

# COMPARISONS OF DIFFERENT DOSAGES OF GONADOTROPIN-RELEASING HORMONE (GnRH) ANTAGONIST, SHORT-ACTING FORM AND SINGLE, HALF-DOSE, LONG-ACTING FORM OF GnRH AGONIST DURING CONTROLLED OVARIAN HYPERSTIMULATION AND *IN VITRO* FERTILIZATION

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## SUMMARY

**Objective:** Both gonadotropin-releasing hormone (GnRH) analogs and antagonists have been used for pituitary desensitization during controlled ovarian hyperstimulation (COH). We aimed to determine the minimum effective daily dose of GnRH antagonist in women undergoing COH. We also compared the efficiency of a GnRH antagonist and a GnRH agonist.

**Materials and Methods:** Women undergoing *in vitro* fertilization/intracytoplasmic sperm injection and embryo transfer were divided into five groups: (1) cetrorelix 0.25 mg ( $n=86$ ); (2) cetrorelix 0.2 mg ( $n=28$ ); (3) cetrorelix 0.15 mg ( $n=30$ ); (4) leuprolide acetate (LA) 0.5 mg/day ( $n=58$ ); (5) single half-dose LA depot 1.88 mg ( $n=49$ ). Cetrorelix was administered daily from menstrual day 8 until the day of human chorionic gonadotropin administration. LA or LA depot was started on day 21 of the previous menstrual cycle.

**Results:** We observed lower gonadotropin (Gn) dosages, estradiol (E2) levels and reduced risk of ovarian hyperstimulation syndrome (OHSS) in the GnRH antagonist groups. A higher risk of luteinizing hormone (LH) surge was noted in cetrorelix 0.2 and 0.15 mg groups. Gn dosages (IU)/E2 levels (pg/mL) in each group were: (1) 1,949.4/1,191.1; (2) 1,869.6/1,010.8; (3) 1,856.7/1,023.6; (4) 2,184.5/1,323.6; and (5) 2,103.5/1,313.5, respectively. LH/OHSS risks were: (1) 3.5%/5.8%; (2) 7.1%/3.6%; (3) 13.3%/3.3%; (4) 3.4%/8.6%; and (5) 2%/8.2%, respectively. Number of oocytes/embryos/grade I, II embryos were: (1) 9.4/7.9/5.8; (2) 7.5/4.2/3.6; (3) 6.3/4.1/3.1; (4) 12.3/8.9/6.6; and (5) 11.8/8.4/6.1, respectively. There was no significant difference in terms of clinical outcomes between groups 1, 4 and 5, except for higher abortion rates (AR) in group 1. Pregnancy rate (PR)/implantation rate (IR) ratios in groups 1, 4, and 5 were statistically higher than those in groups 2 and 3. Chemical PR/IR/AR were: (1) 30.2%/5.9%/7%; (2) 21.4%/5.1%/7.1%; (3) 16.7%/4.1%/10%; (4) 32.8%/5.5%/8.6%; and (5) 30.6%/5.7%/8.2%, respectively.

**Conclusion:** The lowest effective dosage of cetrorelix for pituitary desensitization during COH luteolysis is 0.25 mg, resulting in a comparable PR but a higher AR when compared with GnRH agonist. [*Taiwan J Obstet Gynecol* 2008; 47(1):66-74]

**Key Words:** cetrorelix, GnRH agonist, GnRH antagonist, leuprolide acetate, pituitary suppression



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## Introduction

During the past few decades, gonadotropin-releasing hormone (GnRH) analogs (GnRHa) have been widely used for pituitary desensitization during controlled

ovarian hyperstimulation (COH). The “long protocol” with GnRHa is generally accepted for pituitary suppression, owing to its numerous advantages, including a reduction in cancellation rates, the avoidance of premature luteinization and endogenous luteinizing hormone (LH) surges, an increase in multiple follicular growth [1] and potentially better timing of the treatment cycle [2]. Traditionally, the short-acting GnRHa, leuprolide acetate (LA), is widely used for pituitary suppression. LA has been employed because of concerns over long-acting depot preparations causing profound suppression and luteal phase defects, which adversely affect pregnancy and miscarriage rates [3]. However, the main advantage of the long-acting GnRHa, LA depot, is its greater convenience, and its use is less stressful and more acceptable than that of short-acting LA protocols. In a previous report, we showed that single half-dose LA depot offered a useful alternative for pituitary suppression in ovarian stimulation during *in vitro* fertilization (IVF) [4].

