Influence of Different Gonadotropin-releasing Hormone Agonist Administration Methods on Pregnancy Outcomes of Patients Undergoing *In-vitro* Fertilization-embryo Transfer*

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Summary: This study aimed to investigate the effect of different gonadotropin-releasing hormone agonist (GnRH-a) administration methods on pregnancy outcomes of patients undergoing *in-vitro* fertilization-embryo transfer (IVF-ET). Clinical data of 5217 patients who underwent IVF-ET were retrospectively analyzed. Patients were divided into the long-acting GnRH-a group (n=1330) and the short-acting GnRH-a group (n=3887) based on their various treatment plans. The clinical and laboratory embryo data and clinical pregnancy outcomes were compared between the two groups. The results showed that there were no significant differences in the age, infertility, primary/secondary infertility rate, IVF rate, body mass index (BMI), antral follicle counting (AFC), follicle-stimulating hormone (FSH) level, and the number of transplanted embryos between the two groups (P>0.05). There were no significant differences in the oocyte numbers, MⅡ rate, fertilization rate, cleavage rate and blastocyst formation rate (P>0.05) between the two groups. The gonadotropin (Gn) using days, Gn dose and endometrial thickness were significantly greater in the long-acting GnRH-a group than those in the short-acting GnRH-a group (P<0.01). Additionally, the estradiol (E2) levels, blastocyst freezing rate, embryo utilization rate, transplant cancellation rate and abortion rate were significantly lower in the long-acting GnRH-a group than those in the short-acting GnRH-a group (P<0.01). The clinical pregnancy rate and embryo implantation rate were significantly higher in the long-acting GnRH-a group than in the short-acting GnRH-a group (P<0.01). It was concluded that use of long-acting GnRH-a can effectively reduce the transplant cancellation rate and improve the clinical pregnancy rate of the fresh cycle.

Key words: gonadotropin-releasing hormone agonist; long-acting; short-acting; *in-vitro* fertilization-embryo transfer; clinical pregnancy rate

In-vitro fertilization-embryo transfer (IVF-ET) has been widely used to treat infertility in clinical practice since the first *in-vitro* fertilization (IVF) procedure was conducted by Louis Brown in 1978[1]. In 1983, Warner *et al*[2] demonstrated that continuous administration of gonadotropin-releasing hormone agonist (GnRH-a) could cause pituitary desensitization. In IVF fresh treatment cycle, GnRH-a is commonly used to down-regulate the pituitary-gonadal system and suppress ovarian activity, and gonadotrophins (Gn) are then used to stimulate ovulation in a controlled manner. Among the various types of pituitary down-regulation protocols in use, the long down-regulation protocol achieves the best clinical pregnancy rate[3].

There are two forms of long down-regulation protocols, in which short-acting GnRH-a and long-acting GnRH-a are used respectively. Patients receive daily low-dose injections of short-acting GnRH-a for 14 days in luteal phase in the short-acting GnRH-a group, while those in the long-acting GnRH-a group were administered with only a single injection of high-dose long-acting GnRH-a in follicular phase. A meta-analysis[4] in 2010 showed that the clinical pregnancy rates and ongoing pregnancy rates were similar between the two protocols, but the short-acting GnRH-a group had a higher implantation rate (P=0.02). In the past few years, the short-acting GnRH-a has been widely used and helped many families. However, even with the effective use of short-acting GnRH-a, the clinical pregnancy rate of the fresh cycle could only reach

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50%–60%. How to improve the clinical pregnancy rate of the fresh cycle represents a great challenge for the community of reproductive medicine. Recent studies reported[5, 6] that use of long-acting GnRH-a may improve the clinical fresh cycle pregnancy outcome. So far, it has been controversial which of these two forms of administration is better. In the present study, we retrospectively analyzed the clinical data of patients who underwent the first controlled ovarian hyperstimulation (COH) cycle, with their age ≤35 years, antral follicle counting (AFC) ≥6, and normal follicle-stimulating hormone (FSH). We compared the clinical pregnancy outcomes between the two groups to further evaluate the clinical value of long-acting GnRH-a in long down-regulation protocol.

