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Efficacy and Safety of gonadotropin-releasing hormone (GnRH) Agonists Triptorelin Acetate and Cetorelix Acetate in Assisted Reproduction

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Authors' Contribution:
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Data Collection B
Statistical Analysis C
Data Interpretation D
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Background: The aim of this study was to compare the efficacy and safety of 2 GnRH agonists – triptorelin acetate and cetorelix acetate – in assisted reproduction.





Material/Methods: A total of 182 females who received *in vitro* fertilization and embryo transfer (IVF+ET) from March 2014 to July 2014 were involved, and their clinical data were retrospectively analyzed. Among them, 91 patients received treatment with short-acting triptorelin (group A) and another 91 patients were treated with cetorelix acetate (group B). Fasting blood was extracted from each patient on the day of administration of human chorionic gonadotropin (hCG), and serum levels of luteinizing hormone (LH), estradiol (E2), and progesterone (P) were detected using chemiluminescence method. The number of oocytes, fertilization rate, cleavage rate, and number of obtained embryos were recorded and compared. Pregnancy outcomes and adverse events were observed and compared. Expression level of FSH receptor (FSHR) in endometrial tissues was measured by qRT-PCR.

Results: Serum level of E2 was significantly lower in group B than in group A ($p < 0.05$). Indices, including the number of oocytes, fertilization rate and cleavage rate, number of obtained embryos, and pregnancy rate, were slightly better in group B than in group A, but no significant differences were found. The incidence of ovarian hyperstimulation syndrome (OHSS) was significant higher in group A than in group B ($p < 0.05$). FSHR expression level was significantly lower in group B than in group A.

Conclusions: The effect of cetorelix acetate is superior to that of short-acting triptorelin in assisted reproduction. Our most important finding is that cetorelix acetate reduced the incidence of OHSS.

MeSH Keywords: Embryo Transfer • Fertilization *In Vitro* • Receptors, FSH • Triptorelin Pamoate

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Background

Although no significant changes in the global prevalence of infertility have been observed during the last several decades, the number of couples affected by infertility increased from 42.0 million in 1990 to 48.5 million in 2010 due to population growth [1]. In China, it has been reported that infertility affects about 25% of couples of reproductive age [2]. With a relatively high success rate, *in vitro* fertilization-embryo transfer (IVF-ET) has become more and more popular in the clinical treatment of various types of infertility, and factors that affect the outcomes have also been identified [3–5]. However, the clinical popularization of this technique is still challenged by embryo quality, current available embryo transfer techniques, adverse events, and other factors that can affect embryo transfer [6,7]. The present study focussed on improving the production of oocytes and embryos and reducing the incidence of adverse events.

With the ability to regulate the production and secretion of key sexual hormones, such as luteinizing hormone (LH) and follicle stimulating hormone (FSH), triptorelin acetate, which is a gonadotropin-releasing hormone (GnRH) agonist, has been widely used in assisted reproduction [8]. However, the use of this drug may cause allergic reactions as well as increased blood glucose level and urinary tract infection [9]. In addition, triptorelin acetate may increase the risk of ovarian hyperstimulation syndrome (OHSS) [10], which is a serious complication of IVF+ET [11,12]. Another GnRH antagonist – cetrorelix acetate – has also been widely used to improve pregnancy outcomes [13]. This study aimed to compare the efficacy and safety of triptorelin acetate and cetrorelix acetate in assisted reproduction.

Material and Methods

Patients

A total of 182 women who received *in vitro* fertilization and embryo transfer (IVF+ET) at the Center for Reproductive Medicine, Shandong University, from March 2014 to July 2014 were involved, and their clinical data were retrospectively analyzed. Inclusion criteria were: (1) normal menstrual cycle; (2) normal levels of FSH, LH and E2; (3) without contraindications to IVF+ET; (4) fresh transplantation cycle; and (5) signed informed consent. Exclusion criteria were: (1) polycystic ovary syndrome; (2) severe endometriosis (stage III and above); (3) severe liver and kidney dysfunction; and (4) allergic to drugs used in this study. Those patients were randomly divided into 2 groups: 91 patients received treatment with short-acting triptorelin (group A) and the other 91 patients were treated with cetrorelix acetate (group B). Treatment was performed in a double-blinded manner. No significant differences in general clinical data were found between the 2 groups ($p>0.05$). This study was approved by the Ethics

Committee of the Center for Reproductive Medicine, Shandong University. No significant differences in general information were found between group A and B (see Table 1 for details).

