Am J Transl Res 2021;13(7):8365-8371
www.ajtr.org /ISSN:1943-8141/AJTR0132301

Original Article

Application of antagonist regimen in patients with failed pregnancy assisted by previous long-term regimen during early follicular phase

Yun Huang, Mei Shuai, Linlin Yue, Hua Liao

Department of Reproduction and Genetics, Ganzhou Maternal and Child Health Centre, Ganzhou, Jiangxi Province, China

Received February 22, 2021; Accepted March 28, 2021; Epub July 15, 2021; Published July 30, 2021

Abstract: Objective: To investigate the clinical application of gonadotropin-releasing hormone antagonist (GnRH-ant) in patients with failed pregnancy assisted by the previous long-term regimen during early follicular phase (EFP). Methods: A total of 122 patients with good ovarian function and two previous failed EFP long-term assisted pregnancy were selected from the reproductive center of our hospital for study. All patients were assisted by in vitro fertilization-embryo transfer (IVF-ET) twice. According to the random number table method, the participants were divided into group A (n=61) for subcutaneous injection of gonadotropin-releasing hormone agonist (GnRH-a) and group B (n=61) for GnRH-ant, and the clinical efficacy of the two groups were observed. Results: Group B presented reduced dosage and duration of Gn, increased number and probability of eggs retrieved, and increased number of 2PN, cleavage and transplantable embryos than group A (all P<0.05). Serum estradiol (E2) and luteinizing hormone (LH) levels elevated and T level decreased in group B as compared to group A (all P<0.05). There was no significant difference in follicle-stimulating hormone (FSH) indexes between the two groups (P>0.05). Endometrial thickness and mean ovarian volume (MOV, the mean volume of bilateral ovaries) were not significantly different between group A and group B before treatment (both P>0.05), while were lower in group B than in group A after treatment (both P<0.05). Group B had higher high-quality egg rate (%), fertilization rate (should have numbers here %), cleavage rate (%), high-quality embryo rate (should have numbers here %) and cumulative pregnancy rate (%) than group A (all P<0.05). The incidences of moderate OHSS, early abortion and hydrosalpinx were lower in group B than in group A (all P<0.05), while there was no evident difference between the two groups in the occurrence of severe OHSS and ectopic pregnancy (both P>0.05). Conclusion: GnRH-ant can improve the clinical high-quality embryo rate in patients with the previous failed EFP long-term assisted pregnancy, and reduce the occurrence of OHSS events. Compared with GnRH-a, GnRH-ant is more suitable for clinical application of controlled ovarian hyperstimulation.

Keywords: Fertilization-embryo transfer, antagonist regimen, long-term regimen, pregnancy outcome

Introduction

Clinical studies have demonstrated that there are many factors affecting successful pregnancy in the process of assisted pregnancy, among which embryo quality and uterine receptivity are the top priorities [1]. At present, there are two main methods to accelerate follicular development in the process of in vitro fertilization and embryo transfer (IVF-ET). The first is gonadotropin-releasing hormone agonist (GnRH-a), which is the main method that has been applied in clinical pregnancy. Compared with conventional long-term regimen, GnRH-a can accelerate ovulation, improve endometrial thickness and receptivity, increase the clinical pregnancy probability of subjects, and enhance the clinical treatment effect [2, 3]. GnRH-a therapy is recommended for patients with good ovarian reserve function, which can significantly increase the expression of integrin avβ3, thus bolstering the ability of embryo implantation.

Gonadotropin-releasing hormone antagonist (GnRH-ant), is a molecular polypeptide with lower toxicity and dosage than GnRH-a. GnRH-ant can reduce the expression of gonadotropin (Gn) within several hours and has no ignition

effect [4]. However, it has been shown that some patients treated with GnRH-a had poor oocyte and embryo quality, leading to the failure of IVF-ET [5]. On the premise of ensuring good ovarian function, patients can be re-treated clinically with EFP long-term regimen after the first failure, with a better success rate.; whereas, there are relatively few studies on the improvement of clinical outcomes by GnRH-ant therapy in patients with the above-mentioned treatment failure [6]. Therefore, this study focused on 122 patients with good ovarian function who failed in the previous EFP long-term regimen in our reproductive center to evaluate the influence of GnRH-ant on embryo quality and clinical outcome.