1 SUBJECTS AND METHODS

1.1 Patients

Clinical data of IVF-ET assisted patients who were treated from January 2016 to December 2017 at the Reproductive Center of Tongji Hospital (Wuhan, China) were retrospectively analyzed. A total of 5217 patients in the first COH cycle with their age ≤35 years, AFC ≥6, FSH <10 mIU/mL were included in the study, with 1330 patients in the long-acting GnRH-a group and 3887 patients in the short-acting GnRH-a group. The ethics approval for this study was obtained from the Ethical Committee of Tongji Medical College, Huazhong University of Science and Technology, China.

1.2 COH Cycle

Patients in the short-acting GnRH-a group with regular menstruation received a subcutaneous injection of short-acting GnRH-a for 14 days after follicular discharge (0.1 mg, daily, Diphereline, Ipsen Pharma Biotech, Germany). Those with irregular menstruation or an ovulation disorder received an oral contraceptive for 21 days (2 mg, Diane-35, Bayer, Germany) and then underwent the same treatment as those with regular menstruation. In the long-acting GnRH-a group, a long-acting GnRH-a (3.75 mg, Bonn Nokang, Bonte Pharmaceutical Co., Ltd, China) was subcutaneously administered on the first or second day of menstruation. Serum luteinizing hormone (LH) and estradiol (E2) were detected, and follicular monitoring was performed 14 and 28 days after administration of GnRH-a in the short-acting and long-acting GnRH-a groups, respectively. Then, ovarian stimulation was performed using recombinant FSH (Gonal-F, Merck Serono, Germany) until the down-regulation standard was reached. Follicle development was monitored using transvaginal ultrasound scan, and serum E2, progesterone, and LH levels were measured. When the leading follicle reached a diameter of 18 mm, a 0.25 mg human chorionic gonadotropin (hCG) injection (Ovidrel, Merck Serono, Germany) was administered subcutaneously to trigger the final follicular maturation. After 36–37 h, oocytes were retrieved under the guidance of transvaginal ultrasound. Then, progesterone was administered to support the corpus luteum.

1.3 Embryo Culture

IVF or intracytoplasmic sperm injection (ICSI) was conducted based on the quality of semen. Embryo culture was performed using a G-series sequential culture system (Vitrolife, Switzerland). Three days after fertilization, the embryos were cultured to the cleavage stage. One or two embryos greater than 6 cells and embryos in grade I were selected for transplantation. Meanwhile, the rest of the embryos were frozen or cultured to the blastocyst stage.

1.4 Pregnancy Outcome

On day 14 after the embryo transfer, the serum β-HCG level was detected to assess pregnancy status. Fourteen days later, the ultrasound was used to monitor an embryonic heartbeat to confirm the occurrence of clinical pregnancy.

1.5 Statistical Analysis

The SPSS16.0 software (USA) statistical system was used for data analysis. The data were presented as the means±standard error. The mean of the two groups was compared by *t*-test. The χ^2 test was used to compare the rates between the two groups. All *P* values were 2-sided. A *P* value <0.05 was considered statistically significant.

2 RESULTS

2.1 Comparison of General Data between the Two Groups

The mean age was 29.3±3.45 years in the long-acting GnRH-a group and 29.2±3.13 years in the short-acting GnRH-a group. The infertility years were 3.32±2.23 and 3.29±2.23, respectively. There was no significant difference in the mean age, infertility years, primary/secondary infertility rate, IVF rate, body mass index (BMI), AFC, basal FSH level and embryo transfer number between the two groups (*P*>0.05), as shown in table 1.

