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Comparing the long-acting and short-acting forms of gonadotropin-releasing hormone agonists in the long protocol of IVF/ICSI Cycles: A retrospective study

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Abstract

Aim: This study aimed to compare the efficacy of long- and short-acting gonadotropin-releasing hormone agonist on clinical outcomes of *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) long protocol cycles.

Methods: In this retrospective study, 478 patients were enrolled from October 2012 to November 2014. The pituitary downregulation result, serum hormone levels, gonadotropin (Gn) dose during controlled ovarian hyperstimulation, and outcome of IVF/ICSI–embryo transfer were compared between the two groups.

Results: Compared with the long-acting group, in the short-acting group the duration of downregulation and stimulation was significantly shorter; the total Gn doses, cost of an IVF cycle, rate of ovarian hyperstimulation syndrome, superior-quality embryo rate, and implantation rate were significantly lower; and the serum luteinizing hormone concentrations on the day of Gn and human chorionic gonadotropin administration were significantly higher. The serum estradiol level on the day of human chorionic gonadotropin was higher in the long-acting group. However, no significant differences were noted in other parameters.

Conclusion: The long-acting group was associated with greater amounts of Gn and a longer duration of use for ovarian stimulation. This increased the cost per IVF cycle and may have had a detrimental effect on the pregnancy outcome because of a subsequent increase in the rate of ovarian hyperstimulation syndrome and decrease in the superior-quality embryo rate and implantation rate.

Key words: agonist, GnRH-a, *in vitro* fertilization, intracytoplasmic sperm injection, long protocol, pituitary downregulation.

Introduction

During assisted reproductive technology (ART) cycles, ovulation induction is the most critical and important step. In the past, human gonadotropins were widely used alone, and premature luteinization has increased by about 20%–25%, leading to ovulation before oocyte maturation arrest and cycle cancellation.[1] Therefore, avoiding the spontaneous ovulation induced by the luteinizing hormone (LH) surge has become the main problem of the *in vitro* fertilization (IVF) cycle.[2] In the early 1980s, the gonadotropin-releasing hormone agonist (GnRH-a) was first used in clinics to avoid endogenous LH surge before oocyte retrieval.[3–5] Then, it was widely used for pituitary desensitization during IVF or intracytoplasmic sperm injection (ICSI) treatment. Reducing the level of LH could improve the oocyte development by increasing the estrogen/androgen ratio[4]; moreover, it would improve the endometrial receptivity and wide implantation window.[5–8]

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A Cochrane review showed that the long protocol was associated with the best clinical pregnancy rate among the variety of controlled ovarian hyperstimulation (COH) protocols,[9] and was recommended by the National Institute for Health and Care Excellence. The long protocol has two forms: the long-acting and short-acting long protocol. The short-acting long protocol is widely used; however, it needs daily low-dose injections, making it inconvenient, tiring, and stressful. The use of the long-acting long protocol is much more comfortable for patients, requiring only a single depot dose of GnRH-a in the mid-luteal phase. Usually, it deeply suppresses the pituitary, and, therefore, requires a greater amount of gonadotropin (Gn) and a longer duration of use.[7]

The most effective form of agonist protocol remains controversial. In 2013, a meta-analysis study showed that it was not clear which one was the better protocol for pituitary desensitization in IVF cycles.[8] This retrospective analysis aimed to compare the use of the short- and long-acting long protocols with IVF cycles to study the effectiveness and safety of the two protocols.

Methods

Subjects and study design

A total of 478 patients who underwent the long protocol treatment during the luteal phase of IVF/ICSI cycles from October 2012 to November 2014 were enrolled at Shanghai First Maternity and Infant Hospital. Each patient undertook only one cycle. The main etiology of infertility was tubal factor, ovulation disorder, and/or male factor. Signed informed consent was obtained from all patients before initiating the therapy. All hormones were tested in the same laboratory, using the same radioimmunoassay kit.

The inclusion criteria were: age 22–40 years, serum follicle-stimulating hormone (FSH) < 10 IU/L on day 3 of the menstrual cycle, no previous ovarian surgery, and fewer than three previous IVF/ICSI attempts.

The exclusion criteria were: patients who had received hormone therapy during the last 3 months and those who had suffered major systemic disease, such as uterine abnormality, pelvic tuberculosis, and habitual abortion history.

