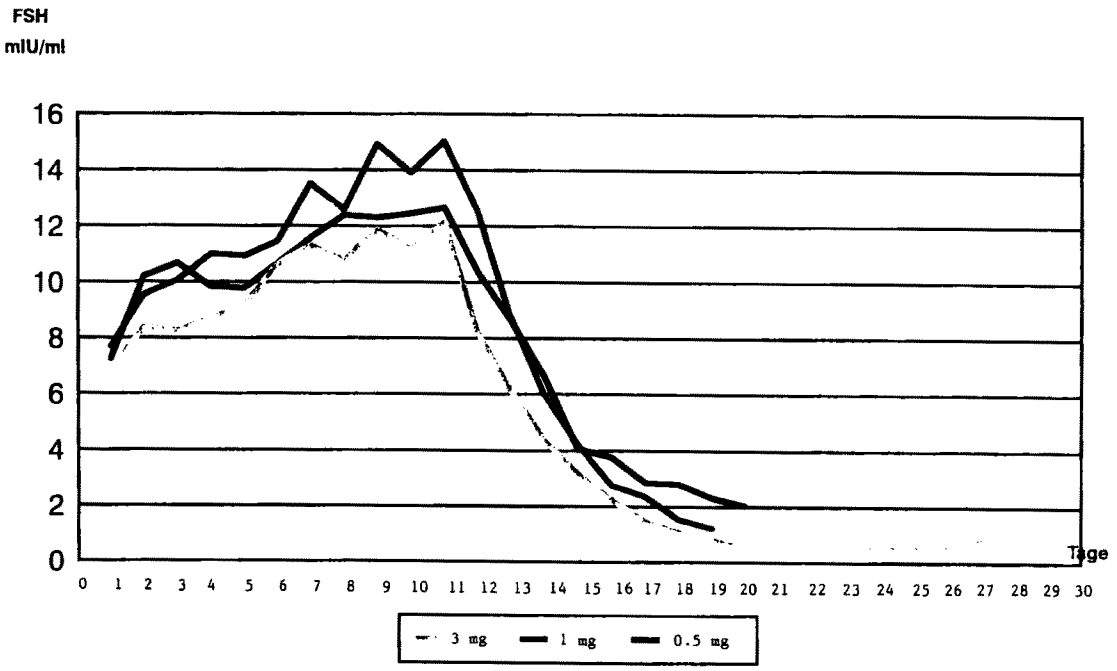

Fig. 2. Ovarian stimulation with hMG and concomitant administration of Cetrorelix at different dosages (3, 1, 0.5 mg/day); mean courses of FSH concentrations (mIU/ml).

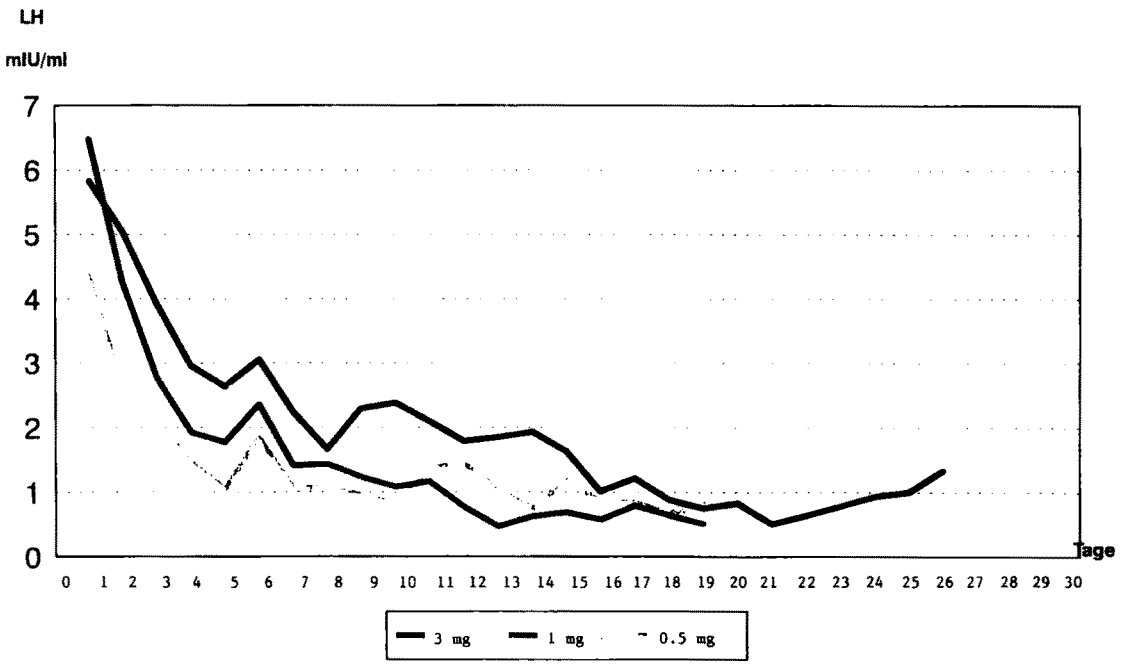

Fig. 3. Ovarian stimulation with hMG and concomitant administration of Cetrorelix at different dosages (3, 1, 0.5 mg/day); mean courses of LH concentrations (mIU/ml).