Treatment methods

Patients in group A were subcutaneously injected with 225 IU recombinant human FSH (rFSH) (approval number: JS20040042, Merck Serono, Merck Switzerland) from the third day of the menstrual cycle to follicle maturation. Subcutaneous injection of triptorelin acetate (0.05mg, approval number: JX20090203, Ferring Pharmaceuticals Ltd., Netherlands) was started in the mid-luteal phase of the previous menstrual cycle (day 21) daily until the injection of human chorionic gonadotropin (hCG) (approval number: X20000133, Merck Serono, Merck Switzerland). In group B, the ovulation induction program was the same as that in group A. Cetrorelix acetate (Baxter Oncology GmbH, Germany) was used instead of triptorelin acetate from the fifth day after the use of rFSH until the injection of HCG.

Three injections of HCG (2000 U/injection, Merck Serono, Germany) were performed when 3 follicles in both ovaries had an average diameter ≥ 20 mm. Eggs were harvested 36 h later and IVF-ET was routinely performed.

Observation indexes

Fasting venous blood (20 ml) was extracted from each patient on the day of the injection of hCG. Serum samples were prepared and serum level of LH, E2, and P were measured by chemiluminescence method. Endometrial thickness was measured by gray-scale ultrasonography. The number of oocytes, fertilization rate, cleavage rate, and number of obtained embryos were recorded and compared. Pregnancy outcomes and adverse events were observed and recorded. Fertilization and embryo evaluation were performed.

QRT-PCR

Trizol reagent (Invitrogen, USA) was used to extract total RNA from endometrial tissues, which were obtained before and after the treatment. RNA samples were tested using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, USA), and the samples with a A260/A280 ratio between 1.8 and 2.0 were used to synthesize cDNA through reverse transcription. The following primers were used in PCR reactions: 5'-TCGCCCTGCACGTGCAGCAATAAC-3' (forward) and 5'-CATAACAGGACCAGCGATGTGAGTG-3' (reverse) for FSHR; GACCTCTATGCCAACACAGT (forward) and AGTACTTGCCTCAGGAGGA (reverse) for β -actin. PCR reaction conditions were: 95°C for 30 s, followed by 40 cycles of 95°C for 12 s and 60°C for 32 s. Data were analyzed using 2^{- $\Delta\Delta$ CT}

Table 1. Comparison of general information.

Groups	Cases	Age (y)	Serum FSH (U/L)	Serum LH (U/L)	FSH/LH
A	91	26–39 (29.09±4.48)	6.48±1.19	5.38±3.73	1.2±1.03
B	91	29–37 (30.56±4.18)	6.43±1.38	5.33±2.72	1.21±0.98

Table 2. Comparison of the levels of hormones between 2 groups on the day of the injection of hCG.

Groups	Cases	LH (U/L)	E2 (pg/ml)	P (ng/ml)
A	91	2.12±1.07	4041.33±2240.23	0.91±0.34
B	91	2.33±0.88	3181.81±1720.34*	1.06±0.28

* Compared with group A, $p < 0.05$.

Table 3. Comparison of pregnancy outcomes between 2 groups.

Groups	Cases	Number of oocytes	Fertilization rate (%)	Cleavage rate (%)	Number of obtained embryos	Pregnancy rate (%)	Endometrial thickness (mm)
A	91	13.13±6.23	63.00 (751/1192)	81.36 (611/751)	4.12±2.13	54.95 (50/91)	1.09±0.20
B	91	14.32±6.64	65.33 (863/1321)	82.86 (551/665)	4.51±2.72	58.24 (53/91)	1.08±0.24

Table 4. Comparison of incidences of adverse event between 2 groups.