GnRH antagonists (GnRH-ant) have been used to prevent the onset of premature LH surges during COH. The major disadvantages of GnRHa are increased gonadotropin (Gn) dosage after prolonged pituitary suppression and a higher risk of ovarian hyperstimulation syndrome (OHSS) [5]. GnRH-ant allow a short and simple treatment regimen for IVF patients. GnRH-ant act by competition with native GnRH for GnRH receptor-binding sites, and result in rapid suppression of Gn. Other advantages of GnRH-ant included the reduction of Gn dosage during COH programs and a lower risk of OHSS [6]. GnRH-ant greatly reduce the duration of pituitary downregulation and prevent adverse events related to flare-up induced by GnRHa.

The development of third- and fourth-generation GnRH-ant has produced favorable clinical results. Cetrorelix (ASTA-Medica, Frankfurt/Main, Germany) or ganirelix (Organon, Oss, The Netherlands) have been used in recent clinical studies [7,8]. Daily administration of GnRH-ant (so-called multiple dose protocol) at its minimum effective dose (0.25 mg/day subcutaneously) has been proven to be safe and effective [9,10]. Clinical studies using cetrorelix started off with relatively high daily dosages of 3 mg and 1 mg [7,8], but the lowest effective daily dose of cetrorelix appeared to be 0.25 mg [11].

In reviewing the MEDLINE database, few studies report trials of GnRH-ant in Asians. Hwang et al [12] demonstrated that the cetrorelix-COH protocol had a similar pregnancy rate (PR) as the GnRHa “long protocol” for women with polycystic ovary syndrome undergoing IVF treatment. Recently, Lee et al [13] demonstrated that both multiple and single dosage GnRH-ant protocols were effective for preventing the LH surge, and

resulted in similar PR compared with LA GnRHa. They also demonstrated that single dosage protocol required further modification to produce favorable folliculogenesis. However, no literature has dealt with the use of GnRH-ant doses of 0.2 mg or less in pituitary suppression of thinner individuals. In general, Asian women are thinner than Caucasian women. Given the racial and ethnic differences, it is logical to suspect that Asians and Caucasians might have different effective GnRH-ant dosages.

To select a more efficient protocol for GnRH-ant for IVF patients, we designed this randomized study to evaluate the follicular development and pregnancy outcome using different dosage protocols for cetrorelix, LA and LA depot, as a GnRHa. In this larger series, we aimed to determine the minimum safe and effective dose of GnRH-ant for pituitary suppression in thin Asian women and evaluated the effects of the different agents on pituitary downregulation. Furthermore, we also compared the clinical differences between GnRH-ant, LA, and LA depot. Only a few studies [13–15] have compared GnRH-ant and LA/LA depot. To our knowledge, this is the largest survey and the first comparison of these protocols in the Asian population.

## Materials and Methods

All patients who received COH, IVF/intracytoplasmic sperm injection (ICSI) and transvaginal embryo transfer (TV-ET) were reviewed. This trial was a phase III, open-label, randomized study to assess the efficacy and safety of GnRH-ant in women undergoing COH. The main inclusion criteria were: age at least 18 years but not older than 39 years; and body weight of 40–70 kg. Approval from the institutional review board was obtained for the analysis of this series.

The patients were divided into five groups: (1) cetrorelix 0.25 mg/day ( $n=86$ ); (2) cetrorelix 0.2 mg/day ( $n=28$ ); (3) cetrorelix 0.15 mg/day ( $n=30$ ); (4) LA 0.5 mg/day ( $n=58$ ); (5) LA depot 1.88 mg ( $n=49$ ). Cetrorelix was administered from menstrual day 8 until the day of human chorionic gonadotropin (hCG) administration. Single dose LA depot (1.88 mg, single subcutaneous dosage; Takeda Chemical Industries Ltd, Japan) or daily LA (0.5 mg/day subcutaneously; Abbott Laboratories, Chicago, IL, USA) were administered on days 21–23 of the previous menstrual cycle.