Treatment protocol

A total of 248 patients in the long-acting group and 230 patients in the short-acting group were studied. Each patient's serum LH, FSH, estradiol (E2), and anti-Müllerian duct hormone levels were measured on day 3 of the menstrual cycle, and all underwent a transvaginal ultrasound scan on the same day. In the mid-luteal phase, patients in the long-acting group received a single dose of intramuscular injection of long-acting triptorelin (1.25 mg); whereas in the short-acting group, patients received a daily dose of subcutaneous injection of short-acting triptorelin (0.05 mg) until the day of administering the human chorionic gonadotropin (hCG) injection. Patients in the long-acting group came back on day 3 of the next menstrual cycle, and patients in the short-acting group came back 14 days later. When the serum LH was lower than 5 IU/L, the serum E2 level was lower than 50 pg/mL and the endometrium thickness was less than 5 mm, which meant downregulation was achieved. Then, ovarian stimulation was performed using a recombinant FSH (Gonal-F). The follicle development was monitored through a transvaginal ultrasound scan and by measuring serum E2, progesterone (P), and LH levels. When the leading follicle reached a diameter of 18 mm or at least two follicles reached a diameter of 17 mm, an hCG injection of 6500 IU was given subcutaneously to trigger the final follicle maturation. After 34–36 h, oocytes were retrieved through transvaginal ultrasound.

IVF or ICSI was performed 40–42 h after the hCG administration. After 16–18 h, the zygote was checked. The embryo grade was recorded according to the criteria published in a previous study.[10] Embryos of grades 1 or 2 were defined as superior quality. About 72 h after fertilization, when the embryos were at the stage of four to eight cells, two or three embryos were transferred to the uterus guided by the transvaginal scan, and the rest of the embryos were frozen. On day 14 after the embryo transfer, a radioimmunoassay kit was used to test β-hCG. A biochemical pregnancy was confirmed if β-hCG was higher than 50 mIU/mL. Then, after 14 days the embryonic heartbeat was monitored by ultrasound to confirm the occurrence of clinical pregnancy.

Each patient received a daily dose of 60 mg progesterone injection and 20 mg oral dydrogesterone from the day of oocyte retrieval for luteal phase support. All patients used these adjuncts until the 8th week after the transfer of embryos unless biochemical pregnancy was ruled out.

Statistical analysis

SPSS 18.0 was used to analyze the data. The independent samples *t*-test was used to test the unpaired result, and the χ^2-test was used to compare the difference for rank data. A P-value < 0.05 was considered statistically significant. The data are presented as means ± standard error (SE) or n/N (%).

Results

The general condition of the two groups and basic endocrine levels were compared and no significant differences were found between the two groups (Table 1).

Table 2 shows that the FSH level on the day of Gn stimulation was significantly lower in the long-acting group compared with the short-acting group (2.25 ± 1.11 mIU/L vs 4.079 ± 1.01 mIU/L, $P = 0.000$). The serum LH level on the day of Gn and hCG administration was significantly lower in the long-acting group than in the short-acting group (1.53 ± 0.92 U/L vs 1.87 ± 0.76 U/L, $P < 0.05$; 0.993 ± 0.766 U/L vs 2.02 ± 1.08 U/L, $P < 0.00\ 1$, respectively). The serum E2 level on the day of hCG administration was significantly lower in the short-acting group than in the long-acting group (2351.29 ± 1607.99 pg/mL vs 2930.73 ± 1423.94 pg/mL, $P < 0.05$), while the level of serum P and P/E2 ratio were not significantly different in the two groups.

The mean length of downregulation and the mean length of stimulation in the short-acting group were significantly lower (12.64 ± 2.024 days vs 14.26 ± 0.869 days, 9.85 ± 1.98 days vs 12.28 ± 2.58 days, respectively). The amount of Gn dosage was also lower in short-acting group (1808.58 ± 602.06 U vs 2316.16 ± 929.87 U, $P < 0.0001$), thus the cost of an IVF cycle was lower in the short-acting group (2.5 ± 1.3 [10 000 yuan] vs 2.9 ± 1.6 [10 000 yuan], $P = 0.03$), while the starting dose of Gn was higher in the short-acting group (162.91 ± 33.98 U vs 176.63 ± 36.92, $P = 0.03$). The E2 level of each follicle on the day of hCG injection was higher in the short-acting group. No significant differences were found in clinical outcomes except the superior-quality embryo rate, implantation rate, and ovarian hyperstimulation syndrome (OHSS) rate. The superior-quality embryo rate and implantation were higher in the short-acting group ($P < 0.05$). The OHSS rate was higher in the long-acting group (8.87% vs 2.61%, $P = 0.003$) (Table 3).