Materials and methods

General information

A total of 122 patients with good ovarian function referred to the reproductive center of our hospital were selected. All patients failed to get pregnant with the long-term regimen twice in the early follicular phase (EFP), and the way of assisted pregnancy was the same. Inclusion criteria: (1) Patients with antral follicle count (AFC) >5; (2) Patients with poor embryo quality as the cause of pregnancy failure; (3) Patients who did not receive GnRH-ant treatment at the first assisted pregnancy; (4) Women ≤38 years old and men <45 years old; (5) Patients with a body mass index (BMI) of 19-27 kg/m^2. Exclusion criteria: (1) Patients with endometriosis (EMS) or polycystic ovary syndrome (PCOS); (2) Patients with malignant or borderline tumors; (3) Patients with uterine organic lesions; (4) Patients with severe uterine malformation or intrauterine adhesion, endometrial tuberculosis or previous history of pelvic tuberculosis; (5) Patients with chromosomal abnormalities or reproductive malformations; (6) Patients with low immune function; (7) Patients with a history of embryonic arrest; (8) Patients with pregnancy failure due to male infertility; (9) Patients participating in other clinical projects. According to the random number method, patients receiving GnRH-a again were included into group A (n=61), while those receiving GnRH-ant therapy were included into group B (n=61). All the enrolled patients and their families understood the study protocol and signed the informed consent after this study was approved by the Medical Ethics Committee of our hospital.

Methods

Long-term regimen for follicle stimulation: On the second day of menstruation, 3.75 mg of GnRH-a drug Enantone (3.75 mg/piece, Takeda (China) Pharmaceutical Company Limited.) was injected subcutaneously. Thirty to five days after injection, B-ultrasound and endocrine examination were performed to detect the expression levels of serum sex hormones follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2) and T and Gn. The recombinant follicle stimulating hormone (75 IU/piece) purchased from Changchun High & New Technology Industry (Group) Inc., China, and/or the injection urinary Gn (75 IU/piece) produced by Yantai Dongcheng Beifang Pharmaceutical Co., Ltd., China were used.

GnRH-ant regimen: On the 2nd or 3rd day after menstruation, recombinant follicle-stimulating hormone (Prospec-Tany, 75 IU/piece) was injected intramuscularly, LH>10 IU/L. When the diameter of the main follicle reached 14 mm, GnRH-a drugs (Pierre Fabre Pharmaceuticals) and cetrorelix acetate for injection (Aquitaine Medical International Co., Ltd; 0.25 mg/piece, 0.25 mg/d) were injected subcutaneously. The rest were the same as the EFP long-term regimen.

Outcome measures

General clinical indicators: The dosage and duration of Gn, the number of eggs retrieved, the rate of MII eggs obtained, as well as the number of MII 2PN, cleavage number, transplantable embryos and transplanted embryos were observed in groups A and B.

Serum E2, LH, FSH and T levels: A TGL-15M desktop micro-high-speed freezing centrifuge (Hunan Pingfan Technology Co., Ltd., China) was used to separate 3 mL of peripheral blood at 3000 r/min for 10 min to collect the serum. Serum levels of E2 (73-17621 pmol/L), LH (0.2-250.0 IU/mL), FSH (0.2-200.00 IU/mL) and T (0.35-55.50 nmol/L) were detected by chemiluminescence immunoassay kits produced by Nanjing Sihongrui Biological Co., Ltd., China.

Uterine receptivity: Three days after menstruation, patients were examined by color Doppler ultrasound diagnostic instrument XH40 (Shanghai Jumu Medical Devices Co., Ltd., China), with probe frequency ranging from 5.0 MHz to 9.0 MHz. The bladder of the patient was emptied, and the endometrial thickness and the mean

Table 1. Comparison of general information of the two groups of patients

	Group A (n=61)	Group B (n=61)	t	*P* value
Average age (years)	37.1±3.3	36.9±3.6	0.319	0.750
Infertility years (years)	4.26±3.20	4.53±2.96	0.484	0.629
BMI (kg/m^2)	23.04±3.22	22.90±3.10	0.245	0.807
Basic FSH (U/L)	10.15±2.55	10.56±2.17	0.956	0.341
Basic LH (U/L)	4.33±1.35	3.98±1.56	1.325	0.188
Basic E2 (pmol/L)	159.20±71.00	161.03±69.36	1.440	0.886
Basic T (ng/DI)	56.33±18.60	57.14±17.66	0.247	0.806
AFC (a)	5.6±2.3	5.8±2.2	0.491	0.624