The COH protocol was as previously described [4]. In brief, during menstrual days 2–7, younger patients (<34 years) in the GnRH-ant/GnRHa groups were administered 150–225 IU/day of recombinant follicle-stimulating hormone (FSH) (Gonal-F; Serono, Rome,

Italy). Older patients ( $\geq 34$  years) in the GnRH-ant/GnRHa groups were administered 225–300 IU/day of Gonal-F. Ultrasound examinations were performed on menstrual days 3, 6, 9, and 12. If the estradiol (E2) level on day 8 was  $< 100$  pg/mL, the daily dose of Gn was increased to 225 IU of Gonal-F in younger patients and to 300 IU of Gonal-F in the older patients. Criteria for cancellation included lower E2 level on menstrual day 8 ( $< 50$  pg/mL) and poor follicle growth during COH (no follicle growth  $> 8$  mm).

The Gn cetrorelix or LA administration continued until two or more follicles of  $\geq 18$  mm were detected; then, hCG (5,000 IU; Serono, Rome, Italy) was administered. Serum LH and E2 concentrations were tested on the day of hCG administration. Oocytes were retrieved transvaginally 34–36 hours later. Oocyte culture, insemination, embryo transfer (ET) and cryopreservation were as previously described [4]. ET was performed 72 hours after oocyte retrieval. A maximum of six embryos were transferred in each patient. Luteal phase was supported with hCG (2,000 IU/day; Serono, Rome, Italy) on days 1, 4 and 7 post-ET and progesterone (400 mg/day; Utrogeston) from day 1 post-ET. Chemical pregnancy was defined as elevated serum  $\beta$ -hCG (above 50 IU/L) 14 days after ET. Clinical pregnancy was determined by visualization of a gestational sac, and fetal viability by ultrasound 4 weeks post-ET.

Personal data (age, body weight, body mass index, cause of infertility), Gn dosage, and serum concentration of LH and E2 on the day of hCG administration were compared between the five groups. Serum E2, LH and hCG levels were measured by means of immunoassay (Immulite 2000; DPC, Flanders, NJ, USA). Retrieved oocyte and embryo numbers, development of OHSS,

embryo quality, and pregnancy rate (PR), implantation rate (IR) and abortion rate (AR) in each group were assessed and compared. The SAS system version 8.1 (SAS Institute Inc., Cary, NC, USA) with ANOVA test were used for statistical analysis. A  $p < 0.05$  was considered statistically significant.

## Results

The mean ages, baseline FSH levels, and the indications for IVF treatment were comparable in each group (Table 1). The body mass index in groups 2 and 3 were lower, but not significantly, compared with groups 1, 4 and 5. Lower Gn dosages and E2 levels were noted in the GnRH-ant groups when compared with the GnRHa groups. Gn dosages (IU)/E2 levels (pg/mL) in each group were: (1) 1,949.4/1,191.1; (2) 1,869.6/1,010.8; (3) 1,856.7/1,023.6; (4) 2,184.5/1,323.6; and (5) 2,103.5/1,313.5, respectively (Tables 2 and 3). A higher risk of LH surge was noted in the lower dosage groups of GnRH-ant (cetrorelix 0.2 mg, 0.15 mg). OHSS risks were lower in the GnRH-ant groups. LH/OHSS risks in each group were: (1) 3.5%/5.8%; (2) 7.1%/3.6%; (3) 13.3%/3.3%; (4) 3.4%/8.6%; and (5) 2%/8.2%, respectively (Tables 2 and 3).

The number of oocytes retrieved/grade I, II embryos in group 1 was higher than those in groups 2 and 3. The 0.25 mg cetrorelix group also produced better qualities of embryos and oocytes. Number of oocytes/embryos/grade I, II embryos in each group were: (1) 9.4/7.9/5.8; (2) 7.5/4.2/3.6; (3) 6.3/4.1/3.1; (4) 12.3/8.9/6.6; and (5) 11.8/8.4/6.1, respectively (Tables 2 and 3). We observed a favorable outcome in the 0.25 mg cetrorelix

