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Original Article

Comparison of the GnRH agonist and antagonist protocol on the same patients in assisted reproduction during controlled ovarian stimulation cycles

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Abstract: Despite the fact that both gonadotropin-releasing hormone (GnRH) agonist and antagonist protocol are effective in suppressing the incidence of premature luteinizing hormone (LH) surges through reversibly blocking the secretion of pituitary gonadotropins, the exact impact of these two distinctive protocols on the clinical setting of patients for in vitro fertilization and embryo transfer (IVF-ET) treatment, however, remained controversial. We thus in the present report conducted a retrospective study to compare the impact of GnRH agonist and antagonist protocol on the same patients during controlled ovarian stimulation cycles. A total of 81 patients undergoing 105 agonist and 88 antagonist protocol were analyzed. We failed to detect a significant difference between two protocols for the difference in duration of ovarian stimulation, number of recombinant FSH (Gonal-F) ampoules used, number of oocytes retrieved, serum levels for estradiol (E2) and progestone (P), thickness of endometrium, and the zygote- and blastocyst-development rate. It is seemly that high quality embryo rate was higher in the antagonist protocol, but the data did not reach a statistical significance. Nevertheless, Implantation rate and clinical pregnancy rate were significantly higher in the antagonist protocol (10.64% and 30.26%, respectively) than that of the agonist protocol (5.26% and 15.82%, respectively). Our data also suggest that the GnRH antagonist protocol is likely to have the advantage for improving the outcome of pregnancy in those patients with a history of multiple failures for the IVF-ET treatment.

Keywords: Gonadotropin-releasing hormone (GnRH), agonist, antagonist, in vitro fertilization, embryo transfer, assisted reproduction, controlled ovarian stimulation cycles

Introduction

It has been well known that ovarian stimulation is an important factor relevant to the success of in vitro fertilization and embryo transfer (IVF-ET) treatment. Therefore, gonadotropin releasing hormone (GnRH) agonist protocol has been developed and employed in the setting of IVF-ET treatment ever since 1980s. The GnRH agonist protocol is designed to suppress the release of pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by desensitizing the pituitary receptors [1, 2]. In late 1990s, the GnRH antagonists have also been found effective for ovarian stimulation by directly binding to the GnRH receptors, and through which they block GnRH receptor activity in a competitive manner [1] and induce an immediate, reversible, and rapid suppression of gonadotropin release [1-4]. As a result, the GnRH antagonist protocol has also been widely employed recently in the clinical settings of women with IVF-ET treatment [5].

GnRH agonists and antagonists are peptides containing 10 amino acids [6]. Despite the fact that GnRH agonist protocol is accompanied by some disadvantages, it has become widely used in clinical IVF-ET treatment, and its application is associated with an increase in the rate of pregnancy [7]. Recently, the development of GnRH antagonist protocol offered another approach for ovarian stimulation by blocking the pituitary receptors. There is evidence that

application of GnRH antagonist protocol decreases the duration of ovulatory stimulus and reduces the incidence of ovarian hyperstimulation syndrome [7-12]. While these observations are exciting and encouraging, controversial results have also been reported [13, 14]. To further address this question, we thus in the current report conducted a retrospective study to compare the impact of GnRH agonist and antagonist protocol on the same patients during controlled ovarian stimulation cycles. We failed to detect a significant difference between the two protocols in terms of the duration for ovarian stimulation, the number of oocytes retrieved, and the rate for fertilization. However, there is suggestive evidence that the GnRH antagonist protocol could possess the advantage over the agonist protocol for improving the outcome of pregnancy in those patients with a history of multiple failure of IVF-ET treatment.

Materials and methods

Subjects

A total of 81 patients and 193 cycles between April 2010 and October 2012 were included for the study. Each of the patients had at least once for the agonist long protocol and once for the antagonist protocol. All patients had regular menstrual cycles (27-33 days), and were undergoing treatment for infertility due to tubal, endometriosis, male and unexplained or mixed factors. In addition, all patients did not process ovarian stimulation 3 months prior to this cycle and did not receive oral contraceptive pill (OCP) pretreatment before this cycle. Comparative study of the clinical and laboratory results was carried out between 105 agonist protocol cycles and 88 antagonist protocol cycles. Consent forms were obtained from all subjects and the studies were approved by the Tongji Hospital Human Assurance Committee.

The GnRH agonist long protocol

All patients undergone GnRH agonist long Protocol were processed for pituitary down-regulation on luteal peak period with triptorelin injection for 14 days. A basic evaluation was then conducted by ultrasound examination and blood test for hormone levels. Medication was then initiated with recombinant FSH (rFSH) (Gonal-F, EMD Serono) at the day of ultrasound examination, in which younger patients (< 35 years old) were prescribed for two ampoules (150 IU) of Gonal-F daily, and elder patients (≥ 35 years old) were administered for three ampoules (225 IU) of Gonal-F daily. The dose was fixed for the first 5 days of stimulation. After 5 consecutive days of medication, transvaginal ultrasound B examination was next performed to monitor the development of follicles, and the dose of rFSH was optimally adjusted based on the number and size of developing follicles.