Note: BMI: body mass index; FSH: follicle-stimulating hormone; E2: estradiol; LL: Luteinizing hormone. T: Testosterone; AFC sinus follicle count.

ovarian volume (MOV, the mean volume of bilateral ovaries) were measured via vagina of the patient in the lithotomy position.

Other clinical outcomes: Other clinical outcomes of patients in groups A and B were observed. High-quality egg rate (%) = number of grade III eggs/number of eggs retrieved ×100%. Fertilization rate (%) = number of fertilization/number of eggs used for fertilization ×100%. Cleavage rate (%) = number of embryos with cleavage on the day of transplantation/number of normally fertilized eggs ×100%. High-qualify embryo rate (%) = 2PN I + II embryo/2PN cleavage number ×100%. Cumulative pregnancy rate (%) = number of pregnancies/number of all egg retrieval cycles ×100%.

Adverse reactions: The incidences of OHSS, early abortion and ectopic pregnancy in groups A and B during treatment were observed.

Statistical analysis

SPSS 23.0 software was used for statistical analysis of the data. Measurement data were expressed as mean ± standard deviation ($\overline{x}$ ± sd) and analyzed by t test. Counting data were described in the form of cases/percentage (n, %) and analyzed by χ^2 test. P<0.05 indicated that the difference was statistically significant.

Results

Comparison of general data between the two groups

There was no significant difference in average age, infertility duration (years), BMI, basal FSH, LH, E2, T and AFC between group A and group B (all P>0.05) (**Table 1**).

Comparison of general clinical data between the two groups

The total dosage and duration of Gn, the number of eggs retrieved, the rate of obtained MII eggs (number of MII eggs/number of eggs retrieved), the number of 2PN, cleavage, transferable embryos and transferred embryos in group B were all better than those in group A (all P<0.05) (**Table 2**).

Comparison of serum levels of E2, LH, FSH and T between the two groups after treatment

Compared with group A, serum levels of E2 and LH in group B were increased, while T levels were decreased, with significant differences (all P<0.05); however, FSH levels showed no evident difference between the two groups (P>0.05) (**Table 3** and **Figure 1**).

Comparison of uterine receptivity between the two groups

Endometrial thickness and MOV were not significantly different between group A and group B before treatment (both P>0.05), while both decreased in the two groups after treatment, with lower values in group B than in group A (both P<0.01) (**Table 4**).

Comparison of clinical outcomes between the two groups

The high-quality egg rate (%), fertilization rate (%), cleavage rate (%), high-quality embryo rate (%) and cumulative pregnancy rate (%) were higher in group B compared with group A, with significant differences (all P<0.05) (**Table 5**).

Comparison of the incidence of adverse reactions between the two groups

The incidences of moderate OHSS, early abortion and hydrosalpinx in group B were lower than those in group A (all P<0.05), while there was no evident difference in the occurrence of severe OHSS and ectopic pregnancy between the two groups (P>0.05) (**Table 6**).

Table 2. Comparison of general clinical data of the two groups of patients

	Group A (n=61)	Group B (n=61)	χ^2/t	*P* value
Gn used total dose (IU)	2750.55±522.02	1960.22±422.28	9.193	<0.001
Gn use time (d)	14.50±3.22	10.05±2.14	8.989	<0.001
Number of eggs captured (n)	4.80±3.25	6.50±4.15	2.519	0.013
Rate of obtaining MII eggs (%)	79.51 (322/405)	90.83 (396/436)	6.817	0.009
2PN number (n)	6.20±2.11	4.59±2.63	3.729	0.001
Number of cleavage (n)	3.66±2.50	4.96±3.44	2.388	0.019
Number of transferable embryos (n)	4.06±2.55	2.90±1.95	2.822	0.006
Number of embryos transferred (n)	2.04±0.60	2.35±0.49	3.125	0.002