**Table 1.** Personal data for patients who received different dosages of gonadotropin-releasing hormone (GnRH) antagonist (cetrorelix 0.25 mg, 0.2 mg, 0.15 mg), long- or short-acting form of GnRH agonists (leuprolide depot, leuprolide acetate) for pituitary suppression during controlled ovarian hyperstimulation\*

	Cetrorelix 0.25 mg (n = 86)	Cetrorelix 0.2 mg (n = 28)	Cetrorelix 0.15 mg (n = 30)	Leuprolide acetate (n = 58)	Leuprolide acetate depot (n = 49)
Age <sup>†</sup> (yr)	33.9 $\pm$ 4.4	32.3 $\pm$ 2.1	31.6 $\pm$ 2.4	30.9 $\pm$ 2.5	32.1 $\pm$ 2.7
BMI <sup>†</sup> (kg/m <sup>2</sup> )	20.6 $\pm$ 1.4	19.0 $\pm$ 1.0	19.5 $\pm$ 1.1	20.7 $\pm$ 2.1	21.1 $\pm$ 1.8
Baseline FSH levels <sup>†</sup> (IU/L)	4.0 $\pm$ 1.8	3.7 $\pm$ 1.6	3.9 $\pm$ 1.3	3.8 $\pm$ 1.4	3.6 $\pm$ 1.8
Infertility causes <sup>†</sup>					
Tubal factor	28 (32.5)	8 (28.6)	7 (23.3)	16 (27.6)	13 (26.5)
Male factor	25 (29.1)	6 (21.4)	6 (20)	13 (22.4)	15 (30.6)
Endometriosis	11 (12.8)	5 (17.9)	4 (13.4)	6 (10.4)	10 (20.4)
Idiopathic	11 (12.8)	7 (25)	7 (23.3)	10 (17.2)	5 (10.2)
Others	11 (12.8)	2 (7.1)	6 (20)	13 (22.4)	6 (12.3)

\*Data are presented as mean  $\pm$  standard deviation or number (%); <sup>†</sup>non-significant difference. BMI = body mass index; FSH = follicle-stimulating hormone.

**Table 2.** Clinical results and laboratory data for patients who received different dosages of gonadotropin-releasing hormone (GnRH) antagonist for pituitary suppression during controlled ovarian hyperstimulation\*

	Cetrorelix 0.25 mg (n=86)	Cetrorelix 0.2 mg (n=28)	Cetrorelix 0.15 mg (n=30)
GnRH antagonist dosage <sup>†</sup> (mg)	1.28 ± 0.29	1.07 ± 0.28	0.763 ± 0.147
Gonadotropin dosage <sup>‡</sup> (IU)	1,949.4 ± 588.7	1,869.6 ± 409.3	1,856.7 ± 383.9
E2 on hCG day <sup>‡</sup> (pg/mL)	1,191.1 ± 329.7	1,010.8 ± 259.1	1,023.6 ± 219.7
LH surge <sup>†</sup>	3 (3.5)	2 (7.1)	4 (13.3)
Ovarian hyperstimulation syndrome <sup>§</sup>	5 (5.8)	1 (3.6)	1 (3.3)
Oocyte no. <sup>‡</sup>	9.4 ± 5.54	7.5 ± 5.1	6.3 ± 6.7
Embryo no. <sup>‡</sup>	7.9 ± 4.14	4.2 ± 2.2	4.1 ± 2.4
Grade I/II embryo no. <sup>‡</sup>	5.8 ± 1.87	3.6 ± 2.2	3.1 ± 1.4
Chemical pregnancy rate <sup>†</sup>	26 (30.2)	6 (21.4)	5 (16.7)
Implantation rate <sup>‡</sup>	5.9%	5.1%	4.1%
Abortion rate <sup>†</sup>	6 (7)	2 (7.1)	3 (10)

\*Data are presented as mean ± standard deviation or number (%); <sup>†</sup>p < 0.05 between each group; <sup>‡</sup>p < 0.05 between cetrorelix 0.25 mg and the other two groups, non-significant difference between cetrorelix 0.2 mg and 0.15 mg groups; <sup>§</sup>non-significant difference between each group. E2 = estradiol; LH = luteinizing hormone.

