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Original Article

Assessing the optimal dose for Cetrorelix in Chinese women undergoing ovarian stimulation during the course of IVF-ET treatment

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Abstract: We conducted a prospective, randomized, and controlled trial to assess the optimal dose for GnRH antagonist, cetrorelix, for Chinese women during the course of ovarian stimulation. The patients were randomly divided into two groups, in which 48 patients were advised to inject 0.25 mg Cetrorelix daily (the 0.25 mg group), while 39 patients were instructed to receive a daily dose of 0.125 mg cetrorelix (the 0.125 mg group). In general, a daily dose of 0.125 mg cetrorelix could be more optimal for Chinese women as manifested by the lower cancellation rate, higher implantation rate and clinical pregnancy rate. Specifically, daily administration of 0.125 mg cetrorelix for patients under 35 years old is associated with a 3-fold higher implantation rate and a 5-fold higher clinical pregnancy rate as compared with that of those patients ≥ 35 years old. On the contrary, higher rates for implantation and clinical pregnancy were noted by daily injection of 0.25 mg cetrorelix in elder patients (≥ 35 years old) as compared with that of young patients (< 35 years old). Together, our data suggest that a daily dose of 0.125 mg cetrorelix could be more optimal for patients < 35 years old, while 0.25 mg/day of cetrorelix are likely conducive to higher implantation and clinical pregnancy rate for those patients ≥ 35 years old. These data could be important for preventing LH surge while maintaining optimal LH levels necessary for embryo implantation for Chinese women during the course of IVF-ET treatment.

Keywords: IVF-ET, GnRH antagonist, cetrorelix, ovarian stimulation

Introduction

In the setting of women with in vitro fertilization-embryo transfer (IVF-ET) treatment, the pituitary gonadotropin-releasing hormone (GnRH) agonist long protocol has been always considered to be the first choice. However, for those elderly patients or patients with repeated failures for the GnRH agonist long protocol, GnRH antagonist protocol could be an alternative choice. GnRH antagonist is a peptide comprising 10 amino acid segments, which binds to the pituitary membrane receptors and directly inhibits the generation of luteinizing hormone (LH) surge [1]. There is evidence suggesting that GnRH antagonist has higher efficacy than GnRH agonist, which leads to the reduction of pituitary desensitization duration and gonadotropins dose [2]. Moreover, GnRH antagonist does not cause "flame up" effect, which renders the patients with lower risk to the development of ovarian hyperstimulation syndrome (OHSS) [3]. As a result, GnRH antagonist has now gradually widely applied in the clinical setting of assisted reproduction.

Interestingly, recent studies have further revealed that GnRH antagonists not only inhibit LH surge, but also attenuate the growth of follicles and endometrium [4], the two factors important for the successful embryo implantation. Therefore, it is important to demonstrate the minimum effective dose for GnRH antagonist during the course of IVF-ET treatment for

Table 1. Demographic and clinical characteristics for the studying subjects

Group/Parameters	0.25 mg group (n=48)	0.125 mg group (n=39)
Age (years)	35.13 ± 4.96	33.97 ± 5.13
Basal FSH (mIU/ml)	8.11 ± 3.92	7.64 ± 2.61
BMI (kg/m^2)	21.54 ± 2.71	21.28 ± 2.00
Duration of infertility (years)	7.68 ± 5.23	7.92 ± 5.33
Primary infertility (%)	16 (33.33)	21 (53.85)
Secondary infertility (%)	32 (66.67)	18 (46.15)
Previous ART times (%)		
1 or 2 times	37 (77.08)	29 (74.36)
More than 3 times	11 (22.92)	10 (25.64)
Causes of infertility (%)		
Tubal factor	33 (68.75)	24 (61.54)
Male factor	7 (14.58)	6 (15.38)
Endometriosis	4 (8.33)	2 (5.13)
Unexplained	1 (2.08)	5 (12.82)
Mixed	3 (6.25)	2 (5.13)

improving clinical pregnancy rate. Yet, some studies have already suggested that the minimum effective dose for GnRH antagonist was 0.25 mg per day in a multiple dose protocol [5, 6]. However, this result was concluded based on a study population of women predominantly with Caucasian origins. Given Asian women are underweight as compared with that of women with European descent, this dose may lead to excessive inhibition of follicles and endometrium. We, therefore, conducted a prospective, randomized and controlled trial to assess the optimal dose of Cetrorelix for the GnRH antagonist protocol in Chinese women during the course of IVF-ET treatment.