The GnRH antagonist protocol

On day 3 of menstrual period, a basic evaluation was conducted by ultrasound examination. Medication was then initiated with recombinant FSH (rFSH) (Gonal-F, EMD Serono) at the day of ultrasound examination as described above, in which younger patients (< 35 years old) were advised to take two ampoules (150 IU) of Gonal-F daily, and elder patients (≥ 35 years old) were arranged to take three ampoules (225 IU) of Gonal-F daily. Similarly, the dose was fixed for the first 5 days of stimulation, and after 5 consecutive days of medication, transvaginal ultrasound B examination was then carried out to monitor the development of follicles. Of note, the dose of rFSH was optimally adjusted based on the ultrasound B results for the number and size of developing follicles. The GnRH antagonist, cetrorelix, was next administered daily by s.c. injection (0.25 mg/d) in the morning (8:00-12:00 AM) from day 6 of the stimulation cycle to the day of human chorionic gonadotropin (HCG) administration. Additional transvaginal ultrasound B examinations were also performed post days 8, 10 and 12 of medication.

Procedures for oocyte retrieval

Gonal-F and cetrorelix were administered continuously until three follicles reached ≥ 17 mm. HCG (10,000 IU, EMD Serono) 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 (ECL2012, Siemens, Germany) as instructed. Oocytes were retrieved 34-38 h after HCG injection and were fertilized in vitro

Table 1. Basic demographic and clinical information for the studying subjects

Parameters	average
No. of patients	81
Age (years)	33.2 ± 4.0
BMI (kg/m^2)	20.9 ± 2.1
Basal FSH concentration (IU/l)	7.1 ± 2.7
Duration of infertility (years)	6.56 ± 4.3
Infertility (%)	
Primary	33 (40.7)
Secondary	48 (59.3)
Causes of infertility (%)	
Tubal factor	54 (66.7)
Male factor	13 (16.1)
Endometriosis	7 (8.6)
Unexplained	6 (7.4)
Mixed	1 (1.2)

BMI: body mass index; FSH: follicle stimulating hormone.

according to the standard procedures as previously reported [15].

Procedures for embryo transfer

Embryo transfer (ET) was carried out 72 h after oocyte retrieval. A maximum of three embryos were transferred into each patient. Progesterone (in oil) was i.m. administered daily (80 mg/day) from day 1 post oocyte retrieval to maintain luteal functionality. Clinical pregnancy was defined as elevated serum β-HCG 14 days after ET and the presence of gestational sac(s) by ultrasonography.

Statistical analysis

The SPSS 17.0 for windows was used for statistical 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 was used for statistical comparisons. Statistical significance was defined as $p < 0.05$.

Results

Demographic information and clinical characteristics for the subjects

All demographic information for the studying subjects is summarized in **Table 1**. The age for the patients ranges from 24 to 43 yr old with an average age of 33.2 ± 4.0 yr. The lowest body mass index (BMI) was noted 16 only, but the highest one reached 28.7 (average 20.9 ± 2.1). Among all 81 subjects recruited, 33 of which (40.7%) were present with primary infertility, and the rest 48 women (59.3%) were associated with secondary infertility. The mean duration of infertility was 6.56 ± 4.3 yr, ranging from 1 to 20 yr. Fifty-four women were diagnosed with tubal problems, 7 with endometriosis, 13 with male factors, and 1 with mixed causes. However, 6 of which failed to reach a confirmative diagnosis, and therefore, they were defined to the category with unexplained factors.

Comparison of the clinical and laboratory results

Clinical and laboratory results between the GnRH agonist long protocol and the antagonist protocol were compared and summarized in **Table 2**. The rFSH duration and dosage in GnRH agonist long protocol were slightly higher than that of the GnRH antagonist protocol, but without a statistical difference. Similarly, a slightly higher oestradiol (E2) was noted for the agonist long protocol as compared with that of the antagonist protocol (1447.21 ± 851.12 vs. 1356.01 ± 785.77) at the day of HCG administration. However, there was no difference for the progesterone level, endometrial thickness, the number of follicles with size ≥ 14 mm, the number of oocytes retrieved, fertilization rate, cleavage rate, and the number of embryos transferred between the two protocols. Nevertheless, higher rates for grade I/II embryos (49.3 vs. 45.8) and available embryos (81.4 vs. 77.7) were noted for the antagonist protocol as compared with that of the agonist long protocol. Of note, the cancellation rate for the antagonist protocol was slightly higher, in which 11 out of 105 cycles (10.48%) were cancelled in the GnRH agonist long protocol, but 12 out of 88 cycles (13.64%) were cancelled in GnRH antagonist protocol. The cancellation rate was defined by the poor quality of embryos and uterine bleeding after medication, while defi-