**Table 3.** Clinical results and laboratory data for patients who received long- or short-acting form of gonadotropin-releasing hormone (GnRH) agonists for pituitary suppression during controlled ovarian hyperstimulation\*

	Cetrorelix 0.25 mg (n=86)	Leuprolide acetate (n=58)	Leuprolide acetate depot (n=49)
Gonadotropin dosage <sup>†</sup> (IU)	1,949.4 ± 588.7	2,184.5 ± 528.4	2,103.5 ± 486.4
E2 on hCG day <sup>†</sup> (pg/mL)	1,191.1 ± 329.7	1,323.6 ± 409.1	1,313.5 ± 427.7
LH surge <sup>‡</sup>	3 (3.5)	2 (3.4)	1 (2)
Ovarian hyperstimulation syndrome <sup>†</sup>	5 (5.8)	5 (8.6)	4 (8.2)
Oocytes no. <sup>†</sup>	9.4 ± 5.54	12.3 ± 4.5	11.8 ± 5.1
Embryo no. <sup>†</sup>	7.9 ± 4.14	8.9 ± 2.7	8.4 ± 2.5
Grade I/II embryo no. <sup>†</sup>	5.8 ± 1.87	6.6 ± 2.3	6.1 ± 2.1
Chemical pregnancy rate <sup>‡</sup>	26 (30.2)	19 (32.8)	15 (30.6)
Implantation rate <sup>‡</sup>	5.9%	5.5%	5.7%
Abortion rate <sup>‡</sup>	6 (7)	5 (8.6)	4 (8.2)

\*Data are presented as mean ± standard deviation or number (%); <sup>†</sup>p < 0.05 between cetrorelix 0.25 mg and other two groups, non-significant difference between LA and LA depot groups; <sup>‡</sup>non-significant difference between each group. E2 = estradiol; LH = luteinizing hormone.

group compared with the LA or LA depot groups. PR/IR in groups 1, 4 and 5 were statistically higher than those in groups 2 and 3. There were no significant differences in clinical outcomes between groups 1, 4 and 5, except for higher ARs in the GnRH-ant groups. Chemical PR/IR/AR in each group were: (1) 30.2%/5.9%/7%; (2) 21.4%/5.1%/7.1%; (3) 16.7%/4.1%/10%; (4) 32.8%/5.5%/8.6%; and (5) 30.6%/5.7%/8.2%, respectively (Tables 2 and 3).

## Discussion

GnRHa have been used for pituitary suppression since the mid-1980s to avoid the adverse effect of a premature LH surge [1,5]. In current practice, GnRHa are routinely used to suppress endogenous Gn during IVF

treatment. The advantages of combining GnRHa with a Gn in COH/IVF-ET using the “long protocol” have been well known [16]. The use of a GnRHa for IVF cycles significantly reduced the cycle cancellation rate and improved the ovarian response [2,3]. Because psychosocial stress may contribute to infertility [17], much attention has been paid to simplifying the cycle program. To overcome the stress and inconvenience induced by the daily administration of short-acting forms of GnRHa, the use of long-acting GnRHa is practical and reasonable. Gianaroli et al [18] have shown that when the convenience, costs and side-effects are taken into account, a single dose of long-acting GnRHa is preferable. Albuquerque et al [19] demonstrated that LA depot was associated with increased requirements for Gn and a longer COH period, but similar PR, compared with the LA protocol. In our previous study, we

demonstrated that the use of low-dose LA depot had the advantages of convenience, less stress and being cost-effective [4].

The daily administration of short-acting GnRHa from the luteal phase of the previous cycle by either injection or intranasal spray is inconvenient, tiring and stressful. However, the clinical effectiveness of long-acting forms of GnRHa remains controversial. Long-acting GnRHa appeared to shorten the pituitary desensitization, while a longer duration of Gn stimulation and higher dosage of Gn were necessary, compared with the short-acting form [18,20]. Furthermore, the long-acting GnRHa possibly impaired embryo development and implantation when compared with short-acting buserelin acetate [3]. In contrast, some investigators have demonstrated the advantage and clinical value of long-acting GnRHa. Dhont et al [21] demonstrated that long-acting GnRHa (goserelin) was reliable as an adjunct to follicular stimulation in COH. Neuspiller et al [22] employed long-acting GnRHa in ovum donation programs and observed that long-acting forms of GnRHa provided similar success rates and more convenient medication compared with short-acting GnRHa. Dada et al [23] also demonstrated that long-acting GnRHa were as effective as short-acting analogs, with no detrimental effects on the luteal phase.