Groups	Cases	Rash	Erubescence	OHSS	Abortion	Total
A	91	1 (1.10)	1 (1.10)	4 (3.30)	2 (2.20)	8 (8.8%)
B	91	1 (2.20)	2 (1.10)	0 (0)*	3 (3.30)	7 (7.7%)

* Compared with group A, $p < 0.05$.

method. Relative expression level of FSHR was normalized to endogenous control β -actin.

Statistical analysis

SPSS19.0 (SPSS, Inc., USA) was used for all statistical analysis. All measurement data are expressed as ($x \pm s$), and comparisons between 2 groups were performed by t test. Count data were assessed by chi-square test. $P < 0.05$ was considered to be statistically significant.

Results

Comparison of the levels of hormones between the 2 groups on the day of the injection of hCG

No significant differences in serum levels of LH and P were found between the 2 groups ($p > 0.05$), while serum level of E2 was significantly lower in group B than in group A ($p < 0.05$) (see Table 2 for details).

Comparison of pregnancy outcomes between the 2 groups

There were slightly more oocytes and obtained embryos in group B than in group A. Fertilization rate, cleavage rate, and pregnancy rate were also slightly higher in group B than in group A. However, no significant differences in those indices were found between the 2 groups ($p > 0.05$). In addition, no significant differences in endometrial thickness were found between the 2 groups ($p > 0.05$) (see Table 3 for details).

Comparison of adverse event incidences between the 2 groups

As shown in Table 4, no significant differences in incidences of rash, erubescence, or abortion were found between group A and B ($p > 0.05$). The total incidence of adverse events in group A was not significantly different from that in group B ($p > 0.05$). However, the incidence of ovarian hyperstimulation syndrome (OHSS) was significantly higher in group A than in group B ($p < 0.01$), and no patients in group B were affected by this disease. OHSS diagnostic criteria were: 1) ovarian

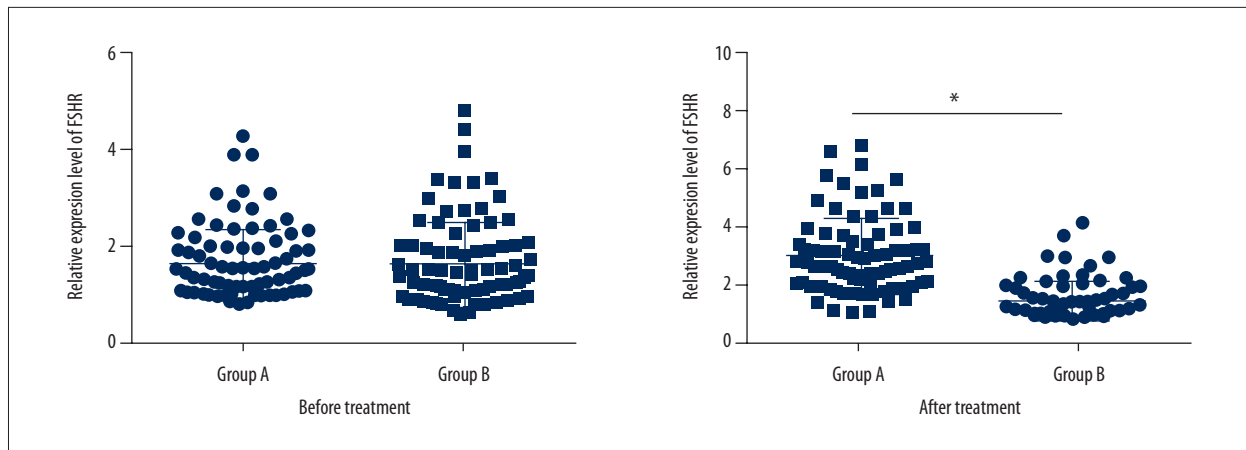


Figure 1. Expression level of FSHR in endometrial tissues of the 2 groups before and after treatment. * Compared with group A, $p < 0.05$.

enlargement with multiple corpus luteum, visible peritoneal pleural effusion; and 2) increased hematocrit and white blood cell count, hyponatremia, and hypoproteinemia.

