LIBYAN JOURNAL OF MEDICINE
2019, VOL. 14, 1652058
https://doi.org/10.1080/19932820.2019.1652058

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REVIEW ARTICLE

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Effect of duration of gonadotropin releasing hormone agonist on the outcome of in vitro fertilization-embryo transfer in a short-acting long regimen

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ABSTRACT

Objective: To investigate the effect of the duration of gonadotropin releasing hormone agonist (GnRH-a) use on the outcome of in vitro fertilization and embryo transfer (IVF-ET) during the short-acting long-term hyperstimulation cycle.

Methodology: Clinical data from 776 patients receiving controlled ovarian stimulation (COS) after short-term regimen downregulation were retrospectively analyzed. According to the duration of GnRH-a, the patients were divided into 3 groups: Group A, 14 days for GnRH-a; Group B, 15–17 days for GnRH-a; and Group C, >18 days for GnRH-a. The clinical data, treatment and clinical outcomes were compared among the groups.

Results: There were no significant differences in fertilization rate, implantation rate, clinical pregnancy rate, abortion rate, ovarian hyperstimulation syndrome (OHSS) rate($P > 0.05$). The total costs in group A were significantly less than those in group B and C($P < 0.001$). The number of eggs and quality embryos generated in group A was significantly higher than that in groups B and C ($P = 0.014$, $P = 0.005$).

Conclusions: In the short-acting GnRH agonist long protocol, satisfactory IVF-ET pregnancy outcome was obtained with the use of GnRH-a for 14 days under the premise of lowering the receptor-regulating standard. Excessive application of GnRH-a will affect the number of eggs and embryos and increase the cost of medical treatment.

ARTICLE HISTORY
Received 19 February 2019
Accepted 29 July 2019

KEYWORDS
Short-acting long protocol; pituitary down-regulation; GnRH-a; clinical pregnancy rate; in vitro fertilization

1. Introduction

Since GnRH-a was first applied to COS in 1984, GnRH-a long protocols have developed into the most mature and commonly used COS protocol for IVF-ET [1]. GnRH-a can competitively occupy the pituitary GnRH receptors, preventing the pituitary from further reacting to endogenous GnRH, inhibiting the endogenous LH peak, avoiding premature ovulation in patients, and improving oocyte retrieval rates and the clinical pregnancy rate [2,3]. However, with the continuous advancement in clinical applications, there are still many problems to be discussed with respect to the use of GnRH-a. These include GnRH-a dose, continuous duration of use, and determination of the Gn start-up time At present, the conventional short-acting long protocol requires that GnRH-a be used until the day of HCG administration. Insufficient duration and dose of GnRH-a may then not inhibit the early LH peak, leading to early ovulation and decline in egg quality, thus affecting the clinical outcome of IVF [4]. And the excessive duration and dose of GnRH-a can cause pituitary hyperinhibition, which can lead to slow ovarian response and luteal insufficiency. Therefore, it is particularly important to determine the optimal dosage and use duration of GnRH. In clinical practice, we found that the short-acting GnRH-a reached the pituitary down-regulation standard after 14 days. However, whether GnRH-a should continue to be used and for how long after it has reached the down-regulation standard so as to initiate Gn has not been confirmed clinically. In this study, we retrospectively analyzed the clinical outcomes of patients receiving a short-acting GnRH agonist long protocol at our Center, and compared the relationships between different days of drug use and subsequent clinical outcomes, in order to explore the optimal duration for pituitary down-regulation.

2. Material and methods

2.1. Subjects

We performed a retrospective comparative study of 776 fresh IVF cycles with a GnRH agonist long protocol used for infertility due to tubal factors or male factors in our hospital between January 2015 and January 2018.

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Supplemental data for this article can be accessed here.

Inclusion criteria: ①Female patient was less than 40 years old; ②the basal follicle stimulating hormone (FSH) < 10 IU/L; ③BMI<30kg/m^2; ④both couples had normal karyotypes. Exclusion criteria: ①karyotype abnormality of couples; ②polycystic ovary syndrome (PCOS) [5]; 3. primary ovarian insufficiency (POI) [6]; ③combined with moderate and severe endometriosis; ④Uterine abnormality or combined with untreated hydrosalpinx .

The duration of GnRH-a use (down-adjustment days) in all patients ranged from 14 to 26 days. It was divided into 3 groups according to the use time for GnRH-a: Group A, the use days of GnRH-a was 14 d; Group B, the use days of GnRH-a was 15–17;. Group C, the use days of GnRH-a was greater than or equal to 18 d. The 3 groups all reached the standard of down-regulation (blood E_2 < 50 pg/mL and the thickness of the endometrium < 5.8 mm) on the 14 to 16 days of GnRH-a use.