Table 2. Comparison of the clinical and laboratory results

Parameters	GnRH Agonist	GnRH Antagonist
Cycles	105	88
rFSH duration (days)	8.99 ± 1.58	7.94 ± 1.47
rFSH dosage (amp)	31.94 ± 12.04	26.48 ± 11.54
HCG day		
Oestradiolv (pmol/l)	1447.21 ± 851.12	1356.01 ± 785.77
Progesterone (ng/ml)	1.32 ± 0.79	1.32 ± 0.54
Endometrial thickness (mm)	10.86 ± 2.27	10.40 ± 2.29
≥ 14 mm follicles	5.58 ± 3.38	5.43 ± 3.21
oocytes retrieved	7.28 ± 5.27	7.39 ± 5.02
Fertilization rate (2PN) (%)	53.9	53.7
Cleavage rate (2PN) (%)	96.23	97.05
Grade I/II embryos	45.8	49.3
Available embryo rate (%)	77.7	81.4
No. of embryos transferred	2.25 ± 0.75	2.33 ± 0.72
Transfer cycles cancelled (%)	10.48 (11/105)	13.64 (12/88)

Available embryos including transferred embryos and frozen embryos.

Table 3. Comparison of the clinical pregnancy outcomes

Parameters	GnRH agonist	GnRH antagonist
Cycles	105	88
Biochemical pregnancy	15	26
Intrauterine pregnancy	7	16
Abortion	7	7
Ectopic pregnancy	3	0
Implantation rate (%)	5.26 (11/209)	15.82 (28/177)*
Clinical pregnancy rate (%)	10.64 (10/94)	30.26 (23/76)*

*P<0.05.

ciency for the number of sperms from husbands was excluded from this category.

Comparison for the outcomes of clinical pregnancy

Eighty-one patients were initiated for the IVF-ET treatment. One hundred five cycles were included in the GnRH agonist long protocol, 7 cases were identified with intrauterine pregnancy, but all pregnancies were stopped by abortion, and 3 cases were found with ectopic pregnancy. In contrast, among 88 cycles included in the GnRH antagonist protocol, 16 cases were identified with intrauterine normal pregnancy, only 7 of which underwent abortion (**Table 3**). In line with these results, the implantation rate (15.82% vs. 5.26%, $p < 0.05$) and clinical pregnancy rate (30.26% vs. 10.74%, $p < 0.05$) were significantly higher in the GnRH antagonist protocol than that in the GnRH agonist long protocol.

Comparison for the times of IVF-ET treatment and pregnancy rate

Fifty-two patients were treated with 1 time of GnRH agonist protocol, and unsuccessful patients were subsequently proceeded with 1 time of GnRH antagonist protocol treatment. Of interestingly note, the clinical pregnancy rate in patients with the first GnRH agonist long protocol was only 9.6%, but the clinical pregnancy rate reached 21.2% for those unsuccessful patients with the subsequent GnRH antagonist protocol treatment. Twenty-seven patients received 3 times of IVF-ET treatment, in which 46 cycles were included for the agonist long protocol, and 35 cycles were conducted for the antagonist protocol (**Table 4**). Remarkably, only 5 out of 46 cycles were identified with successful clinical pregnancy in patients received the agonist long protocol treatment, while 10 out of 35 cycles were identified with successful clinical pregnancy in patients proceeded with the antagonist protocol, and the pregnancy rate was significantly higher than that of the agonist long protocol (28.6% vs. 10.9%, $p < 0.05$, **Table 4**). It is noteworthy that two patients received 4 times of IVF-ET treatment including 3 times of GnRH agonist long protocol and 1 time of GnRH antagonist protocol. None of the 6 cycles in the agonist long protocol reached successful pregnancy, while these two patients displayed clinical pregnancy after receiving the antagonist protocol treatment (**Table 4**). Altogether, our data suggest that the antagonist protocol likely possesses advantage to reach successful pregnancy for those patients with a history of multiple failures of IVF-ET treatment.