Materials and methods

Study subjects

A total of 87 patients who had at least one episode of failure for the long GnRH agonist protocol treatment at the Center for Assisted Reproduction in Tongji Hospital between May 2012 to July 2013 were included for the study. The study was designed as prospective, randomized and controlled manner. Inclusion criteria were age from 25 to 45 years, regular menstrual cycles (25-32 days), presence of normal uterus with ovaries on both sides, 19-24 kg/m^2 for body mass index (BMI), and no ovarian stimulation 3 months prior to this cycle. Patients were randomly divided into two groups by flipping a coin way, either receiving 0.25 mg/day Cetrorelix (Cetrotide, Serono, Geneva, Switzerland) (n=39) or 0.125 mg/day (n=48). All demographic information for the studying subjects is summarized in **Table 1**. Informed consent was obtained from all studying subjects, and the study was approved by the Human Assurance Committee at the Tongji Hospital of Huazhong University of Science & Technology.

The GnRH antagonist protocol

On day 3 of menstrual period, a basic evaluation was conducted by ultrasound examination. Starting dose was identified according to each patient's age and antral follicles number. In general, young patients (< 35 years old) were advised to take 150-225 IU/day of recombinant FSH (recFSH; Gonal F®, Serono, Italy), and elder patients (≥ 35 years old) were instructed to take 225-300 IU of recFSH daily. After 5 days of stimulation, transvaginal ultrasound examination was carried out to monitor the development of follicles, and the dose of recFSH was adjusted appropriately according to the development of follicles. Once the largest follicle size reached 14 mm, cetrorelix (0.25 mg/d or 0.125 mg/d) was then applied by S.C. injection daily, up to the day of human chorionic gonadotropin (HCG) administration.

Procedures for oocyte retrieval

Recombinant FSH and cetrorelix were administered continuously until three follicles reached 17 mm, HCG (10,000 IU, EMD Serono, USA) was then administrated, and serum concentrations for estradiol (E2), LH, and progestone (P) were tested on the day of HCG administration. The hormones were determined using an Immulite Automated Analyser System (ECL-2012, Siemens, Germany) according to the manufacture's instruction. Oocytes were retrieved 34-38 h after HCG injection and were fer-

Table 2. Results for clinical manifestations of all studying subjects

Group/Parameters	0.25 mg group (n=48)	0.125 mg group (n=39)
Retrieved oocyte cycles	48	37
Transfer cycles	42	35
D3 antral follicle count (AFC)	8.00 ± 4.68	7.72 ± 4.01
rFSH duration (days)	8.48 ± 1.61	8.32 ± 1.58
rFSH dosage (amp)	31.08 ± 11.67	31.18 ± 13.29
Antagonist dosage (amp)	4.04 ± 1.17	2.04 ± 0.44
Day of antagonist administrated	8.54 ± 1.27	8.59 ± 1.27
Antagonist duration (days)	4.02 ± 1.12	4.03 ± 0.87
HCG day		
Oestradiol (pmol/l)	1557.16 ± 1055.82	1613.68 ± 931.10
Progesterone (ng/ml)	1.17 ± 0.47	1.35 ± 1.45
LH (IU/L)	3.01 ± 2.65	5.07 ± 4.8
≥ 14 mm follicles	4.74 ± 2.28	5.26 ± 2.56
Endometrial thickness (mm)	10.34 ± 2.62	10.16 ± 2.36
Patients with LH > 10 IU/L (%)	1 (2.08%)	3 (7.69%)

tilized in vitro according to the standard procedures as previously reported [7, 8].

