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Original Article

Low-dose GnRH antagonist protocol is as effective as the long GnRH agonist protocol in unselected patients undergoing *in vitro* fertilization and embryo transfer

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Abstract

Objective: The present retrospective and controlled comparative study was designed to evaluate the pregnancy rate achieved using a modified, fixed, multiple-dose 0.125 mg gonadotropin-releasing hormone (GnRH) antagonist protocol with the long GnRH agonist protocol as the control group.
Materials and methods: One hundred and twenty unselected women between 30 and 40 years of age, in their first cycle of IVF/ICSI, with a baseline follicle-stimulating hormone (FSH) <10 IU and an antral follicle count >3 were assigned into two groups: (1) the study group received 0.125 mg of cetrorelix daily starting on Day 6 of stimulation; and (2) the control group received leuprolide daily starting in the mid-luteal phase of the preceding cycle. Both groups were given a flexible dose of recombinant FSH for stimulation. An ongoing pregnancy rate of more than 12 weeks was the primary outcome measure of the study.
Results: Primary and secondary outcomes were comparable in both groups. A shorter duration of stimulation, a lower dosage of recombinant FSH consumption and a thinner endometrium on the day of human chorionic gonadotropin administration were all observed in the GnRH antagonist group.
Conclusion: A dosage of 0.125 mg GnRH antagonist protocol was effective for these unselected patients during IVF/ET.

Keywords: GnRH antagonist; Long protocol; Modified flexible multiple-dose regimen; Pregnancy rate

Introduction

A long protocol using a gonadotropin-releasing hormone (GnRH) agonist suppressing pituitary function to prevent a premature LH surge has been used commonly to stimulate ovulation. However, oversuppression of the pituitary function may result in a higher overall dose of gonadotropin and a longer duration of stimulation [1], especially for those women who respond poorly to reproductive treatment. A decade ago, the role of antagonists in stimulation of ovulation was evaluated [2]. The advantages of a GnRH antagonist are that (1) a shorter time is required to prevent the LH surge [3]; (2) there is a reduced requirement for exogenous gonadotropin; and (3) there is a shorter duration of stimulation [4]. For these reasons, many studies have aimed to evaluate the role of antagonists in stimulation of ovulation for poor responders, i.e., those women with repeated failures or poor responses in previous ovulation-stimulation cycles. The results have been encouraging [4–6].

Moreover, the role of GnRH antagonists in normal responders or in non-selected patients is still under debate because there was a lower rate of clinical pregnancy and fewer oocytes were retrieved [7]. The necessity of further studies aimed at more flexible GnRH antagonist regimens, such as the timing of initiation and the dosage of the GnRH antagonist, as well as the timing of the human chorionic gonadotropin

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(hCG) in the antagonist protocol has been discussed. Therefore, we designed a retrospective comparative study to assess whether the GnRH antagonist protocol is suitable for unselected patients. The demographics, protocol pattern, laboratory results and pregnancy outcome were analyzed.

Materials and methods

The study was performed between January 2008 and July 2009. Women between 30 and 40 years of age, in their first cycle of *in vitro* fertilization (IVF) or intracytoplasmic semen injection (ICSI), with a basal follicle-stimulating hormone (FSH) <10 IU and a total early antral follicle count >3 were enrolled in this study. Patients were excluded from this study if: (1) they had undergone oocyte cryopreservation due to a medical condition; or (2) ICSI was performed for preimplantation diagnosis (or exclusion) of inherited disease.

Protocol design

GnRH agonist, 0.5–1 mg/day, (Lupron, Takeda, Osaka, Japan) was administered subcutaneously in the mid-luteal phase of the menstrual cycle in the GnRH agonist group. Patients received recombinant FSH (r-FSH) (Gonal-F, Serono, International, Geneva, Switzerland or Puregon; N.V. Oganon, Oss, Netherlands) in a variable dosage until the day of hCG administration after downregulation of the pituitary function, with a serum E2 level <45 pg/mL. In the GnRH antagonist group, 0.125 mg/day of GnRH antagonist (Cetrotide; Serono International, Geneva, Switzerland) was administered intramuscularly on Day 6 of stimulation until the day of administration of hCG.