2.2. Protocol

Basal (cycle day 3) serum levels of follicle-stimulating hormone (FSH), luteinizing hormone(LH), estradiol (E_2), and testosterone (T) were determined before entering an IVF cycle. All patients were treated with 0.1 mg of short-acting GnRH-a (Triptorelin, dabigat®, Ipsen Pharma Biotech, France or dairylin®, Ferring pharmaceutical Co., LTD, Switzerland) from the mid-luteal phase of the last menstrual cycle for downregulation. Human recombinant follicle-stimulating hormone(Gonal-f®, Merck Serono, Switzerland; or Puregon®, Merck Sharp & Dohme, USA) was administered daily with a fixed dose of 150–225 IU according to the size of the ovarian follicles and the level of FSH, LH, E_2, and after 4–7 days, Gn dose was adjusted according to the follicular development and hormone level of the patients, and r-LH (Luveris®, Merck, Switzerland) or hMG (menotrophin, Livzon Pharmaceutical Group Inc, China) was added in a timely fashion. When the diameter of 2 follicles ≥ 18 mm or 3 follicles ≥ 17mm, HCG (chorionic gonadotropin, Livzon Pharmaceutical Group Inc, China) was given at a dose of 6000–10,000 IU to induce oocyte maturation, On the day of HCG injection, serum LH, E_2 and P levels were measured. Thirty-six h after injection, eggs were obtained by puncture guided by vaginal B-ultrasonography. Embryos were graded by two professional embryologists according to the number of blastomeres and degree of fragmentation and transferred on day 3. Embryos of good quality were defined as having at least 2–4 cells on day 2, 6–8 cells on day 3, and the amount of fragmentation was less than 20%. Patients with OHSS risk (i.e. 15 eggs were obtained, E_2 > 3000 pg/mL on the day of retrieval and abdominal distension, or both ovaries showed diameters >70 mm on the transplantation day) underwent embryonic freezing, and we choose the appropriate time for frozen embryo transfer.

2.3. 0bvervational index

The primary observational indicators were LH level on the HCG day and clinical pregnancy rate. Secondary observation indicators were E_2、LH、and FSH levels on the day of Gn initiation; dosage of Gn used; duration of Gn used; E_2、P、LH levels on the HCG day; number of oocytes retrieved, fertilization rate, implantation rate, abortion rate, and OHSS rate. The economic observation indicators were down-regulation expenses and total expenses (including the down-regulation expenses and the Gn expenses).

2.4. Statistical analysis

All data were analyzed using statistical package SPSS version 23.0. Continuous data are presented as means ± standard deviation ($\bar{x} \pm s$) and categorical data as percentages (%).The differences were analyzed with independent-samples test and χ^2 test. P < 0.05 was considered to be significant.

3. Results

There was no significant difference in age, duration of infertility, proportion of primary infertility, BMI, basal sex hormone level or choice of assisted pregnancy among the groups (P > 0.05). (Table 1)

No cycles were cancelled due to ovarian hyporesponsiveness in any of the 3 groups, and no early LH peak and follicular luteinization occurred in any of the three groups, and no early ovulation occurred in any of the three groups. There was no significant difference in serum E_2 level of patients on the starting day (P > 0.05).The serum LH and FSH levels were as follows; group A > group B > group C; serum LH and FSH levels were significantly higher in patients from group A relative to patients from groups B and C (P < 0.05), while there was no statistically significant difference between group B and group C (P > 0.05). There were also no significant differences in dosage of Gn used, duration of Gn used, or dosage of HMG used. The serum hormone levels of LH (group A > group C > group B), E_2 (group A > group B > group C), P (group A > group C > group B) were significantly different among the three groups (P < 0.05). There were also significant differences among the three groups (P < 0.001) in the down-regulation cost and total cost (group C > group B > group A) (Table 2).

There were no significant differences in fertilization rate, fresh embryo transfer rate, number of available embryos, implantation rate, clinical pregnancy rate, abortion rate, OHSS rate among the three groups (P > 0.05). However, there were significant differences among the three groups in the number of oocytes retrieved and the total good quality embryo rate (group A > group B > group C) (Table 3).

Table 1. General information of patients (Mean ± SD).