Table 3. Comparison of serum E2, LH, FSH and T levels in the two groups

	Group A (n=61)	Group B (n=61)	t	*P* value
FSH (U/L)	8.50±1.63	8.33±1.70	0.564	0.574
LH (U/L)	1.60±0.28	3.15±0.42	23.98	<0.001
E2 (pmol/L)	7753.02±948.24	9620.04±1850.65	7.012	<0.001
T (ng/dL)	44.30±4.69	39.56±5.10	5.343	<0.001

Note: FSH: follicle stimulating hormone; E2: estradiol; LL: Luteinizing hormone. T: Testosterone.

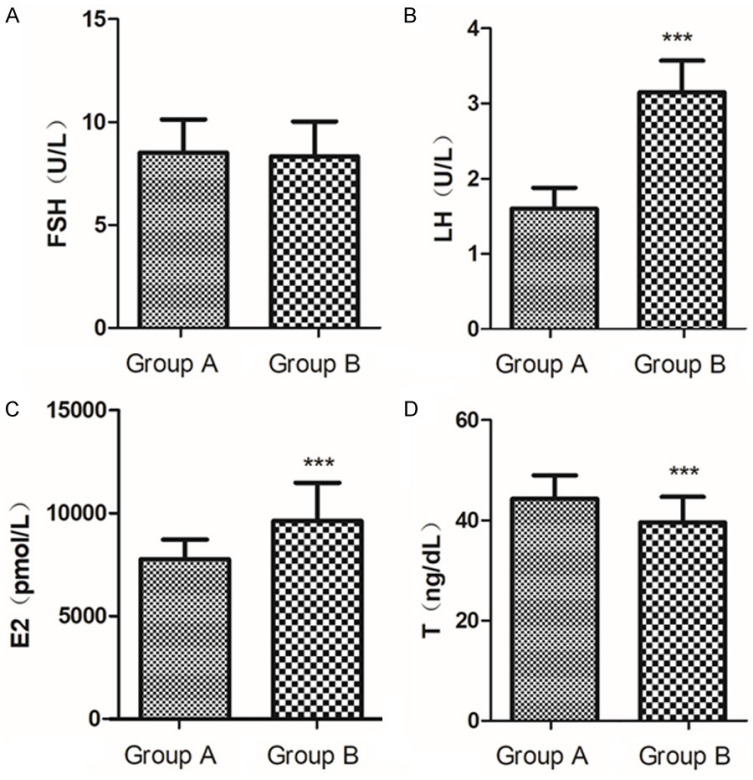

Figure 1. Comparison of serum levels of E2, LH and T. A: FSH level; B: LH level; C: E2 level; D: T level. Compared with group A, ***P<0.001. FSH: follicle-stimulating hormone; LH: luteinizing hormone; E2: Serum estradiol.

Discussion

Increasing the success rate of IVF is the therapeutic purpose of reproductive doctors. GnRH-ant and GnRH-a, both of which are GnRH analogues, can improve the success rate of IVF and the expression of FSH and LH in pituitary cells to enhance the therapeutic effect [6, 7].

This study shows that patients in group B had reduced duration and dosage of Gn and improved ability of egg division and embryo transfer after GnRH-ant treatment, which is significantly different from those in group A treated with GnRH. GnRH-ant can competitively bind to GnRH-IR in the pituitary gland, inhibit GnRH from secreting downstream protein kinase, and stimulate Gai to reduce the proliferation of peripheral germ cells. In addition, GnRH-ant can inhibit Gn and estrogen in a dependent way, without affecting the pituitary gland [8]. And it has a short half-life *in vivo*, which can reduce the dosage of GN and shorten the time of GN use. GnRH-a, through internal and external stimulation, leads to reduced stimulation sensitivity, which promotes the desensitization of the pituitary gland for a long time, resulting in a long action time of Gn, while increasing the dosage is easy to damage the pituitary gland function [9, 10]. It has been shown that GnRH-ant can increase the number and rate of eggs retrieved and the number of transferable embryos in patients with IVF, with a better clinical efficacy compared with GnRH-a

Table 4. Comparison of endometrial thickness and average volume of bilateral ovaries between the two groups

