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CLINICAL ARTICLE

Effect of a lower-dose cetorelix acetate protocol on in-vitro fertilization outcome

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Luteinizing hormone
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Lower dose

Abstract

Objective: To determine whether a low initial dosage of cetorelix acetate could prevent a premature luteinizing hormone (LH) surge in women undergoing controlled ovarian stimulation. **Method:** Treatment with a recombinant follicle stimulating hormone was started on Day 3 of the menstrual cycle, and 0.125 mg of cetorelix was injected daily from Day 5 of the ovarian stimulation until the diameter of the dominant follicle reached at least 16 mm. The dosage was then doubled and maintained at 0.250 mg/day until the day before the injection of human chorionic gonadotropin. **Result:** There was a significant decrease in serum LH concentration 1 day after doubling the cetorelix dosage, and the LH concentration remained low during the follicular phase. Clinical pregnancy occurred in 18 women (42.8%), with 2 intrauterine fetal deaths before the 12th week. **Conclusion:** Increasing the cetorelix dosage from 0.125 to 0.250 mg/day when the follicular size is appropriate can prevent a premature LH surge.

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1. Introduction

The administration of gonadotrophin-releasing hormone (GnRH) antagonists was introduced to prevent a premature

rise of luteinizing hormone (LH) during ovarian stimulation for in-vitro fertilization (IVF) [1,2]. The suggestion in 1994 by Diedrich et al. [1] that multiple daily injections of cetorelix acetate, a GnRH antagonist, might be effective was later supported by the findings of a phase 3 clinical trial [2] of the most commonly used dosage, 0.250 mg/day. This trial reported a low, 0.9% incidence of premature LH surges.

Lower pregnancy rates have been reported following GnRH antagonist-stimulated cycles than following the long

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GnRH agonist protocol [3], however, and increasing the GnRH-antagonist dosage was shown to be associated with lower implantation and fertilization rates [4,5]. Therefore, using a lower dose of a GnRH antagonist in IVF cycles may enhance reproductive outcome.

Deep suppression of LH during controlled ovarian stimulation is associated with adverse reproductive outcome [6,7], and women with a relatively slower follicular maturation may be especially affected by a premature administration of a GnRH antagonist [8]. Thus, if we are to opt for the least harmful effects on reproductive outcome, and therefore take into account individual response to ovarian stimulation, it could be of value to develop a minimum-dose GnRH-antagonist protocol based on follicular size.

We designed a novel GnRH-antagonist protocol starting with 0.125 mg/day of cetrorelix, and increasing the dosage to 0.250 mg/day to prevent an LH surge when the diameter of the dominant follicle reached a minimum of 16 mm. The present study investigated whether this low initial dosage could prevent an LH surge while providing good reproductive outcome.

2. Materials and methods

This nonrandomized observational study was carried out with 42 women to investigate the efficacy of a new GnRH-antagonist protocol for the prevention of a premature LH surge in women undergoing IVF cycles. It was approved by the Ethics Committee of Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan, and all couples signed informed consent or study participation forms.

The participants were aged between 24 and 37 years (mean $\pm$ SD, 31.8 ± 3.4 years). They had a mean body weight of 54.7 ± 7.1 kg; a mean body mass index (calculated as the weight in kilograms divided by height in meters squared) of 21.6 ± 2.8 ; and a mean baseline follicle stimulating hormone level (FSH) of 6.3 ± 1.2 mIU/mL.

The cause of infertility pertained to the man in 12 cases (28.6%); it was due to a tubal factor in 11 cases (26.2%), to endometriosis in 6 cases (14.3%), and remained unexplained in 13 cases (30.9%).

The exclusion criteria were age older than 38 years, Day 3 FSH level higher than 15 mIU/mL, irregular menstrual cycle, and more than 3 past attempts at IVF. An ultrasound evaluation excluded the presence of an ovarian cyst greater than 12 mm.

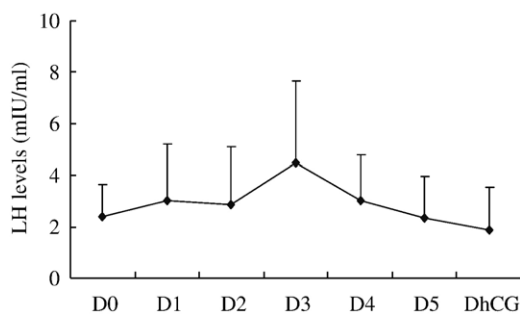


Figure 1 Mean serum LH concentrations during controlled ovarian stimulation. Values are presented relative to the first day (D0) of cetrorelix acetate administration.