Procedure for embryo transfer

Embryo transfer (ET) was carried out 72 h after oocyte retrieval as described previously [7, 8]. In brief, a maximum of three embryos were implanted into each patient. Progesterone (in oil) was i.m. administered daily (80 mg/day) from day 1 after oocyte retrieval to maintain luteal functionality. Clinical pregnancy was confirmed by the elevation of serum β-HCG 14 days after implantation along with the presence of gestational sac(s) through ultrasonography exam.

Statistical analysis

The SPSS 17.0 for windows was employed for data analysis. Data on age, body mass index (BMI), basal FSH concentration, the number of antral follicles, the duration/dosage of rFSH used, serum concentrations for E2, LH and progesterone, the number of follicles with size ≥ 14 mm, the endometrial thickness on the day of HCG administration, the number of oocytes aspirated/fertilized were recorded. The patients were also followed for the number of good quality embryos and the rates of fertilization, implantation and the presence of clinical pregnancy. All values were expressed as mean ± SD. Student's *t*-test and Chi-square test were employed for statistical comparisons. In all cases, $p < 0.05$ was considered with statistical significance.

Results

Demographic information and clinical characteristics for the studying subjects

All demographic information for the studying subjects is summarized in **Table 1**. There were 48 patients included to receive 0.25 mg/day of cetrorelix, while 39 patients were randomly assigned to receive 0.125 mg/day of cetrorelix. We failed to detect a significant difference between the two study groups in terms of mean age, basal FSH, BMI and duration of infertility. In general, tubal factor was the main cause of infertility for all studying subjects, which accounted for 68.7% for the patients in the 0.25 mg group and 61.5% for the patients in the 0.125 mg group.

Results for clinical manifestations

For patients in the 0.125 mg group, 2 cycles were cancelled due to early ovulation, and 2 patients cancelled embryo transplantation due to poor quality of embryos (5.4% cancellation rate). In contrast, much higher cancellation rate (12.5%) was noted for patients in the 0.25 mg group, in which 6 patients underwent cancellation for embryo transplantation due to poor embryo quality and uterine bleeding. We failed to detect a significant difference between the two groups in terms of antral follicle count (AFC), rFSH duration and dosage, antagonist duration, HCG day serum hormone levels and endometrial thickness. However, the antagonist dosage for patients in the 0.25 mg group was significantly higher than that of patients in the 0.125 mg group (4.04 ± 1.17 amps vs. 2.04 ± 0.44 amps, $p < 0.05$, **Table 2**). Also, a slightly higher rate for the premature LH rises (LH ≥ 10 m IU/ml) was noted in patients in the 0.125 mg group (3 out of 39 patients, 7.69%) as compared with that of patients in the 0.25 mg group (1 out of 48 patients, 2.08%, **Table 2**).

Table 3. Comparison of clinical indexes and IVF-ET results

Group/Parameters	0.25 mg group (n=48)	0.125 mg group (n=39)
Oocytes retrieved	6.52 ± 3.84	6.68 ± 4.22
Mature oocytes	5.85 ± 3.54 (89.78%)	6.03 ± 4.11 (87.80%)
Immature oocytes	0.42 ± 0.82 (6.39%)	0.59 ± 0.93 (8.66%)
Fertilization rate (2PN) (%)	56.55	57.48
Cleavage rate (2PN) (%)	98.31	96.50
Grade I/II embryos	1.83 ± 1.86	1.71 ± 1.75
No. of embryos transferred	1.92 ± 0.99	2.22 ± 0.82
No. of frozen embryos	0.81 ± 1.54	1.00 ± 1.75
Implantation rate (%)	13.04	18.29
Clinical pregnancy rate (%)	21.4	28.6

Comparison for the clinical indexes and IVF-ET results

All clinical results for patients in the 0.25 mg and 0.125 mg cetrorelix groups were summarized in **Table 3**. We failed to observe significant differences between the two groups of patients with respect to the number of oocytes retrieved, number of mature oocytes, 2 pronuclei (2PN) fertilization rate, and number of grade I/II embryos (**Table 3**). Interestingly, higher rates were noted for patients in the 0.125 mg group as compared with that of patients in the 0.25 mg group in terms of implantation rate (18.29% vs. 13.04%) and clinical pregnancy rate (28.6% vs. 21.4%), although the differences did not reach a statistical significance (**Table 3**).