Time (s)	Group A (n=61)	Group B (n=61)	t	*P* value
Endometrial thickness (mm)				
Before treatment	8.05±1.33	7.96±1.51	0.349	0.728
After treatment	6.93±1.12	6.20±0.85	4.055	0.001
t	5.031	7.933		
P value	<0.001	<0.001		
Average bilateral ovarian volume (m^2)				
Before treatment	11.20±2.51	11.64±2.33	1.003	0.318
After treatment	9.60±2.69	8.10±1.88	3.570	0.001
t	3.397	9.235		
P value	0.001	<0.001		

Table 5. Comparison of clinical outcomes between the two groups

	Group A (n=61)	Group B (n=61)	χ^2	*P* value
High-quality egg rate (%)	71.60% (290/405)	86.46% (377/436)	4.177	0.041
Fertilization rate (%)	65.43% (255/405)	75.92% (331/436)	3.900	0.046
Cleavage rate (%)	62.96% (230/265)	91.54% (303/331)	3.745	0.049
High-quality embryo rate (%)	35.85% (95/265)	41.09% (136/331)	3.855	0.047
Cumulative pregnancy rate (%)	27.86% (17/61)	47.54% (29/61)	5.025	0.025

Table 6. Comparison of the incidence of adverse reactions between the two groups

	Group A (n=61)	Group B (n=61)	χ^2	*P* value
Moderate OHSS	12 (19.67%)	3 (4.92%)	6.157	0.013
Severe OHSS	1 (1.64%)	0 (0.00%)	2.330	0.127
Early abortion	7 (11.47%)	1 (1.64%)	4.816	0.028
Hydrosalpinx	4 (6.57%)	0 (0.00%)	4.136	0.042
Ectopic pregnancy	3 (4.92%)	1 (1.64%)	0.771	0.380

regimen, which is similar to the results obtained in this study [11]. Therefore, GnRH-ant can not only block GnRH-induced pituitary excitability and reduce the expression of sex hormones and steroids, but act quickly and recover the pituitary function quickly, and increase the number of eggs retrieved and the implantation rate.

This study showed that compared with group A, E2 and LH in serum of patients in group B increased and T level decreased, with a similar FSH level. FSH is a sensitive indicator secreted by pituitary gland that can predict ovarian function. Evidence has shown that FSH expression is abnormal in serum of patients with ovarian dysfunction, and the detection of FSH level changes in serum of patients on the 3rd day of menstrual cycle can better reflect ovarian function. LH is indispensable to maintain the activity of the luteum itself and its production of steroid hormones. While the change of E2 level reflects the growth and maturation of follicles. Elevated serum E2 is a manifestation of multiple follicular development, but too high a level may increase the risk of severe ovarian hyperstimulation syndrome, which may damage embryo quality and endometrial receptivity [12]. Ararooti T et al. has reported that GnRH-ant regimen can improve patients' assisted pregnancy and sex hormone E2 level compared with GnRH-A long regimen, which is similar to the research results in this paper [13]. GnRH-a can reduce the expression of endogenous LH and FSH, and play a role in controlling ovulation artificially. For patients who failed with GnRH-a for the first time, the pituitary gland of patients would rapidly release endogenous GN after the second administration, and the secretion of LH and FSH was gradually reduced after 5-7 days of medication to achieve the effect of pituitary down-regulation. Serum E2 expression of patients increased after GnRH-ant administration, suggesting that

GnRH-ant regimen can promote endometrium development in the early luteal phase, leading to reduced endometrial receptivity, which may indirectly improve embryo implantation during the fresh transplantation cycle [14].

Lower endometrial thickness and MOV were determined in group B in this study. Endometrial receptivity is a key factor in clinical pregnancy. Embryos can be positioned and adsorbed in the endometrium, so good endometrial receptivity can improve the ability of embryo implantation. Doppler ultrasound can better reflect the uterine receptivity of patients, and parameters such as endometrial thickness and MOV are commonly used to evaluate endometrial receptivity [15]. Previous literature suggests that GnRH-ant regimen is inferior to GnRH-a in endometrial receptivity, because antagonist has luteolysis effect, which affects endometrium and embryo implantation rate. Compared with GnRH-a regimen group, the endometrial receptivity of GnRH-ant regimen group is closer to the natural cycle, while there is no difference between them in uterine receptivity [16].