2.2 Clinical Treatment and Oocytes Collection

The total dose of GnRH-a was 3.75 mg in the long-acting GnRH-a group, significantly greater than that in the short-acting GnRH-a group (*P*<0.01). Meanwhile, the number of days for Gn use, the total amount of Gn and the thickness of endometrium were significantly increased in the long-acting GnRH-a group as compared with those in the short-acting GnRH-a group (11.45±1.88 *vs.* 9.96±1.53 days, 2169.21±831.75 *vs.* 2028.75±698.25 IU, 12.05±2.57 *vs.* 11.79±2.54 mm, *P*<0.01). Furthermore, the E2 levels on the trigger day were significantly lower in the long-acting GnRH-a group than those in the short-acting GnRH-a

group (3089.87±1783.87 *vs.* 4462.88±2312.91 pg/mL, $P<0.01$). In addition, no significant difference was noted in the number of follicles >14 mm and the number of oocytes obtained between the two groups ($P>0.05$) (table 2).

2.3 Laboratory Data and Pregnancy Outcomes

In the long-acting GnRH-a group, 1330 cycles were performed, and 19 463 oocytes were obtained, of which 16 714 oocytes were mature MⅡ. A total of 920 IVF patients had a 2PN rate of 62.6%, and 410 ICSI patients had a 2PN rate of 71.2%. On the third day after fertilization, 10 069 embryos were cultured, and 6715 blastocysts were then formed. In contrast, a total of 56 384 oocytes were obtained from the 3887 cycles in the short-acting GnRH-a group, and 47 993 mature MⅡ eggs were obtained. The 2PN rate of the 2708 patients with IVF was 62.2%, while that in the ICSI group was 69.8%. Meanwhile, 18 946 blastocysts were formed in the 28 639 embryos that were cultured. Furthermore, there were no significant differences in MⅡ rate, 2PN rate, cleavage rate and blastocyst formation rate between the two groups ($P>0.05$, table 3).

Additionally, as shown in table 3, the blastocyst freezing rate was 58.53%, and the embryo utilization rate was 48.58% in the long-acting GnRH-a group, significantly lower than those in the short-acting GnRH-a group (the blastocyst freezing rate: 60.43%; the embryo utilization rate: 50.20%) ($P<0.01$). Among the 1330 cycles in the long-acting GnRH-a group, 427 patients were abolished in the fresh-cycle, and the transplant cancellation rate was 32.11%, which was significantly lower than that in the short-acting GnRH-a group (37.02%, $P<0.01$). The pregnancy rate and the implantation rate were significantly higher in the long-acting GnRH-a group than in the short-acting GnRH-a group (68.22% *vs.* 58.86% and 53.44% *vs.* 43.96%, respectively, $P<0.01$), and the abortion rate was significantly lower in the long-acting GnRH-a group than in the short-acting GnRH-a group (5.54% *vs.* 9.37%, $P<0.01$).

Table 1 Comparison of general information between the two groups

Parameter	Short-acting GnRH-a group (*n*=3887)	Long-acting GnRH-a group (*n*=1330)	*P*
Mean age (years)	29.2±3.13	29.3±3.45	0.33
Infertility years (years)	3.29±2.23	3.32±2.23	0.67
Primary/secondary infertility rate (%)	67.9	68.3	0.81
IVF rate (%)	69.2	69.7	0.22
BMI (kg/m^2)	21.7±2.94	21.8±2.97	0.29
FSH (mIU/mL)	6.98±1.63	6.90±1.56	0.12
AFC (*n*)	14.57±5.41	14.37±5.20	0.24
Embryo transfer number (*n*)	1.83±0.31	1.82±0.38	0.34

Table 2 Clinical treatment and oocytes collection in the two groups

Parameter	Short-acting GnRH-a group (*n*=3887)	Long-acting GnRH-a group (*n*=1330)	*P*
Total dose of GnRH-a (mg)	1.85±0.28	3.75±0*	0.000
Days for Gn use (day)	9.96±1.53	11.45±1.88*	0.000
Total amount of Gn (IU)	2028.75±698.25	2169.25±831.75*	0.000
E2 (pg/mL)	4462.88±2312.91	3089.87±1783.87*	0.000
Intima thickness (mm)	11.79±2.54	12.05±2.57*	0.001
Number of follicles >14 mm (*n*)	12.77±5.23	12.64±8	0.50
Number of oocytes obtained (*n*)	14.51±6.56	14.84±6.47	0.11