Table 1 Serum concentrations of luteinizing hormone (LH) and dosage of cetrorelix acetate in 42 women treated according to a novel, 2-dosage protocol

Day of measurement of LH level and dose of cetrorelix received on that day	Serum LH level, mIU/mL
1 day before treatment initiation with 0.125 mg/d	2.37 ± 1.2
1st day of treatment with 0.125 mg/d	3.03 ± 2.2
Last day of treatment with 0.125 mg/d	3.51 ± 2.7^a
First day of treatment with 0.250 mg/d	2.51 ± 2.3^a

Values are given as mean $\pm$ SD; they were normally distributed.

^a The difference between these values were significant ($P < 0.05$ by the paired *t* test).

Controlled ovarian stimulation was started on Day 3 of the menstrual cycle, approximately between 6:00 and 8:00 PM, with 225 IU/day of recombinant FSH (Gona-F; Serono, Geneva, Switzerland) for 4 days, and the dosage was then adjusted according to follicular response. On the evening of Day 5 of gonadotropin stimulation, treatment with cetrorelix (Cetrotide; Serono) was initiated with a subcutaneous injection of 0.125 mg, and this daily dose was increased to 0.250 mg when the dominant follicle size reached at least 16 mm. This dosage was maintained until the day before ovulation was induced with the administration of 10,000 IU of human chorionic gonadotropin (hCG) (Pregnyl; NY Organon, Oss, The Netherlands). Ovulation was induced when at least 2 follicles had reached 18 mm and serum estradiol (E_2) levels were found to be appropriate. Oocyte retrieval was performed 36 h after hCG administration and IVF or intracytoplasmic sperm injection was then performed, followed by embryo transfer on the second or third day. The luteal phase was supported by 600 mg of vaginally administered micronized progesterone (Utrogestan; Piette International S. A., Brussels, Belgium). An LH surge was defined as a serum LH level of 10 mIU/mL or higher, with a progesterone level of 1.0 ng/mL or higher.

Table 2 Characteristics of 3 patients with high serum LH concentration before the dominant follicle reached 16 mm

Characteristic	Patient 1	Patient 2	Patient 3
LH concentration, mIU/mL			
Day of LH rise	11.3	10.9	13
1 day after first 0.25-mg cetrorelix injection	9.5	6.8	8.2
Day of hCG administration	3.9	1.8	5.2
P ₄ level on day of LH rise, ng/mL	1.3	0.6	0.6
Size of leading follicle on day of LH rise, mm	13	15	14.5
No. of oocytes retrieved	11	6	9
Outcome	No pregnancy	No pregnancy	Singleton pregnancy

Abbreviations: hCG, human chorionic gonadotropin; LH, luteinizing hormone; P₄, progesterone 4.

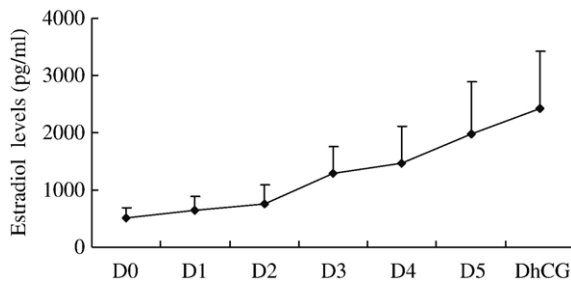


Figure 2 Serum estradiol values relative to the first day (D0) of cetrorelix administration. DhCG indicates the day human chorionic gonadotropin was administered.

An LH level of 10 mIU/mL or higher without an increase in the progesterone level was defined as an LH elevation [9].

Transvaginal ultrasound was performed daily from the first day of the cetrorelix treatment. Serum concentrations of LH, E_2 , and progesterone (P_4) were measured on the morning of Day 5 of gonadotropin stimulation until the day of hCG administration. Serum levels of LH, E_2 , and P_4 were measured using Immulite kits (Diagnostic Products Corp., Los Angeles, California, USA). The sensitivity of the immunoassay for LH was 0.1 mIU/mL. The sensitivity of the competitive immunoassay used to measure estradiol and progesterone levels was 15 pg/mL for E_2 and 0.2 ng/mL for P_4 .

Values are expressed as mean $\pm$ SD. The paired t test was used for statistical analysis where appropriate. Statistical analyses were performed using the SPSS statistical package for Windows, version 10.0 (SPSS Inc., Chicago, Illinois, USA). $P < 0.05$ was considered statistically significant.