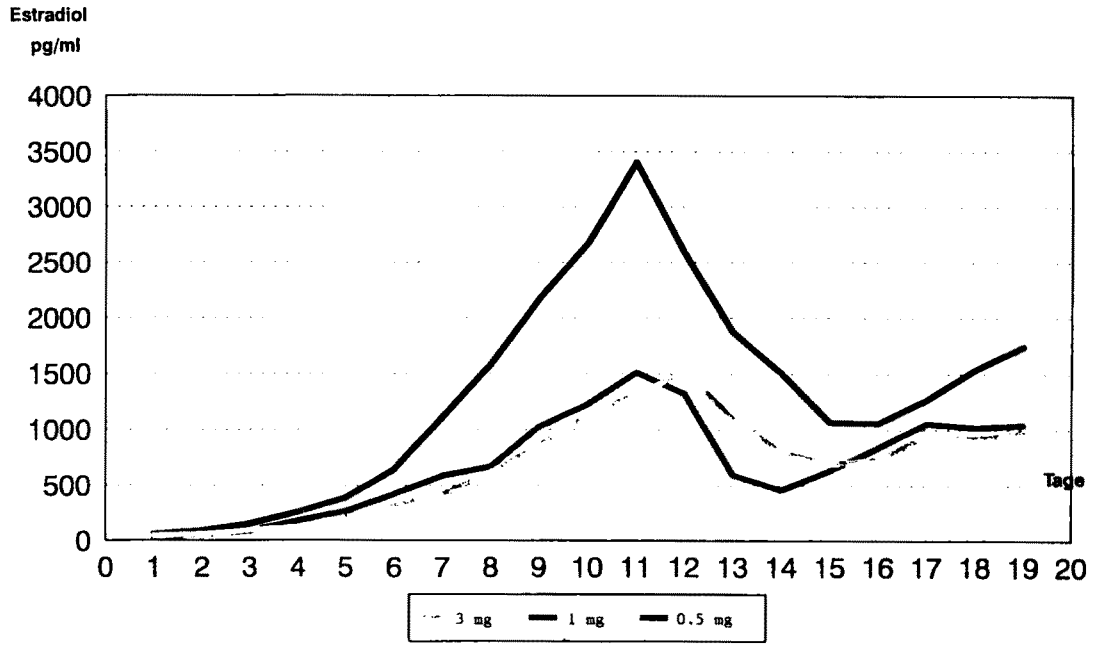

Fig. 4. Ovarian stimulation with hMG and concomitant administration of Cetrorelix at different dosages (3, 1, 0.5 mg/day); mean courses of estradiol concentrations (pg/ml).

Table I. Ovarian Stimulation with hMG and Cetrorelix (3, 1, or 0.5 mg)[a]

Estradiol level		
3 mg	1 mg	0.5 mg
852.25 ± 325.19	1022.5 ± 602.86	2164.91 ± 2102.93

[a] Ovarian stimulation with hMG and concomitant administration of Cetrorelix at different dosages (3, 1, 0.5 mg/day); concentrations of estradiol (pg/ml) at cycle day 10 (mean values and SE).

Table II. Ovarian Stimulation with hMG and Cetrorelix (3, 1, or 0.5 mg)[a]

	3 mg	1 mg	0.5 mg
No. of oocytes	106	94	127
No. of oocytes per retrieval	8.83	7.83	12.7
Fertilization rate	45.3%	53.2%	67.7%
No. of embryos	30	28	27
Excellent embryos	36.7%	53.6%	37%

[a] Number of oocytes retrieved, fertilization rates, and number and quality of embryos replaced in ovarian stimulation with hMG and concomitant administration of Cetrorelix at different dosages (3, 1, 0.5 mg/day).

The average use of HMG ampoules was 30 in the 3-mg group 27 in the 1-mg group, and 26 in the 0.5-mg group.

DISCUSSION

The premature LH surge can be prevented by 3 mg Cetrorelix per day as well as 1 and as 0.5 mg per day. Probably the amount of Cetrorelix applied could be reduced even further. Lower dosages still have to be tested and will probably have implications for the necessity of luteal support. Until now, to the best of our knowledge, nobody knows if luteal support is really necessary and beneficial under GnRH antagonist treatment. After ceasing antagonist treatment with Nal–Glu and after spontanous ovulation has taken place in women with normal cycles, the luteal phase was normal (10). Having translated the experiences of the agonist protocols to our Cetrorelix protocol, we also performed luteal-phase support by the administration of 5000 IU hCG i.m. on days 2 and 5 after oocyte retrieval (19). Due to this, interpretation of the lower progesterone levels observed in the luteal phase under lower dosages of antagonists is difficult. Probably the effect of exogenous hCG is deminished because of less interference with presumptive paracrine regulatory systems. Regarding the average maximum of estradiol levels at cycle day 10 in the three dosage groups,