*$P<0.01$ *vs.* short-acting GnRH-a group

Table 3 Laboratory data and pregnancy outcomes of IVF/ICSI

Parameter	Short-acting GnRH-a group (*n*=3887)	Long-acting GnRH-a group (*n*=1330)	*P*
MⅡ rate (%)	85.12	84.66	0.12
IVF 2PN rate (%)	62.2 (*n*=2708)	62.6 (*n*=920)	0.57
ICSI 2PN rate (%)	69.8 (*n*=1179)	71.2 (*n*=410)	0.07
Cleavage rate (%)	98.40	98.42	0.9
Blastocyst formation rate (%)	66.15	66.69	0.33
Blastocyst freezing rate (%)	60.43	58.53*	0.0065
Embryo utilization (%)	50.20	48.58*	0.0028
Transplant cancellation rate (%)	37.02	32.11*	0.0014
Clinical pregnancy rate (%)	58.86	68.22*	0.0000
Implantation rate (%)	43.96	53.44*	0.0000
Abortion rate (%)	9.37	5.54*	0.001

*$P<0.01$ *vs.* short-acting GnRH-a group

3 DISCUSSION

GnRH-a has been widely used in cycles of IVF. Among the various types of GnRH-a ovarian stimulation protocols, the long down-regulation protocol represents the best clinical pregnancy rates per cycle initiated. There are two types of GnRH-a administration used clinically to lead to hypophysis desensitization in the IVF cycle in the long down-regulation protocol: daily subcutaneous injection of low-dose short-acting GnRH-a, and administration of long-acting analogues at higher dose (depot).

Devreker *et al*[7] examined the efficacy of long-acting *versus* short-acting GnRH-a in long down-regulation protocol in IVF-ET. They found that the implantation and delivery rates were significantly lower in patients given long-acting GnRH-a than those administered with short-acting GnRH-a (21.1% *vs.* 32.8%; 29.1% *vs.* 48.9%, respectively, $P<0.05$). They speculated that long-acting GnRH-a may interfere with the luteal phase and embryonic development, and they believed that short-acting GnRH-a should be used preferentially in IVF-ET. Albuquerque *et al*[8] in their study found that there was no statistically significant difference in the primary outcome, clinical pregnancy rates per woman (OR 0.94, 95% CI 0.65 to 1.37), between the use of depot GnRH-a and daily GnRH-a; use of depot GnRH-a for pituitary desensitization in IVF cycles increased the number of Gn ampoules (WMD 3.30, 95% CI 1.27 to 5.34) and the duration of ovarian stimulation (WMD 0.56, 95% CI 0.31 to 0.81), as compared with use of daily GnRH-a. Over the past few decades, doctors tend to use short-acting GnRH-a for patients with good ovarian reserve.