All patients received r-FSH in variable dosages and durations based on the ultrasound appearance of the follicles and the serum estradiol (E2) levels during the stimulation of ovulation. Serial transvaginal ultrasonography was arranged to monitor the follicular growth and endometrial thickness every 2 to 3 days after beginning the stimulation cycle. The serum level of E2 was checked on the day of ultrasonography. When the leading follicle reached a diameter of 17 mm, 10000 IU hCG (Ovidrel, Serono International, Geneva, Switzerland) was administrated to trigger ovulation. Oocyte retrieval was performed 36 hours later. Retrieved oocytes were graded as mature if a corona radiata and the first polar body were visible. Oocytes were inseminated with either IVF or ICSI, according to clinical needs. No more than four embryos were transferred on Day 3 after oocyte retrieval. A urine pregnancy test was performed 2 weeks after the embryo transfer. For those patients with a negative pregnancy test result, serum hCG levels were checked to confirm the result. For those with a positive urine pregnancy test, an ultrasound scan was carried out 3 weeks later.

Data analysis

Results were presented as the mean ± standard deviation. A *t* test and a χ^2 test were used to calculate the *p* value, as appropriate. A two-sided *p* value of <0.05 was considered statistically significant. Data were analyzed using the commercially available software package SPSS (SPSS, Inc., version 14, Chicago, IL, USA).

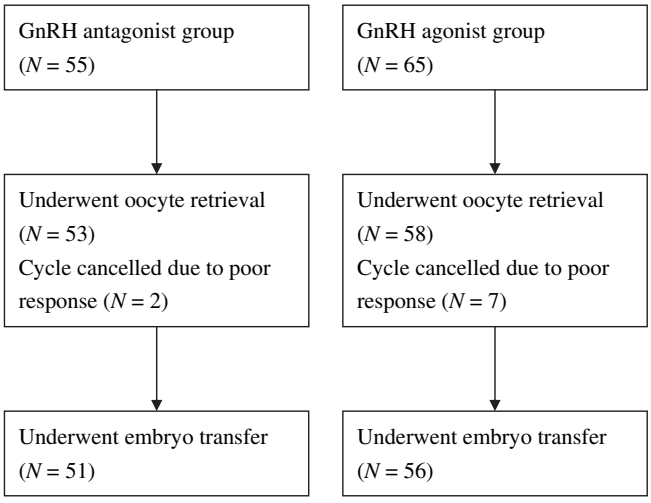

Fig. 1. Study flow chart and outcomes.

The primary outcome measures were the ongoing pregnancy rate. Secondary outcome measures were cycle cancellation rate, fertilization rate, implantation rate and the incidence of ovarian hyperstimulation syndrome (OHSS).

Results

Fig. 1 depicts the study flow chart. A total of 55 patients in the GnRH antagonist protocol and 65 patients in the long GnRH agonist protocol were enrolled for analysis. The rate of cancellation of cycles in the GnRH antagonist group was less than that in the GnRH agonist group, but the data did not achieve statistical significance (0.04 in the antagonist group vs. 0.11 in the agonist group, $p = 0.14$). Fifty-one patients in the antagonist group and 56 patients in the agonist group underwent embryo transfer.

Table 1 shows the demographic characteristics of both groups. All were on their first cycle of IVF-ET cycle. The patient's age, baseline FSH level, early antral follicle count, duration of infertility and body mass index were similar in both groups. Infertility causes were mainly combined factors (22%), tubal factors (25%) and male factors (21%) in the antagonist group, and male factors (43%) and tubal factors (24%) in the agonist group.

Table 2 shows stimulation and cycle outcome. The average duration of r-FSH stimulation in the antagonist group was

Table 1
Patient demographics.

	Antagonist group	Agonist group	*p* values[a]
Age (y)	34.6 ± 4.3	33.8 ± 3.5	0.27
Duration of infertility (y)	4.1 ± 3.0	3.2 ± 2.3	0.24
Body mass index (kg/m^2)	22.1 ± 3.0	23.6 ± 5.4	0.12
FSH on days 2–3 (IU/L)	7.2 ± 2.7	7.4 ± 2.7	0.76
Antral follicle count	7.6 ± 3.4	8.5 ± 4.1	0.17

Note: values expressed as mean ± SD.
[a] Calculated by *t* test.

Table 2
Stimulation and cycle outcomes.