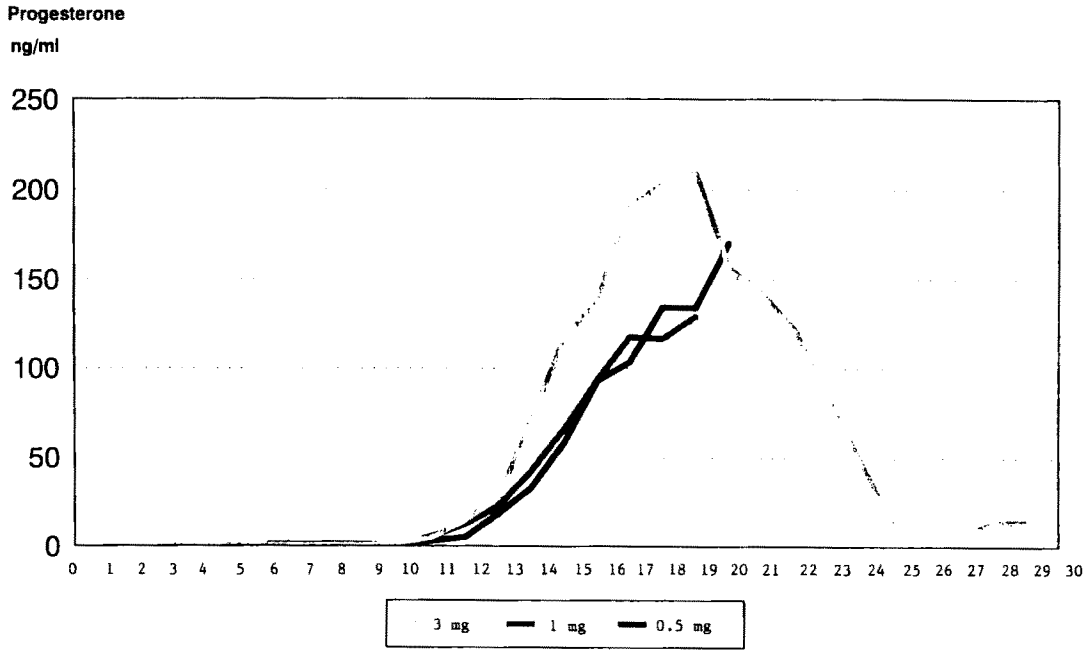

Fig. 5. Ovarian stimulation with hMG and concomitant administration of Cetrorelix at different dosages (3, 1, 0.5 mg/day); mean courses of progesterone concentrations (ng/ml).

the SEM is quite high and thus the interpretation of observed differences is difficult, but the $P = 0.07$ on the Wilcoxon rank test in a matched-pair comparison of the concentration increase "*A*" (estradiol level = *A* × day) between cycle day 7 and cycle day 11 in the groups treated with 0.5 and 1.0 mg/day of Cetrorelix shows a clear trend to difference, while the same test performed on those patients treated with 1.0 and 3.0 mg/day of Cetrorelix, with $P = 0.650$, does not. This analysis is retrospective and performed "a posteriori," thus its results cannot be taken for granted. For the moment it is just a clinical observation. Nevertheless, the ovarian response at the lowest dosage seems to be slightly more sensitive than in patients treated with higher dosages of cetrorelix. But actually there is no striking explanation for this observation, as the gonadotropin courses are not concordant. This question must be elucidated in further prospective studies. Probably paracrine and intraovarian mechanisms as have been described for agonistic compounds should be looked at properly (20,21). For the GnRH antagonist Nal–Glu a direct effect on the granulosa cell of the follicle has been discussed (22). Regarding the number of cumulus–corona complexes obtained, fertilization rates and use of hMG, the lower dosage seems to be the most favorable, again indicating an inverse relationship of both level of ovarian response and fertilization with the antagonist dose. The differences in the average amount of hMG ampoules used is not significant but have to be compared with the average amount needed in the long agonistic protocol, which is about 40 ampoules per cycle (19). Analyzing the data as estradiol response per ampoule hMG administered, the relation in the group treated with 3 mg Cetrorelix/day was 28.4 ± 15.7 pg/ml/amp, that in the group treated with 1 mg Cetrorelix/day 34.6 ± 23.7 pg/ml/amp, and that in the group treated with only 0.5-mg Cetrorelix/day 71.7 ± 80.7 pg/ml/amp, again reflecting the above-mentioned inverse relationship between ovarian response and antagonist dose.

CONCLUSIONS

Cetrorelix, 0.5 mg/day, administered in accordance with the described "Lübeck protocol" is enough to prevent completely the premature LH surge during controlled ovarian hyperstimulation (COH). Nevertheless, the minimal effective dosage of Cetrorelix per day remains undefined. Perhaps even 0.25 or 0.10 mg/day would be enough. Not to be taken for granted and estimated as a simple clinical observance, the ovarian response at this lower dosage to stimulation with hMG seems to be slightly more sensitive than in patients treated with higher dosages of antagonists. Further clinical studies are required, using the mentioned even lower dosage of 0.25 or 0.10 mg/day for elucidation of this question.

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