To further address the above question, we next subdivided patient populations into young subgroup (patients with age < 35 years old) and elder subgroup (patients with age ≥ 35 years old) in each study group as summarized in **Table 4**. For those young patients (< 35 years old), HCG day progesterone and LH levels were noted slightly higher in the 0.125 mg group as compared with that in the 0.25 mg group, but without a statistical difference. More importantly, a 60% higher implantation rate (25.49% vs. 15.91%) and a 63% higher clinical pregnancy rate (42.86% vs. 26.32%) were noted for those young patients in the 0.125 mg group as compared with that of young patients in the 0.25 mg group (**Table 4**). Of importantly note, for patients in the 0.125 mg group, young patients demonstrated a 3-fold higher implantation rate (25.49% vs. 6.45%, $p < 0.01$), and a 5-fold higher clinical pregnancy rate (42.86% vs. 7.14%, $p < 0.01$) than that of elder patients. In sharp contrast, for patients in the 0.25 mg group, those young patients only manifested a 53% higher implantation rate (19.51% vs. 10.42%) and a 51% higher clinical pregnancy rate (26.32% vs. 17.39%) than that of elder patients. In line with these results, an opposite effect was noted in elder patients, in which elder patients in the 0.25 mg group displayed a higher implantation rate (10.42% vs. 6.45%) and a higher clinical pregnancy rate (17.39% vs. 7.14%) than that of elder patients in the 0.125 mg group. Together, our data support that 0.125 mg/day of cetrorelix could be the most optimal dose for young Asian women (< 35 years old) during the course of IVF-ET treatment, while 0.25 mg/day of cetrorelix could be better for those women with age ≥ 35 years old.

Discussion

A major problem relevant to the relatively low efficacy of ovarian stimulation in women undergoing IVF-ET treatment is the development of premature luteinizing hormone (LH) surge [9]. Recent studies have consistently demonstrated that unlike GnRH agonists, GnRH antagonists are potent to suppress LH surge without initial flare effect [10]. Indeed, GnRH antagonists have been demonstrated to induce a rapid, reversible suppression of gonadotropin release by competitively blocking the GnRH receptors, and through which they provide protection for women against LH surges during ovarian stimulation [11]. Studies have also revealed that the half-life for GnRH antagonist is relatively short, with probably only 13 h, and the maximum inhibitory effect for a GnRH antagonist is about 4 h after its administration [12]. Once the patients stopped to receive GnRH antagonist, their pituitary function would rapidly recover [13]. Therefore, by optimizing the dose of GnRH antagonist, the depth of pituitary suppression for a particular patient can be appropriately adjusted, which would be associated with higher implantation rate and clinical pregnancy rate.

In general, the optimal dose for a particular GnRH antagonist should be effectively inhibiting the LH surge, but without a discernable

Table 4. Comparison of IVF-ET results between young and elder patients

Group/Parameters	0.25 mg group (n=42)		0.125 mg group (n=35)	
Age subgroup (years)	< 35	≥ 35	< 35	≥ 35
Number of patients	19	23	21	14
rFSH duration (days)	8.35 ± 1.50	8.57 ± 1.46	8.26 ± 1.58	8.40 ± 2.01
rFSH dosage (amp)	26.52 ± 10.47	34.33 ± 11.03	30.42 ± 13.22	32.27 ± 11.32
HCG day oestradiol (pmol/l)	1656.25 ± 1023.25	1496.85 ± 985.10	1661.43 ± 971.22	1539.88 ± 994.4
HCG day progesterone (ng/ml)	1.07 ± 0.52	1.24 ± 1.23	1.60 ± 0.41	1.03 ± 0.26
HCG day LH (IU/L)	2.05 ± 1.22	3.64 ± 1.75	6.59 ± 4.32	3.33 ± 1.96
Oocytes retrieved	8.40 ± 4.31	5.18 ± 3.65	7.43 ± 5.29	5.93 ± 3.90
Implantation rate (%)	15.91	10.42	25.49	6.45
Clinical pregnancy rate (%)	26.32	17.39	42.86	7.14