	Antagonist group	Agonist group	p values[a]
Duration of stimulation (d)	8.5 ± 1.3	9.0 ± 1.7	0.03
Total FSH dosage (IU)	1697.3 ± 547.4	1922.8 ± 561	0.03
Endometrial thickness (cm) at hCG day	1.0 ± 0.2	1.1 ± 0.2	0.02
E2 (pg/mL) at hCG day	1688.7 ± 1030.1	1929.0 ± 1248.1	0.28

Note: values expressed as mean ± SD.
[a] Calculated by t test.

shorter (8.5 ± 1.3 days vs. 9.0 ± 1.7 days, $p = 0.03$), the average total r-FSH dosage was less (1697.3 ± 547.4 IU vs. 1922 ± 561.0 IU, $p = 0.03$), and the endometrium on hCG day was thinner (1.0 ± 0.2 cm vs. 1.1 ± 0.2 cm, $p = 0.02$) than that of the agonist group. The serum E2 level on the day of hCG administration was lower in the antagonist group, but the difference did not achieve statistical significance.

Table 3 shows the laboratory and pregnancy outcomes. In the antagonist group, the mean number of retrieved oocytes (10 ± 6.5 vs.12.6 ± 8.2, $p = 0.08$) and the mean number of mature oocytes (7.8 ± 4.8 vs. 9.9 ± 6.5, $p = 0.06$) were not significantly less than in the agonist group. The fertilization rate in both groups was similar (49% in the antagonist group vs. 51% in the agonist group, $p = 0.69$). The mean number of transferred embryos was 2.8 (±0.9) in the antagonist group and 3.0 (±1.0) in the agonist group ($p = 0.38$). The implantation rate in the antagonist group was 21% as compared to 23% in the agonist group ($p = 0.69$). The overall pregnancy rate per cycle (42% vs. 40%), the pregnancy rate per oocyte retrieval (43% vs. 45%), the pregnancy rate per embryo transfer (45% vs. 46%) and the ongoing pregnancy rate per embryo transfer (39% vs. 36%) are shown in Table 3. There was no statistically significant difference between the two groups in these outcomes. The incidence of OHSS was 0.04 in the antagonist group and 0.05 in the agonist group, and there was no statistically significant difference ($p = 0.79$).

Discussion

To the best of our knowledge, our study was the first to evaluate the clinical efficacy of 0.125 mg GnRH antagonist in a modified, fixed, multiple-dose protocol for an unselective population. The results showed that this protocol had comparable pregnancy rates to the GnRH agonist protocol.

Table 3
Laboratory and pregnancy outcome.

	Antagonist group	Agonist group	p values[a]
No. of oocytes retrieved	10.0 ± 6.5	12.6 ± 8.2	0.08
No. of mature oocytes	7.8 ± 4.8	9.9 ± 6.5	0.06
Fertilization rate	49%	51%	0.69
No. of transferred embryos	2.8 ± 0.9	3.0 ± 1.0	0.38
Implantation rate	0.21	0.23	0.69
Pregnancy rate per oocyte retrieval	43%	45%	0.88
Pregnancy rate per embryo transfer	45%	46%	0.89
Ongoing pregnancy rate per embryo transfer	39%	36%	0.71

Note: values expressed as mean ± SD.
[a] Calculated by t test or χ^2 test.

The pharmacodynamic effect of a 0.125 mg GnRH antagonist was shown to be similar to that of a 0.25 mg dose, but the serum hormone levels were suppressed more profoundly in the latter group [8]. Doses of GnRH antagonist that were too low resulted in an increased incidence of premature LH surge [9]. Consequently, the recommended dose of GnRH was at least 0.25 mg. Recently there was a report addressing the use of 0.125 mg GnRH antagonist in a controlled ovarian hyperstimulation/intrauterine insemination, and the clinical results were satisfactory [10]. In this study we also tried to evaluate the clinical effect of 0.125 mg GnRH antagonist, and our results showed a compatible retrieved oocyte number and matured oocyte number between the 0.125 mg GnRH antagonist group and the GnRH agonist group, and the pregnancy rates were also comparable in both groups. In our study, we could not exclude the phenomenon of premature LH surge, since we did not monitor the serum LH and progesterone levels during the ovulation stimulation and on hCG day. Based on the fact that an adequate number of matured oocytes were retrieved in the GnRH antagonist group and the morphological observation of the oocytes through microscopy, we did not find evidence showing either premature luteinization or the premature rupture of follicles in our patients.