Indices	Group A	Group B	Group C	P value
No. of cases	288	315	173	
Age(Year)	31.2 ± 4.6[1]	31.1 ± 4.4	31.8 ± 4.8	0.192
Infertility duration(year)	3.15 ± 2.24	3.38 ± 2.23	3.31 ± 2.54	0.243
Percentage of primary infertility(%)	46.9(135/288)	49.5(156/315)	42.8(74/173)	0.359
Body mass index(kg/m^2)	23.82 ± 3.78	23.28 ± 3.65	23.25 ± 3.86	0.094
Basal FSH (mIU/mL)	6.79 ± 1.67	6.68 ± 1.59	6.80 ± 1.47	0.557
Basal LH (mIU/mL)	5.47 ± 2.36	5.33 ± 2.15	5.22 ± 2.23	0.584
Basal E2 (pg/mL)	39.43 ± 16.89	38.92 ± 15.71	42.62 ± 18.18	0.079
Basal P (ng/mL)	0.44 ± 0.25	0.43 ± 0.28	0.42 ± 0.28	0.301
Method of ART				
IVF(%)	79.9%(230/288)	70.8%(223/315)	72.3%(125/173)	0.567
ICSI(%)	20.1% (58/288)	29.2% (92/315)	27.7%(48/173)	

Table 2. Clinical data and treatment costs relative to the down-regulation protocol (Mean ± SD).

Indices	Group A	Group B	Group C	P value
No. of case	288	315	173	
Down-regulation duration (d)	14.00 ± 0.00	15.01 ± 0.59[1)]	15.00 ± 0.59[1)2)]	<0.001
Total days of GnRH-a used(d)	14.0 ± 0.0	16.3 ± 0.8[1)]	20.4 ± 2.2[1)2)]	<0.001
Starting day				
E_2 (pg/mL)	26.53 ± 10.87	26.27 ± 10.92	25.64 ± 11.68	0.701
LH (mIU/mL)	2.07 ± 0.84[2)3)]	1.94 ± 0.84	1.87 ± 0.60	0.020
FSH(mIU/mL)	3.94 ± 1.08[2)3)]	3.76 ± 1.01	3.67 ± 1.35	0.022
Dosage of Gn used (IU)	2577 ± 1085	2390 ± 1040	2502 ± 1067	0.094
Duration of Gn used (d)	11.64 ± 2.14	11.92 ± 2.40	11.13 ± 2.54	0.052
hCG day				
E_2 (pg/mL)	3537 ± 1425[2)3)]	3121 ± 1376	3116 ± 1381	<0.001
P (ng/mL)	1.19 ± 0.60[2)3)]	1.52 ± 1.67	1.32 ± 0.71	<0.001
LH (mIU/mL)	1.86 ± 2.75[2)3)]	1.17 ± 1.15[3)]	1.38 ± 1.29	0.003
Down-regulation expenses(¥)	885 ± 135	1285 ± 224[1)]	1804 ± 4405[1)2)]	<0.001
Total expenses (¥)	4340 ± 966	4701.±858[1)]	5339 ± 4535[1)2)]	<0.001

1)P < 0.05 vs. Group A; 2)P < 0.05 vs. Group B; 3)P < 0.05 vs. Group C;

Table 3. Laboratory indicators and pregnancy outcomes(Mean ± SD).

Indices	Group A	Group B	Group C	P value
No. of case	288	315	173	
No. of oocytes retrieved	14.05 ± 8.00	13.54 ± 7.40	12.12 ± 7.76	0.014
Fertilization rate (%)				
IVF	62.8	64.3	65.1	0.227
ICSI	78.4	80.1	82.8	0.207
Total good quality embryo rate (%)	52.1	49.0	46.6	0.005
	(976 + 187/1820 + 413)	(889 + 304/1684 + 751)	(449 + 133/877 + 373)	
Fresh embryo transfer rate (%)	41.7(120/288)	42.2(133/315)	39.9(69/173)	0.892
Frozen embryo transfer rate (%)	58.3 (168/288)	57.8(182/315)	60.1(104/173)	
No. of available embryos	1.7 ± 0.5	1.8 ± 0.4	1.8 ± 0.5	0.496
Implantation rate(%)	39.1(186/476)	41.2(222/539)	36.2(112/197)	0.364
Clinical pregnancy rate (%)	47.2(136/288)	53.0(167/315)	45.1(78/173)	0.178
Abortion rate (%)	3.7(5/136)	2.4(4/167)	6.4(5/78)	0.298
OHSS rate (%)	5.6(16/288)	3.8(12/315)	3.5(6/173)	0.463

4. Discussion

Porter first reported the application of Gn combined with GnRH-a for COS in 1984 [7], After more than 30 years of application and improvement, the short-acting GnRH-a long protocol is now widely used in clinical practice because of its high number of retrieved eggs [8], and has gradually developed into one of the most commonly used COS protocols in IVF-ET. GnRH-a exhibits a structure of amino acids substitutions in the 6th and 10th positions of the natural GnRH decapeptide. These changes make the molecule difficult to undergo cleavage by endopeptidase in vivo. The stability of GnRH-a is thereby enhanced, its half-life is prolonged, and its affinity with the GnRH receptor is greatly increased. Short-term stimulation occurs at the initial stage of administration, and then, with GnRH-a persisting, most of the receptors are occupied and migrate to the cells, such that the loss of GnRH receptors on the surface of the pituitary cells cannot be replenished [2,9].