3. Results

A total of 42 women underwent oocyte retrieval and embryo transfer. The daily injection of 0.125 mg of cetrorelix, started on the evening of Day 5 of gonadotropin stimulation and increased to 0.250 mg when the dominant follicle diameter was at least 16 mm, effectively suppressed the LH surge until the injection of hCG. Serum LH concentration was constantly low during the follicular phase (Fig. 1). The difference in serum LH levels was significant for those measured on the last day of the half dose (0.125 mg) of cetrorelix and the first day of the full dose (0.250 mg); the difference, however, was not

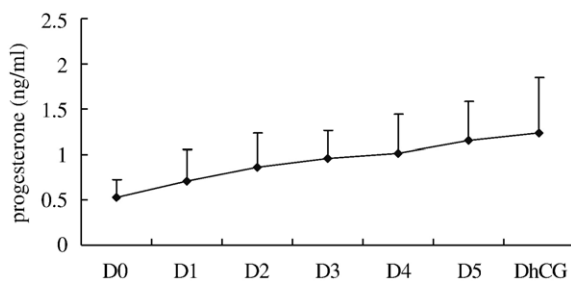


Figure 3 Serum progesterone values relative to the first day (D0) of cetrorelix administration. DhCG indicates the day human chorionic gonadotropin was administered.

Table 3 Characteristics of the in-vitro fertilization cycle

Characteristic	Values
No. of ampoules of rFSH administered	25.0 $\pm$ 4.1
No. of patients undergoing oocyte retrieval	42
No. of patients undergoing embryo transfer	42
Days with cetrorelix acetate	5.5 $\pm$ 1.3
No. of treatment days with 0.125 mg of cetrorelix	3.6 $\pm$ 1.3
No. of treatment days with 0.250 mg of cetrorelix	1.8 $\pm$ 0.9
Total dose of cetrorelix, mg	0.9 $\pm$ 0.2
No. of days of ovarian stimulation	9.4 $\pm$ 1.3
No. of oocytes retrieved	13.4 $\pm$ 7.0
No. of oocytes with 2 pronuclei	11.2 $\pm$ 6.6
No. of clinical pregnancies	18 (42.8%)
No. of ongoing pregnancies	16 (38.1%)

Abbreviation: rFSH, recombinant follicle stimulating hormone. Values are given as number, number (percentage), or mean $\pm$ SD.

significant between levels measured on the eve of the first administration of the half dose and the day of the first administration of the half dose (Table 1). Therefore, a half dose of cetrorelix prevented an LH surge but it did not suppress LH production, which a full dose would not have done.

In 3 patients, the cetrorelix dosage was increased to 0.250 mg/day before the dominant follicle reached 16 mm because their serum LH concentration was 10 mIU/mL or higher (Table 2). Two of these patients had a LH elevation and the remaining one had an LH surge. The LH levels decreased immediately after the first 0.250-mg injection of cetrorelix in all 3 patients, one of whom became pregnant.

Serum E_2 levels steadily increased during the cetrorelix treatment (Fig. 2), and there was a trend toward a P_4 elevation near the day of the hCG injection (Fig. 3). Table 3 summarizes the clinical results, with the amount of gonadotropin used, the duration of the cetrorelix treatment, the total dose of cetrorelix administered, the number of oocytes retrieved, the number of oocytes with 2 pronuclei, and the clinical and ongoing pregnancy rates. There were 18 clinical pregnancies (42.8%) among the 42 study participants, but 2 intrauterine fetal deaths occurred before the 12th week of pregnancy.

4. Discussion

Gonadotrophin-releasing hormone antagonists immediately and reversibly block the pituitary receptors of GnRH, thereby preventing a premature LH surge and making a short and simple protocol possible for IVF cycles [1]. However, most controlled studies using GnRH antagonists have reported a slight but significant trend toward lower pregnancy rates compared with the long GnRH agonist protocol [3]. This difference, it has been suggested, may be due to a deleterious effect of GnRH antagonists on oocyte quality, embryo development, or the endometrium [10]. In this light, lowering the dosage of the GnRH antagonist would improve outcome in pregnancies in IVF cycles.

Previous studies demonstrated that a fixed scheme of 0.20 mg/day of cetrorelix was effective in preventing a premature LH surge regardless of follicular size [11,12]. The present study obtained LH surge suppression using a novel GnRH-antagonist protocol with a lower dose of cetrorelix (0.125 mg) starting on the evening of Day 5 of ovarian stimulation, and increased to 0.250 mg once an appropriate follicular size was reached. In addition, the mean amount of cetrorelix administered per patient in this study, 0.9 mg, was less than the 1.0 mg used in one of the previous trials of 0.20 mg in a fixed scheme [11]. To the best of our knowledge, the treatment protocol in this study used the lowest dose of GnRH antagonist reported in clinical trials.

In the present study, 3 patients had an increase in LH concentrations before the dominant follicle reached 16 mm, the diameter needed to increase cetrorelix dosage according to the protocol. Because of the potentially detrimental effect of high LH levels [13], the dosage of cetrorelix was increased to 0.250 mg per day before the leading follicle reached 16 mm. The LH concentrations decreased immediately in all 3 patients, and one of them became pregnant. Because response to ovarian stimulation varies, especially among women with polycystic ovarian syndrome, this novel GnRH-antagonist regimen could be valuable.