Comparison of expression level of FSHR between 2 groups

Expression levels of FSHR in endometrial tissues collected from patients were measured by qRT-PCR. As shown in Figure 1, no significant differences in expression level of FSHR in endometrial tissues were found between the 2 groups before treatment ($p > 0.05$). However, after treatment, expression levels of FSHR were significantly lower in group B than in group A ($p < 0.05$).

Discussion

In the use of IVF-ET, a luteinizing hormone (LH) surge may occur before the diameter of the leading follicle reaches the optimum size, and these premature LH surges inhibit the induction of multiple follicular maturation in many women [14]. GnRH agonists (GnRHa) play an important role in inhibiting the occurrence of premature LH surges by reversibly blocking the secretion of pituitary gonadotrophin. With the effects of GnRH agonists, cancellation of assisted conception cycles is inhibited and the pregnancy rate is increased [14]. As a type of GnRH agonist, triptorelin acetate has been widely used in assisted reproduction [15]. Cetrorelix acetate is also a commonly used GnRH antagonist for the treatment of different pregnancy-related diseases [16]. Our study shows that cetrorelix acetate is more effective in reducing serum level of E2 than is triptorelin acetate. However, the mechanism underlying the stronger ability of cetrorelix acetate to reduce E2 levels remains unclear and warrants further investigation.

As a receptor of FSH, FSHR mediates FSH signaling, and FSH gene polymorphisms are closely correlated with infertility [14].

In addition, abnormal expression patterns of FSHR were observed in women with infertility compared to normal women [18]. Therefore, controlling FSHR is a major challenge in treatment of infertility. Triptorelin acetate and cetrorelix acetate have been shown to regulate the expression of FSHR [19]. However, our study shows that cetrorelix acetate is superior to triptorelin acetate in inhibiting the expression of FSHR, which also explains the slightly improved indexes, including the number of oocytes, fertilization rate, cleavage rate, number of obtained embryos, and pregnancy rate, in patients treated with cetrorelix acetate compared to patients treated with triptorelin acetate. Our results may provide guidance for the selection of these 2 drugs for controlling FSHR expression.

OHSS is one of the most serious consequences of induction of ovulation, and it is common in women who received IVF+ET [11,12]. Triptorelin acetate has been widely used to prevent this disease [10], but its efficiency is unsatisfactory. In our study, OHSS occurred in 4 women who received triptorelin acetate treatment, which accounts for 1/30 of all patients. However, OHSS was not observed in patients treated with cetrorelix acetate. These data suggest that cetrorelix acetate can effectively inhibit the occurrence of OHSS in patients who underwent IVF+ET. Rash, erubescence, and abortion are also commonly observed in patients treated with IVF+ET [20]. However, in the present study, no significant differences in the incidences of those complications were found between patients treated with triptorelin acetate and those treated with cetrorelix acetate. Therefore, our future research will focus on reducing the occurrence of rash, erubescence, OHSS, and abortion after IVF+ET. However, we failed to characterize the molecular mechanisms underlying the actions of cetrorelix acetate, which is also another direction of our future studies.

Conclusions

Triptorelin acetate and cetrorelix acetate showed no significant differences in reducing serum levels of LH and P, but cetrorelix acetate is superior to triptorelin acetate in reducing serum level of E2 and expression level of FSHR. Compared with triptorelin acetate, cetrorelix acetate was slightly better at improving indexes, including the number of oocytes, fertilization rate,

cleavage rate, number of obtained embryos, and pregnancy rate. Therefore, we conclude that the efficacy of cetrorelix acetate is superior to that of short-acting triptorelin in assisted reproduction. One of our most important findings is that cetrorelix acetate reduced the incidence of OHSS. Our study is limited by its small sample size. Future studies with larger sample sizes are needed to confirm our conclusions.

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