Possible explanations for these results could be that (1) our study population was all Asian women with thin frames (BMI: 22.1); and (2) the hCG was administered earlier, when the dominant follicle reached 1.7 cm in diameter, before the clinical rise of the LH. In addition, the body weight, rather than the body mass index (BMI), was the most common parameter for determining the dosage in routine pharmacokinetic studies. Patients with the same BMI but different body heights may have very different body weights. The significant body weight difference between Asian and Western women explains our reason for choosing the 0.125 mg daily dose of cetrorelix in this study. We suggest that a 0.125 mg daily dose of cetrorelix could achieve comparable treatment outcomes without inducing premature luteinization in patients with body weights less than 56 kg, or with a BMI less than 22 kg/m^2. Further randomized prospective study to evaluate the efficacy of a 0.125 mg GnRH antagonist is absolutely necessary to establish the clinical value of this protocol.

Our study showed two clinical advantages of the GnRH antagonist protocol: (1) the stimulation duration was shorter; and (2) there was less usage of r-FSH. Current consensus is that a higher dose leads to the retrieval of more oocytes but similar pregnancy rates in standard patients (younger than 40 years of age, having two ovaries, a normal menstrual cycle and a normal basal FSH level) [11]. The recommended dose of FSH was 100 to 250 IU/day for the standard population in the agonist protocol and 150 IU/day or 200 IU/day in the GnRH antagonist cycles [12]. An increased dose did not compensate for the age-related decline in ovarian function. Based on this reasoning, both the antagonist group and the agonist group were treated with the recommended dose of r-FSH in our study and showed comparable outcome. Shorter stimulation duration

was not only friendlier to the patients receiving the treatment, but also decreased their economic burden. The average cost of medication during ovulation stimulation in patients accepting the GnRH antagonist protocol was 625 USD as compared to 750 USD in the agonist group. More specific analysis should be done in the future on the cost effectiveness of this modified, fixed, multiple-dose 0.125 mg GnRH antagonist protocol.

Endometrial thickness was thinner on hCG day during the GnRH antagonist cycle in our study. Characteristics of the endometrium measurable by ultrasound, such as endometrial thickness, played a role in implantation, and had a strong negative value in setting some minimal criteria in the artificial reproductive technique protocol [13]. An endometrium thickness of less than 0.8 cm was believed to relate to a low rate of pregnancy. The etiology of decreased endometrial thickness in the antagonist protocol was unclear. We could not prove that decreased endometrial thickness had any effect on the pregnancy rate, but caution should be exercised in the use of a GnRH antagonist in those patients with a poor endometrial condition, history of repeated induced abortion, or scant menstrual flow.

A lower E2 serum level on hCG day in the GnRH antagonist protocol has been described in previous studies [14−16]. Endometrial receptivity is a crucial factor in determining the success of IVF. A high estradiol concentration on the day of hCG administration is detrimental to uterine receptivity [17]. Excessively high estradiol concentration has been associated with the dyssynchrony of endometrial glands and stroma, representing a suboptimal environment for implantation [18]. *In vitro* study has also demonstrated that a high level of estradiol itself has a direct toxic effect on the embryo by affecting the cleavage stage [19]. Therefore, we might assume that a lower E2 level may contribute to a better treatment outcome using the antagonist protocol. In our study, however, the E2 level on hCG day in the GnRH antagonist group was lower than in the agonist group, but this difference did not reach statistical significance. The limited number of cases and the standard r-FSH dosage for the unselective general population in our study were two key factors in explaining the difference. In addition, our study design was retrospective, rather than prospective and randomized. Both of these factors affected the strength of this study.

In conclusion, the 0.125 mg GnRH antagonist modified, flexible, multiple-dose protocol had a pregnancy rate comparable to that of the GnRH agonist group in our unselected population. This dose reduced the consumption of r-FSH, shortened the duration of treatment and proved to be a good protocol for an unselected population aged between 30 and 40 years at their first IVF-ET cycle. Adverse effects on the endometrium should be considered when treating the subfertile patient with a possible unfavorable endometrial condition.

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