Discussion

Main findings

The long-acting long protocol offers the advantages of better compliance and convenience for patients along with less stress of injections. However, it causes extrapituitary side-effects.[10–12] Porter *et al.* first used GnRH-a to suppress ovarian activity in 1984.[2] Kondaveeti-Gordon *et al.* found that the suppression needed at least 14 days.[13] Wang *et al.* found that if the duration of GnRH-a downregulation reached 10 days, the endometrium thickness and the serum endocrine level tended to be stable.[14] In 2014, Ren *et al.* also analyzed that prolonged downregulation of GnRH-a in the long-acting long protocol might increase live-birth rates, but would decrease the number of oocytes and embryos, and reduce the serum LH level on the starting day of Gn

Table 1 General condition of patients receiving COH in the long- and short-acting groups

	Long-acting group ($n = 248$)	Short-acting group ($n = 230$)	*P*-value
Age (years)†	28.47 ± 3.61	27.41 ± 4.08	0.06‡
Body mass index (kg/m^2)†	21.79 ± 2.8	21.67 ± 3.03	0.75‡
Mean duration of infertility†	6.92 ± 3.25	6.99 ± 3.13	0.89‡
Infertility cause, *n* (%)			0.43§
Tubal factor (%)¶	(136/248) 54.8	(142/230) 61.7	
Ovulation obstacle (PCOS) (%)¶	(18/248) 7.3	(16/230) 6.96	
Male factor (%)¶	(73/248) 29.4	(54/230) 23.5	
Endometriosis factor (%)¶	(8/248) 3.23	(10/230) 4.35	
Unexplained factor (%)¶	(13/248) 5.24	(8/230) 3.48	
Basal antral follicle count†	13.28 ± 4.22	12.27 ± 4.15	0.09‡
Method of fertilization, *n* (%)			0.03§
IVF (%)¶	(154/248) 62.1	(167/230) 72.6	
ICSI (%)¶	(78/248) 31.5	(48/230) 20.9	
IVF (Rescue ICSI)¶	(16/248) 6.45	(15/230) 6.52	
Basic endocrine levels			
AMH (ng/mL)†	4.87 ± 1.72	5.61 ± 1.94	0.32‡
D3-FSH (IU/L)†	8.4 ± 3.0	7.7 ± 2.5	0.305‡
D3-LH (IU/L)†	5.77 ± 1.2	5.54 ± 1.1	0.23‡
D3-E2 (pg/L)†	48.98 ± 22.46	58.84 ± 53.47	0.74‡

†Data are presented as means ± standard error. ‡Independent samples *t*-test. §χ^2-test. ¶Data are presented as *n*/*N* (%). AMH, anti-Müllerian duct hormone; COH, controlled ovarian hyperstimulation; E2, estradiol; FSH, follicle-stimulating hormone; ICSI, intracytoplasmic sperm injection; IVF, *in vitro* fertilization; LH, luteinizing hormone; PCOS, polycystic ovary syndrome.

Table 2 Comparison of laboratory variables of the two groups

	Long-acting group (n = 248)	Short-acting group (n = 230)	P-value
Serum endocrine level on Gn stimulation day			
FSH (mIU/L)†	2.25 ± 1.11	4.079 ± 1.01	0.000‡
LH (mIU/L)†	1.53 ± 0.92	1.87 ± 0.76	0.004‡
E2 (pg/L)†	18.49 ± 13.02	24.17 ± 11.4	0.001‡
Serum endocrine level on hCG injection day			
LH (mIU/L)†	0.99 ± 0.77	2.02 ± 1.08	0.000‡
P (ng/L)†	0.75 ± 039	0.78 ± 0.38	0.591‡
E2 (pg/L)†	2930.73 ± 1423.94	2351.29 ± 1607.99	0.004‡
P/E2†	0.34 ± 2.44	0.30 ± 1.77	0.13‡

†Data are presented as mean ± standard error. ‡Independent samples *t*-test. E2, estradiol; FSH, follicle-stimulating hormone; Gn, gonadotropin; LH, luteinizing hormone; P, progesterone.

Table 3 Comparison of clinical outcomes between the two groups

	Long-acting group (n = 248)	Short-acting group (n = 230)	P-value
Endometrium thickness on the day of the hCG injection (mm)†	10.36 ± 2.67	9.98 ± 2.94	0.310‡
Downregulation days (days)†	12.64 ± 2.024	14.26 ± 0.87	0.000‡
Starting dose of Gn†	162.91 ± 33.98	176.63 ± 36.92	0.03‡
Gn dosage (U)†	2316.16 ± 929.87	1808.58 ± 602.06	0.000‡
Gn duration (days)†	12.28 ± 2.58	9.85 ± 1.98	0.000‡
Total cost per cycle (10 000 yuan)†	2. 5 ± 1. 3	2.9 ± 1.6	0.03‡
E2 level of each follicle on the day of the hCG injection (pg/mL)†	193.0 ± 70.81	248.90 ± 80.29	0.000‡
No. of oocytes retrieved (n)†	11.27 ± 5.41	10.66 ± 5.51	0.606‡
Fertilization rate (%)§	(1991/2710) 73.5	(1808/2454) 73.7	0.924¶
Cleavage rate (%)§	(1784/1991) 89.6	(1668/1868) 89.3	0.0.793¶
Implantation rate (%)§	(115/385) 29.9	(130/348) 37.4	0.034¶
Clinical pregnancy rate (%)§	(80/198) 42.10	(76/176) 43.20	0.601¶
Cycle cancellation rate (%)§	(44/248) 17.7	(52/230) 22.61	0.209¶
OHSS occurring rate (%)§	(22/248) 8.87	(6/230) 2.61	0.003¶
Superior-quality embryo rate (%)§	(1160/1782) 65.09	(1156/1668) 69.30	0.009¶
Early abortion rate (%)§	(6/80) 7.5	(8/76) 10.53	0.582¶
Ectopic pregnancy rate (%)§	(8/80) 10	(3/76)3.95	0.211¶