In conclusion, this study showed that a premature LH surge can be prevented with a daily cetrorelix acetate dose of 0.125 mg initially, followed by an increase to 0.250 mg when the dominant follicle size reaches at least 16 mm. Although the overall GnRH dose is dependent on follicular size and therefore varies with individual need, the overall amount of injected cetrorelix was lower in this study than in a similar one, and a lower amount might reduce the potentially adverse effect of the GnRH antagonist on pregnancy rates following IVF. Large prospective randomized controlled studies are needed to compare ovarian stimulation and pregnancy outcome using our novel protocol and a fixed, 0.250-mg regimen of cetrorelix.

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References

- [1] Diedrich K, Diedrich C, Santos E, Zoll C, al-Hasani S, Reissmann T, et al. Suppression of the endogenous luteinizing hormone surge by the gonadotrophin-releasing hormone antagonist Cetrorelix during ovarian stimulation. *Hum Reprod* 1994;9:788–91.
- [2] Felberbaum RE, Albano C, Ludwig M, Riethmuller-Winzen H, Grigat M, Devroey P, et al. Ovarian stimulation for assisted reproduction with HMG and concomitant midcycle administration of the GnRH antagonist cetrorelix according to the multiple dose protocol: a prospective uncontrolled phase III study. *Hum Reprod* 2000;15:1015–20.
- [3] Al-Inany HG, Abou-Setta AM, Aboulghar M. Gonadotrophin-releasing hormone antagonists for assisted conception. *Cochrane Database Syst Rev* 2006;19:3CD001750.
- [4] Ganirelix Dose-Finding Study Group. A double-blind, randomized, dose-finding study to assess the efficacy of the GnRH antagonist Ganirelix (Org 37462) to prevent premature luteinizing hormone surges in women undergoing controlled ovarian hyperstimulation with recombinant follicle stimulating hormone. *Hum Reprod* 1998;13:3023–31.
- [5] Felberbaum R, Reissmann T, Kupker W, Al-Hasani S, Bauer O, Schill T, et al. Hormone profiles under ovarian stimulation with human menopausal gonadotropin (hMG) and concomitant administration of the gonadotropin releasing hormone (GnRH)-antagonist Cetrorelix at different dosages. *J Assist Reprod Genet* 1996;13:216–22.
- [6] Coppola F, Poti ER, Barusi L, Ferrari B, Salvarani MC, Vadora E. Profound luteinizing hormone suppression induces a deleterious follicular environment during assisted reproduction technology. *Fertil Steril* 2003;79:459–60.
- [7] Esposito MA, Barnhart KT, Coutifaris C, Patrizio P. Role of periovulatory luteinizing hormone concentrations during assisted reproductive technology cycles stimulated exclusively with recombinant follicle-stimulating hormone. *Fertil Steril* 2001;75:519–24.
- [8] Escudero E, Bosch E, Crespo J, Simon C, Remohi J, Pellicer A. Comparison of two different starting multiple dose gonadotropin-releasing hormone antagonist protocols in a selected group of in vitro fertilization-embryo transfer patients. *Fertil Steril* 2004;81:562–6.
- [9] Olivennes F, Belaisch-Allart J, Emperaire JC, Dechaud H, Alvarez S, Moreau L, et al. Prospective, randomized, controlled study of in vitro fertilization-embryo transfer with a single dose of a luteinizing hormone-releasing hormone (LH-RH) antagonist (cetrorelix) or a depot formula of an LH-RH agonist (triptorelin). *Fertil Steril* 2000;73:314–20.
- [10] Hernandez ER. Embryo implantation and GnRH antagonists: embryo implantation: the Rubicon for GnRH antagonists. *Hum Reprod* 2000;15:1211–6.
- [11] Chen HJ, Lin YH, Hsieh BC, Seow KM, Hwang JL, Tzeng CR. Is a lower dose of cetrorelix acetate effective for prevention of LH surge during controlled ovarian hyperstimulation? *J Assist Reprod Genet* 2006;23:289–92.
- [12] Chang YL, Hsieh YY, Tsai HD. Preliminary report on the effect of a lower dose of gonadotropin-releasing hormone antagonist (cetrorelix) on ovarian hyperstimulation in lower-weight Asian women. *Taiwan J Obstet Gynecol* 2006;45:317–20.
- [13] Kolibianakis EM, Albano C, Kahn J, Camus M, Tournaye H, Van Steirteghem AC, et al. Exposure to high levels of luteinizing hormone and estradiol in the early follicular phase of gonadotropin-releasing hormone antagonist cycles is associated with a reduced chance of pregnancy. *Fertil Steril* 2003;79:873–80.