Discussion

The enhancement of sensitivity for patients in response to controlled ovarian hyperstimula-

Table 4. Comparison of the IVF-ET treatment times and clinical pregnancy rate

IVF-ET Times	patients No.	GnRH agonist		GnRH antagonist	
		cycles	pregnancy rate	cycles	pregnancy rate
2	52	52	9.6% (5)	52	21.2% (11)
3	27	46	10.9% (5)	35	28.6% (10)*
4	2	6	0 (0)	2	100% (2)*

*P<0.05.

tion is a pivotal factor associated with successful clinical pregnancy during the IVF-ET treatment [16]. A poor ovarian response could be caused either by the idiopathic factors or other factors relevant to the condition of patient health such as the age, diminished ovarian reserve, endometriosis and prior ovarian surgery [17]. Our studies in the current report failed to detect a significant difference between the GnRH agonist long protocol and the antagonist protocol in terms of the duration of ovarian stimulation, number of recombinant FSH (Gonal-F) ampoules used, number of oocytes retrieved, serum levels for estradiol (E2) and progestone (P), thickness of endometrium, and the zygote- and blastocyst-development rate. However, implantation rate and clinical pregnancy rate were noted significantly higher in patients proceeded with the antagonist protocol (10.64% and 30.26%, respectively) as compared with that of patients proceeded with the agonist protocol (5.26% and 15.82%, respectively). Similarly, the GnRH antagonist protocol could be more efficient for improving the outcome of pregnancy in those patients with a history of multiple failures for the IVF-ET treatment.

In the process of controlled ovarian hyperstimulation (COH), GnRH agonists and antagonists are manifested by the inhibition of the endogenous luteinizing hormone (LH) peak, in which they specifically bind to the membrane receptors on pituitary cells. Upon binding to the receptors on pituitary cells, GnRH agonist stimulates copious amount of luteinizing hormone (LH) and follicle stimulating hormone (FSH) secretion, a phenomenon so-called "flame up effect" [18-20]. However, after 7-10 days of consecutive stimulation, GnRH receptors on pituitary cells are exhausted along with desensitization, which then suppresses FSH and LH release, a phenomenon called "down-regulation" [1, 2]. In contrast, GnRH antagonists possess a similar structure as the natural GnRH, and therefore, they function as a GnRH receptor blocker. Previous studies demonstrated that those GnRH antagonists can produce inhibitory effect on LH after 6-8 h of binding to the receptors [21, 22]. Since the GnRH antagonist protocol is simple, convenient and flexible along with the absence of functional ovarian cyst formation and "menopausal" symptoms commonly seen in the agonist protocol, it has become a preferential choice by clinical doctors and patients [23-25]. However, data from some randomized clinical trials revealed that the antagonist protocol retrieves less number of oocytes along with lower pregnancy rates than the agonist long protocol [26, 27]. More recently, some meta-analysis based studies failed to suggest a significant difference in terms of pregnancy outcomes between these two protocols [28, 29]. By studying the subjects in our dataset, we demonstrated suggestive evidence that the antagonist protocol may possess the advantage for improving the implantation rate and pregnancy rate over the agonist long protocol, particularly in those patients with a history of multiple failures of IVF-ET treatment. Given the fact that our dataset only contains limited number of patients, future studies with more subjects and stimulation cycles would be necessary to further confirm those observations.

As aforementioned, our dataset contains subjects with a history of multiple failures for the IVF-ET treatment including some poor ovarian responders, elder and polycystic ovary syndrome (PCOS) patients. All patients proceeded with the two distinctive protocols received similar clinical medication and displayed similar laboratory results and number of oocytes recruited. However, the rFSH duration and dose were slightly lower for the antagonist protocol over the agonist long protocol, which is consistent with the other reports [30]. Of note, the E2 level on HCG day in the antagonist protocol was slightly lower than the agonist protocol, which is probably due to that the antagonist repressed endogenous GnRH secretion in the follicle growth period [31]. Similarly, our quality embryo rate and available embryo rate in the antagonist protocol are slightly higher over the agonist

long protocol, suggesting that the antagonist protocol may have the advantage for improving embryo quality in patients with repeated failures in IVF-ET agonist treatment [32]. In line with the data reported by Takahashi and colleagues, the implantation rate and clinical pregnancy rate in the antagonist protocol were significantly higher than that of the agonist long protocol [32], demonstrating that the GnRH antagonist protocol could be a more effective for elder patients with repeated failures for the agonist long protocol.

In summary, GnRH agonists have been widely used in controlled ovarian hyperstimulation during the IVF-ET treatment. In contrast, the clinical application time for GnRH antagonists is relatively short, and their impact on the outcome of IVF-ET treatment, however, is yet to be fully elucidated. The studies in our dataset demonstrated feasible advantage for the antagonist protocol over the agonist long protocol in terms of implantation rate and pregnancy rate, particularly in those patients with multiple failures for the agonist protocol. However, additional studies with more subjects and stimulation cycles would be necessary to further confirm these data. It would be also necessary to optimize the protocol and to conduct studies for better understanding its effect on endometrium. In the clinical settings of patients with IVT-ET treatment, we should evaluate the IVF-ET outcomes not only limited to the level of pregnancy rate, but more attention should be paid to the short and long-term effect on patients resulted from the ovulation induction protocols. Therefore, the antagonist protocol provides us a choice for individualized IVF-ET treatment in clinical settings.

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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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