Few investigators have studied the clinical effects of lowering the dose of long-acting GnRHa. Simon et al [24] reported that lowering the dose of LH-releasing hormone (LHRH) analog to 0.1 mg/day during folliculogenesis had no adverse effect on the COH and IVF results. Balasch et al [25] showed that the full dose (3.75 mg) of D-Trp-6-LHRH depot and its half dose (1.87 mg) were comparable in pituitary desensitization. According to our past experiences, after the adjustment of the LA depot dosage, we observed that half-dose LA depot had similar effects on pituitary desensitization, Gn dosage and PR, compared with the short-acting LA [16,26]. However, both LA and LA depot required a rather long treatment period to achieve pituitary downregulation and required the administration of larger dosages of Gn to achieve adequate follicular growth [16]. Higher Gn dosages, higher serum E2 on the day of hCG administration, and multiple follicular responses were identified as the major risk factors for OHSS [17].

GnRH-ant have been developed in parallel with GnRHa, but their development history has been plagued by a high incidence of histamine release following injection. Over the past few years, progress in the prevention of this histamine-releasing activity has been made. Third-generation GnRH-ant (cetrorelix and ganirelix) have been administered in a multiple dose regimen in

women undergoing COH. Until recently, GnRHa were the only choice available to physicians for the prevention of premature LH surges in women undergoing COH. The recent approval of GnRH-ant for this indication gives clinicians some new options. Major advantages of GnRH-ant include a shorter duration of recruitment and COH, reduced dosage of Gn, and a lower risk of OHSS compared with GnRHa [13].

It is suggested that GnRHa have a direct effect on ovarian steroidogenesis, which is independent of their action on the pituitary [27]. This unwanted effect and other possible drawbacks of GnRHa are thought to be eliminated with the use of GnRH-ant. The mechanism of action of GnRH-ant is through competitive blocking of the GnRH receptor, which results in a rapid but reversible suppression of Gn secretion. Because GnRH-ant immediately suppress gonadotropins by blocking GnRH receptors, treatment may be restricted to those days when a premature LH surge is likely to occur. Serum GnRH-ant concentrations increased in a linear dose-proportional manner, while serum LH decreased in a dose-proportional manner [28,29].

In several trials, the GnRH-ant regimens have been associated with slightly lower PR and IR than the established GnRHa protocols [30]. Although several studies have indicated a slight reduction in PRs with GnRH-ant, when compared with GnRHa, this problem may be rectified by developing flexible regimens designed for individual patients [31]. GnRH-ant can suppress the premature LH surge completely within a few hours, allowing luteolysis by mid-cycle administration. Introducing flexible GnRH-ant regimens aimed at improving clinical outcomes should be an area for research in the near future [32].

Two major protocols using GnRH-ant have been developed, including a multiple dose (MD) regimen and a single dose protocol (SD). In general, most investigators found more favorable results with the MD protocol than with the SD protocol [13]. The SD protocol might result in greater suppression of serum LH than the MD protocol [33]. The SD protocol was associated with a shorter duration of Gn use, smaller numbers of developing follicles, lower serum E2 levels on the day of hCG administration, and a smaller number of zygotes [13,34]. A fixed multi-dose GnRH-ant protocol is feasible for patients who are poor responders to a long agonist protocol [32]. The maximum endogenous LH suppression occurs about 4 hours after GnRH-ant administration [29]. Moreover, rapid recovery of pituitary function was observed after discontinuation of GnRH-ant [35]. This was due to the relative short elimination half-life (about 13 hours) of GnRH-ant [9]. These observations indicated that the degree of

pituitary suppression could be adjusted by changing the GnRH-ant dose according to the size of the leading follicle. Starting the GnRH-ant according to the size of the leading follicle (16 mm) was as effective as starting on a fixed day, and reduced GnRH-ant administration [36].