This study confirmed that the rates of high quality eggs, fertilization, cleavage, high-quality embryo rate and cumulative pregnancy were higher while the incidences of moderate OHSS, early abortion and hydrosalpinx were lower in group B as compared to group A. GnRH-ant does not need to desensitize the pituitary gland of patients before use, which reduces the economic cost of patients and the adverse reactions of drug stimulation [17]. In the research of Cai Lisi et al., two different regimens (GnRH-a long-term regimen vs. GnRH-ant) can both improve the clinical pregnancy rate of patients [18, 19]. However, some scholars hold the opposite attitude. First of all, since embryos need to be frozen during GnRH-ANT treatment, thawing and transplantation after suitable uterine conditions can significantly increase the clinical pregnancy rate [20, 21]. In addition, premature induction of progesterone receptor expression before luteinization should be avoided during the use of GnRH-ant, which can indirectly improve endometrial receptivity and facilitate embryo implantation. In a self-controlled study, the endometrial receptivity in the GnRH-ant regimen group was closer to the natural cycle than that in the GnRH-a regimen group, while there was no significant difference in clinical implantation rate and clinical pregnancy rate, which is different from the results of this study. The deviation may be resulted from experimental sample size, basic physical quality of patients and other factors, so the sample size should be further expanded in the future.

Considering that this study is a single-centered with a small sample size, we will cooperate with other related research units to increase the sample size in the later stage, so as to provide evidence-based basis for clinical treatment. To sum up, GnRH-ant can increase the high-quality embryo rate and improve the pregnancy outcome of patients with the previous failed EFP long-term assisted pregnancy, and is more suitable for clinical application of controlled ovarian hyperstimulation compared with GnRH-a.

Acknowledgements

This work was supported by the Science and Technology Plan Project of Jiangxi Provincial Health Commission (202140699).

Disclosure of conflict of interest

None.

Address correspondence to: Hua Liao, Department of Reproduction and Genetics, Ganzhou Maternal and Child Health Centre, No. 100 Zhangjiang North Avenue, Ganzhou 341000, Jiangxi Province, China. Tel: +86-18779789239; E-mail: liaohua39@21cn.com

References

[1] Chen T, Zhao F, Wang Q, Liu C, Lan Y, Wang S, Xin Z and Yang X. Salpingectomy may decrease antral follicle count but not live birth rate for IVF-ET patients aged 35-39 years: a retrospective study. J Ovarian Res 2020; 13: 80.

[2] Yang S, Liang L, He YL, Chen XN, Wang Y, Li HZ, Li R and Wang HY. Gonadotropin releasing hormone agonist and standard dosage of human chorionic gonadotropin trigger improve the in vitro fertilization and embryo transfer/intracytoplasmic sperm injection treatment prognosis of patients with history of poor fertilization outcomes. Chin J Reprod Contracept 2019; 3: 185-190.

[3] Ma JR and Liu CY. Effect of gonadotropin-releasing hormone agonist on ovarian function in patients undergoing ovarian radical resection. Shaanxi Med J 2020; 1: 24-26, 36.

[4] Liu XH, Wu XH and Yang S. Changes and correlations of anti-MÜLlerian hormone and stem-cell factors in different ovarian reserve patients during gnrh-antagonist protocol and the effects on controlled ovarian hyperstimulation outcomes. Arch Gynecol Obstet 2019; 300: 1773-1783.

[5] Klobučar M, Pavlić SD, Car I, Severinski NS, Milaković TT, Badovinac AR and Pavelić SK. Mass spectrometry-based glycomic profiling of the total igg and total proteome N-glycomes isolated from follicular fluid. Biomol Concepts 2020; 11: 153-171.

[6] Orvieto R, Kirshenbaum M, Galiano V, Zilberberg E, Haas J and Nahum R. Stop GnRH-agonist combined with multiple-dose GnRH-antagonist for patients with elevated peak serum progesterone levels undergoing ovarian stimulation for IVF: a proof of concept. Gynecol Obstet Invest 2020; 85: 357-361.