†Data are presented as mean ± standard error. ‡Independent samples t-test. §Data are presented as n/N (%). ¶χ^2-test. E2, estradiol; Gn, gonadotropin; hCG, human chorionic gonadotropin; OHSS, ovarian hyperstimulation syndrome.

and the day of hCG administration.[15] In the present study, the level of serum LH was lower in the long-acting group, which might be caused by extrapituitary side-effects. Several studies showed that the low LH level induces a negative effect on oocyte maturation, fertilization, and embryo development.[16–19] In 2002, Balasch and Fabregues[20] put forward the concept of the LH "threshold" and LH "ceiling." They suggested that an appropriate LH hormone level during the follicular phase of menstrual and induced cycles is prime to a favorable pregnancy outcome, and that women with too high or too low LH concentrations achieve a poor pregnancy rate in ART.[21] An LH level < 1 IU/L is considered below the LH threshold level, which would reduce the E2 concentration in follicular fluid, affect the oocyte maturation and fertilization, and induce a negative effect on the egg quality and pregnancy rate.[22] An unacceptably low level of serum LH would require more Gn, hence increasing the cost of COH. In this study, the LH serum level on the day of Gn stimulation and hCG injection was significantly different in the two groups. It was lower in the long-acting group on the day of Gn stimulation, although it was within the LH "threshold" level (1–6 mIU/mL); however, on the day of the hCG injection, the LH level was 0.99 ± 0.77 mIU/mL in the long-acting group, which was below the LH threshold. The superior-quality embryo rate in the long-acting group was 65.09%, while it was 69.30% in the short-acting group, which was significantly different. This finding implied that a lower LH serum level might have a negative influence on the embryo quality.

In this study, no severe hyperstimulation syndrome occurred; the incidence of mild-to-moderate OHSS was significantly higher in the long-acting group (9.1% vs 2.6%). According to a previous study,[23] the long-acting long protocol needed more Gn for ovarian stimulation compared with the short-acting long protocol. In this study, the Gn dosage in the two groups was 2316.16 ± 929.87 IU in the long-acting group vs 1808.58 ± 602.06 IU in the short-acting group, which was significantly different. The long-acting GnRHa group increases the cost of an IVF cycle compared with the short-acting GnRHa group, because it lengthens the period of ovulation and requires higher doses of Gn. Furthermore, the increasing Gn dosage caused the higher E2 level on the day of hCG injection in the long-acting group, which was the main reason for the occurrence of OHSS.[24]

In this study, the E2 level on the day of hCG injection was higher in the long-acting group, while the ratio of E2 to the number of follicles with ≥14-mm diameter on the day of hCG injection was significantly different in the two groups, at 193.0 ± 70.81 pg/mL in the long-acting group versus 248.90 ± 80.29 pg/mL in the short-acting group (P = 0.000). These findings demonstrate that the oocytes had a higher quality in the short-acting group, which contributed to the embryo quality and uterine receptivity; the embryo implantation rate in the short-acting group was 37.4%, which was higher than that in the long-acting group, similar to the Ozdegirmenci report.[25]

The Gn duration was also found to be longer in the long-acting group, causing an increase in patients' expenses. The Gn dosage is associated with the degree of pituitary downregulation.

In conclusion, the clinical outcomes of the superior-quality embryo rate and implantation rate were significantly higher in the short-acting group. The mean duration of downregulation was more appropriate in the short-acting group. Although the long-acting group can effectively reach the downregulation standard, it seemed too strong, with a lower serum LH concentration. The long-acting group required a greater Gn dosage and increased the days of Gn stimulation. The short-acting group required a lower amount of gonadotropin and is more flexible; thus, the short-acting group was more cost-effective, and it should be recommended.

Disclosure

No potential conflict of interest relevant to this article is reported.

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