impact on the development of follicles and endometrium. In order to determine the minimal effective dose, Albano and colleagues conducted comparative studies in women prescribed with different doses of cetrorelix, and suggested a minimum effective daily dose of 0.25 mg cetrorelix [14]. In contrast, studies carried out by Chen and colleagues suggest that a daily dose of 0.2 mg cetrorelix would be more optimal to prevent premature LH surge [15], while studies from Tiboni and co-workers demonstrate that a cetrorelix dose of 0.125 mg/day is effective as that of 0.25 mg/day in preventing premature LH rise during controlled ovarian stimulation [16]. Together, these studies suggest that the optimal dose for cetrorelix varies a lot due to the differences of originality of patient populations. We thus conducted this prospective study to define the optimal cetrorelix dose for Women with Chinese descent. In general, our data revealed that patients administered with 0.125 mg/day of cetrorelix manifest lower cancellation rate, but higher implantation and clinical pregnancy rates than that of patients injected with 0.25 mg/day of cetrorelix. Particularly, a daily dose of 0.125 mg cetrorelix could be beneficial for patients under 35 years old, while for those patients ≥ 35 years old, a daily dose of 0.25 mg cetrorelix could be more optimal.

There is evidence indicating that LH levels impact the successful rate for embryo implantation. In a double-blind, randomized study, the ganirelix dose-finding study group revealed that the increase of antagonist dose on the one hand can enhance LH suppression; low LH levels on the other hand are associated with lower pregnancy rate [17]. Indeed, Studies in our patients revealed that higher dose of cetrorelix (0.25 mg vs. 0.125 mg) in young patients (< 35 years old) is associated with a 3-fold reduction for implantation rate and a 5-fold reduction for clinical pregnancy rate. To our surprise, an opposite effect was observed for patients ≥ 35 years old, in which higher dose of cetrorelix is associated with a higher implantation rate and a higher clinical pregnancy rate. This discrepancy is likely caused by the differences of cetrorelix sensitivity between young and elder patients. It is plausible to assume that young patients are more sensitive to cetrorelix, while elder patients are less sensitive. In support of this assumption, we detected almost 1-fold higher LH levels at the HCG day in young patients as compared with that of elder patients in the 0.125 mg group (6.59 ± 4.32 vs. 3.33 ± 1.96, **Table 4**), further supporting that certain levels of LH at HCG day are essential for implantation. On the contrary, a slightly higher LH levels were noted in elder patients as compared with that of young patients in the 0.25 mg group (3.64 ± 1.75 vs. 2.05 ± 1.22, **Table 4**). These results are actually in consistent with the observation that elder patients in the 0.25 mg group manifested higher rates for implantation and clinical pregnancy as compared with that of elder patients in the 0.125 mg group. Together our data support that a daily dose of 0.125 mg cetrorelix would be beneficial for young patients, while 0.25 mg/day of cetrorelix are probably more conducive to embryo implantation for elder patients.

In summary, we have demonstrated evidence supporting that the optimal dose for GnRH antagonist, cetrorelix, is an important factor relevant to higher implantation and clinical pregnancy rate. In general, a daily dose of 0.125 mg cetrorelix is optimal for Chinese

women during ovarian stimulation. Specifically, daily administration of 0.125 mg cetrorelix for patients < 35 years old is associated with a 3-fold higher implantation rate and a 5-fold higher clinical pregnancy rate as compared with that of those patients ≥ 35 years old. In contrast, higher rates for implantation and clinical pregnancy were noted by daily injection of 0.25 mg cetrorelix in elder patients (≥ 35 years old) as compared with that of young patients (< 35 years old). All together, our data support that a daily dose of 0.125 mg cetrorelix could be more optimal for young Chinese women, while administration of 0.25 mg/day of cetrorelix could be more conducive to a successful IVF-ET treatment for those patients ≥ 35 years old.

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Disclosure of conflict of interest

The authors declare no competing financial interests. All authors have read and agreed the content within the manuscript.

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