There is still controversy about the real efficacy of GnRH-ant administration. Some investigators claimed that an equivalent PR was achievable using GnRH-ant protocols and GnRHa protocols [9,37–40]. Using GnRH-ant may offer a favorable alternative for IVF poor responder patients [41]. The GnRH-ant can provide short and simple treatment and are particularly attractive for administration in women undergoing COH, achieving comparable PR compared with the “long protocol” regimen. Zikopoulos et al [42] demonstrated that GnRH-ant facilitated a short, simple treatment and resulted in comparable PR when compared with GnRHa in couples with unexplained infertility and/or mild oligozoospermia and undergoing COH. Furthermore, GnRH-ant usage improved the PR in patients with a history of multiple failures of IVF using a GnRHa protocol, possibly because of improvement in the quality of the blastocysts generated [43]. Roulier et al [33] compared SD cetrorelix (3 mg) and single full dosage of GnRHa (Decapeptyl Retard 3.75 mg) in COH luteolysis, and found fewer recovered oocytes but similar PR in the GnRH-ant group compared with the GnRHa group.

In contrast, some investigators demonstrated lower levels of serum E2, fewer small follicles/oocyte and decreased PR in GnRH-ant cycles, when compared with GnRHa [44–46]. The MD GnRH-ant protocol is a short and simple protocol with a significant reduction in incidence of OHSS but a lower PR compared with the GnRHa long protocol [47]. GnRH-ant injection during the early follicular phase would likely disturb the growth of cohort follicles [48]. MD cetrorelix administration might result in greater suppression of LH, which produces lower serum levels of E2 when Gn devoid of LH are used [49]. Therefore, some LH supplement might need to be considered during cetrorelix administration. However, the addition of recombinant LH might prevent a decrease in estradiol during GnRH-ant administration, but does not positively influence the clinical outcome in terms of oocyte number, maturation, embryo quality, fertilization rate, PR, or IR [50].

Therefore, minimal dose adjustment of GnRH-ant to suppress LH release without impairing the oocyte development and embryo implantation might be considered in these situations. Concerning racial differences, most Asian women appeared to be thinner than Caucasians. As 1.88 mg instead of 3.75 mg LA depot has been proved to be an adequate dosage for pituitary

suppression in Asians [4,26], it is logical to suspect this lower adjustment of cetrorelix dosage would also apply for pituitary suppression in Taiwanese. To select the minimum effective daily dose of GnRH-ant, a multicenter, double-blind, randomized, dose-finding study was performed on 333 women, using six different dosages ranging from 0.0625–2 mg [44,14]. Albano et al [10] demonstrated that the minimum effective dose of cetrorelix able to prevent premature LH surge in COH cycles was 0.25 mg/day in Caucasian individuals. A dose of 0.25 mg/day cetrorelix was considered to be a safe, short and convenient treatment regimen in women undergoing COH and resulted in a good clinical outcome [44].

Borm and Mannaerts [9] demonstrated that a daily dose of 0.25 mg GnRH-ant prevented an LH surge and led to a favorable outcome (37% ongoing pregnancy rate). LH is effective in stimulating E2 secretion in granulosa cells that have acquired LH-binding sites [51]. During COH, a relative elevation of serum LH level can be observed, which might be suppressed immediately by the GnRH-ant. The severe suppression by cetrorelix might interrupt the folliculogenesis and decrease serum E2 elevation. However, different GnRH-ant dosages (cetrorelix 0.5 mg or 0.25 mg) have no different impact on the luteal phase of IVF/ICSI cycles when hormonal support is given [10].

In women aged 40 years and older with abnormal FSH levels, Weghofer et al [15] demonstrated that COH with 0.25 mg of GnRH-ant resulted in favorable outcomes. Escudero et al [52] demonstrated comparable results with administration of GnRH-ant on different days (administration on stimulation day 6 or when the leading follicle was  $\geq 14$  mm). Engel et al [53] demonstrated that body weight did not influence cetrorelix plasma concentrations, and they, therefore, suggested that cetrorelix modification was not necessary for individuals with different body weights treated with cetrorelix during COH. In contrast, Al-Inany and Aboulghar [54] reported that serum levels of GnRH-ant exhibited a linear inverse relationship to body weight, such that it would seem likely that smaller women would probably require lower doses of GnRH-ant for preventing the LH surge. A dose of 2.5 mg cetrorelix was effective at achieving pregnancy in a clinical study in Taiwanese women, and it was concluded that the difference could be due to racial differences between Caucasian and Asian women [55].