[7] Sun HP and Yin HQ. Effect of GnRH antagonist immobilization regimen and gnrh agonist low-dose long-term regimen on initial IVF/ICSI therapy in patients with normal ovarian function infertility. Chin Matern Child Health Assoc 2020; 35: 3263-3266.

[8] Wang X Zhen JR, Sun ZY, Deng CY, Zhou YZ, Wang HB, Xiao YL and Yu Q. Comparison of low dose gnrh agonist long protocol and gnrh antagonist protocol in patients with diminished ovarian reserve. J Reprod Med 2020; 29: 311-316.

[9] Zhu MS and Zhong PW. Study on the effect of glucocorticoid combined with gnrh-α in the patients with infertility caused by mild endometriosis and influence for the sex hormones. Chin J Hum Sexuality 2019; 28: 51-55.

[10] Liang D, Zhang SY, Zhong PW, Zhu MS and Li WR. Evaluation on curative effect of glucocorticoid combined with gnrh-A on reducing recurrent early abortion in patients with mild endometriosis. Chin J Clin Obstet Gynecol 2020; 21: 145-149.

[11] Zhang J, Zhou XY, Chen YX, Zhang QY, Li Y, She J, Chen X and Chen SL. Effects of cetrorelix versus ganirelix in gonadotropin-releasing hormone antagonist cycles for preventing premature luteinizing hormone surges and on clinical outcomes of IVF-ET cycles. J South Med Univ 2019; 39: 1207-1212.

[12] Gobikrushanth M, Dutra PA, Bruinjé TC, Colazo MG, Butler ST and Ambrose DJ. Characterization of the variability and repeatability of gonadotropin-releasing hormone-induced luteinizing hormone responses in dairy cows within a synchronized ovulation protocol. J Dairy Sci 2017; 100: 6753-6762.

[13] Ararooti T, Niasari-Naslaji A, Razavi K and Panahi F. Comparing three superovulation protocols in dromedary camels: FSH, eCG-FSH and hMG. Iran J Vet Res 2017; 18: 249-252.

[14] Gao XX, Reng LP, Zhang AJ, Zhang SH, Wang H and Wang SY. Effects of short protocol with GnRH-a and GnRH-ant on pregnancy outcome in vitro fertilization. Chin J Birth Health Hered 2014; 22: 113-114, 140.

[15] Li ZS, Lu HO, Zhang YM, Wang YH and Yu YX. Effect of endometrial receptivity evaluation on clinical pregnancy rate in infertile patients on the day of transplantation. Chin J Pract Gynecol Obstet 2020; 36: 264-266.

[16] Xing W, Lin H, Li Y, Yang D, Wang W and Zhang Q. Is the GnRH antagonist protocol effective at preventing OHSS for potentially high responders undergoing IVF/ICSI? PLoS One 2015; 10: e0140286.

[17] Huang YN, Wang Y, Pei FL, Zheng CC, Zeng YY and Zeng B. Roche in different side of III~IV joint GNRH - a endometriosis infertile patients postoperative recurrence rate and pregnancy rate affect retrospective study. J Guangzhou Univ Tradit Chin Med 2020; 37: 850-855.

[18] Cai LS, Ou JP, Xing WJ, Cai LH, Li T and Tao X. Comparison of pregnancy outcomes between fixed and flexible gonadotropin-releasing hormone antagonist protocol in the fresh embryo transfer cycle. J New Med 2019; 50: 388-393.

[19] Ljubobratović U, Péter G, Sándor Z, Kugyela N and Rónyai A. The effect of hormonal preparation (Gonadotropins Vs. Gonadoliberins) on pre-seasonally obtained eggs and larvae quality in pikeperch (Sander lucioperca L.). Aquac Int 2019; 27: 1009-1024.

[20] Wei H, Li D, Wang L and Shao XG. Effect of GnRH antagonist on the endometrial receptivity in fresh embryo transfer cycle: a retrospective study. J Int Reprod Health/Fam Plann 2018; 37: 36-40.

[21] Guan WZ and Wang XX. Comparison of the effectiveness between gonadotropin-releasing hormone antagonist protocol and gonadotropin-releasing hormone agonist long protocol in patients older than 35 years old with normal ovarian response. Pract Pharm Clin Rem 2018; 21: 1166-1169.