The traditional long protocol implies that GnRH-a be used from the mid-secretory phase to the HCG administration days [2]; however, there is still controversy regarding the duration of GnRH-a in the clinic. If short-acting GnRH-a is used for a brief period or in a small amount, this results in incomplete down-regulation

and early-onset LH peak, leading to an increase in the cycle-canceling rate. However, if short-acting GnRH-a is used for too long or in a much greater amount, it may inhibit LH surge release and reduce ovarian reactivity, reduce the quality of eggs, affect the function of the corpus luteum, and even affect the secretion of ovarian hormones [10]. Studies have shown that appropriately extending the administration time of GnRH-a can increase the synchronization of follicular development [11] and improve the quality of oocytes and embryos [12]. Other studies have shown that increasing the duration of GnRH-a use can lead to prolonged ovarian stimulation and increased Gn dosage requirements [13].According to the patient's situation, the dose of GnRH-a is usually 0.05 mg or 0.1 mg, and the duration of use varies from 14 to 26 days. Moreover, the Gn is started after 14 to 16 days of GnRH-a used when sufficient down-regulation occurs .

The results show that all the patients in the 3 groups obtained satisfactory effects of down-regulation and had no precocious LH peak or early ovulation. The total amount of Gn used in group A was higher than that in groups B and C, but there was no significant statistical difference ($P = 0.094$). The probable reason may be the promotion of Gn levels with deeper pituitary inhibition so as to restore them to a certain extent, increasing the sensitivity to Gn and thus reducing the dosage of Gn; this is consistent with the findings of De Placido et al [14]. In group A, the levels of FSH and LH on Gn startup days and HCG days were higher than those in groups B and C, and the difference was statistically significant. This indicates that the long-term application of GnRH-a has stronger inhibitory effect on the pituitary. In the process of follicular growth, LH plays two roles: first, it directly acts on theca cells to produce androgen, as a substrate, and androgens enhance the activity of aromatase and stimulate granulosa cells to produce estrogen; and second, in the middle stages of follicular growth, the paracrine function of the ovary is induced, promoting the growth and development of theca cells, thereby promoting egg maturation [15,16]. In our study, the number of eggs obtained and the quality embryo rate in group A were higher than these indices in groups B and C, and the difference was statistically significant ($P < 0.05$). The results showed that the long usage of GnRH-a could inhibit LH excessively and decrease ovarian responsiveness, egg quality and embryo quality, supporting the concept of LH 'threshold' proposed by Balasch and Fabregues [17]. Although the LH level of group A was higher than that of groups B and C at the start day of Gn, the progesterone level of group A on the day of HCG administration was lower than that of groups B and C, which was inconsistent with previous studies [18]. During follicular development, LH acts on theca cells and granulosa cells to promote the transformation of cholesterol into progesterone. In this study, the level of LH in groups B and C was lower on the Gn start day, but the added dose of HMG (75IU FSH,75IU LH) was higher than that in group A, which may be the reason for the higher P levels in groups B and C. In addition, the simultaneous development of multiple follicles leads to enhanced activity of granulosa cells, which further promotes the generation of P [19].

Although the duration of GnRH-a used was different, as was the degree of pituitary inhibition – which led to the differences in serum hormone levels on the Gn and HCG start days, we observed no early LH peak or early ovulation in any of the three groups, and there were no significant differences in embryo implantation rate, clinical pregnancy rate, abortion rate, or OHSS incidence among the three groups ($P > 0.05$). In terms of cost, group A was lower than groups B and C, and the difference was statistically significant ($P < 0.001$).

In summary, in the process of superovulation where a short-acting GnRH-a long protocol is used, GnRH-a can be used for only 14 days to obtain a satisfactory pregnancy outcome under the premise of achieving appropriate down-regulation. Excessive down-regulation may affect the number of eggs obtained and the quality of the embryo, and can increase the patient's cost with COS. It is the responsibility of every clinician to choose an effective COS protocol while reducing the cost to patients and saving medical resources. Using GnRH-a down-regulation for 14 days and discontinuing after initiating Gn may not only ensure a good clinical outcome but also reduce the economic burden to the patient. Although this protocol comprises a flexible ovulation-stimulation scheme, our results still need to be confirmed in a large-sample, prospective clinical study.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The present study was supported in part by Natural Science Foundation of China (Grant No.81373676; 81674018) and the Key Development Program of Shandong Province (NO.2017G006016).

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