However, in recent years, a number of studies found that long-acting GnRH-a therapy was superior to short-acting one in terms of clinical pregnancy rates and implantation rates[9]. A prospective randomized trial[10] reported that patients who received the long-acting GnRH-a regimen had significantly higher ongoing pregnancy rates (80% *vs.* 53.85%) and a trend toward higher implantation rates (42.68% *vs.* 30.38%). Several studies[11, 12] reported similar results. In addition, a great number of studies[5, 6] found that the utilization of long-acting GnRH-a achieved good pregnancy rates in patients with endometriosis, polycystic ovary syndrome, advanced age, and in patients who had failed antagonistic treatment. In the present study, we retrospectively analyzed the patients who were in the first COH cycle, with their age ≤35 years, AFC ≥6, and FSH<10 (mIU/mL) in our hospital. We further compared the clinical and laboratory data between the two groups and evaluated the clinical application of long-acting down-regulation drugs in the follicular phase. Our results showed that there was no statistically significant difference between the two groups in the mean age, infertile years, primary/secondary infertility rate, IVF/ICSI rate, basal FSH level, AFC, BMI and embryo transfer number, which means that the groups were comparable. The long-acting GnRH-a group had longer pituitary desensitization time, stimulation time, and duration of Gn, and higher doses of Gn than the short-acting GnRH-a group did, which is consistent with those reported by Duan[13]. There was no significant difference in the number of eggs, M Ⅱ rates, 2PN rates, cleavage rates and blastocyst formation between the groups, and Gao *et al* reported the similar results[14]. On the triggering day, the E2 levels in the long-acting GnRH-a group were decreased significantly, and so did the transplant cancellation rate of the fresh cycle. Use of long-acting GnRH-a resulted in the production of more high-quality embryos that could be transplanted in the fresh cycle, and it reduced the unpredictable damage caused by embryo freezing. In addition, the thickness of endometrium, clinical pregnancy rates and implantation rates of patients in the long-acting GnRH-a group were significantly enhanced as compared with those in the short-acting GnRH-a group (12.05±2.57 *vs.* 11.79±2.54 mm; 68.22% *vs.* 58.86% and 53.44% *vs.* 43.96%, respectively, $P<0.01$), and the abortion rate was significantly lower in the long-acting GnRH-a group than in the short-acting GnRH-a group. These findings may be due to the biological effects of the long-acting GnRH-a (Bonn Nokang) used in the long-acting GnRH-a group.

Bonn Nokang, a long-acting GnRH-a that was used in the long-acting GnRH-a group, is a highly active luteinizing hormone releasing hormone (LH-RH) derivative. It is stronger than LH-RH due to its resistance to proteolytic enzymes and its affinity for LH-RH receptors. Use of Bonn Nokang can produce a transient pituitary-gonadal system excitatory action, then greatly inhibit the function of the pituitary-gonadal system and gonadotropin release, and further inhibit the ovarian response to gonadotropin, thereby reducing E2 production. In the long-acting GnRH-a group, release of GnRH-a sustained for 4 weeks, and the long-acting GnRH-a was constantly released into the blood, which could continuously reduce the ovarian response and produce a strong inhibition of the pituitary-gonadal system[15]. Therefore, the long-acting GnRH-a can inhibit LH and E2 more effectively and continuously reduce the E2 level of the fresh cycle, improve endometrial receptivity, and avoid the ovarian hyperstimulation syndrome (OHSS) and implantation window closing due to excessive E2 levels, thereby increasing the clinical pregnancy rate of the fresh cycle, reducing the abortion rate and achieving better pregnancy outcomes.

In addition, some study found that for patients with endometriosis (EMT), use of prolonged GnRH-a could significantly improve their clinical pregnancy

outcomes, which may be due to the endometrial atrophy induced by prolonged down-regulation of pituitary-gonadal system[16]. Moreover, in 90% of EMT patients, the cancer antigen 125 (CA125) level was found to be decreased to normal range, suggesting that long-acting pituitary desensitization drugs can ameliorate the adverse factors affecting follicular development in the pelvic and ovarian microenvironment, and improve endometrial tolerance[16].

Nevertheless, the blastocyst freezing rate and embryo utilization rate in the long-acting GnRH-a group were found to be significantly lower (58.53% *vs.* 60.43% and 48.58% *vs.* 50.20%, respectively, $P<0.01$) than in the short-acting GnRH-a group. Therefore, the effects of long-acting GnRH-a on the cumulative pregnancy rate are undetermined, although it improved the clinical pregnancy rate and implantation rate of the fresh cycle and reduced the abortion rate.

In summary, our study documented that for patients with good ovarian reserve, long-acting GnRH-a can achieve ideal clinical pregnancy outcomes in the fresh cycle, shorten the time from first clinical consultation to pregnancy and reduce the psychological burden of the patient. Since use of long-acting GnRH-a led to decreased blastocyst rate and embryo utilization rate, further study is still needed to examine whether long-acting GnRH-a adversely affects the cumulative pregnancy rate.

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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