Despite the convenience of GnRH-ant application during COH, the use of GnRH-ant rather than GnRHa co-treatment for IVF is not widely accepted. One possible concern is that corpus luteum function seems to be impaired in IVF cycles with GnRH-ant [56]. However,

COH is associated with elevated progesterone levels in the late follicular phase and accelerated endometrial maturation in the subsequent luteal phase. Saadat et al [57] demonstrated that non-significant differences in pre-retrieval serial serum progesterone levels and luteal phase endometrial histology existed between cycles utilizing GnRHa or GnRH-ant. Luteal support is essential when a long-acting GnRHa is used [58]. Adequate luteal support compensates for luteolysis induced by GnRH-ant and assures good clinical outcome. Herman et al [58] have demonstrated that mid-luteal hCG addition helped to preserve corpus luteum function. In our unit, we routinely administer 2,500 IU of hCG on days 1, 4 and 7 post-ET, to prevent the negative effects of GnRHa or GnRH-ant on the corpus luteum or the endometrium. The reduced dose of long-acting GnRHa and the luteal supplement of hCG may contribute to the similar luteal desensitization and clinical results found with short-acting GnRHa.

In this series, to the best of our knowledge, we have demonstrated the largest application of MD cetrorelix in Asians. We first tried a lower dosage of cetrorelix for Asians, but observed that the risk of an LH surge was still high in the lower dosage groups (0.2 mg and 0.15 mg). The LH surge risk of 0.15–0.2 mg daily was higher than that in 0.25 mg trials. We, therefore, concluded that individuals with lower body weights (<50 kg) still required a 0.25 mg daily dosage of cetrorelix. We suggest that the 0.2 mg and 0.15 mg cetrorelix doses are not suitable for LH suppression, even in the thinner individuals.

We noted that PR/IR in the 0.25 mg group appeared higher than in the 0.2 mg and 0.15 mg groups. The number of oocytes retrieved/grade I, II embryos was higher in group 1 than in groups 2 and 3 (10.5/7.8 vs. 8.3/3.9). A lower incidence of LH surge and higher E2 levels on the day of hCG administration were observed in group 1 than in groups 2 and 3. There was a non-significant difference between the 0.25 mg cetrorelix and the LA/LA depot groups regarding prevention of an LH surge. We observed non-statistical differences between cetrorelix groups (0.25 mg, 0.2 mg, 0.15 mg) in Gn dosage and OHSS. The 0.25 mg cetrorelix resulted in similar PR but higher AR compared with those of the LA/LA depot groups [59]. The patients from the cetrorelix group produced fewer follicles compared with individuals from the LA/LA depot groups. The results might be due to the absence of early pituitary downregulation and synchronization of follicles during COH, lower Gn dosage, as well as higher level of ovarian suppression (reduced follicular development and depressed serum E2 levels) and severe LH interruption during the later folliculogenesis stages [13].

In conclusion, 0.25 mg of cetrorelix is the lowest effective dosage for pituitary suppression during COH. A regimen of 0.25 mg MD cetrorelix showed comparable pituitary suppression and clinical results with those of LA/LA depot. Because of a higher risk of LH surge and poorer clinical outcome, a lower dosage of cetrorelix (0.2 mg, 0.15 mg) was unsuitable for pituitary suppression during COH. AR was still higher in 0.25 mg cetrorelix administration. In view of the limited number of patients studied, larger cohort recruitment is required for further clarification. Furthermore, the influence of GnRH-ant on the PR, synchronization of follicles, as well as oocyte and embryo qualities, merits further study. The real effect of cetrorelix upon the luteal phase also merits further investigation. A bright future for the application of GnRH-ant is expected. Further application of GnH-ant will allow a short and simple treatment regimen for IVF patients undergoing COH, and it is expected that the availability of GnRH-ant will lead to a shorter, cheaper and safer protocol. Clinical outcomes may be improved by developing more flexible antagonist regimens, an approach that requires further evaluation. As clinicians gain experience with larger applications of GnRH-ant, optimal treatment paradigms will likely emerge.

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